# PHYSICOCHEMICAL PROPERTIES AND PROTEIN DIGESTIBILITY OF TUNA BY-PRODUCTS OBTAINED THROUGH ENZYMATIC HYDROLYSIS

by

**HERPANDI**

**Thesis Submitted in fulfillment of the requirements for the degree of Doctor of Philosophy**

**February 2014**

# ACKNOWLEDGEMENTS

I sincerely thank Allah, my God, the Most Gracious and Most Merciful for enabling me to complete my Ph.D. successfully and for often putting so many good people in my way. In completing my Ph.D. thesis I owe a great debt to many people. I wish to extend my deep thanks gratitude and appreciation to everyone who has contributed to the successful completion of my thesis. First and foremost, I would like to express my sincere thanks, gratitude and deep appreciation to my supervisor, Prof. Madya. Dr. Nurul Huda and my co-supervisors Prof. Madya. Dr. Rosma Ahmad and Puan Wan Nadiah Wan Abdullah, for their sincere effort, interest and time they have kindly spent to guide my research, also for excellent supervision, intellectual guidance and invaluable comment, without which it would have been impossible to complete my thesis. I would like to gratefully acknowledge and thank the Universiti Sains Malaysia Fellowship, the Universiti Sains Malaysia Research University Postgraduate Research Grant Scheme (USM-RU-PGRS) for funding my research project. A heartfelt thank you goes out to all the laboratory assistants of School of Industrial Technology, Universiti Sains Malaysia, especially Mr. Maaruf, Mrs. Mazura, Mr. Rahim, Mr. Abdul Ghoni, Mr. Asmaizan, Mr. Alfendy, Mr. Firdaus, for assisting me in various laboratory activities and providing valuable suggestions for my research. My research could not have been completed without the contributions of my laboratory mates and friends, especially Mr. Ariefandi, Mrs.Tina, Mrs. Nopi, Ms. Pales, Mr. Kurnia, Mrs. Titik who stood by me through thick and thin, and generously assisted me in any way possible. I deeply appreciated

all my friends in Indonesian Student Association, especially Nofrizal, Arnawan, Elviandi, Rina, Wiedya, Ina, Erwin, Raye, Dody, Aan, Bambang and Dolok for all the moment that we spent together, for all the comforting words through rough times in our study. My sincere thank goes to my beloved wife, Mrs. Rini Marlina, for the incredible patience and support she showed me in my time of need, also to my wonderful children (Faiz and Keyla), for always becoming my source of strength and inspiration. I also extend my thanks to my family, including my in-laws, who have always supported me throughout my study. Last but not least, no words are ever sufficient to express my everlasting gratitude, appreciation and thanks to my beloved, wonderful mother and my late father, my parent in-law, your prayers and unwavering supports of everything I do have helped make me who I am today.

Herpandi

Penang, January, 28, 2014

# TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# LIST OF PLATES

# LIST OF ABBREVIATION

| | |
|---|---|
| AABA | Alpha-amino butiric acid |
| AAI | Antioxidant activity indexes |
| AAS | Atomic absorption spectrophotometer |
| ABAB | 2,2'-azo-bis(2-aminopropane) |
| ABTS | 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid |
| ACE | Angiotensin-converting enzyme |
| ALB | Albacore |
| ANOVA | Analysis of variance |
| AOAC | Official Methodes of Analysis of the Association of Agricultural Chemists |
| BCTFA | British Columbia Tuna Fishermen's Association |
| BET | Bigeye tuna |
| BFT | Atlantic bluefin tuna |
| BHA | Butylated hydroxyanisole |
| BHT | Butylated hydroxytoluene |
| BV | Biological value |
| CCD | Central composites designs |
| C-PER | Calculated protein efficiency ratio |
| CRFD | Completely randomized factorial design |
| CV | Coefficients of variations |
| Da | Dalton |

| | |
|---|---|
| DH | Degree of hydrolysis |
| DHA | Docosahexanoic acid |
| DNA | Deoxyribonucleic acid |
| DPPH | 2,2'-Diphenyl-1-picrylhydrazil |
| EAA | Essential amino acid |
| EAAI | Essential amino acid index |
| EAI | Emulsifying activity index |
| EC | Emulsifying capacity |
| EPA | Eicosapentanoic fatty acid |
| ES | Emulsifying stability |
| ESI | Emulsion stability index |
| ET | Electron transfer |
| FADs | Fish Aggregation Devices |
| FAO | Food and Agriculture Organization |
| FC | Foaming capacity |
| FCC | Food Chemical Codex |
| FECFA | Joint FAO/WHO Expert Committee on Food Additives |
| FPH | Fish protein hydrolysates |
| FPH-A | Skipjack dark flesh protein hydrolysate produced using Alcalase® 2.4L FG |
| FPH-C | Commercial fish protein hydrolysate |
| FPH-P | Skipjack dark flesh protein hydrolysate produced using Protamex® |
| FRAP | Ferric reducing antioxidant power |
| FS | Foam stability |
| FT | Free tryptophan |

| | |
|---|---|
| FTIR | Fourier transform infrared |
| GPC | Gel permeation chromatography |
| HPLC | High performance liquid chromatography |
| IUBMB | The International Union of Biochemistry and Molecular Biology |
| NOAA | National Oceanic and Atmospheric Administration |
| NPR | Net protein ratio |
| NPU | Net protein utilization |
| OHC | Oil holding capacity |
| OPA | Osmometric, *o*-phthaldialdehyde |
| ORAC | Oxygen radical absorbance capacity |
| PBF | Pacific bluefin tuna |
| PDCAAS | Protein digestibility-corrected amino acids score |
| PDI | Protein dispersibility index |
| PER | Protein efficiency ratio |
| PPDI | Pensin-pancreatin digest index |
| PSI/NSI | Protein or nitrogen solubility index |
| PUFA | Polyunsaturated fatty acid |
| RFD | Completely randomized factorial design |
| RNS | Reactive nitrogen species |
| RNV | Relative nutritive value |
| ROS | Reactive oxygen species |
| RSA | Radical scavenging activity |
| RSM | Response Surface Methodology |
| SBF | Southern bluefin tuna |
| SKJ | Skipjack tuna |

| | |
|---|---|
| SN-TCA | Soluble nitrogen-trichloroacetic acid |
| TCA | Trichloroacetic acid |
| TEAC | Trolox equivalent capacity |
| TIS | Tripsin indigestible |
| TNBS | 2,4,6-trinitrobenzenesulfonic acid |
| TPTZ | Tripyridyltriazine |
| TRAP | Trapping antioxidant parameter |
| USA | United States of America |
| VCEAC | Vitamin C equivalent antioxidant capacity |
| WDP | Water-dispersible protein |
| WHC | Water holding capacity |
| WHO | World Health Organization |
| WSP | Water-soluble protein |
| YFT | Yellowfin tuna |

# CIRI-CIRI FIZIKOKIMIA DAN KEBOLEHCERNAAN PROTEIN HASIL SAMPINGAN TUNA YANG DIPEROLEH MELALUI HIDROLISIS BERENZIM

## ABSTRAK

Penghasilan protein hidrolisat ikan merupakan kaedah alternatif penggunaan hasilan sampingan ikan yang semakin giat dijalankan. Protein hidrolisat ikan yang dihasilkan daripada hasilan sampingan ikan berpotensi untuk diguna dalam industri makanan. Namun begitu, penggunaannya masih terhad disebabkan rasanya yang pahit. Empat jenis enzim Neutrase® 1.5MG, Flavourzymes® 500 MG, Protamex® dan Alcalase® 2.4L FG telah dipilih untuk menentukan kesannya terhadap proses hidrolisis bahagian otot gelap ikan tuna skipjack. Enzim Alcalase® 2.4L FG dan Protamex® didapati lebih baik berbanding enzim Neutrase® 1.5MG dan Flavourzyme® 500 MG di mana hidrolisat protein ikan yang dihasilkan mempunyai darah hidrolisis yang tinggi dan kandungan tryptophan-bebas yang rendah. Proses pengoptimaan menggunakan kaedah response permukaan berpusat menggunakan empat faktor (pH, kepekatan, suhu, dan masa hidrolisis) mendapati keadaan optimum hidrolisis Protamex® ialah pada suhu 58 °C, pH 6.57 dengan kepekatan 3% selama 4 jam menghasilkan 18.48% darjah hidrolisis (DH) dan nilai tryptopan-bebas (FT) yang boleh diterima iaitu 72.10 mg kg-1. Sebaliknya, pengoptimuman Alcalase® 2.4L FG pada suhu 65.41 °C, pH 8.87 kepekatan 2.04% selama 5.73 jam menghasilkan 20.0% DH dan 107.20 mg kg-1 FT. Hidrolisis Alcalase® 2.4L FG dan Protamex® menghasilkan FPH yang berwarna lebih terang, kandungan protein yang tinggi dengan masing-masing 76.52 dan 70.88% dan kandungan natrium dan

magnesium yang tinggi. FPH-P mempunyai lebih banyak penyebaran peptide (91%) berat molekul sederhana dalam julat 1000-4000 Da (91%) tanpa peptida berat molekul rendah (<1000 Da), manakala FPH-A hampir 80% terdiri daripada peptida berat molekul rendah (<1000-3000 Da). Kedua-dua FPH-A dan FPH-P mempunyai kebolehlarutan yang sangat baik pada semua pH yang dikaji dan keupayaan memegang air yang tinggi, dengan kapasiti membuih lebih daripada 120% dan menunjukkan kecenderungan yang sama dalam ciri-ciri mengemulsi. FPH-A dan FPH-P mempunyai kualiti protein yang baik (profil asid amino, skor kimia, indeks penting asid amino, nisbah kecekapan protein, nilai biologi) dengan penghadaman protein in-vitro FPH-P sehingga 63.8% dan FPH -A sehingga 64.9%. Kedua-dua sampel tersebut juga menunjukkan aktiviti antioksidan dan '*chelating*' yang baik. Kajian ini telah berjaya menyelesaikan masalah utama protein hidrolisat ikan dengan menghasilkan produk dengan kurang rasa pahit yang lebih berpotensi untuk digunakan dalam makanan dan makanan haiwan.

# PHYSICOCHEMICAL PROPERTIES AND PROTEIN DIGESTIBILITY OF TUNA BY-PRODUCTS OBTAINED THROUGH ENZYMATIC HYDROLYSIS

## ABSTRACT

Fish protein hydrolysate (FPH) is one of the emerging alternatives of the utilization of fish by-product, with a potential application in food industry. However, the application is still limited due to the occurrence of intense bitterness. Four commercially available enzymes including Neutrase® 1.5MG, Flavourzymes® 500 MG, Protamex® and Alcalase® 2.4L FG were chosen determine their ability to hydrolysis skipjack tuna dark flesh. Alcalase® 2.4L FG and Protamex® were found to give higher degree of hydrolysis and low content of free tryptophan in the FPH, compared to Neutrase® 1.5MG and Flavourzymes® 500 MG. Optimization process using response surface methodology employing four factors (pH, concentration, temperature, and time of hydrolysis) suggested an optimal condition of protamex hydrolysis for 4 hours at 58 °C. pH 6.57 using 3 % Protamex®, resulting in 18.5% DH and an acceptable value of FT of 72.10 mg $kg^{-1}$. Optimization using Alcalase® 2.4L FG resulted in 20.0% of DH and 107.20 mg $kg^{-1}$ of FT at the optimum condition of 65.4 °C, pH 8.87 using 2.04% alcalase for 5.73 hours. The FPH obtained from the optimal condition of hydrolysis by Alcalase® 2.4L FG and Protamex® exhibited bright color, high protein content with mean percentage of 76.52 and 70.88%, respectively and had high content of sodium and magnesium. FPH-P had higher molecular distribution (91%) of medium molecular weight

peptides at the range of 1000-4000 Da without low molecular peptides (<1000 Da), while FPH-A mostly composed (almost 80%) of low to medium molecular weight peptides (<1000-3000 Da). Both FPH-A and FPH-P had excellent solubilities at all pH studied and had high water holding capacity, with foaming capacity more than 120% and similar emulsifying properties. Both FPH-A and FPH-P had good protein quality (amino acid profile, chemical score, essential amino acid index, protein efficiency ratio, biological value) with the in vitro protein digestibility of 63.8% and 65% for FPH-P and FPH-A respectively. They also showed good antioxidant activity and chelating activity. Thus, this study successfully able to produce low bitterness fish protein hydrolysate which have potential to be used for feed and food application.

# CHAPTER 1 INTRODUCTION

## 1.1 Background

Hydrolysates can be defined as protein that are chemically or biologically broken down into peptides of varying sizes. Although chemical hydrolysis is more commonly used in industrial practice, biochemical hydrolysis holds the most promise for the future because it results in food grade products of high functional and nutritive value. Biochemical hydrolysis is performed by utilizing enzymes to hydrolyze peptide bonds. This can be done via proteolytic enzymes already present in fish viscera or by adding enzymes from other sources. The process of using enzymes offers many advantages because it allows good control of the hydrolysis at a low cost with good properties of resulting products. By applying enzyme technology, it may be possible to produce a broad spectrum of food ingredients or industrial products for a wide range of applications (Kristinsson and Rasco, 2000b).

Protein hydrolysates are produced for a wide variety of uses in the food industry, including milk replacers, protein supplements to cereal food, soups, bread and crackers, stabilizer in beverages and flavour enhancers in confectionary products (Venugopal and Shahidi, 1994). Fish protein hydrolysates could find potential use as functional food ingredients as emulsifier and binder agents (Wasswa *et al.*, 2007). Pacheco-Anguilar *et al.* (2008) reported that hydrolysates from Pacific whiting muscle produced by commercial protease have good functional properties indicating their possible use in different food systems. However, further research including real

food system is recommended. The use of commercial enzymes for production of highly functional hydrolysate from marine species of low commercial value can be a feasible technology to make the most of a vast underutilized resource and for use as a food ingredient for direct human consumption. Klompong *et al.* (2007) found that when the degree of hydrolysis increased, the interfacial activities (emulsion activity and stability index, foaming capacity and stability) of hydrolysates decreased, possibly caused by the shorter peptide chain length. At the same degree of hydrolysis, the functionalities of protein hydrolysates depended on the enzyme used. Enzymatic modification was responsible for the changes in protein functionality.

Fish protein hydrolysates (FPH) from cod, salmon and saithe contained high levels of taurine, potassium and B-vitamins. Salmon FPH was particularly rich in niacin and panthothenic acid (Liaset and Espe, 2008). The cod and saithe insoluble peptide fractions contained high levels of the indispensable amino acid including tryptophan and of trace elements selenium, iron and zinc. Research using animal studies for nutritional evaluation had also been attempted. Liaset *et al.* (2000) reported that the nutritional evaluation of the FPH made from cod frame hydrolyzed by alcalase and subsequently by kojizyme resulted in high nitrogen balance, net protein utilization, biological value and protein digestibility at 10% FPH-N inclusion level. The FPH was rich in low molecular-weight peptides and low in free amino acid.

In addition, protein hydrolysates from fish sources also have been found to possess antioxidant activities (Klompong *et al.*, 2007, 2009; Je *et al.*, 2005; 2007; 2008, Thiansilakul *et al.*, 2007; Dong *et al.*, 2008, Raghavan and Kristinsson, 2008). Moreover, preliminary data suggested that hydrolysated fish protein could represent

an interesting source of anticancer peptides (Picot *et al.*, 2006), anti anemia agent (Shang-gui, 2004; Dong *et al.*, 2005) and components of microbial growth media (Guerard *et al.*, 2002; Aspmo *et al.*, 2005; Martone *et al.*, 2005; Vasileva-Tonkova *et al.*, 2007; Vanquez *et al.*, 2008).

Enzymes used to hydrolyze fish protein have at least one common characteristic: they have to be food grade and if they are of microbial origin, the producing organism has to be non-pathogenic (Pedersen, 1994). The variety of food-grade proteolytic enzymes is wide and offers enzymologists good opportunity to produce fish by-product hydrolysates. The most common commercial proteases reported used for the hydrolysis of fish protein are from plant sources such as papain (Hoyle and Maerrit, 1994; Shahidi *et al.*, 1995) or from animal origin, such as pepsin (Vieira *et al.*, 1995), chymotrypsin and trypsin (Simpson *et al.*, 1998). Enzymes of microbial origin have been applied to the hydrolysis of fish proteins. In comparison to animal or plant derived enzymes, microbial enzymes have other several advantages including a wide variety of available catalytic activities, as well as greater pH and temperature stabilities (Diniz and Martin, 1997). From a technical and economical point of view, microbial enzymes such as alcalase operating at alkaline pH have been reported to be most efficient in the hydrolysis of fish proteins (Dufosse *et al.*, 2001). Other enzyme preparations have shown excellent potential for hydrolyzing fish protein to make highly functional fish protein hydrolysates including Protamex (Choi *et al.*, 2009), Flavourzyme, Corolase (Kristinsson and Rasco, 2000a), Umamizyme (Guerard *et al.*, 2002) and Kojizyme (Nilsang *et al.*, 2005).

There are several reports about enzyme application for hydrolysis of different fish processing by-products and the under-utilized fish species. These include Mullet (Rebeca *et al.,* 1991), Shark (Onodenalore and Shahidi, 1996; Diniz and Martin, 1997), Herring (Hoyle and Merrit, 1994), Sardine (Dong *et al.*, 2005), Pollack (Je *et al.*, 2005), Capelin (Shahidi *et al.*, 1995), Mackerel (Wu *et al.*, 2003), Salmon (Kristinsson and Rasco, 2000a; Liazet *et al.*, 2000), Pacific whiting (Benjakul and Morrissey, 1997; Pacheco-Anguilar *et al.*, 2008), Yellowfin tuna (Guerard, 2001, 2002), Cod (Gilmartin and Jervis, 2002; Aspmo *et al.*,, 2005; Slizyte *et al.*, 2005), Hake (Martone *et al.*, 2005), Catla-catla ( Bhaskar *et al.*, 2008), Gold carp (Sumaya-Martinez *et al.*, 2005), Silver carp (Dong *et al.*, 2008), Grass carp skin (Wasswa *et al.,* 2007), Round scad (Thiansilakul *et al.*, 2007), Small croaker (Choi *et al.*, 2009), Black tilapia (Abdul Hamid *et al.*, 2002) and Threadfin bream (Normah *et al.*, 2005). However, there is still a lack of research work carried out on hydrolysis of fish protein by-product.

Meanwhile, tuna (*Thunus* sp) and tuna-like species are economically very important and significant source of food. Their global production has tended to increase continuously from less than 0.6 million ton in 1950 to almost 5 million ton today (FAO, 2013d). Approximate contributions of individual principal market tuna species to their 2011 total catch are : Albacore (ALB) 5.4%, Atlantic bluefin tuna (BFT), Pacific bluefin tuna (PBF) and Southern bluefin tuna (SBF) less than 1%, Bigeye tuna (BET) 10%, Yellowfin tuna (YFT) 24% and the highest production of all is Skipjack tuna (SKJ) with percentage production of 59.1%. Tuna is generally processed for raw meat and marketed as loins/steaks or as a canned food. Due to global competition, the profit margin on tuna loins/steaks is limited. In the canning

process, only about one-third of the whole fish is available for value addition. The tropical species of skipjack and yellowfin are mostly used for canning. Because of that situation, they fetch lower prices than the *tuna* used for sashimi such as bluefin and bigeye (bigeye is tropical species). Guerard *et al.* (2002) reported that solid wastes from the canned fish processing industry composed of muscle after loins are taken, fish viscera, gills, flesh dark/dark muscle, head, bone, and skin, can be as high as 70% of the original material. Sultanbawa and Aksnes (2006) reported processing discards from tuna canning industry are estimated at 450000 million tons annually. They concluded that the tuna industry must, therefore, look at avenues to add value to tuna processing discards. Although tuna industry in Malaysia is relatively smaller compared to other countries like Thailand and Indonesia, the government's pioneering efforts to make the country a major player in international tuna trade is commendable. To this end, the Malaysian government has identified one of the ports in Penang Island as an international tuna port as a catalyst for tuna industry in Malaysia (Binyamin, 2006).

Protein-rich by-products from the canning industry, especially dark flesh of the fish, have limited uses due to their darker color, susceptibility to oxidation and off flavour. Consequently, they are discarded or processed into low market-value products, such as fish meal and fertilizer. Recovery and alteration of fish protein present in the by-product material, and use as fish protein functional ingredient in food systems is a very exciting and promising alternative. Hydrolysis process is one of the methods that have been developed to convert fish by-products and under-utilized fish into the marketable and acceptable forms (fish protein hydrolysates) which can be widely used in food systems (Kristinsson and Rasco, 2000b).

The main goal of fish by-products hydrolysis is to obtain the maximum possible recovery of all valuable components while maintaining a high quality hydrolysate. Bitterness is a major problem affecting the sensory acceptability of protein hydrolysates. However, some commercial enzymes can minimize the bitterness in the hydrolyzed product (Liaset *et al.*, 2000). Enzymatic hydrolysis of protein is common a way to improve the properties of protein. The properties of protein hydrolysates are determined by the degree of hydrolysis and by the structure of the peptides produced. These in turn are dependent on the nature of the protein and the specificity of the enzyme used, as well as on the hydrolysis conditions, particularly pH and temperature. The choice of enzyme for a given application depends on the substrate and the desired properties of the final hydrolysates. Degradation of protein renders it more soluble. Other functional properties, such as emulsifying, foaming, viscosity, gelatinization and water absorption capacity are also affected by the hydrolysis. Thus, one of alternative technique to utilize this by-product is by converting it to become FPH as dark flesh skipjack tuna which still has functional properties. Yet, the scientific information regarding the FPH from dark flesh skipjack tuna are still lacking. Thus, this research will analyse the potential production of FPH from dark flesh skipjack tuna as an alternative functional fish by-product.

## 1.2 Objective

The main objectives of this project were to evaluate enzymatic preparations of low-bitterness protein hydrolysates from tuna (*Thunnus* spp.) by-products and to evaluate physicochemical characteristics, digestibility and antioxidative properties of the hydrolysates.

The specific objectives were:

1. To study the effect of different industrial proteases on the hydrolysis of skipjack tuna by-products from the fish-processing industry
2. To optimized the combined effects of pH, temperature, time and enzyme concentration of a selected protease on the DH and FT content during the hydrolysis of skipjack tuna by-products from the fish-processing industry
3. To evaluate the physicochemical properties of the resulted hydrolysate from skipjack tuna by-products
4. To evaluate the functional properties of the resulted hydrolysate from skipjack tuna by-products
5. To assess the protein quality and digestibility of the resulted hydrolysate from skipjack tuna by-products
6. To analyze the antioxidative properties of the resulted hydrolysate from skipjack tuna by-products
7. To determine the sensory attribute (bitterness) of the resulted hydrolysate from skipjack tuna by-products

# CHAPTER 2 LITERATURE REVIEW[1]

## 2.1 Tuna Fishing Industry

### 2.1.1 Tuna distribution

Tuna (*Thunnus* spp.) and tuna-like species have long been known as a main commodity of fisheries. The principal market of tuna frequently is divided into tropical tuna (i.e., bigeye (*T. obesus*), skipjack (*Katsuwonus pelamis*), yellowfin tuna (*T. albacares*)) and temperate tuna (i.e., albacore (*T. alalunga*), Atlantic bluefin tuna (*T. thynnus*), Pacific bluefin tuna (*T. orientalis*), and southern bluefin tuna (*T. maccoyii*)). In addition to the principal market tuna, many other types of tuna are more neritic and live in seas over the continental shelf (e.g., longtail tuna (*Thunnus tonggol*), blackfin tuna (*Thunnus atlanticus*), and black skipjack tuna (*Euthynnus lineatus*)). Important tuna-like species that are caught in recreational and sport fisheries include billfishes (*Istiophoridae*), king mackerels (*Scomberomorus cavalla*), and butterfly kingfish (*Gasterochisma melampus*). They swarm in oceans all over the world (Majkowski, 2007).

---

[1]Part of this chapter has been published. Herpandi, Huda, N., Rosma, A., & Wan Nadiah, W. A. (2011). The Tuna Fishing Industry: A New Outlook on Fish Protein Hydrolysates. *Comprehensive Reviews in Food Science and Food Safety*, *10*(4), 195-207.

The vertical distribution of most species of tuna is influenced by the thermal and oxygen structures of the water column (FAO, 2013b). Small-sized tuna species and juveniles of species that attain large sizes tend to live near the surface, whereas adults of large species inhabit deeper waters. The use of deep longlines showed that bigeye tuna can be found at depths as great as 300 m. Albacore are also caught using Fish Aggregation Devices (FADs) at depths to about 200 m. Acoustic telemetry has shown that billfishes are found near the sea surface during the day, but they frequently descend to greater depths at night (FAO, 2013b).

Most tuna and tuna-like species are highly mobile and in many instances undertake extensive migrations. Skipjack tuna is a pelagic species that can be found in tropical, subtropical, and warm temperate waters. It migrates extensively between the central Pacific and the coastal waters of both the Eastern Pacific and Japan. Moreover, it can be found from Massachusetts to Brazil, including the Gulf of Mexico and the Caribbean in the Atlantic. Southern Bluefin tuna, which lives only in the southern hemisphere, migrates from spawning areas around Australia to the Atlantic, Pacific, and Indian Oceans. In South Australia, southern Bluefin tuna is captured in the wild between December and March and then is farmed for 6–9 months in an open water environment (Cleanseas, 2010; FAO, 2013c; NOAA, 2010).

The Atlantic Bluefin tuna, also known as northern Bluefin tuna, is a subtropical pelagic fish. It is distributed mainly in Western Atlantic areas such as Canada, the Gulf of Mexico, and the Caribbean Sea to Venezuela and Brazil. In addition, it is found around the Lofoten Islands off Norway to Canary Island, the Mediterranean, and the southern part of the Black Sea. In the Pacific Ocean, northern Bluefin tuna migrates between the near-shore waters off Canada, Mexico, and the

United States and Japanese waters. In contrast, albacore is a highly migratory cosmopolitan fish that can be found in tropical and temperate waters of all oceans and the Mediterranean Sea (except at the sea surface between 10°N and 10°S). Although yellowfin and bigeye tuna undertake migrations of several thousand miles, these migrations are not as extensive as those of the other principal market species. Many of the secondary market species also appear to be less migratory than the principal market species. However, some species of billfish migrate several thousand miles (FAO, 2013c; Froese and Pauly, 2010a,b).

The principal market *tuna* are distributed in the Atlantic Ocean (North, South, Western, Eastern, and Mediterranean Sea), Indian Ocean, Pacific Ocean (North, South, Eastern, Western, and Central), and Southern Ocean. Each ocean has its own particular species, such as the Pacific Bluefin tuna, which is usually found in the Pacific Ocean, and the southern Bluefin tuna in the Southern Ocean. However, bigeye tuna, albacore, yellowfin tuna, and skipjack tuna can be caught in the Atlantic, Pacific, and Indian Oceans. Table 2.1 shows the occurrence of tuna species in different oceans.

### 2.1.2 Tuna production

According to FAO (2013d), the total catch of the commercial tuna species increased from 162,980 metric tons in 1950 to more than 4.4 million metric tons in 2011 (Figure 2.1). The total catch increased greatly from 2.5 million metric tons in 1986 to 4.5 million metric tons in 2005. Various problems led to a 6% decline to 4.2 million metric tons in 2007. Therefore, subsequent years are expected to have experienced further reduction in tuna catches to just above 4 million metric tons. The

observed increase in tuna catch can be attributed to new applization of fishing technology. In the early 1950s, fish trap, pole, and line fishing were the primary methods used by fishermen. In later years when large-scale industrial fisheries began to operate, new methods, such as purse seining, were developed. Other modern technologies (e.g., FADs) have significantly influenced the exploitation of tuna species.

Table 2.1. Occurrence of tuna species in different oceans (*FAO, 2013c*)

| Common name | Scientific name | Areas of occurrence |
|---|---|---|
| Skipjack | *Katsuwonus pelamis* | Worldwide |
| Yellowfin tuna | *Thunnus albacores* | Worldwide |
| Bigeye tuna | *Thunnus obesus* | Worldwide |
| Albacore tuna | *Thunnus alalunga* | Worldwide |
| Atlantic bluefin tuna | *Thunnus thynnus* | Atlantic Ocean |
| Pacific bluefin tuna | *Thunnus orientalis* | Pacific Ocean |
| Southern bluefin tuna | *Thunnus maccoyii* | Southern parts of Atlantic, Indian and Pacific Ocean |
| Longtail tuna | *Thunnus tonggol* | Indian Ocean, western Pacific Ocean |
| Blackfin tuna | *Thunnus atlanticus* | Western Atlantic Ocean |
| Kawakawa | *Euthynnus affinis* | Indian, western and central Pacific Oceans |
| Black skipjack | *Euthynnus lineatus* | Eastern Pacific Ocean |
| Little tunny | *Euthynnus alleteratus* | Atlantic Ocean |
| Bullet tuna | *Auxis rochei* | Worldwide |
| Frigate tuna | *Auxis thazard* | Indian and Pacific Oceans |
| Slender tuna | *Allothunnus fallai* | Southern Ocean |

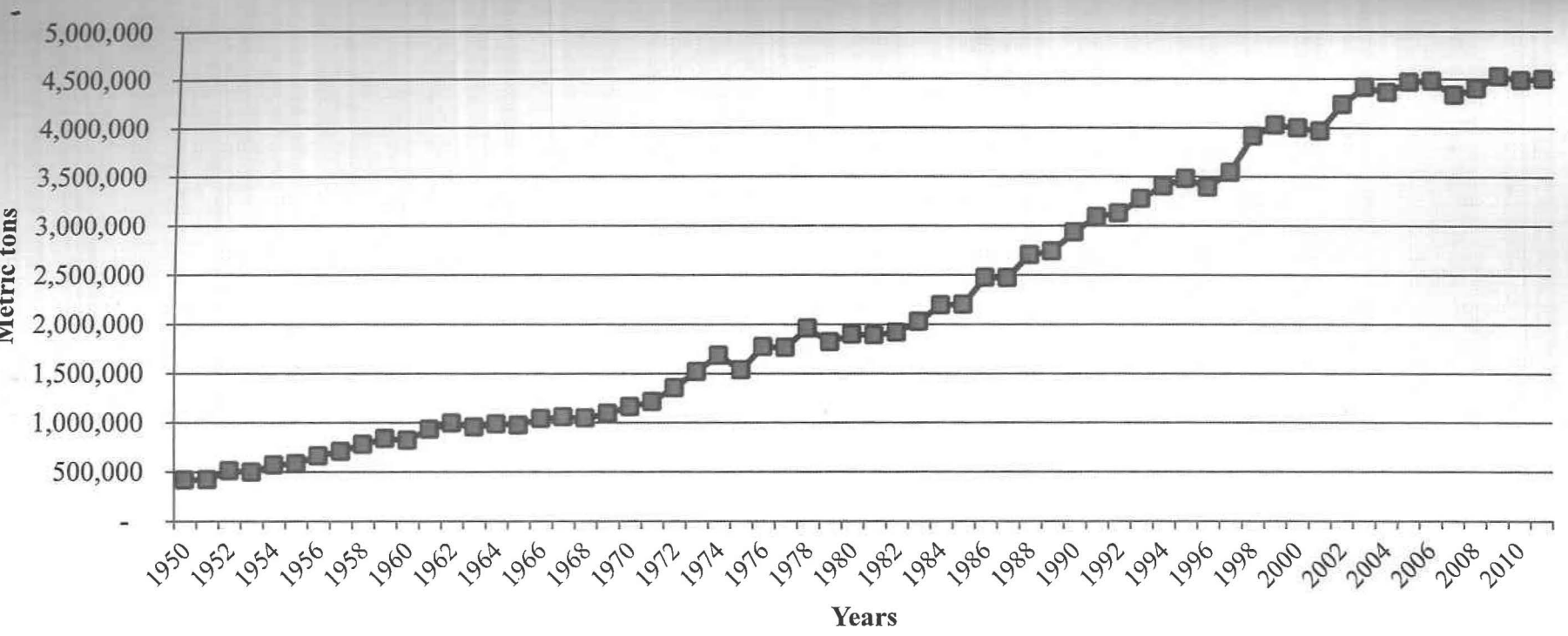


Figure 2.1. World catches of commercial tuna, 1950–2011 (*FAO, 2013d*)

The main fishing grounds for commercial tuna species are located in the Pacific Ocean, which provided ~70% of catches of commercial tuna from 1950 to 2011 (FAO, 2013c). The main tuna catching nations are concentrated in Asia; Japan and Taiwan are the main producers (Table 2.2). Other important tuna catching nations in Asia are Indonesia, the Republic of Korea, and the Philippines. Although Japan continues to be the worlds major tuna catching country, its catches have declined in recent years: In 2011, Japanese tuna production was 463,069 metric tons, whereas a peak of 780,000 metric tons occurred in 1986 and 1993 (FAO, 2013d).

In Taiwan, the catch in 2001 (439,251 metric tons) was more than double that of the years in the period 1990-1998 (FAO, 2013d). Catches declined to 373,461 metric tons in 2006 and then declined again to 316,252 metric tons in 2011. The loss of the tuna fishing grounds in the Central Eastern Pacific due to the tuna/dolphin issue led to a substantial decline (> 60%) in US tuna production in 2000. Thus, US production declined from fifth highest in the world in 1999 to fourteenth in 2004. Spain and France are also important tuna fishing countries, and they mainly fish in the Indian Ocean. At present, Spain is ranked at number five among the main tuna fishing nations and France is number eight (FAO, 2013d).

Based on tuna species, skipjack is the main species caught, and catches of this species doubled during the past 15 years (Figure 2.2). In 2000, skipjack catches reached > 2 million metric tons and in 2007 they reached 2.6 million metric tons. Yellowfin tuna, which is the second major species caught, also showed increased catches over time. This species is generally higher priced than skipjack, and it also is used in canning. In 2003, yellowfin catches reached a record of 1.42 million metric tons, which was an increase from the 1 million metric tons in the mid-1990s.

However, like catches of skipjack, yellowfin catches declined by as much as 125,896 metric tons in 2004. Albacore catches have remained stable over the years. Catches of bigeye tuna increased dramatically until 2002. There is concern about over-fishing of bigeye tuna, especially in fisheries using Fish Agregating Devices (FADs). In 2003 and 2004, catches of bigeye tuna decreased for the first time. Fisheries managers hope that this decline is because of protection measures and not a result of over-fishing and declining resources.

## 2.2 Tuna Industry Waste

### 2.2.1 Tuna anatomy

According to FAO (2013c), common characteristics of tuna are the same among members of the Scombridae and billfish families. *Tuna* have two distinct dorsal fins that generally are separated; the first one is supported by spines and the second only by soft rays. The pelvic fins are inserted below the base of the pectoral fins. The caudal fin is deeply notched. All scombrids and billfishes except swordfish have a pair of caudal keels in the middle of the caudal peduncle at the base of the caudal fin; the swordfish has only a large median caudal keel. The more advanced members of the Scombridae family also have a large median keel anterior to the pair of caudal keels. The body of all scombrids is robust, elongate, and streamlined. The first dorsal and first anal fins of all scombrids and billfishes, except swordfish, can fold down into grooves and the pectoral and pelvic fins into depressions when the fish is swimming rapidly. All scombrids and billfishes have four gill arches on each side. The gill filaments are ossified as gill rays.

Like most fish, tuna have white and red muscle. However, the proportion of red muscle in tuna is much higher than that of other fishes (Dickson, 1995), which allows tuna to swim at high speeds for long periods without fatigue (Joseph *et al.*, 1988; Bushnell and Holland, 1997). White muscle, which can work in both aerobic and anaerobic conditions, is present in a lower proportion in tuna compared to other fish. The red muscles are located deep within the body, extending from the vertebral column to a lateral subcutaneous position, and appearing to be more important at the anterior part of the fish. Graham *et al.* (1983) also noted that the proportion of red muscle seems not to increase with the size of tuna due to the greater efficiency and labor sharing between red and white muscles in tuna compared to other fishes. The heart and white muscle aerobic capacities are significantly greater in tuna than in billfishes and other scombrids.

The size of commonly captured tuna species ranges from 30 to 200 cm (Table 2.3), with maximum size and weight ranges of 70–300 cm and 9–650 kg. The largest size and weight belongs to Atlantic Bluefin tuna, and the smallest values belong to black skipjack.

Table 2.2. Main tuna catching countries/entities, 2000–2011 (*FAO, 2013d)*

| Country/Entity | Quantity (metric tons) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **2000** | **2001** | **2002** | **2003** | **2004** | **2005** | **2006** | **2007** | **2008** | **2009** | **2010** | **2011** |
| Japan | 635,812 | 572,718 | 570,434 | 585,084 | 482,621 | 536,604 | 496,067 | 548,396 | 535,851 | 485,994 | 531,584 | 463,069 |
| Taiwan Province of China | 435,946 | 439,251 | 495,855 | 439,268 | 458,706 | 408,584 | 373,461 | 401,356 | 328,909 | 328,217 | 335,810 | 316,252 |
| Indonesia | 421,749 | 385,127 | 381,660 | 348,130 | 378,256 | 331,705 | 380,393 | 432,276 | 462,150 | 508,447 | 485,598 | 590,575 |
| Spain | 302,384 | 256,681 | 279,130 | 311,638 | 274,285 | 286,579 | 313,480 | 209,130 | 256,001 | 251,592 | 259,509 | 284,669 |
| Republic of Korea | 218,197 | 230,510 | 257,570 | 229,375 | 231,320 | 252,190 | 288,834 | 294,363 | 281,088 | 319,726 | 311,925 | 244,038 |
| Philiphine | 206,193 | 190,725 | 211,901 | 269,627 | 277,905 | 285,244 | 312,952 | 360,612 | 430,622 | 412,804 | 389,351 | 331,661 |
| Other nei | 190,722 | 159,589 | 172,825 | 189,901 | 94,438 | 44,627 | 18,554 | 9,823 | 9,965 | 13,446 | 11,918 | 8,206 |
| France | 151,650 | 143,044 | 162,962 | 174,456 | 165,668 | 164,459 | 143,341 | 100,647 | 102,578 | 91,716 | 88,834 | 85,846 |
| Mexico | 120,558 | 144,717 | 160,227 | 142,486 | 146,592 | 150,341 | 107,114 | 118,926 | 116,512 | 133,465 | 120,490 | 118,763 |
| United States of America | 152,361 | 149,743 | 154,153 | 112,451 | 99,917 | 79,643 | 91,729 | 94,230 | 135,245 | 201,208 | 240,110 | 226,571 |

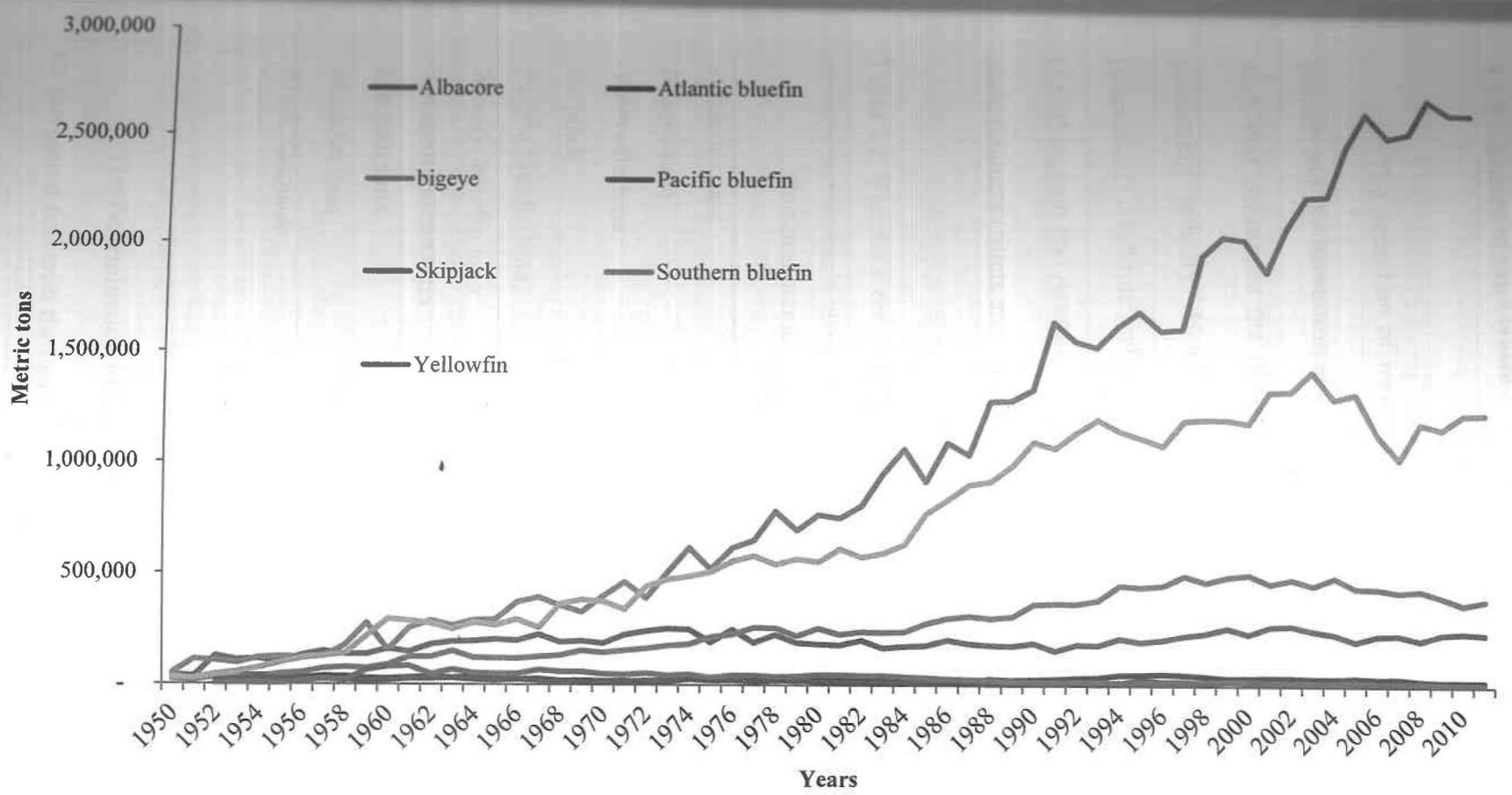


Figure 2.2. World catches of commercial tuna by species, 1950–2011 (*FAO, 2013d)*

### 2.2.2 Source of tuna waste

The definition of waste or by-product in the fish industry varies with fish species and the harvesting and processing methods used. Generally, the main body flesh that constitutes the fillets is considered to be the main product in the tuna processing industry. Head, backbones, trimmings or cutoffs, skin, and guts (intestines) constitute what is generally thought of as by-product or waste (Kristbergsson and Arason, 2007). Plate 2.1 shows a cross-section of a tuna with the main products (fillets and loins) and waste material labelled.

Table 2.3. Various sizes and weights of tuna (*Source: FAO, 2013c*)

| Common name | Common size (cm) | Maximum size (cm) | Maximum Weight (in kg) |
|---|---|---|---|
| Albacore tuna | 40–100 | 127 | 40 |
| Bigeye tuna | 70–180 | 230 | 200 |
| Yellowfin tuna | 60–150 | 200 | 175 |
| Skipjack | 40–80 | 108 | 33 |
| Pacific bluefin tuna | 200 | 300 | 450 |
| Atlantic bluefin tuna | 80–200 | 300 | 650 |
| Southern bluefin tuna | 160–200 | 225 | 160 |
| Longtail tuna | 40–70 | 130 | 35 |
| Blackfin tuna | 40–70 | 100 | 19 |
| Black Skipjack | 30–65 | 70 | 9 |

The determination of yield in the fish processing industry generally is based on the gutted fish with the head. According to Arason (2003), a gutted fish with head contains 62% edible flesh and 46% skinless tuna fillet. Fish heads contain relatively little meat and are usually discarded or utilized as animal feed. However, certain

parts of the tuna head (i.e., tongue, cheeks, collar or nape, and upper head) can be consumed as a meat source. The tongues and cheeks are considered by some consumers to be delicacies due to their unique taste and excellent texture.

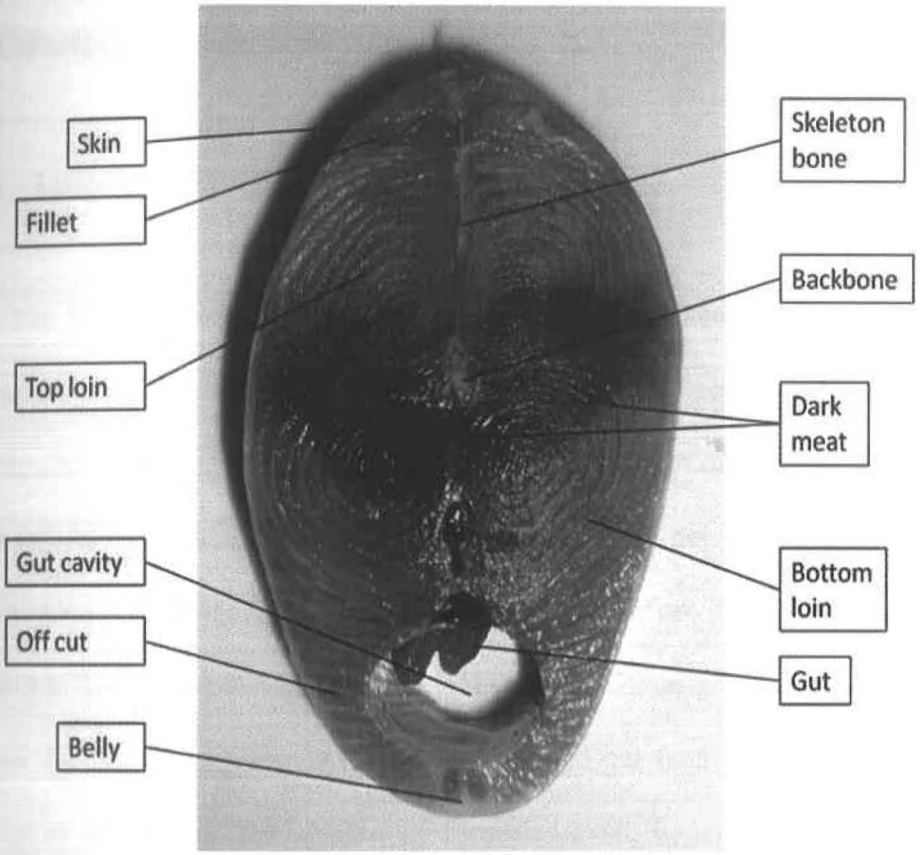


Plate 2.1. Cross-section of a tuna showing main products (fillets and loins) and waste material

Stone (2007) reported that tuna loins and fillets generally constituted 37.1% and 17.9% of a headless tuna, respectively. Both are main parts extracted in the tuna industry. However, use of only these parts leaves a great deal of waste from a single tuna. Stone (2007) reported that bones and dark meats, which are considered to be waste, made up 17.9% of a headless tuna, and skin and guts (viscera) constituted 13%, the belly 6.2%, and scrap from the frame 7.9%. The viscera, including both the liver and roe (or milt), may constitute 10–25% of the net weight of a whole tuna

depending on maturity and season. Other parts of the guts, such as the pyloric caeca, normally are not consumed but may serve as a source of bioactive compounds such as enzymes, which can be used for various applications.

### 2.2.3 Utilization of tuna waste

#### 2.2.3(a) Tuna dark muscle as source of pet food

Pet food products that are tuna based account for about 5% of canned pet food in most major markets. Blood meat (dark tuna muscle) accounts for about 12% of raw tuna butchered for canning and is the main ingredient of tuna-based pet food. A major use of blood meat from tuna is to give flavor to pet feed. This dark meat, which lies next to the backbone, is trimmed from tuna before it is canned for human consumption. Gourmet pet feed, which is essentially human-grade tuna, is produced in limited quantities from whole tuna loins. Canned pet feed tuna is processed the same way as other tuna, and dozens of formulas exist, including being packed in water or jelly with vitamin and mineral pre-mixes, vegetable oils, antioxidants, coloring agents, and sometimes pulverized tuna frames to boost calcium content. There may be opportunities for the Canadian albacore tuna industry to sell dark meat to pet food manufacturers for niche markets (BCTFA, 2001).

#### 2.2.3(b) Tuna oil

Tuna oil is becoming an important by-product of the tuna processing industry. Unused parts of tuna that are processed for the tuna canning industry are used to make refined oil, which has a low odor and light yellow color. Usually only

the head, meat, and bones, but not the viscera, are used in tuna oil production. Tuna livers are not processed into oil. Crude tuna oil is produced from tuna waste by steam followed by purification. This first-stage oil is a darker color than that of the finished product. Oil separation equipment at canneries is used to extract water, solids, and metal ions as quickly as possible. The product is then shipped to a refinery to undergo a four-step process that involves neutralization, bleaching, and winterizing to remove crystallized fats, followed by a deodorizing process to remove odor-causing contaminants. The oil then is either shipped in bulk or packaged and sent to end users, including the pharmaceutical industry and other manufacturers.

Tuna oil is a source of polyunsaturated fatty acids (PUFAs), especially EPA (eicosapentanoic fatty acid, C22:5n3) and DHA (docosahexanoic acid, C22:6n3), which are omega 3 fatty acids. The oil contains approximately 5.7% EPA and 18.8–25.5% DHA (Chantachum *et al.*, 2000; Wongsakul *et al.*, 2003). The PUFAs play an essential role in human health and nutrition, as they can reduce the risk of coronary disease, prevent certain cancers, and improve immune function. A convenient method for delivery of omega 3 fatty acids is the use of oil-in-water emulsions (Shen *et al.*, 2007). However, long chain PUFAs in tuna oils are highly unsaturated and therefore are highly susceptible to oxidation. Lipid oxidation in tuna oils can be reduced by adding antioxidant to the oil or by encapsulation of the oil (Klinkesorn *et al.*, 2005; 2006). Use of encapsulation technologies to retard the oxidation of tuna oils has been reported and has drawn considerable attention in the food industry.

Generally, fish oil—including tuna oil—contains a complex mixture of fatty acids with varying chain lengths and degrees of unsaturation. Overconsumption of fish oils to obtain omega 3 PUFAs may increase the intake of cholesterol and other

saturated fatty acids by consumers (Shahidi and Wanasundara, 1998). Concentration or enrichment of omega 3 PUFAs in tuna oil could help to avoid this problem (Klinkesorn *et al.*, 2004). Some studies indicate that PUFA concentrates that are devoid of more saturated fatty acids are better for human consumption than fish oils themselves, as they allow the daily intake of total lipid to be kept as low as possible.

### 2.2.3(c) Tuna collagen and gelatin

Collagen and gelatin are different forms of the same macromolecule. Collagen, which is one of the most abundant animal-derived proteins, is the precursor of gelatin (gelatin is the partially hydrolyzed form of collagen). Collagen and gelatin are widely and diversely used in food, medicine, cosmetics, and cell cultures, and the consumption of collagen and gelatin has increased with the development of new industrial applications (Karim and Bhat, 2009). Collagen and gelatin used in commercial products are mainly obtained from cows and pigs, but mammalian diseases (e.g., bovine spongiform encephalopathy and foot/mouth disease) present safety problems because of the risk of transferring the disease to humans. In addition, certain religions prohibit the use of cow and pig products. In contrast, the risk of transferring pathogens is low in fish collagen and gelatin, and these products do not contradict Islamic food laws and Hindu/Buddhist religious sensitivities.

Fish skin, bone, and fins can be used as sources of collagen and gelatin. Although they are dumped as waste, their yield of collagen is very high (about 36–54%) (Nagai and Suzuki, 2000b). Collagen accounts for about 30% of the total protein of most organisms (Woo *et al.*, 2008). Nagai and Suzuki (2000a) reported

that the collagen contents of the fish skin waste of Japanese sea bass, chub mackerel, and bullhead shark were 51.4%, 49.8%, and 50.1% (dry basis), respectively. The yields of collagen in fish bone also were very high: skipjack tuna (53.6%), Japanese sea bass (42.3%), ayu (40.7%), yellow sea bream (40.1%), and horse mackerel (43.5%) (on the basis of lyophilized dry weight).

Production of fish gelatin is not a new phenomenon, as it has been produced since 1960 by acid extraction. To date, most of it has been used for industrial applications (Norland 1990). Detailed extraction procedures and characterization of the properties of fish gelatin were described by Grossman and Bergman (1992) in a United States patent. According to Karim and Bhat (2009), many researchers have studied extracts from the skin and bones of various cold-water (e.g., cod, hake, Alaska pollock, and salmon) and warm-water (e.g., tuna, catfish, tilapia, Nile perch, shark, and megrim) fish. In order to be applied in the food and pharmaceutical industries, fish gelatin must possess the following characteristics. First, a large quantity of by-product and its economical collection are essential for continuous production in industry. Second, gelatin from fish by-products must have rheological properties (e.g., gel strength, gelling, and melting points) that are comparable to those of mammalian gelatin.

Traditionally, gelatin made from marine species was thought to have inferior physicochemical properties compared to mammalian gelatin, and this was especially true for gelatin made from cold-water fish species (Leuenberger, 1991; Gudmundsson and Hafsteinsson, 1997; Haug *et al.*, 2004). However, recent studies showed that certain fish gelatins might have similar quality characteristics as those of mammalian gelatin and that the quality depends on the species from which the

gelatin was extracted and the processing conditions used (Choi and Regenstein, 2000; Cho *et al.*, 2005; Zhou *et al.*, 2006; Yang *et al.*, 2007). For example, Cho *et al.* (2005) reported that the gel strength of yellowfin tuna skin gelatin (426 Bloom) was higher than that of bovine and porcine gelatins (216 Bloom and 295 Bloom, respectively), whereas the gelling and melting points were lower. The tuna skin gelatin also had dynamic viscoelastic properties that were similar to those of mammalian gelatins. Moreover, the collagen extracted from yellowfin tuna dorsal skins had good solubility and viscosity qualities (Woo *et al.*, 2008). As for thermal characteristic, Rahman *et al.* (2008) reported that a disadvantage of tuna gelatin was it showed lower glass transition compared to mammalian gelatin when equilibrated at the same constant relative humidity. The variation in gelatin characteristics has been shown to be correlated with the proportion of proline and hydroxyproline in the original collagen. Haug *et al.* (2004) reported that mammalian gelatin contained considerably large amounts of proline and hydroxyproline, whereas fish gelatin contained lower amounts.

### 2.2.3(d) Tuna bone powder

The organic component of fish bone, which accounts for 30% of the total, is made of collagen (Nagai *et al.*, 2004). The remaining 60–70% consists of inorganic substances, mainly calcium phosphate and hydroxyapatite. Thus, fish bone is rich in useful inorganic substances that contain a balance of calcium and phosphorus that can be used as a calcium food supplement (Yoon *et al.*, 2005). However, to date fish bone has been used chiefly in animal feed.