Analysis of volatile compounds, proximate composition, and fatty acids in Pacific bluefin tuna (*Thunnus orientalis*)

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ABSTRACT

Pacific bluefin tuna (PBT; *Thunnus orientalis*) has grown significantly in popularity in recent years due to the globalization of Japanese cuisine. PBT is highly sought after for sushi and sashimi products due to its great quality and taste. Wild populations of this species have been affected by their increasing popularity, pushing innovators in the food industry to create meat alternative versions of PBT. The muscle composition of PBT varies, leading to different types (cuts) of meat in a way that is analogous to various cuts of beef. This study evaluated the differentiation amongst the 6 distinct cuts, including *otoro*, *ventral akami*, *dorsal akami*, *ventral chu-toro*, *dorsal chu-toro*, and *wakaremi* conducting volatile analysis, proximate analysis, and fatty acid analysis. The results from these analyses can then be used as a base standard for companies seeking to create alternatives versions of PBT. Samples analyzed in this study were cultured PBT species that were caught as juveniles and raised in captivity on a PBT farm in Mexico. Volatile analysis was conducted using a SPME GC/MS method. Overall, 41 aroma compounds were identified in PBT that met the identification criteria, including 9 aldehydes, 7 alcohols, 14 alkanes, 2 ketones, 4 alkenes, 3 aromatic compounds, and 2 miscellaneous compounds. Proximate analyses were conducted using standard methods. Significant differences ($p < 0.05$) were found between each cut for the proximate analysis. The fatty acid analysis determined that there were twenty-two identifiable fatty acids found in the different cuts. The *omega*-3 fatty acids

eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) with DHA being present at a higher amount than EPA in each cut. Overall, there are similarities and differences among the different cuts of bluefin tuna that researchers would need to mimic to provide adequate nutritional and sensorial properties of PBT.

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GENERAL AUDIENCE ABSTRACT

Pacific bluefin tuna (PBT; *Thunnus orientalis*) is a fish that has gained tremendous popularity over the years due to the globalization of Japanese cuisine. This tuna species is synonymous with high quality and great taste, making it key for sushi and sashimi dishes. The increased demand for this species has caused wild populations to decrease; therefore, the food industry has sought to create meat alternatives for the species. The PBT has 6 distinct cuts that make up the composition of the fish, similar to the differences that can be found in beef cuts. These 6 distinct cuts are *otoro*, *ventral akami*, *dorsal akami*, *ventral chu-toro*, *dorsal chu-toro*, and *wakaremi*. The purpose of this study is to determine the aroma composition, fatty acid composition, and fat, moisture, ash, and protein contents of the different cuts. To measure the aroma composition, gas-chromatography mass spectroscopy (GC-MS) was used; it is a machine that can be used to identify and measure the aroma compounds of products. Standard procedures were used for the other analyses. Overall, 41 aroma compounds were identified in PBT that met the identification criteria, consisting of aldehydes, alcohols, alkanes, ketones, alkenes, aromatic compounds, and some miscellaneous compounds. The cuts displayed distinguishable differences in their fat, ash, moisture, and protein contents. The fatty acid analysis concluded that there were twenty-two identifiable fatty acids found in the different cuts. In this study, close attention was paid to *omega*-3 fatty

acids due to their health benefits for consumers. The *omega*-3 fatty acids eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) were found in all cuts with DHA being present at a higher amount than EPA in each cut. From this study, researchers have the foundation for understanding the composition of PBT to create a meat alternative that meets consumer expectations.

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Chapter 1. Introduction

Bluefin tuna is a fish that is popular in Japanese cuisine it is a prized fish for sushi. Specifically, the Pacific Bluefin tuna (*Thunnus orientalis,* PBT) species is highly sought after for its quality and delicious taste. This has led to extreme pressure being placed on the stocks of bluefin tuna (BT) of all three species (Longo 2011; Craig et al. 2017). In addition, the increasing globalization of Japanese cuisine means that this species is not only popular with Japanese consumers which is responsible for nearly 90% of the market share, but with many around the world with emerging markets in the US and Southeast Asia (Bestor 2000; Lovatelli et al. 2008). With the increased interest and consumption of this fish, there are concerns about the fate of the species.

Aquaculture or fish-farming has been one of the ways that the industry has adapted to meet the growing demand of consumers. However, it is challenging to implement aquaculture across all species of fish. For example, bluefin tuna cannot be easily farmed from egg to fish; like some other fish species, they have to be caught in the wild, and then they can be grown in captivity (Charles 2014). There has been a study conducted in Japan that has determined a way to produce Pacific bluefin tuna in captivity successfully, but there are still challenges that have prevented this method of raising bluefin tuna from becoming a widely implemented practice (Sawada et al. 2005). The result is that the farming of PBT is still quite limited.

Therefore, the food industry is pushing to create alternatives to this sought-after fish to provide the product to consumers before the species becomes extinct and is no longer able to be consumed. One way the industry has decided to mimic this species is by creating plant-based meat alternatives (PBMAs). PBMAs are products meant to mimic traditional meat products while being completely made of vegetables and other plant-based

components. Recently the impact of meat consumption on human health and the environment has come under scrutiny (Evan et al. 2020), further increasing consumer interest in foods that will replace meat in their diets. Currently, there are various PBMAs for beef and chicken that can be purchased in stores and in restaurants for consumers to enjoy. Many companies, including Beyond Meats, Impossible Foods along with several others, have been able to provide products that can satisfy vegetarians, vegans, flexitarians, and in some cases, skeptical meat-eaters. However, there are still some people who do not view these alternatives as providing the same satisfaction standard as traditional meat products, leading the industry to create other products that could possibly satisfy those consumers.

Another method the industry is researching focuses on growing meat via cell culture in a production facility, potentially having less effect on the environment while still giving consumers real "meat." Most recently, *in vitro* cell culture techniques have been used to create "artificial" hamburger meat, providing hope that this can be useful to the food industry (Evan et al. 2020). It may seem that food production is meeting a science fiction era; however, creating tissue from culture is far from new. It was initially introduced in the late 1980s and was eventually used in the 1990s, where it was used to replace biological tissues (Noor et al., 2016). Thus, while the process has been around for some time, there are still challenges that need to be addressed so products can be produced on a mass scale.

Current research has mainly focused on replacing beef; however, plant-based meats and culture-based meats are not limited to beef alone and there are companies looking at other source of meat including seafood. In the case of alternative seafood

products, there is an added challenge due to the widely disparate species (fish, crustaceans, shellfish). Since there are many different varieties to choose from, companies in this research area are focusing on different species. There are various companies from across the globe (Russia, USA, Hong Kong, Singapore) working to create alternatives for different species, whose products are at different stages of development (Halpern et al. 2021). Bluefin tuna is among the focus species and providing an alternative to wild caught tuna is crucial to decrease pressure on wild stock, to hopefully protect the declining wild population of the species.

While there is still a great deal of work to be done in terms of creating the finished plant-based and cell-based meat alternatives, several factors of bluefin tuna need to be determined to create products with the highest consumer acceptance. These factors include understanding the volatile composition, proximate composition, and fatty acid profiles, so cultured products can better copy products from the wild. **The aim** of this research is to determine the compounds responsible for the aroma profile in different cuts of bluefin tuna including otoro, ventral akami, dorsal akami, ventral chu-toro, dorsal chu-toro, and wakaremi. As well, we aim to determine the proximate and fatty acid composition of the different cuts. The null hypothesis is that composition of the tuna will not differ based on cut (location on the animal). The alternate hypothesis is that composition of the tuna will be different, based on cut.

Research Objectives

The objectives of this research are as follows: 1. To determine the volatile compounds in the different cuts of bluefin tuna responsible for its distinct aroma profile using SPME-GC-MS; 2. To determine the proximate composition and the fatty acid composition of the different bluefin tuna cuts using standard methods.

The studies used to support these objectives are as follows:

Study 1: GC-MS analysis of the six cuts of bluefin tuna;

Study 2: Approximate analysis of the six cuts of bluefin tuna that included moisture, ash, protein, fat, and fatty acid analyses.

Chapter 2. Review of Literature

Pacific Bluefin Tuna

The Pacific bluefin tuna (*Thunnus orientalis*; PBT) is a highly prized fish. This species in particular has a high market value and is highly sought after for human consumption, which affects wild populations (Madigan et al. 2017; Ijima et al. 2019). While the ocean accounts for a large majority of world surface, its resources are not limitless and, due to the ocean vastness, it is challenging to accurately measure the populations of fish species; this may lead to many species populations being endangered well before there are methods established to protect them (Yan et al. 2021). For PBT, that risk is increasing due to the growing interest in PBT in cuisine. There is increased global interest in sushi and sashimi making bluefin tuna a staple for many (Bestor 2000). Like many of the oceans organisms including bluefin tuna (BT) are also suffering from the effects of climate change on increasing sea water temperature (Bestor 2000; Ijima et al. 2019).

Capture Based Aquaculture (CBA): Sea Cages

According to the Food and Agriculture Organization (FAO), aquaculture is defined as the "farming of aquatic organisms including fish, mollusks, crustaceans and aquatic plants; with some form intervention that in the rearing process to enhance production, such as regular stocking, feeding, protection from predators, etc." (Lovatelli et al. 2008). There a several different methods used in aquaculture that are used in the rearing of aquatic organisms. For PBT and other species of BT, the most common method used involves capturing juveniles. Capture-based aquaculture (CBA) is a method in which marine organisms are captured from the wild and then rendered in captivity (Lovatelli et al. 2008). The age at which the PBT and other BT are captured can vary from juveniles (<25 kg) to adults (>30kg); in the eastern pacific the fisheries have access to only to some age ranges

while the western pacific has access to all age groups (Metian et al. 2014; Madigan et al. 2017). Besides location, another factor that contributes to the age BT is captured depends on the fisheries' particular needs to meet market demand. There are two different types of rearing methods in CBA one known as "farming" which is when immature specimens are kept for a longer time (20 months) and "fattening" when mature specimens are kept for a shorter time (usually 4-9 months) and is linked to market demand for the product (Lovatelli et al. 2008; Metian et al. 2014). For PBT specifically, they are typically caught as juveniles and raised until maturity, with the exception of one fishery in Japan that has been able to establish a complete life cycle process for PBT in captivity (Sawada et al. 2005).

For all species of BT, no matter the age at which they are caught they are all kept in what is known a sea cage. These are open-sea net floating cages that allow the fish room to swim; the cages can vary in size from 30-90 m diameter with a depth of 15-30 m the size is mainly controlled by sea location (Lovatelli et al. 2008). For PBT whose life cycle has been established completely in captivity once they passed the nursery stage they were also transferred to sea cages (Sawada et al. 2005). The practice of using sea cages as a way for rendering these species is widespread across the globe. The process has been industrialized in the Mediterranean for Atlantic bluefin tuna (*Thunnus thynnus*), in Australia for Southern bluefin tuna (*Thunnus maccoyii*), and in Japan and Mexico for PBT (Sawada et al. 2005). The fish will be kept in the sea cages until they reach a desired weight that is suitable for market sale, which could vary country to country.

Progression and Challenges of Seafood Alternatives

Plant-based foods have come a long way in that they can not only replace seafood nutritionally, but they can also mimic seafood. The need for the product to mimic meat is

high on consumers' need's list to lead them to consume these products instead of traditional meats. In a systematic review conducted by Fiorentini et al. (2020), researchers discussed the importance of color, overall appearance, taste, flavor, aroma, and texture of PBMAs as attributes that need to be addressed to meet consumer expectations. It can be said that consumers eat with all their senses, therefore, it is vital that all these attributes are satisfactory to consumers. Ingredients, manufacturing process, and packaging will all play a huge role in the final outcome of these sensory attributes; that is why it is essential to have products evaluated analytically and by untrained and trained sensory panelists (Fiorentini et al. 2020; Kazir and Livney 2021). Overall, to create a product that meets consumer expectations, food scientists across various concentrations (chemistry, sensory, processing, microbiology; etc.) will have to work in conjunction with one another.

For cell-based seafood alternatives, there are several hurdles in the process that need to be overcome before becoming a staple in consumers' kitchens. These hurdles include cell-line establishment, creating growth media, problems with low reproducibility, accessibility concerns, high cost, and regulations. Unlike plant cells cultures where a single cell can be used to grow a complete plant, currently animal cell lines can only grow portions of the animal meaning there will need to be different cell lines for muscle and adipocyte cells (Loyola-Vargas and Ochoa-Alejo 2012). Cell-lines are crucial for cell-based meat products' success. Successful cell-lines are cells that can be grown in culture (outside of the organism) for many generations. For fish, there have not been many successful cell lines that can survive many generations of reproduction. However, one study shows that there are now at least 283 cell lines have been established for several fish species (Lakra et al. 2011). However, all these cell-lines are not applicable for large scale multi-generation

production of cells for consumption. It is the case for most diploid cells derived from mammal tissues only have a limited life span in culture because they will eventually reach a point of senescence (Hu 2020). The same phenomenon can be applied to cells derived from other animal species. Human cancer cells have been able to be grown *in vitro* due to the contribution of Henrietta Lacks; however, some normal animal cells have achieved “immortalization” through viral or oncogene transformation which allows them to grow continuously, but they will have a different morphology than the cells from which they were initially derived (Masters 2002; Hu 2020). Currently, only nine cell lines of fish muscle have been able to reach “immortalization” that allow them to continuously grow (Rubio et al. 2019). Therefore, companies need to establish their own cell lines of the species they are working to create.

To have a successful cell line, another vital factor for developers to consider is the growth media. The growth media will supply the cell with the nutrients they need to survive and multiple outside of the animal. Traditional growth media used for mammalian cells currently contains fetal bovine serum (FBS) to help the cells grow; however, FBS is taken from a cow's fetus during the slaughtering process, which contradicts some companies' decisions to move away from animal cruelty (Lakra et al. 2011). FBS is also very expensive. Therefore, some companies choose to use media that does not contain FBS. For example, some scientists have been able to produce serum from maitake mushroom extract instead of FBS, and it was used to grow the cells successfully (Benjaminson et al. 2002). While this serum looks promising, it is unclear whether it will work well with other cell-lines.

Additionally, cell-based meat production currently faces reproducibility problems as well as the need for expensive technical equipment requiring skilled professionals, making it harder for developing nations to enter the market (Bhat and Bhat 2010; Cartín-Rojas and Ortiz 2018). Regulations also must be established before these products can make it to market. Development of regulations for products that have never been created can be challenging, which is why many companies must work with regulators to establish what regulations are needed to verify that these products are safe to consume. All of these hurdles need to be addressed before consumers can purchase cell-based meat.

Flavoring of Seafood Products

There have been patents that determined that some compounds can be added to other foods to give it a seafood flavor. Daniel Sortwell determined that S-methyl methionine chloride is preferred over other salts because it can increase scallop flavor (1976). Instead of adding flavors to foods directly, flavor precursors are converted to flavor compounds during the cooking process. The primary flavor compound of scallops is dimethyl sulfide; however, this compound is extremely volatile and can decrease in storage and be completely lost after cooking (Sortwell 1976). Therefore, it was important to discover a compound that would be transformed into dimethyl sulfide during cooking but was more stable and would not decrease over time. Later in 1983, a patent was approved that established the addition of *cis*-4-heptenol could be added to foods to impart a pleasant seafood aroma and flavor (Parliment and Herzing-Giordano 1983). The application of this invention gives and overall seafood-like flavor, but not a distinctive flavor recognition like the previous patent mentioned above. The researchers discovered that the added *cis*-4-heptenol was stable in food items, and it was soluble in both nonaqueous and aqueous

solutions (Parliment and Herzing-Giordano 1983). Therefore, the addition of *cis*-4-heptaneol could be applicable to a variety of food items.

Researchers and companies looking to successfully apply flavoring components to their products should consider mimicking the technologies of the seafood product surimi. Surimi is commonly known as “artificial crab” and was first made from leftover fish scraps and deboned mince to increase utilization of fish waste. Surimi products are usually shaped and flavored to mimic the flavor of shellfish (Park 2005). Surimi is a product whose technology originated in Japan, but it has gained a lot of popularity in American cuisine. Traditionally, surimi was made from Alaskan pollock; however, a decrease in Alaskan pollock's harvesting has shifted the production to other species (Park 2005). While the fish source may have changed, the flavor needed to remain the same. In the case of surimi, while it is often associated with crab, it gets its flavor profile from crab, shrimp, lobster, and some other seafood the flavoring mostly consists of natural flavoring that have been extracted from the primary seafood with a mix of some artificial flavoring (Park 2005).

It should be noted that volatile flavor components can be derived from different precursors. In seafood, some are derived from lipid degradation that occurs through enzyme-mediated reactions and is responsible for a mild melon-like or seaweed-like attribute found in fresh seafood; however, when oxidative deterioration occurs, it will create the presence of fishy flavors (Josephson et al. 1985; Park 2005). The fishy flavors are not desirable in high quality seafood products. Enzymatically derived compounds play a role in flavor perception; it is critical to establish the degree to which they are essential. Additionally, volatile aroma compounds can be derived oxidatively, microbially, and thermally (Park 2005). To create the perfect flavor application, it is vital to understand

how these different processes can affect flavor. Particularly in fresh seafood, the degradation due to microbes is likely to occur faster than degradation due to oxidation. It should be reiterated that not all oxidation is negative; some oxidation caused by the lipoxygenase enzyme degradation of polyunsaturated fatty acids (PUFA) to carbonyl compounds has been linked to positive volatile development in seafood (Hsieh and Kinsella 1989). A study conducted by Kuo and Pan (1991) determined that lipoxygenase was responsible for over 40% of the volatile compounds found in shrimp. However, further oxidation can lead to prominent rancid flavor characteristics; specifically one by-product of PUFA autoxidation includes hydroperoxides which are unstable and can breakdown into rancid fishy volatile components (Hsieh and Kinsella 1989; Park 2005; Varlet et al. 2007; Liu et al. 2021). These rancid flavors are undesirable, so their presence should be monitored when analyzing the fish for its volatile compounds and the potential final products since the components can be susceptible to the same degradation as they would in the fish naturally. One of the main reasons for a difference in the production of desired aroma versus fishy odors is linked to storage conditions; therefore, proper storage conditions need to be strictly followed. In surimi, thermally derived seafood compounds play the most prominent role (Park 2005). When processing a product, the time at which the flavoring is added is crucial because heating the product can result in a change or loss in flavor. In the current research area, there does not seem to be significant research on the change of seafood flavors after thermal processing. For culture-based fish products this will also need to be established if they want to sell products not only to mimic raw seafood, but cooked seafood as well.

Factors Affecting Fish Flavor Quality

Enzymes have a significant impact of fish flavor development. Specifically, the production of trimethylamine (TMA), which contributes to an overly fish flavor, has been linked to proteases and trimethylamine-N-oxide reductase (Schwimmer 1981). TMA has a distinctive "fishy" aroma and trimethylamineoxide (TMAO) is an osmoregulatory compound found in many fish species. Careful consideration should be taken when analyzing flavor components of fish to not activate these enzymes because undesirable changes could occur.

While enzymes can contribute to off-flavor production, there are some enzymes that can be utilized to reduce the production of off-flavors. In fish, ribonuclease can reduce the amount of odor formation (Schwimmer 1981). The addition of ribonuclease may not be possible when trying to understand the complex flavors of fish, but storage is a factor that can be more easily controlled. It has been found that oxidative rancidity can occur in fish products that have not been processed, leading to the production of off-flavors after several days in storage at 1°C (Mendenhall 1972). To curtail the production of these off-flavors, storing at cooler temperatures could be beneficial. The creation of off-flavors in unprocessed fish is because of enzyme pathways that act on the lipids found in the fish (Schwimmer 1981).

Flavor of fish can also be altered based on the welfare of the fish during the time of its processing. Different stressors can influence the rigor mortis of the fish, and if a fish is processed before, during, or after rigor mortis there can be effect on the flavor and other sensory perceptions. Rigor mortis occurs when creatine phosphate is broken down, followed by a decreasing amount of adenosine triphosphate (ATP). Once the amount of ATP has reached 0.1 μmol/g rigor will onset (Wang et al. 1998; Oliveira et al. 2004).

During rigor mortis, the alternating cross-linkages that occur between myosin and actin cease and are replaced by permeant linkages causing the muscle to become stiff. Once rigor is resolved, the muscle will become soft and the muscle will become meat (Wang et al. 1998). It has been determined that the at the end of an animals' life, the pre-slaughter and slaughter conditions can influence the final quality that will be expressed and the changes in organoleptic properties during storage (Poli et al. 2005). While these can also be applied to wild-caught fish, it is of particular importance with farmed fish because these animals are at an increased chance of experiencing stressful conditions. When a fish is experiencing stressful conditions, there will be endocrine responses that lead to an increase in the fishes' heartbeat, increased oxygen uptake, higher energy source mobilization, and an increase in plasma glucose (Poli et al., 2005). These attributes can be used to test the stress levels of fish. It has been shown that managing the stress to the fish before slaughtering will delay the onset of rigor mortis, which means the fish could be processed before rigor sets and which would lead to an increase in fillet yields and cause less damage to the flesh (Poli et al. 2005).

Slaughter can be one of the most stressful times for a fish. Traditionally there are three methods used to stun the fish, including a manual technique (blow to the head, a stab to the neck), asphyxiation by CO_2, and electric shock (Marx et al. 1997). Each of these methods have a different stress effect on the fish. Marx et al. (1997) determined that the best method depends on the type of fish; for the species studied in this work, trout, carp, and eel, it was determined that CO_2 works for trout, but was inefficient for carp and eel. Therefore, it is important to recognize that some stressors vary from species to species, and it is critical to establish what method works best each species because managing the stress

to the animal can lead to better quality product. In contrast, stressful conditions before and during killing will lead to an increase in lactic acid production, which will increase the rate of rigor mortis onset, affecting the quality of the flesh of the fish (Poli et al. 2005). The well-being and handling of fish can affect the quality and sensory attributes of fish, so this must be considered when evaluating fish samples.

Lastly, to fully understand the volatile compounds responsible for seafood's flavor, several factors need to be met, including that the seafood is of good quality and is appropriately stored during the analysis process (Park 2005). It is crucial to have good quality fish because, if not, the products that would be created will be of lower quality, and potentially undesirable to consumers. Another factor to keep in mind is that fresh raw seafood will differ in flavor composition from cooked seafood (Park 2005). It is important to keep in mind the final product because that dictate which kind of volatile compounds are necessary for the product.

Diet Effect on Proximate and Fatty Acid Composition

Diet plays a key role in the final nutritional composition of fish; it is of particular interest to understand the effects of diet on final proximate and fatty acid composition of cultured fish (rendered in captivity) since their diets are controlled. It has been noted that there are both similarities and differences than can be found in the composition of wild and cultured fish species (Roy et al. 2010). For BT, this is important to note since many are wild as juveniles and are then cultured until they are sold to the market. Typically, a BT larvae will feast upon zooplankton usually a mix of crustacean, jellyfish, mollusks, and other fishes' larvae (Lovatelli et al. 2008). In the wild, juveniles and adult BT consume mostly squid, fish, and crustacean (Lovatelli et al. 2008). In captivity this diet is adapted to help meet the BT dietary needs while also being cost effective for operation procedures.

Often in captivity BT species will be fed a compound diet of that consist of a mix of small pelagic species such as a variety of mackerel (several species), sardines (*Sardinella aurita, Sardinops melanostics*), and squid (*Illex sp*.) (Sawada et al. 2005; Lovatelli et al. 2008). There has also been some interest on whether there can be substitutions made to the diet of cultured BT. Some researchers have determined that there is the possibility of decreasing the reliance of small pelagic species by including alternative protein sources such as soybean meal and soya protein concentrate, in addition to new fatty acid sources (Smullen 2009; Biswas et al. 2011).

While diet can affect all compositional aspects of the BT, protein and fat content are the most closely examined. Therefore, the dietary protein and fat are of the utmost considered when trying to establish a diet for the fish. Due to the importance of dietary proteins being the source of important amino acids that are utilized to synthesize new proteins for growth, reproduction, and replacement of existing protein (Mourente and Tocher 2009). Dietary fat will influence fat content and fatty acid composition, thus affecting organoleptic proteins and nutritional aspects of the fish. It has been discovered that consumers believe that wild fish are better in quality and flavor than cultured species, but many wild species have been found to be leaner than their cultured counterparts (Mourente and Tocher 2009; Roy et al. 2010). Fat content in fish meat is of particular importance to consumers. The primary reason for consumers' interest is because of the fatty acid (FA) profile of fish with a specific interest in eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) the latter of which can be found in high levels in tuna (Mourente and Tocher 2009; Roy et al. 2010). It is essential that the dietary

requirements of the BT are meet, not only to yield successful rendering, but to meet the expectations set by consumers.

Previous Research on the Volatile Analysis of Tuna

The flavor of canned tuna has been analyzed. Changes in volatile flavor compounds over canned tuna shelf-life in the refrigerator at 4°C was studied (Kim and Lindsay 1992). The volatile flavors presence in the beginning would indicate what consumers would deem acceptable, but over time those flavors can change and produce off-flavors which are undesirable to consumers. Over the course of 4 weeks, once a week a mixed tuna sample (Albacore; *Thunnus alalunga*) was removed and a sensory evaluation and an analysis of the volatile compounds was completed (Kim and Lindsay 1992). Volatile compounds were assessed using gas chromatography flame ionization detector (GC-FID), which is commonly used. Once the samples were prepared, they were then analyzed. Volatiles were then redistilled with diethyl ether in a tube where the extracts were concentrated under a stream of nitrogen, the volatiles were separated by using a Carbowax 20M capillary column (60m x 0.32 mm, i.d., 0.25μm coating thickness) at an oven temperature programmed to be 50°C to 250°C; helium was used as the carrier gas (Kim and Lindsay 1992). Analyzing volatiles this way is useful; however, other methods of analysis can also indicate the presence of volatiles, therefore, running more than one method can indicate more information. In this study, gas-chromatography mass spectroscopy (GC-MS) was also used with the same conditions as mentioned previously (Kim and Lindsay 1992). These two analyses allowed researchers to compare results of compounds found in samples. In the end, the researchers found 126 compounds in canned tuna, of which 54 were determined by mass spectra. The compounds found in the highest concentration were 1-penten-3-ol, benzaldehyde, and 1, 4-dimethyl benzene, but each decreased over storage. An increase in

some compounds such as diethyl-trisulfide, 1,2 dichlorobenzene, and 2-heptylthiophene occurred, suggesting that they affect quality parameters for canned tuna (Kim and Lindsay 1992). From the research we can conclude they are many aroma compounds found in canned tuna, but it is less clear the extent to which each compound plays a role in positive or negative aroma/flavor quality.

Furthermore, research on the nonvolatile and volatile compounds found in cooked, canned, and raw yellowfin tuna (*Thunnus albacores*) has been conducted. Using headspace solid-phase microextraction (SPME) paired with GC-MS, the volatile compounds were analyzed and the nonvolatile compound were determined such as free amino acids, organic acids, and 5'-nucleotides (Zhang et al. 2019). For this analysis, samples were minced and allowed to equilibrate at 70°C for 15 min. From the researchers' preliminary experimental results, it was established that the optimal volatile extraction occurred at 70°C for 40 min using a divinylbenzene/ carboxen/ polydimethylsiloxane (DVB/CAR/PDMS, 50/30 μm) film SPME fiber (Zhang et al. 2019). Other SPME fibers could potentially be used to extract the compounds from the tuna samples, however the DVB/CAR/PDMS fiber is commonly used because of its ability to bind polar and nonpolar volatile aroma compounds. The volatile compounds were analyzed using a TR-35 MS column (0.25 mm × 30 m, 0.25 μm; Thermo Fisher Scientific, MA,USA) with helium as the carrier gas; the temperature program used started at 30°C then ramped up to 92°C at a rate of 4°C until it finally reached a final temperature of 200°C at a rate of 5°C/min (Zhang et al. 2019). From this, the researchers were able to establish the aroma compounds found in the different tuna samples. It was determined that a total of 7, 9, and 7 volatile compounds responsible for aroma were found in raw, cooked, and canned tuna respectively (Zhang et al. 2019). The

list of compounds included a variety of aldehydes, alcohols, ketones, hydrocarbons, aromatics, furans, and others. As summary of those compounds can be found in Table 2-1. While some compounds are distinctive for raw, cooked, or canned product, there are several compounds that overlap such as octanal and nonanal, which might suggest that these compounds can play an important role in the flavor perception of tuna despite the difference in preparation method before consumption.

Type of Tuna	**Compounds Found**
Raw tuna	Decanal, nonanal, octanal, (E)-2-nonenal
Cooked Tuna	Octanal, nonanal, 2-pentylfuran, 1-Octen-3-ol, hexanal, 2-ethyl-furan
Canned Tuna	2-Methyl-3-furan-thiol, octanal, nonanal

Table 2-1: Summary table of the major compounds found in raw, cooked, and canned yellowfin tuna (*Thunnus albacores*) (Zhang et al. 2019).

Chapter 3. Analysis of volatile compounds in Pacific bluefin tuna (*Thunnus orientalis*) by SPME-GC-MS

Abstract

Volatile composition of a food has a huge impact on the overall acceptance of food products. Volatile composition can affect sensory perception, both directly through sniffing and indirectly through mastication and swallowing. The purpose of this research was to determine the volatile composition of the 6 different cuts of Pacific bluefin tuna (PBT: *Thunnus orientalis*), *dorsal akami*, *ventral akami*, *dorsal chu-toro*, *ventral chu-toro*, *wakaremi*, and *otoro*. Determination and identification of compounds was done by using gas chromatography-mass spectroscopy (GC-MS). This study identified 41 compounds found among the 6 different cuts of bluefin tuna that met the identification requirements: including 9 aldehydes, 7 alcohols, 14 alkanes, 2 ketones, 4 alkenes, 3 aromatic compounds, and 2 miscellaneous compounds. This research can serve as a starting point for the method development and analysis of the different cuts of PBT.

Keywords: Pacific bluefin tuna, volatile analysis, GC-MS

Introduction

There are several factors that can affect consumers perception of foods. Volatile composition is one of those factors. Due to the creation of gas chromatography-mass spectroscopy volatile compounds can be separated via the gas chromatograph and subsequently identified by the mass spectrometer (Grayson 2016), thus, allowing researchers the ability to identify compounds that are responsible for consumers recognition of food products.

Volatile compounds affect consumers sensorial perception of foods in two pathways: orthonasal and retronasal. In the orthonasal process consumers are sniffing directly with their noses, while in the retronasal process consumers are perceiving odors during mastication and swallowing in which odors travel through the the pharynx until they reach the nose (Bojanowski and Hummel 2012). For fish, especially fish that will be consumed raw, the volatile compound composition is especially crucial because there is no masking involved through a secondary process (i.e., cooking etc.). It has been said that volatile aroma compounds have been associated with the freshness of fish, therefore, they have a huge impact of consumer acceptance of fish products (Mansur et al. 2003).

Some research has been done to analyze the volatile composition of other bluefin tuna (BT) species, but there is an absence of research done to analyze the volatile composition of Pacific bluefin tuna (PBT; *Thunnus orientalis*). This study aims to identify the volatile composition of the 6 different cuts of cultured PBT.

Materials and Methods

Samples

For preliminary experimentation, sushi-grade yellowfin tuna was purchased from Oasis World Market (Blacksburg, Va) and Kroger (Blacksburg, Va). Bluefin tuna (*Thunnus orientalis*) from a farm in Mexico was shipped on dry ice to Finless Foods located in Emeryville, California. Researchers at Finless Foods then cut the fish into its different cuts: *otoro, ventral akami*, *dorsal akami*, *ventral chu-toro*, *dorsal chu-toro*, and *wakaremi*. The pieces were portioned, vacuum-sealed, frozen at -80°C, and then shipped overnight on dry ice to researchers at Virginia Tech in Blacksburg, Va. Once the pieces of bluefin tuna arrived, they were immediately placed into -80°C freezer (Thermo Scientific Asheville, NC, USA) until further testing. Bluefin tuna samples used in this experimentation were vacuumed-sealed on April 28th, 2021, and May 27th, 2021.

Sample Preparation and Volatile Extraction

Yellowfin tuna were kept in a standard freezer at -18°C. Bluefin tuna samples were stored in a -80°C freezer until analysis, to preserve quality before analysis. We used the yellowfin tuna to establish methods for gas chromatography analysis, to prevent wasting the more expensive bluefin tuna. Once methods were established, the following procedure was used to extract the volatile compounds from bluefin tuna. Individual samples were weighed to approximately 33 g using a balance. Then 33 mL of boiled distilled water and 8.25g of ashed NaCl were added to the sample before blending. Samples were blended in a NutriBullet Nutrient Extractor (Los Angeles, CA, USA) for 6 sec, to create a paste. Following that, 11 μL of diluted 2,3-dimethylpyrazine (1 mL/1000mL of D.I. water) was added as the internal standard to the sample and then blended again for 6 sec. Using a plastic transfer pipette, 6 grams of paste were placed in the amber-tinted 20 mL headspace vials (Supelco Inc, Bellefonte, PA, USA) to create five replicates for analysis for each cut.

Vials were then sealed with an 18 mm Teflon-lined silicone septum. The volatiles were collected using HS-SPME method according to Zhang et al. (2019). From preliminary experimentation, it was determined that the optimal conditions for vial equilibration was for 15 min at 40°C. After samples were allowed to equilibrate, a volatile extraction occurred using a 1 cm SPME fiber coated with a divinylbenzene/carboxen/ polydimethylsiloxane (DVB/CAR/PDMS) for 50 min at 40°C. Once volatiles were extracted, the fiber was inserted into the GC inlet, with a GC injection port temperature of 240°C for 5 min before separation on the GC-MS.

Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis

A GC-MS QP-2010 Ultra Shimadzu (Shimadzu, Kyoto, Japan) was used to analyze the volatile compounds in the bluefin tuna. The autosampler (AOC-5000 Plus) was used to assist in running the samples. A Zebron ZB-5 MS column (0.25 mm x 30 m, 0.25 μm; Phenonemex Torrance CA) was used. Helium (purity 99.999%) was used as the carrier gas at a linear flow velocity of 25 cm/sec. The injection mode was set to splitless. The column oven temperature program was set to start at 40°C for 5 min and then increased at a rate of 5°C/min until it reached 240°C and was held at this temperature for 5 min, for a total run time of 50 min. The MS settings conditions were set up using the following program: ion source temperature, 230°C; detector interface temperature, 200°C. The start time for the EI source was set to 1.10 min and the end time to 50 min. The acquisition mode was set to 1 scan speed of 1250, solvent cut time, 0.6 min, a mass charge ratio (*m/z*), 40-400. For best results, a blank vial was run before a bluefin tuna sample, then again after every two vials of a sample.

Statistical Analysis

In this test, 5 replicates (n=5) were run. Identification of the volatile compounds present was done by comparing calculated linear retention indices (LRI) to LRIs found in the literature (Flavornet, Pherobase, etc) and library searches (NIST Database). JMP software Pro 16 (©2021 SAS Institute Inc., Cary, NC, USA) was used to run one-way ANOVA at a significance level of $a = 0.05$. The post hoc test Tukey's HSD was conducted to identify whether samples were significantly different from each other at a p-value of $\alpha = 0.05$. In addition, 2,3-dimethylpyrazine was used as an internal standard to be used in the quantification of volatile compounds. The peak ratio was calculated by dividing the peak area of the compound by the peak area of the internal standard. The average and standard error of the peak ratio was reported to compare to other research done on tuna.

Results and Discussion

There were 41 compounds found among the 6 different cuts of bluefin tuna that met the identification requirements. These compounds included 9 aldehydes, 7 alcohols, 14 alkanes, 2 ketones, 4 alkenes, 3 aromatic compounds, and 2 miscellaneous compounds (Table 4-1). Peak ratio of each compound was also determined using the peak area of the compound divided by the peak area of the internal standard (Table 4-2). Several aldehydes' including hexanal, octanal, nonanal and decanal were also found to be present in raw yellowfin tuna (*Thunnus albacores*) (Zhang et al. 2019). However, other additional aldehydes were identified as being in PBT that were not present in yellowfin tuna including: butanal, (E)-2-pentenal, heptanal, (E,E)-2,4-heptadienal, and dodecanal. The aldehydes octanal and nonanal are thought to be important to the aroma of tuna because they have been found to have low odor thresholds (Jiang et al. 2017; Zhang et al. 2019). Some studies suggest that some of these aldehydes including heptanal, octanal, and nonanal

may be linked to off-odors that bind easily to myosin in fish (Gu et al. 2020; Xu et al. 2020). However, several of these compounds are also associated with with green and floral notes (Selli et al. 2009).

The alcohols 1-penten-3-ol and 1-octen-3-ol found in PBT were also found to be in raw yellowfin tuna (Zhang et al. 2019). Formation of these alcohols has been linked to the oxidation of polyunsaturated fatty acids (PUFAs) and in some instances they have been linked to potential off-flavors found in fish (Selli et al. 2009; Zhang et al. 2019; Gu et al. 2020). The ketone compounds found in PBT were not ones that were found in raw yellowfin tuna. In the PBT samples the ketones present were 2,3-pentanedione and 2,3-octanedione. These compounds have been found in other fish species such as raw rainbow trout and 2,3-pentanedione was said to provide buttery, caramel, and fruity aroma, while 2,3-octanedione had a metallic aroma (Ma et al. 2020). Furans have been linked to be of importance in cooked tuna samples; however, at low levels it can be found in raw tuna in the study conducted on yellowfin tuna found the furan 2-ethylfuran an found the compound 2-pentylfuran and cooked samples (Zhang et al. 2019). In PBT 2-pentylfuran was found in the leaner dorsal akami cut. Degradation of linoleic acid is associated with the formation of 2-penthylfuran (Gayoso et al. 2017), however, 2-pentylfuran was also found in raw samples of raw rainbow trout and found to have an orange or licorice aroma (Turchini et al. 2004; Ma et al. 2020).

Most of the alkanes were found in the *ventral chu-toro* cut. In some species of fish like rainbow trout there has been a link found between the metabolism between of n-alkanes and their subsequent appearance in lipids of the fish (Cravedi and Tulliez 1986). The alkenes and aromatic compounds were not the same as those found in the yellowfin

tuna studied previously (Zhang et al. 2019). A reason for this different could be because PBT cuts contain varying levels of fat while yellowfin tuna is known for being almost completely lean.

	Dorsal Akami	Ventral Akami	Dorsal Chu-toro	Ventral Chu-toro	Wakaremi	Otoro	p-value
Aldehydes							
Butanal	9.50E+05	1.07E+07	8.30E+05	1.10E+06**	1.12E+06	8.27E+05*	0.9257
2-Pentenal, E	1.81E+05*b	ND	3.39E+05b	3.51E+05**b	6.48E+05ab	1.13E+06a	0.0040
Hexanal	1.28E+07	1.28E+07	6.77E+06	9.02E+06	2.98E+07	8.24E+06	0.3475
2-Hexenal, E	ND	ND	ND	ND	ND	9.25E+05	
Heptanal	2.00E+06ab	2.10E+06a	6.01E+05c	6.94E+05bc	1.36E+06abc	9.54E+05bc	0.0008
Octanal	1.58E+06ab	2.12E+06a	8.11E+05b	1.33E+06ab	1.51E+06ab	1.25E+06ab	0.0087
2,4-Heptadienal, (E,E)	5.73E+05	8.88E+05	5.76E+05	9.35E+05	8.86E+05	1.35E+06	0.0624
Nonanal	1.80E+06a	1.70E+06a	3.83E+05b	5.59E+05b	8.47E+05b	3.74E+05b	<.0001
Decanal	5.87E+04	ND	ND	ND	ND	ND	
Dodecanal	ND	ND	ND	ND	ND	2.13E+05	
Alcohols							
1-Penten-3-ol	1.58E+07b	1.69E+07b	1.45E+07b	2.07E+05b	1.57E+07b	3.27E+07a	<.0001
1-Pentanol	6.08E+05	7.15E+05	3.11E+05	ND	5.57E+05	ND	0.2822
2-Penten-1-ol, Z	1.77E+06b	2.04E+06b	1.33E+06b	3.15E+06b	2.15E+06b	5.65E+06a	0.0001
1-Hexen-3-ol	3.77E+05*	4.19E+05	ND	ND	4.20E+05**	ND	0.9493
1-Heptanol	1.20E+06**ab	1.70E+06a	ND	ND	7.66E+05b	5.79E+05*b	0.0177
1-Octen-3-ol	2.97E06ab	3.42E+06a	9.58E+05b	1.12E+06b	1.85E+0ab	1.65E+06ab	0.0068
2-Ethyl-1-hexanol	ND	3.22E+04*	ND	ND	ND	ND	

Alkanes							
2-Methylhexane	ND	ND	ND	3.53E+05*	ND	ND	
3-Methylhexane	ND	ND	9.64E+04	9.16E+04	7.61E+04*	ND	0.6933
Methyl-cyclohexane	ND	ND	6.85E+04**	ND	4.84E+04**	ND	
2,3,4-Trimethylpentane	ND	ND	1.95E+05b	4.36E+05a	1.48E+05bc	6.97E+04c	<.0001
3-Methylheptane	ND	ND	1.27E+05*	ND	1.11E+05*	ND	
3-Methylnonane	ND	ND	ND	3.94E+058*	ND	1.47E+05**	<.0001
2,6,10-Trimethyldodecane	ND	ND	ND	ND	1.20E+05	ND	
2,5-Dimethylhexane	ND	ND	ND	2.07E+05	ND	ND	
3,3-Dimethylhexane	ND	ND	ND	5.18E+05*	ND	ND	
2-Methylheptane	ND	ND	ND	1.24E+06	ND	ND	
2,4-Dimethylhexane	ND	ND	ND	2.85E+05*	ND	ND	
2,2,5-Trimethylhexane	ND	ND	ND	9.73E+05*	ND	ND	
Ethyl-cyclohexane	ND	ND	ND	5.76E+04*	ND	ND	
2,5-Dimethylheptane	ND	ND	ND	4.05E+05**	ND	ND	
Ketone							
2,3-Pentanedione	8.60E+06abc	9.18E+06ab	4.64E+06c	4.95E+06bc	4.21E+06c	1.15E+07a	<.0001
2,3-Octanedione	2.37E+06ab	2.83E+06a	7.33E+05b	ND	ND	1.22E+06ab	0.0090
Alkene							

3,5,5-Trimethyl-2-hexene	3.71E+06*	3.57E+06	ND	ND	2.05E+06	2.75E+06	0.2875
4-Methyl-1-heptene	ND	ND	1.03E+05**	ND	ND	ND	
3,5-Octadiene (Z,Z)	ND	ND	1.49E+05**	ND	2.50E+05	ND	
1,3-Dimethyl-1-cyclohexene	ND	ND	ND	ND	4.98E+04*	ND	0.4428
Aromatic							
Toluene	1.16E+05*c	ND	1.15E+06b	ND	1.47E+06a	ND	<.0001
Ethylbenzene	ND	ND	ND	ND	4.61E+05**	ND	
Mesitylene	1.87E+05**	ND	ND	ND	ND	1.88E+05	0.9848
Miscellaneous							
2-Pentylfuran	1.61E+05*	ND	ND	ND	ND	ND	
2-Methyldecalin	ND	ND	ND	ND	4.03E+04**	ND	

Table 3-1: Summary table of the volatile compounds found in the different cuts of bluefin tuna. The average peak area is reported in scientific notation. ND means not detected. *Indicates compound only found in 4 samples. **Indicates samples found in only 3 samples. Different letters indicate samples that are significantly different from each other at ($p < 0.05$) by Tukey’s HSD.

	Dorsal Akami	Ventral Akami	Dorsal Chu-toro	Ventral Chu-toro	Wakaremi	Otoro
Aldehydes						
Butanal	0.69±0.28	0.42±0.11	0.45±0.10	0.61±0.12**	0.37±0.10	0.58±0.07*
2-Pentenal, E	0.12±0.04*	ND	0.18±0.05	0.24±0.13**	0.22±0.07	0.77±0.08
Hexanal	8.97±2.79	5.10±1.21	3.68±0.71	5.60±0.40	11.15±7.50	5.67±0.37
2-Hexenal, E	ND	ND	ND	ND	ND	0.63±0.14
Heptanal	1.39±0.43	0.84±0.20	0.32±0.07	0.43±0.02	0.47±0.09	0.66±0.04
Octanal	1.10±0.33	0.84±0.19	0.44±0.08	0.82±0.06	0.52±0.08	0.86±0.05
2,4-Heptadienal, (E,E)	0.39±0.11	0.35±0.08	0.31±0.13	0.59±0.12	0.30±0.07	0.92±0.08
Nonanal	1.20±0.30	0.66±0.11	0.19±0.03	0.35±0.03	0.29±0.05	0.26±0.01
Decanal	0.04±0.01	ND	ND	ND	ND	ND
Dodecanal	ND	ND	ND	ND	ND	0.14±0.00
Alcohols						
1-Penten-3-ol	10.80±2.93	6.57±1.20	7.78±1.37	12.90±0.83	5.31±0.72	22.51±1.40
1-Pentanol	0.43±0.14	0.28±0.06	0.17±0.05	ND	0.19±0.06	ND
2-Penten-1-ol, Z	1.26±0.40	0.82±0.19	0.72±0.21	1.94±0.15	0.73±0.21	3.85±0.46
1-Hexen-3-ol	0.28±0.06*	0.17±0.04	ND	ND	0.14±0.05**	ND
1-Heptanol	0.96±0.17**	0.68±0.16	ND	ND	0.62±0.19	0.41±0.03*
1-Octen-3-ol	2.05±0.62	1.36±0.36	0.52±0.15	0.70±0.08	0.62±0.19	1.12±0.16
2-Ethyl-1-hexanol	ND	0.01±0.00*	ND	ND	ND	ND
Alkanes						

2-Methylhexane	ND	ND	ND	0.20±0.05*	ND	ND
3-Methylhexane	ND	ND	0.05±0.01	0.06±0.01	0.03±0.01*	ND
Methyl-cyclohexane	ND	ND	0.05±0.02**	ND	0.02±0.01**	ND
2,3,4-Trimethylpentane	ND	ND	0.10±0.02	0.27±0.03	0.05±0.01	0.05±0.02
3-Methylheptane	ND	ND	0.07±0.01*	ND	0.04±0.00*	ND
3-Methylnonane	ND	ND	ND	0.25±0.01*	ND	0.11±0.01**
2,6,10-Trimethyldodecane	ND	ND	ND	ND	0.04±0.00	ND
2,5-Dimethylhexane	ND	ND	ND	0.13±0.02	ND	ND
3,3-Dimethylhexane	ND	ND	ND	0.33±0.04*	ND	ND
2-Methylheptane	ND	ND	ND	0.83±015*	ND	ND
2,4-Dimethylhexane	ND	ND	ND	0.19±0.02*	ND	ND
2,2,5-Trimethylhexane	ND	ND	ND	0.58±0.07*	ND	ND
Ethyl-cyclohexane	ND	ND	ND	0.03±0.00*	ND	ND
2,5-Dimethylheptane	ND	ND	ND	0.25±0.01**	ND	ND
Ketone						
2,3-Pentanedione	5.99±1.71	3.62±0.72	2.54±0.75	3.01±0.14	1.47±0.23	7.98±0.39
2,3-Octanedione	1.68±0.53	1.14±0.30	0.41±0.13	ND	ND	0.84±0.06
Alkene						
3,5,5-Trimethyl-2-hexene	2.74±0.57*	1.41±0.33	ND	ND	0.69±0.18	1.86±0.48

4-Methyl-1-heptene	ND	ND	0.06±0.01**	ND	ND	ND
3,5-Octadiene (Z,Z)	ND	ND	0.08±0.01**	ND	0.08±0.03	ND
1,3-Dimethyl-1-cyclohexene	ND	ND	ND	ND	0.02±.0.00*	ND
Aromatic						
Toluene	0.06±0.01*	ND	0.61±0.09	ND	0.50±0.05	ND
Ethylbenzene	ND	ND	ND	ND	0.16±0.02**	ND
Mesitylene	0.14±0.05**	ND	ND	ND	ND	0.13±0.01
Miscellaneous						
2-Pentylfuran	0.11±0.04*	ND	ND	ND	ND	ND
2-Methyldecalin	ND	ND	ND	ND	0.01±0.00**	ND

Table 3-2: Summary table of the peak ratio of the volatile compounds found in the different cuts of bluefin tuna. Values are reported as mean ± SE. ND means not detected. *Indicates compound only found in 4 samples. **Indicates samples found in only 3 samples.

Conclusion

This study was able to identify the volatile compounds that make up the different cuts of PBT. It illustrated that while there is some overlap of compounds that can be found in all 6 cuts, there are some compounds that are only found in specific cuts. In total 41 compounds found among the 6 different cuts of bluefin tuna that met the identification requirements including 9 aldehydes, 7 alcohols, 14 alkanes, 2 ketones, 4 alkenes, 3 aromatic compounds, and 2 miscellaneous compounds. Some of the compounds identified could have either a pleasant or unpleasant aroma based on their quantity in the samples. Further research should be done with gas chromatography-olfactometry (GC-O) should be conducted to better understand their role in these samples. Additionally, fatty cuts likely have additional volatile compounds that play a role in the sensorial perceptions of those cuts; therefore, additional adjustments could be made to the analysis methods of those cuts to better identified and separate those compounds. This research can serve as a starting point for the method development and analysis of the different cuts of PBT.

Chapter 4. Proximate Composition and Fatty Acid Analysis of Pacific bluefin tuna (*Thunnus orientalis*)

Abstract

The proximate composition and fatty acid (FA) analysis of the *dorsal akami*, *ventral akami*, *dorsal chu-toro*, *ventral chu-toro*, *wakaremi*, *otoro* cuts of cultured Pacific bluefin tuna (PBT;*Thunnus orientalis*) was conducted in this study. To evaluate the differences in composition of the different cuts. These cultured PBT were caught have juveniles and the raised in captivity until they reached market size. Statistical differences ($p < 0.05$) were found for the moisture, ash, protein, and fat content between each cut of cultured PBT. Additionally, a significant difference ($p < 0.05$) was found for eicosapentaenoic acid (EPA, 20:5n-3). Docosahexaenoic acid (DHA, 22:6n-3) was found in higher amounts than EPA in each cut, but there was not a significant difference between cuts. Overall, the fat content of cultured PBT is vital to the organoleptic and nutritional aspects of PBT.

Keywords: bluefin tuna, proximate composition, fatty acid composition, DHA, EPA, *omega*-3, *omega*-6

Introduction

The Pacific bluefin tuna (PBT, *Thunnus orientalis*) is an important species not only for the balance of the world's oceans, but also for the consumption of humans. The consumption of PBT and other bluefin tuna (BT) species has increased over the years due to a spike in global consumption of sushi and sashimi (Bestor 2000). Since more people are consuming the species, it has started to decline in number, making the fish an interest to companies creating seafood alternatives.

To create a seafood alternative that consumers will enjoy, companies need to understand the proximate and fatty acid composition of PBT, to properly meet or exceed consumer expectations of nutrition. Since diet can affect final composition, it is crucial to examine the protein, ash, moisture, and fat contents. Carbohydrates are not analyzed due to their minimal contribution to fish carcass mass (Johnston et al. 2002) and for proximate analysis, they are determined by difference. Fat content and subsequent fatty acid composition are of high importance to consumers due to their proposed health benefits. Fish can contain essential fatty acids (EFAs) that are needed for brain development in the early stages of life docosahexaenoic acid (DHA) specifically has been associated with neural development, however, the benefits can also continue into the later stages of life as continued consumption of long chain n-3 fatty acids (LCn3) is associated with lower risk of coronary heart disease (Kris-Etherton et al. 2002; Innis 2007; Innis and Friesen 2008). Thus, it is critical that seafood alternatives contain these fatty acids at equal or greater levels.

Composition of wild and cultured (full-cycle) PBT have been analyzed to determine the effects of diet on final proximate and fatty acid composition (Roy et al. 2010). There has been little research conducted on the proximate and fatty acid composition of PBT that

have been raised partially in the wild as juveniles and then raised in captivity in sea cages, which now makes up most of the PBT market. The purpose of this study is to determine the proximate and fatty acid composition of PBT that have been raised both in the wild and in captivity.

Materials and Methods

Moisture Content

The moisture content of the different cuts of bluefin tuna was determined by following an oven method (modified AOAC, 24.003a, 1984). Approximately 3 g (± 0.05g) of the sample was weighed into a pre-dried glass crucible (without a lid) and was dried at 105°C for 16-18 hr in an oven (Thermo Scientific, Dubuque, IA, USA). This analysis was conducted in triplicate. The crucibles were retrieved from the oven and placed into desiccators to cool before they were weighed. The resulting weight loss was used to calculate moisture content.

Ash Content

The ash content of the bluefin tuna cuts was done by dry ashing the samples previously used in the moisture analysis (modified AOAC 31.012, 1984). Samples were inserted into a temperature-controlled furnace (Lindberg, Watertown WI) at a temperature of 550°C overnight. Once light gray ash was achieved the crucibles were removed and placed into a desiccator. After the crucibles reach room temperature they were weighed. The change in weight was used to determine the ash content.

Protein Content

The Kjeldahl method was used to used determine the protein content of the bluefin tuna cuts (991.36, AOAC 1998) based on nitrogen determination. Two replications were performed on each cut. Roughly 2 g (±0.05 g) of the sample was weighed into a glass tube containing filter paper (Whatman #541, no-nitrogen). A packet of Kelmate'N Kjeldahl

Digestion Mixture 200 (containing 10.0 g K_2SO_4 and 0.3 g $CuSO_4$), along with 3 mL 30-35% hydrogen peroxide, and 15 mL concentrated sulfuric acid was added to the sample. To hydrolyze the sample, the sample was digested in a block digester for 50-60 min, then cooled for 10 min. Following that 50-70 mL of distilled water and 50-60 mL of 10 N sodium hydroxide was be added. The glass tubes were then inserted into the distillation unit (FOSS Kjeltec 8100; Hillerød, Denmark). In an Erlenmeyer flask, a boric acid solution was added (250 mL) to the receiver for distillation. The resulting collected distillate was titrated with 0.20 N HCl. The conversion factor to calculate the estimated protein content was 5.6 instead of the standard 6.25 because it is calculated conversion factor for fish and shrimp (Mæhre et al. 2018).

Fat Content

The fat content of the tuna cuts was measured using a modified Folch, Lees, and Sloane Stanley method (1957) as reported by Christie (1982). During a trial test of the method, we determined that 1 g of sample, 10 mL of methanol, and 20 mL of dichloromethane were not able to reach the blades of the blender jar, therefore, adjustments were made to correct overcome this challenge. Accurate measurement of 5 g (±0.05 g) of the sample was weighed and homogenized for 1 min with 50 mL of methanol in a Waring blender jar (Waring Commercial, Stamford, CT, USA). After initial homogenization 100 mL of dichloromethane was added and the mixture was homogenized for another 2 min. The mixture was then vacuumed filtered using a Büchner funnel and filter paper (Whatman No. 4 Dia. 90 mm; Maidstone, United Kingdom) into a Büchner flask. The cake was reextracted using 20 mL of dichloromethane, and 10 mL of methanol. The volume of the remaining product after extraction was taken then the addition of 25% of the total volume of 0.88 % KCl was combined and allowed to separate in a separatory funnel. The lower

lipid-containing layer was removed, and the volume was measured. The lipid was washed by adding 25% of the total volume of distilled water. The lipid was dried by using anhydrous sodium sulfate which was allowed to sit in samples until the liquid became clear. The clear liquid was then filtered to separate from the hydrated sodium sulfate. Sodium sulfate was washed using filter paper (Fisherbrand Qualitative P4 Dia. 24 cm; Pittsburgh PA, USA) and dichloromethane. The lipid was removed from the dichloromethane extracts using a rotary evaporator with a water temperature below 55°C. The round-bottom flask was weighed before the addition of the extract to ensure accuracy. The round-bottom flask was weighed after extraction to determine the % lipid in the sample.

Fatty Acid Analysis

Preparation of Fatty Acid Methyl Esters (FAME)

The American Oil Chemists' Society Official Method Ce-1b-89 "Fatty Acid Composition by GLC – Marine Oils" was used to prepare and identify fatty acid methyl esters. Using the fat extracted from the previous analysis, a 2-step methylation process was conducted to create FAME. The internal standard tricosanoic methyl ester (C23:0, 99+% purity, ACROS Organics; New Jersey, USA) was used in this analysis. Approximately 50 mg of the internal standard was added to a 50 mL volumetric flask that was brought to volume by the addition of isooctane. An accurate measurement of ~25 mg of fat was placed into 10 mL centrifuge tubes for each sample. Then 1.5 mL of 0.5 N NaOH in methanol was added to each tube and then capped with nitrogen before heating at 98°C for 5 min. The tubes were briefly placed in water to cool before adding 2 mL of 10% BF_3/methanol reagent (Sigma-Aldrich), flushing tubes with nitrogen then capping the tubes and heating at 98°C for 30 min. After placing them in water to cool again, 1.0 mL of the isooctane solution was added, the tubes were flushed with nitrogen, capped and shaken forcefully for 30 sec.

Next 3 mL of saturated NaCl solution was added to the tubes and they were flushed with nitrogen, capped, and then shaken thoroughly. The clear isooctane layer was then transferred to GC vials (10 mm Screw Thread, Fisherbrand, Pittsburgh PA, USA). The vials were then loaded into the GC autosampler tray.

Gas Chromatography-Mass Spectroscopy (GC-MS) for Fatty Acid Analysis

For this analysis, a GC-2010 MS TQ-8030 (Shimadzu, Kyoto, Japan) was used to separate and identify FAMEs extracted from the bluefin cuts' oil samples. The autosampler (AOC-20i+s) was used to assist in running the samples. The amount of sample injected into the GC was 1.0 µL for each FAME sample. A Carbowax column (Zebron ZB-Wax 60m x 0.25 mm .d., 0.25 µm film) was used the GC-MS along with helium (purity 99.999%) as carrier gas with a total flow rate of 495.9 mL/min and a column flow rate of 2.19 mL/min, linear velocity of 40 cm/sec, and a split ratio of 225:1. The column oven temperature program was set to where the GC injection port temperature was set to 250°C, followed by a temperature program that was as follows: the initial temperature of the column was 175°C with no hold time, then the temperature increased at a rate of 1.0°C/min until it reached a final temperature of 240°C with no hold time. The MS settings were as follows: the ion source temperature was 230°C, the interface temperature was 220°C, the MS was set to acquisition mode Q_3 scan, start at 40 *m/z* and end at 450 *m/z*, and a scan speed of 2000. The GC total run time was 70 min. Fatty acids were then identified by using calculated ECL (equivalent chain lengths), MS library search (NIST), and comparing results from FAME standard and cod liver oil (CLO) sample run using the same program.

Statistical Analysis

The statistical analysis was conducted using JMP software Pro 16 (©2021 SAS Institute Inc., Cary, NC, USA). For the proximate analysis the tests were conducted in

triplicate (n=3), with the exception of the protein content analysis which was conducted in duplicate (n=2), and ash content of the otoro cut which used (n=5) in its statistical analysis due to the variation in measurement of that cut samples were run again a second time. The samples were analyzed using one-way ANOVA at a significant level of $\alpha = 0.05$. The post hoc test Tukey's HSD was conducted to identify whether samples were significantly different from each other at a p-value of $\alpha = 0.05$.

For the fatty acid analysis, the FAMEs were quantified using the gas chromatography mass-spectroscopy. All FAME were analyzed in triplicate (n=3). The %weight were analyzed using a one-way ANOVA with a significant level of $\alpha = 0.05$ followed by the post hoc test Tukey's HSD to test the significant difference between samples at p-value of $\alpha = 0.05$.

Results and Discussion

Moisture Content

The moisture content was a substantial portion of the composition of the cultured PBT. Especially, for the leaner cuts that were around 70.27% to 71.15% which represent ventral and dorsal akami respectively (Figure 4-1). The moisture content of the other cuts was around 44.7 % to 52.2 % (Figure 4-1). The trend coincides with the research conducted on cultured (full cycle) PBT (Roy et al. 2010). However, the values of moisture content of the culture PBT in this study, have a similar moisture content to wild PBT in the study conducted by Roy with the akami cuts having around 68.6 to 69.1 (g/100 g wet meat) moisture compared to 62.7 to 62.9 (g/100 g wet meat) (2020). Other research studies have shown that moisture can be affected by location on the fish whether in the front or the rear and that moisture content changes as the fish grows (Nakamura et al. 2005, 2007). Moisture content from these studies was comparable to what was seen in our experiment. A one-way

ANOVA showed statistical significance ($p < 0.05$) for the moisture content of different cuts (Table 4-1). A Tukey's HSD post hoc test was conducted to determine which cuts were significantly different from one another. In this study it was determined that the leaner *akami* cuts were significantly different from the fatty cuts, but while the mixed cuts *wakaremi*, *dorsal chu-toro* and *ventral chu-toro* were significantly different from the akami cuts, ventral chu-toro was not significantly different from the otoro cut (Figure 4-1). This is a difference from what was observed previously where the dorsal and *ventral akami* cuts were not significantly different from the *dorsal chu-toro*, *ventral-chu-toro*, and *wakaremi* cuts in all three sample types for moisture content (Roy et al. 2010). The difference may be due to the nature in which our samples were raised compared to being strictly cultured or wild they are a hybrid of both. The moisture content will likely have an effect on the organoleptic properties of the fish.

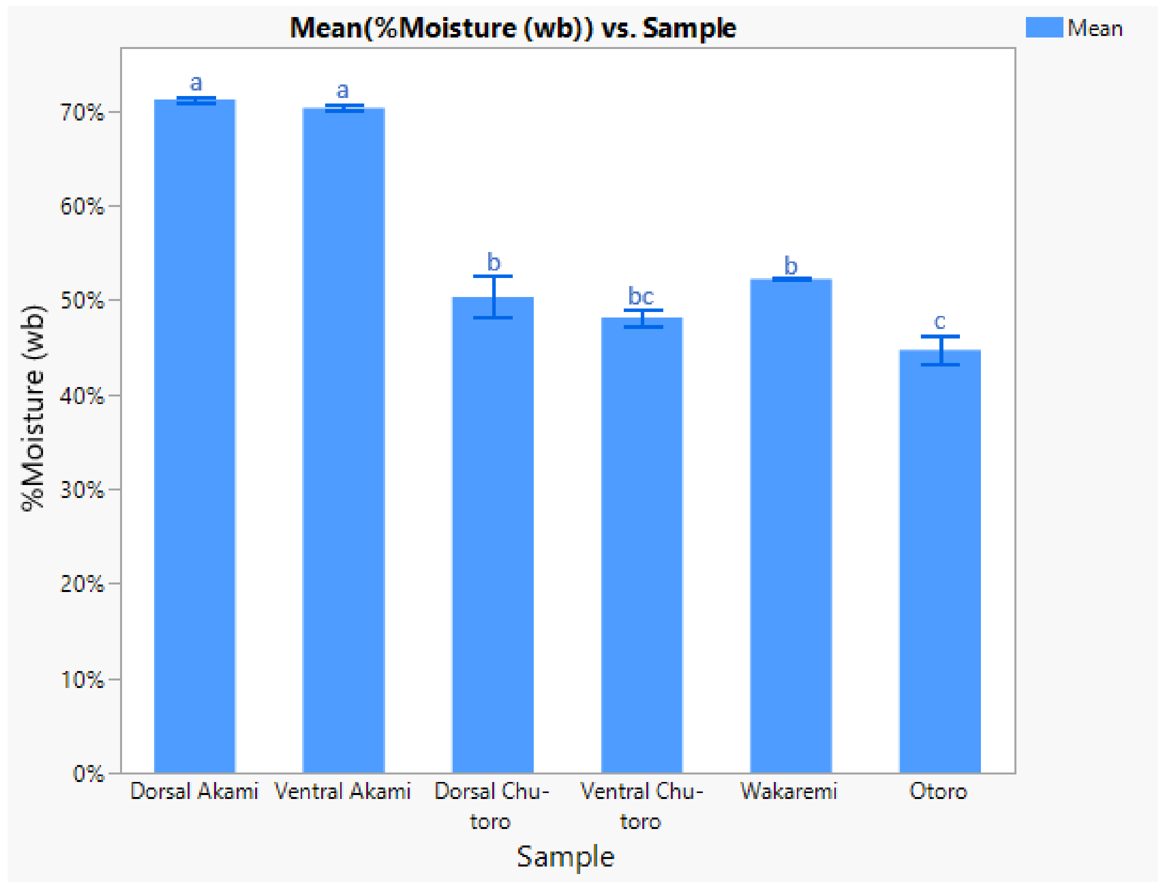

Figure 4-1: Moisture content of the different cuts of Pacific bluefin tuna with standard error bars. Different letters indicate samples that are significantly different from each other at ($p < 0.05$).

Source of Variation	Sum of Squares	d.f.	Variance	F	p-value
Between Groups	2012.2963	5	402.459	100.3953	<.0001
Within Groups	48.1049	12	4.009		
Total	2060.4012	17			

Table 4-1: F-test table for the moisture content of the different Pacific bluefin tuna cuts.

Ash Content

The ash content of the cultured PBT did not differ between samples, there was no true trend like the moisture content that we observed. Ash contents do not to seem to vary

as much between various cuts like other proximate compositions such as fat and moisture content that are distinct to each cut (Haard 1992; Shearer 1994). The ash content of the fish ranged from 0.82 % to 1.46 % in the otoro and ventral akami cut respectively (Figure 4-2). Results were similar to those determined by Roy et al. where there was not a true trend that was seen between cuts the ash content was between 1.1 to 1.3 (g/100g wet meat) in cultured (full cycle) tuna in the *dorsal chu-toro* and 3 different cuts (*dorsal akami*, *wakaremi*, and *otoro*) respectively, but got as high as 2.0 (g/100g wet meat) for wild the *ventral chu-toro* cut and as low as 0.9 (g/100g wet meat) for culture-fasted *otoro* cut (2020). Similar trends were also seen in studies that focused on location and growth phases (Nakamura et al. 2005, 2007). A one-way ANOVA was also conducted and was found to be significant ($p < 0.05$) (Table 4-2). Therefore, and Tukey's HSD post hoc test was also conducted to determine which cuts were significantly different from one another (Figure 4-2).

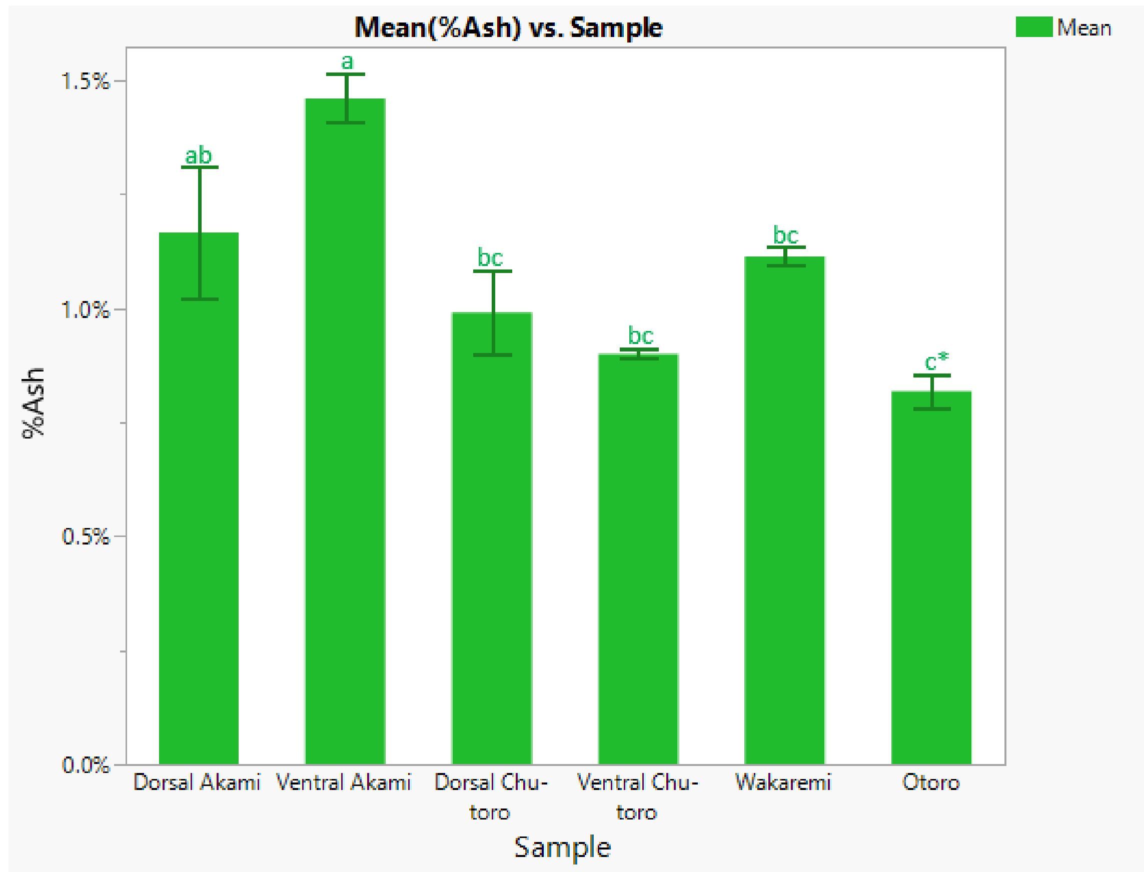

Figure 4-2: Ash content of the different cuts of Pacific bluefin tuna with standard error bars. Different letters indicate samples that are significantly different from each other at ($p < 0.05$). * Contains n=5 unlike other samples in column.

Source of Variation	Sum of Squares	d.f.	Variance	F	p-value
Between Groups	0.9010	5	0.1802	11.4667	0.0002
Within Groups	0.2200	14	0.0157		
Total	1.121	19			

Table 4-2: F-test table for the ash content of the different Pacific bluefin tuna cuts.

Protein Content

The protein content of the cultured PBT analyzed ranged from 16.79 % to 26.86% for the *otoro* and *ventral akami* cuts respectively (Figure 4-3). The protein content found in cultured (full cycle) tuna previously supported these results (Roy et al. 2010). However, protein content amongst all the cuts were not seen as significantly different from one another except for *otoro*; similarly this was also seen when comparing the front and back cuts of the fish (Nakamura et al. 2005; Roy et al. 2010). In this study, there were significant differences found between cuts except for *dorsal chu-toro* and *wakaremi* (Figure 4-3). This may be due to the difference in raising of the animal or because different conversion factors were used in the calculation of the protein content. The studies used a previous conversion factor of 6.25 that is commonly used to determine protein content in meat instead of a conversion factor determined by Mæhre et al. to be more accurate for fish and shrimp of 5.6 (2018). Using one factor or the other will result in a difference of around 10% but this shouldn't affect differences between samples. A one-way ANOVA was conducted and was found to be significant ($p < 0.05$) (Table 4-3). A Tukey's HSD post hoc test was used to determined which cuts were significantly different ($p < 0.05$) from one another (Figure 4-3).

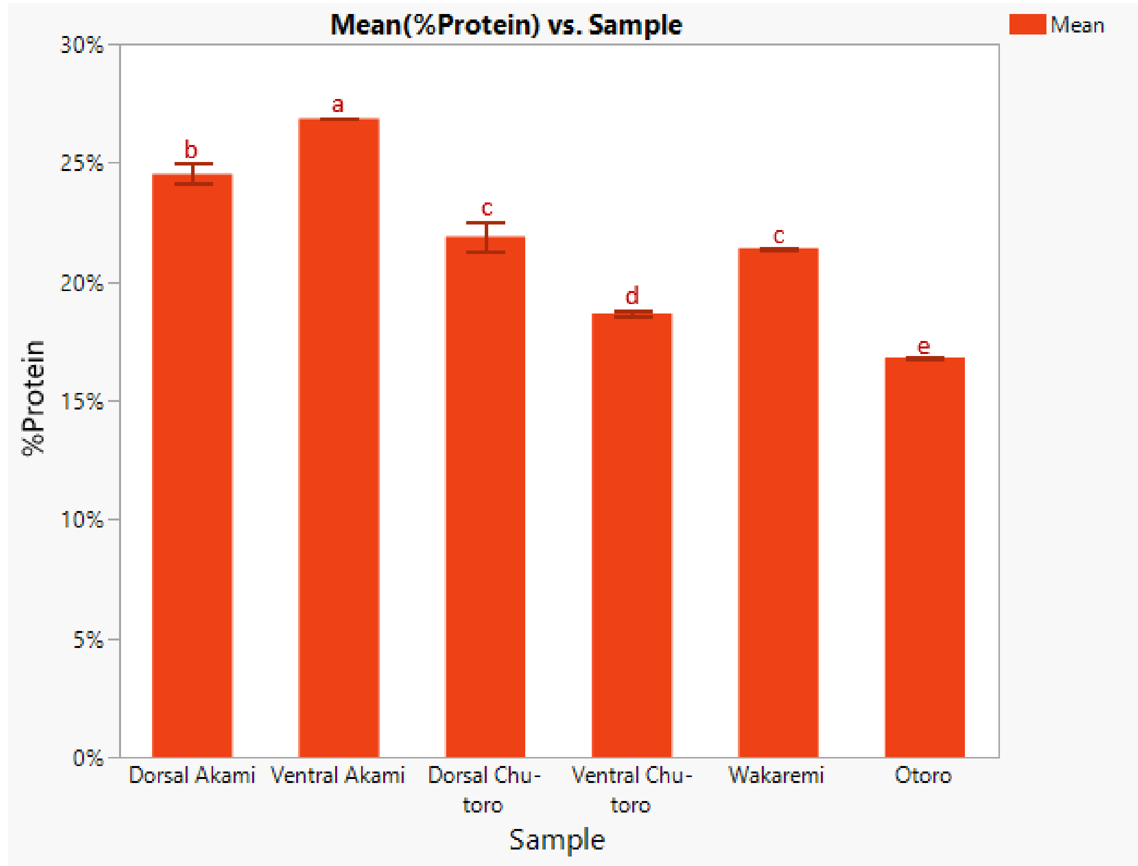

Figure 4-3: Protein content of the different cuts of Pacific bluefin tuna with standard error bars. Different letters indicate samples that are significantly different from each other at ($p < 0.05$).

Source of Variation	Sum of Squares	d.f.	Variance	F	p-value
Between Groups	0.0136	5	0.0027	133.2409	<.0001
Within Groups	0.0001	6	0.0000		
Total	0.0137	11			

Table 4-3: F-test table for the protein content of the different Pacific bluefin tuna cuts.

Fat Content

The fat content of the cultured PBT was determined to be around 4.89% to 49.29% in ventral akami and otoro respectively (Figure 4-4). The trend is supported by previous

research done on cultured (full cycle) PBT; however, the lipid content did appear to be slightly higher for the leaner cuts, but lower for the *otoro* cut (Roy et al. 2010). The differences in diets may be a factor in why these values vary. A one-way ANOVA was conducted, and it was found to be significant ($p < 0.05$) (Table 4-4). Subsequently, a Tukey's HSD post hoc test was also conducted to determine which cuts were significantly different ($p < 0.05$) from one another (Figure 4-4). It has been observed that the moisture content and fat content are inversely proportional to one another in fish muscle (Wheeler and Morrissey 2003; Jankowska et al. 2007). The same phenomenon was seen in our samples where the leaner *akami* cuts having the highest moisture content and the lowest amount of fat and vice versa for the *otoro* cut. The fat content is of main interest to consumers not only due to the health benefits of fats found in fish, but also because of the affect that fat has on mouthfeel when consuming.

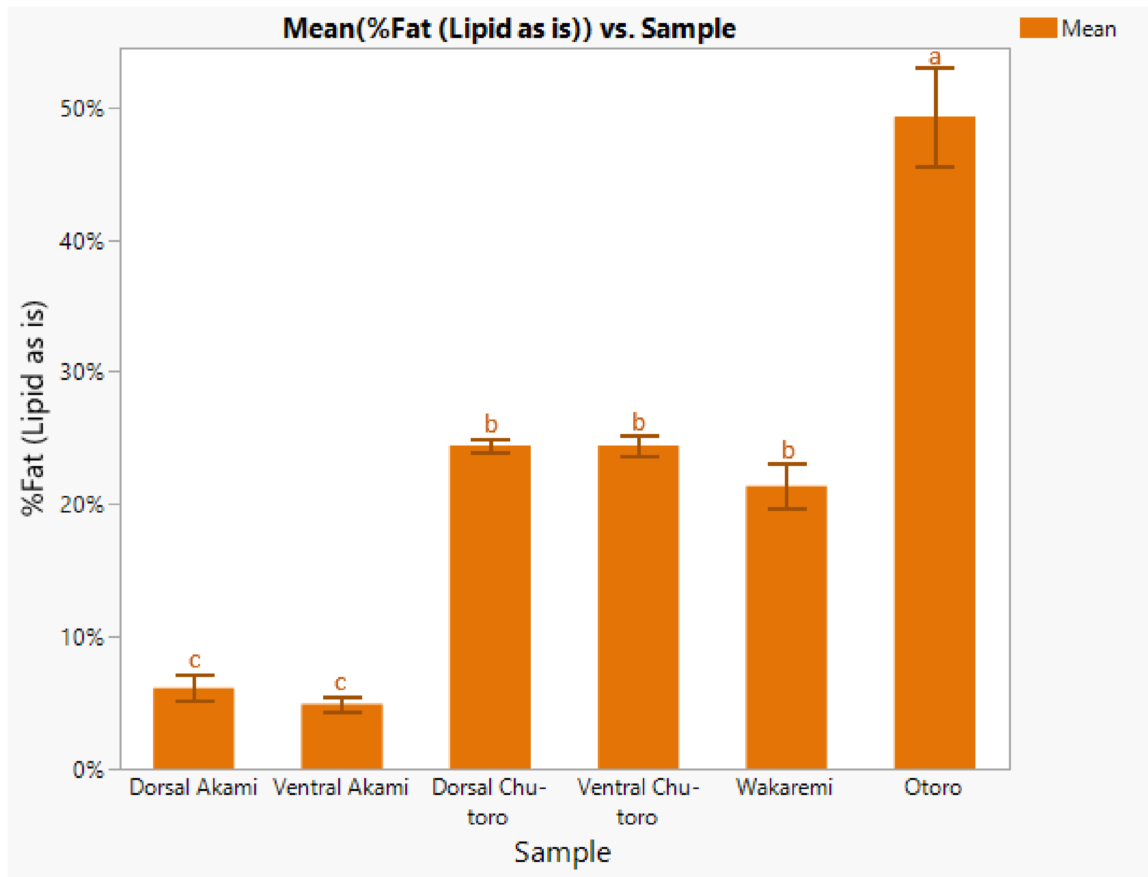

Figure 4-4: Fat content of the different cuts of Pacific bluefin tuna with standard error bars. Different letters indicate samples that are significantly different from each other at ($p < 0.05$).

Source of Variation	Sum of Squares	d.f.	Variance	F	p-value
Between Groups	3904.9204	5	78.0984	83.2886	<0.0001
Within Groups	112.5221	12	9.377		
Total	4017.4425	17			

Table 4-4: F-test table for the fat content of the different Pacific bluefin tuna cuts.

Summary

An overview of the proximate composition is displayed below (Table 4-5). For most of the compositional aspects (moisture, fat, and protein content) of the fish there is a distinct difference between the different cuts. According to the USDA Food Data Central, Fish, tuna, fresh, bluefin, raw was said to have a moisture content around 38.1 (g/100g), an ash content of 1.18 (g/100g), a protein content 23.3 (g/100g), and a fat content of 4.9 (g/100g) (USDA 2019). It is hard to compare directly to the data collected in this study as the USDA does not describe the cut that was examined for these results, but based on the numbers it is likely that one of the leaner akami cuts was used. The data from the USDA agree with what was found in the dorsal and ventral akami cuts.

Samples	Moisture Content (%)	Ash (%)	Protein Content (%)	Fat Content (%Lipid as is)
Dorsal Akami	71.15±0.23a	1.16±0.14ab	24.53±0.00b	6.11±0.95c
Ventral Akami	70.27±0.29a	1.46±0.05a	26.86±0.00a	4.89±0.54c
Dorsal Chu-Toro	50.28±2.19b	0.99±0.09bc	21.89±0.01c	24.42±0.55b
Ventral Chu-Toro	48.12±0.96bc	0.90±0.01bc	18.66±0.00d	24.42±0.76b
Wakaremi	52.20±0.09b	1.11±0.02bc	21.41±0.00c	21.36±1.66b
Otoro	44.67±1.48c	0.82±0.04c*	16.79±0.00e	49.29±3.73a

Table 4-5: Summary table of the proximate analysis of each Pacific bluefin tuna cut reported as mean ± SE. Different letters going down a column means samples are significantly different from each other at ($p < 0.05$). * Contains n=5 unlike other samples in column.

Fatty Acid Composition

A summary table of all the fatty acids found in the different cuts of PBT can be found below (Table 4-6). Some trace fatty acids (FA) (<0.1%) were excluded from table.

Those fatty acids include 12:0, 18:3n-6, and others in all samples. Additionally, the akami cuts had trace amounts of some FA that were found in other cuts at higher levels (Table 4-6). The highest report FA in the samples tested were 16:0, 18:1n-9, 20:5n-3, and 22:6n-3 (Table 4-6). These fatty acids were also reported as being the highest FA by previous researchers (Saito et al. 1999; Nakamura et al. 2007; Roy et al. 2010). Roy et al. (2010) observed that wild PBT had higher FA concentrations of these FA than cultured (full cycle) PBT; in contrast in our study the cultured PBT had a similar FA concentration to the wild PBT with the exception of 22:6n-3 which was similar in value to cultured (full-cycle) PBT. In the study conducted by Nakamura et al. (2007), the FA concentration for 16:0, 18:1n-9, and 22:6n-3, for cultured (full cycle) were similar to what was found in our study with the exception of 20:5n-3. The samples in this study were found to have higher amounts of FAs than the ones in the comparative study. This variation may be due to a difference in the GC detector in this study a MS was used compared to a flame ionization detector (FID) used in other studies (theoretical response factors are required for accurate FID FAME results but are rarely used). Additionally, while the amount of FA is higher in wild PBT the cultured PBT contained more fat, therefore, supplying more of the FAs because the quantity of fat is higher despite the portioned of fat being smaller than wild PBT (Roy et al. 2010).

Eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) were compared for each cut (Figure 4-5). A one-way ANOVA was conducted, and EPA was found to be significantly different ($p < 0.05$) between cuts, thus a Tukey's HSD post hoc test was conducted to determine which cuts were significantly different from one another (Figure 4-5). The amount of EPA was higher in the samples in this studied compared to

cultured (full cycle) PBT (Roy et al. 2010). It is believed that the diet of wild fish is the reason for increased levels of n-3 FA over cultured fish species (Alasalvar et al. 2002). It is possible then that the diet of the cultured PBT in this study was high enough in EPA to provide higher levels of EPA in the muscle (fish are monogastric and diet fatty acid level affects muscle fatty acids). DHA was higher than EPA in all the cuts which is supported by previous studies and this trend can also be seen in other species of BT (Wheeler and Morrissey 2003; Nakamura et al. 2007; Roy et al. 2010). One reason that the concentration of DHA is higher in fish may be due to its importance in marine fish for their growth and survival (Kanazawa et al. 1979).

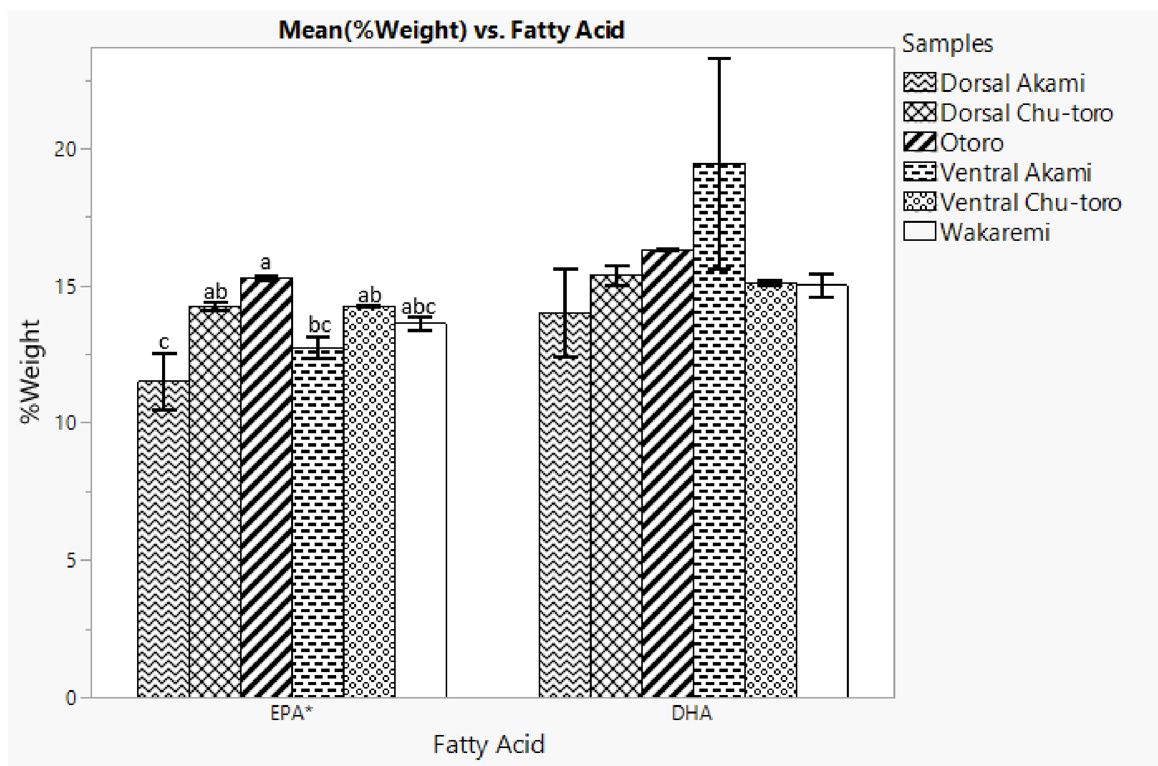

Figure 4-5: % Weight of the long chain polyunsaturated fatty acids EPA and DHA by cut of Pacific bluefin tuna. The error bars represent the standard error for each cut. *Signifies a significance at ($p < 0.05$). Different letters going down a column means samples are significantly different from each other at ($p < 0.05$).

Fatty Acid	Dorsal Akami	Ventral Akami	Dorsal Chu-toro	Ventral Chu-toro	Wakaremi	Otoro	p-value
14:0	3.99±0.22	3.42±0.41	3.53±0.18	3.39±0.06	3.66±0.22	3.76±0.15	0.4905
15:0	0.32±0.02	0.31±0.02	0.29±0.02	0.28±0.01	0.31±0.01	0.34±0.01	0.1634
16:0	21.54±0.99a	21.27±0.31a	17.00±0.47b	16.86±0.20b	17.59±0.60b	16.82±0.43b	<.0001
16:1n-7	6.29±0.20	4.99±0.87	5.81±0.13	5.63±0.03	5.93±0.22	6.06±0.08	0.2957
17:0	0.39±0.03	0.36±0.03	0.37±0.01	0.38±0.01	0.35±0.02	0.39±0.02	0.7242
17:1n-8	0.26±0.07*	0.19±0.02*	0.18±0.02	0.18±0.03	0.26±0.09	0.24±0.05	0.7422
18:0	8.66±0.27a	9.42±0.43a	7.39±0.02b	7.48±0.01b	7.35±0.14b	6.98±0.06b	<.0001
18:1n-9	20.70±0.90ab	17.18±1.94b	21.09±0.16ab	21.33±0.11ab	21.71±0.13a	19.94±0.09ab	0.0339
18:1n-7	4.27±0.20	3.65±0.35	4.07±0.04	4.15±0.01	4.17±0.02	4.10±0.03	0.2202
18:2n-6	0.83±0.02	0.97±0.10	0.94±0.03	0.89±0.02	0.91±0.02	1.02±0.03	0.1521
18:3n-3	0.52±0.25	Trace	0.31±0.02	0.34±0.01	0.33±0.01	0.36±0.06	0.7113
20:0	Trace	0.33±0.09*	0.23±0.03*	0.35±0.02	0.25±0.01	0.23±0.05	0.1875
20:1n-9	1.49±0.07ab	0.95±0.27b	1.68±0.07a	1.68±0.05a	1.62±0.05a	1.36±0.05ab	0.0079
20:2n-6	0.28±0.05	Trace	0.41±0.01	0.50±0.03	0.39±0.05	0.39±0.16*	0.2367
20:3n-3	0.60±0.05b	Trace	0.83±0.03ab	0.85±0.05a	0.82±0.05ab	0.92±0.08a	0.0209
20:4n-6	0.44±0.13*	0.42±0.12*	0.58±0.03	0.71±0.06	0.55±0.07	0.50±0.06	0.1330
20:5n-3	11.53±1.02c	12.73±0.38bc	14.26±0.15ab	14.26±0.02ab	13.62±0.27abc	15.27±0.07a	0.0015
22:1n-9	0.73±0.09	0.90±0.02*	1.10±0.08	0.93±0.15	1.08±0.02	0.82±0.07	0.0917
22:2n-6	Trace	Trace	0.49±0.06	0.47±0.07	0.49±0.08	0.59±0.04	0.5990
22:5n-3	2.53±0.25	2.66±0.09	3.54±0.08	3.52±0.02	2.89±0.61	3.28±0.05	0.0861
22:6n-3	14.02±1.62	19.47±3.83	15.40±0.36	15.10±0.09	15.02±0.43	16.30±0.06	0.3537
24:1n-9	0.55±0.05	0.59±0.05	0.51±0.06	0.69±0.09	0.58±0.09	0.57±0.12*	0.7268

Table 4-6: Summary table of the %weight of fatty acid found in each cut of Pacific bluefin tuna. Fatty acids with less than 0.1% weight were excluded from the table. Different letters within a row signify a significant difference by Tukey's HSD at ($p < 0.05$). *Signifies that the fatty acid was only found in two samples instead of all three.

Conclusions

Understanding the proximate and fatty acid composition of cultured PBT is crucial in creating seafood alternatives that can meet or exceed consumer expectations. Since these products will be consumed as sushi or sashimi products there is no masking errors from consumers. While are all proximate components are important to organoleptic properties of cultured PBT; for the leaner cuts the moisture content is likely the most influential it is organoleptic properties because it is over half of the composition of the cut. For the fattest cut otoro the fat content is likely the most influential for its organoleptic properties because it is approximately half of its composition. The quantity of EPA and DHA are critical because of their linkage to health benefits. These FAs should be incorporated into seafood alternatives at equal or greater amounts, if possible, into the seafood alternative, so that these products can be seen as having an advantage over the seafood products found in the marketplace currently.

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