THE EFFECT OF COOKING METHOD ON PROXIMATE COMPOSITION, TOTAL IRON, HEME IRON AND NON-HEME IRON IN HARUAN (*CHANNA sp.*), CATFISH (*CLARIAS sp.*), SIAKAP (*LATES CALCARIFER*) AND TILAPIA (*OREOCHROMIS sp.*)

By

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ABSTRACT

Nowadays, the freshwater fish industry has increased in line with the increased demand from the consumer due to its richness nutrient especially protein, mineral and vitamin. In Malaysia, fish are rarely eaten raw and mostly will be cooked with various types of cooking method but this process are involves thermal treatment which will affect the nutrient content in fish especially iron. However, studies on the effect of cooking method on total iron, heme iron and non-heme iron in fish specifically in freshwater fish was limited. So the aim of this study was to determine the effect of difference cooking method on proximate composition, total iron, heme iron and non-heme iron in Haruan (*Channa sp*), Catfish (*Clarias sp*), Siakap (*Lates calcarifer*) and Tilapia (*Oreochromis sp*). The samples was bought at Pasar Siti Khadijah and each types of freshwater fish was divided in five group that are raw, fried, baked, boiled and steamed method. The proximate composition (moisture, ash, lipid and protein), total iron, heme and non-heme iron composition was studied. Iron (II) standard graph was used to determine the value of total iron and heme iron in all samples. The amount of non-heme iron was obtained through differences between total iron and heme iron. Effect of cooking method on proximate composition was significantly different especially in fried method; where it causes a significant decreased of moisture especially in Tilapia (67.8 %). At the same time, fried method increased the ash, protein and lipid content in all species except for catfish. Meanwhile the steamed method showed the significant increase in moisture, lipid and protein in certain type of fish. All sample showed a significant different on the amount of total iron between fresh and cooked sample. The amount of heme iron in Haruan and Tilapia showed a significant different while catfish and Siakap are otherwise. However the effects of cooking method on the amount of heme iron are different in Haruan and Tilapia. Then the values of non-heme iron in all samples are significant at $p<0.05$. Briefly, the changes of nutrient due to cooking method are also depends on the type of fish.

ABSTRAK

Pada masa kini, industri ikan air tawar telah meningkat selaras dengan peningkatan permintaan daripada pengguna kerana ia kaya dengan nutrisi terutamanya protein, mineral dan vitamin. Di Malaysia, ikan jarang dimakan mentah dan kebanyakanya akan dimasak dengan pelbagai jenis kaedah memasak tetapi proses ini melibatkan rawatan haba yang akan memberi kesan kepada kandungan nutrisi dalam ikan terutamanya zat besi. Walau bagaimanapun, kajian tentang kesan kaedah memasak kepada jumlah besi, besi heme dan besi bukan heme dalam ikan khususnya ikan air tawar adalah terhad. Jadi tujuan kajian ini adalah untuk menentukan kesan perbezaan kaedah memasak terhadap komposisi proksimat, jumlah zat besi, zat besi "heme" dan zat besi bukan "heme" dalam Haruan (*Channa sp*), Keli (*Clarias sp*), Siakap (*Lates calcarifer*) dan Tilapia (*Oreochromis sp*). Kesemua sampel dibeli di Pasar Siti Khadijah dan setiap jenis ikan dibahagikan kepada lima kumpulan iaitu kaedah mentah, goreng, bakar, rebus dan kukus. Komposisi proksimat, jumlah zat besi, zat besi "heme" dan zat besi bukan "heme" dikaji. Graf standard zat besi (II) digunakan untuk mengenalpasti nilai zat besi dan zat besi "heme" dalam semua sampel. Jumlah zat besi bukan "heme" telah diperolehi melalui perbezaan antara jumlah zat besi dan zat besi "heme". Kesan kaedah memasak kepada komposisi proksimat ketara berbeza terutamanya dalam kaedah goreng; di mana ia menyebabkan penurunan ketara kelembapan terutama di Tilapia (67.8%). Pada masa yang sama, kaedah goreng meningkatkan kandungan abu, protein dan lipid dalam semua spesies kecuali ikan keli. Sementara itu kaedah kukus menunjukkan peningkatan yang ketara dalam kelembapan, lipid dan protein dalam jenis ikan tertentu. Semua sampel menunjukkan perbezaan yang signifikan ke atas jumlah keseluruhan zat besi antara sampel segar dan dimasak. Jumlah zat besi "heme" dalam Haruan dan Tilapia menunjukkan perbezaan yang signifikan manakala ikan keli dan Siakap adalah sebaliknya. Walau bagaimanapun kesan kaedah memasak kepada jumlah zat besi "heme" adalah berbeza di Haruan dan Tilapia. Kemudian nilai-nilai zat besi bukan "heme" dalam semua sampel adalah signifikan pada $p < 0.05$. Secara ringkas, perubahan nutrien kerana kaedah memasak juga bergantung kepada jenis ikan.

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CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

Fish is the primary sources of animal protein and its consumption has been increased in recent decades. In the working paper 2011-65 by The World Fish Center (2011) indicated that the demand for fish is expected to increase significantly especially in South and South-East Asia where the current global per-capita supply of fish is 17 kg per year. Fish consumption among Malaysian has been reported increased from 49 kg per capita in 2000 to 53 kg in 2005 (Abdi et al., 2012). Based on a report by The Star , prepared by Aruna, (2014) he stated that "Malaysians are among the world's top fish consumers, eating at least 56.5 kg of fish per person each year" and "the statistic also shows that the major species consumes in Malaysia included mackerel, shrimp, squid, tilapia and catfish (*keli*)". The consumption of fish increased due to the increasing world population, higher living standard and positive image of fish (Ayeloja et al., 2013). Onyia et al. (2013) in their article entitled , " Comparison of Nutrient Values of Wild and Cultured *Heterobranchus Bidorsalis* and *Clarias Gariepinus*" stated that UNICEF (2006) had mentioned that fish has become the key ingredient on the global menu, not only in Malaysia, but also worldwide.

In Malaysia, consumers not only consume marine fish but also consume freshwater fish especially those who live near to river and lake and make it as their main protein sources. Based on Mat Jais (2007), freshwater fish has been associated with our daily life from a long time ago and some of the freshwater animal especially fish has their own legendary story to shows how people largely consumed freshwater fish. Nowadays, the freshwater fish industry in Malaysia is growing rapidly due to technology advancement and governmental support (Endinkeau & Kiew, 1993). One of the most common freshwater fish consumed by Malaysian is catfish due to its taste and low market prices (Abdi et al., 2012). There are two species of catfish that highly consumed in Malaysia that are *Clarias Gariepinus (C. gariepinus)* and *Hemibagrus Nemurus (H.nemurus)* (Abdi et al., 2012). However, *C.gariepinus* is by far most cultivated by using a suitable technique (Pouomogne, 2008). *C.gariepinus* or also known as North African Catfish is usually dark and spotted greyish coloured and it has thick skin (Pouomogne, 2008).

Another most common freshwater fish is tilapia (*Oreochromis* sp.). Based on Aruna (2014), it is stated that tilapia is popular among Indian and Chinese consumers in Malaysia. Tilapia demand in local and foreign markets increased in Asia and Latin America (FAO Globefish, 2014). Haruan (in Malay) or snakehead fish that belong to *Channidae* family is one of the freshwater fish that has been commercially introduced worldwide as a fish for medical purpose. According to the Endinkeau and Kiew (1993), Zuraini et al. (2006), Mat Jais (2007) and Annasari et al. (2013), Haruan is not only been consumed as a food but it also well known for its positive effect on wound healing. Instead of Catfish, Tilapia and Haruan, Siakap (in Malay) or in other word, also known as Asian Sea bass or Barramundi is one of the famous freshwater fish that is consumed by Malaysian. Badiani et al., (2013) has highlighted in their journal that Fuentes et al. (2010), Grigorakis (2007), Orban et al. (2002), Özden & Erkan (2008) and Yildiz (2008) state that Siakap has received attention for its macronutrient and micronutrient composition.

In general, fish including marine and freshwater fish has been considered as an excellent source of animal protein in the human diet. It is not only consumed due to its protein but also due to other nutrients and minerals (Marimuthu et al., 2012). Mineral are essential nutrients that present in small amount in human body and its deficiency can lead to serious problem (Devi & Sarojnalini, 2012). One of the important trace mineral that present in significant amount in fish is iron, especially when eaten with bones (Sub-Committee on Fish Trade, 2014). Iron is an important mineral which may cause anaemia due to iron deficiency. This health problem can bring more serious effects especially in children and women (Roos et al., 2007). Although iron is one of the most abundant trace element in the human body but the insufficient daily intake can result in anaemia (Hosseini et al., 2014). In developing countries, it shows that the amount of iron intake not only depends on the amount of iron supplies daily, but also the form of iron (heme and non-heme) and the composition of the diet which is consumed (Kongkachuichai et.al., 2002). There are two primary forms of iron that can be found in food which are heme and non-heme iron. The animal product such as red meats contain both form and the plant product consists of one form that is non-heme iron (Kongkachuichai et al., 2002). Heme iron comes from animal sources that contain meat factor such as beef, pork, lamb, fish, chicken and turkey. It is the type of dietary iron that is absorbed the best while non-heme iron mostly found in plant and bean and it is not well absorbed by our body as compared to heme iron (Clinical Nutrition Services Department UWH, 2009).

In Malaysia, fish are rarely eaten raw and most of them will be cooked by using various cooking method including frying, baking, grilling, steaming and boiling. The cooking process will involve thermal treatment which will affect the nutritive value in fish (Marimuthu et al., 2012; Turhan et al., 2004). As stated above fish is also an important sources of iron and as cooking process take place, it can decreased the bioavailability of the iron in food (Singla, 2011). Turhan et al. (2004) and Pourkhalili et al. (2013) mention that during cooking, oxidation will take place in the presence of oxygen that will result in oxidative cleavage of porphyrin ring of heme. This will then result in decrease of the heme iron in food containing meat-factor and cause some of the free iron that released from the oxidation process converted into non-heme iron and increased non-heme iron in food. So there are clear relationship between the cooking methods with the heme iron in the food-containing meat-factor especially fish.

1.2 RATIONALE OF STUDY

Iron is one of the important micro-mineral in the body and based on RNI (2005), recommended intakes of iron was approximately 35 mg/kg in adult women and 45 mg/kg in adult men respectively. In periodic table, iron is in group 8 and period 4 elements with atomic weight 55.845 (Gokhale, 2011). It is necessary for growth development especially for cellular functioning and synthesis of some hormone and for metabolism. Iron has a crucial function to the body where it is needed to transport oxygen to tissues throughout the body via haemoglobin and myoglobin. Haemoglobin and myoglobin is a protein component of iron including cytochrome and enzymes that are involve in redox reaction (Australian National Health and Medical Research Council (NHMRC), 2006). In fact about 65 to 75 % of the body's iron is in the blood that is in form of haemoglobin (Anderson & Fitzgerald, n.d.). Iron also can exist in form of oxidation states in the body that are in form of divalent iron, Fe II and trivalent iron, Fe III states (Gokhale, 2011). Iron also is a pro-oxidation metal which can accelerate autoxidation by becoming an initiator and this interaction will take place from its interaction with oxygen or peroxides (Sickler, 2000).

Iron requirements of humans vary based on age and gender. In addition, the deficiency and the excessive intakes of iron both can result in detrimental effect. Iron can be obtained from the diet and supplement and these dietary sources will influences the efficiency of iron absorption, ranges from <1% to >2%. There are two types of iron in food that are heme and non-heme iron. Heme iron is derived primarily from the haemoglobin and myoglobin of flesh food such as poultry, meat, and fish while non-heme iron is found in plant origin (RNI, 2005). Heme has a high bioavailability compare to non-heme iron (Australian National Health and Medical Research Council (NHMRC), 2006) because it is soluble at the pH of the small intestine (RNI, 2005). The inadequate iron intake can lead to varying degree of deficiency including low iron stores, early iron deficiency and iron deficiency anemia. Cozzolino (as cited in Pereira et al., 2014) stated that the iron deficiency in diet can lead to anemia that affecting more than two billion people worldwide and most of them are pregnant women and children. Kongkachuichai et al. (2002) also mention that iron deficiency is one of the world's most common disorder that resulting from the insufficient intake of iron. Iron deficiency can associated with poor diet, malabsorptive disorder and blood loss (Office of Dietary Supplements, 2014). This deficiency can affect mental development and reduce the attention span and learning in children while in women, it will lead to the serious impact on health and morbidity.

This study evaluated the influence of cooking on the proximate composition, heme and non-heme iron content in freshwater fish. This is because the heme iron and non-heme iron content in food can be decreased due to several factors including cooking. Based on Singla, (2011) cooking decreased the bioavailability of iron in food. Heme iron will be converted into less available non-heme with different heat treatment and these involve the oxidation of the porphyrin ring (Singla, 2011). Then the oxidation of the heme will result in increased of non-heme iron content in food after heat treatment. As mention above, non-heme iron does not has good absorption into the body as compared to heme iron. In fact, this non-heme iron needs heme iron to increase its absorption into the body. So this can affect the iron intake of the individual. For some reason, it is important to take into account that heme iron is transformed into non-heme iron when heat is applied to the food. Unfortunately most of the available scientific data only provided the heme and non-heme iron content from raw food. Scant data was available which considering the loss of the heme iron during cooking (López & Martos, 2004). Some researcher (Cross et al., 2012; Lombardi-Boccia et al., 2002; Pereira et al., 2014; Pourkhalili et al., 2013; Purchas et al., 2003) has previously studied the effect of cooking method on heme and non-heme iron content but their focus was on meat and poultry. While there are other researcher (Adeyemi et al., 2013; Devi & Sarojnalini, 2012; Gokoglu et al., 2004; K Marimuthu et al., 2012) has previously studied on the effect of cooking on proximate and mineral composition of fish. So this study was conducted to gain more information about the retention of heme and non-heme iron content and also the proximate composition due to the cooking method in freshwater fish.

1.3 RESEARCH OBJECTIVE

1.3.1 GENERAL OBJECTIVE

- To investigate the effect of different cooking method on the proximate composition, total iron, heme iron and non-heme iron in Catfish (*Clarias* sp.), Haruan (*Channa* sp.), Siakap (*Lates calcarifer*) and Tilapia (*Oreochromis* sp.).

1.3.2 SPECIFIC OBJECTIVE

- To determine the differences of proximate composition (moisture, ash, protein and lipid) between fresh and cooked Catfish (*Clarias* sp.), Haruan (*Channa* sp.), Siakap (*Lates calcarifer*) and Tilapia (*Oreochromis* sp.).

- To determine the differences of total iron, heme iron and non-heme iron content in fresh and cooked Catfish (*Clarias* sp.), Haruan (*Channa* sp.), Siakap (*Lates calcarifer*) and Tilapia (*Oreochromis* sp.).

1.4 RESEARCH QUESTION

- Is there any difference in proximate composition (moisture, ash, lipid and protein) between fresh and cooked Catfish (*Clarias* sp.), Haruan (*Channa* sp.), Siakap (*Lates calcarifer*) and Tilapia (*Oreochromis* sp.)?

- Is there any difference in total iron, heme iron and non-heme iron content between fresh and cooked Catfish (*Clarias* sp.), Haruan (*Channa* sp.), Siakap (*Lates calcarifer*) and Tilapia (*Oreochromis* sp.)?

1.5 HYPOTHESIS

HYPOTHESIS 1

Null hypothesis, H_0

There are no significant different between the proximate content of fresh and cooked Catfish (*Clarias sp.*), Haruan (*Channa sp.*), Siakap (*Lates Calcarifer*) and Tilapia (*Oreochromis sp.*).

Alternative hypothesis, H_A

There are a significant different between the proximate content of fresh and cooked Catfish (*Clarias sp.*), Haruan (*Channa sp.*), Siakap (*Lates Calcarifer*) and Tilapia (*Oreochromis sp.*).

HYPOTHESIS 2

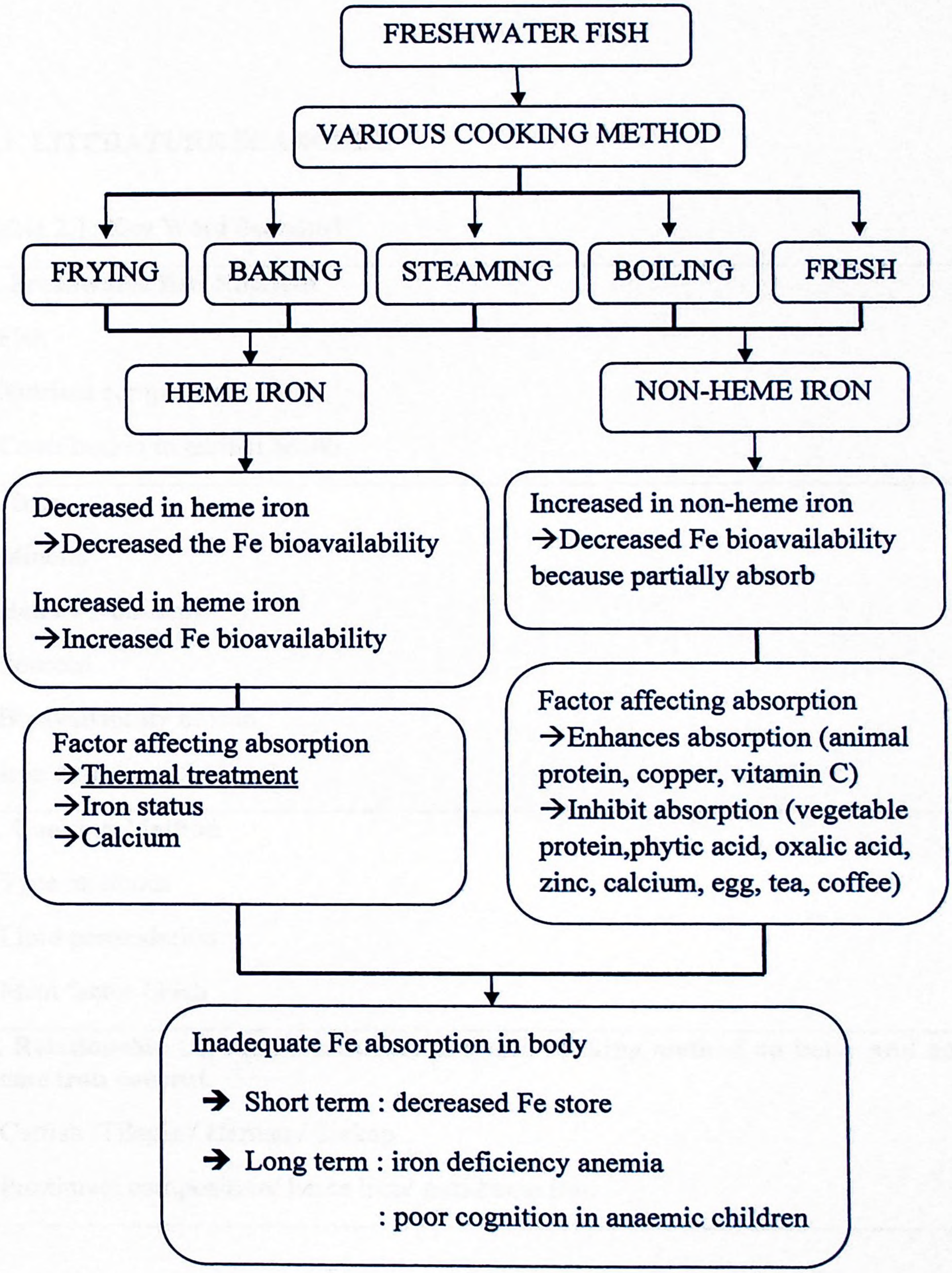
Null hypothesis, H_0

There are no significant difference in total iron, heme iron and non-heme iron content between fresh and cooked Catfish (*Clarias sp.*), Haruan (*Channa sp.*), Siakap (*Lates Calcarifer*) and Tilapia (*Oreochromis sp.*).

Alternative hypothesis, H_A

There are a significant difference in total iron, heme iron and non-heme iron content between fresh and cooked Catfish (*Clarias sp.*), Haruan (*Channa sp.*), Siakap (*Lates Calcarifer*) and Tilapia (*Oreochromis sp.*).

1.6. CONCEPTUAL FRAMEWORK



CHAPTER 2

LITERATURE REVIEW

2.1. LITERATURE SEARCHED

Table 2.1: Key Word Searched

1. Freshwater fish Nutrient - Fish - Nutrient component - Contribution to human health
2. Iron - Mineral - Heme / Non-heme - Sources - Bioavailability of iron - Iron deficiency / Anemia
3. Cooking Method - Type /methods - Lipid peroxidation - Meat factor / Fish
4. Relationship between freshwater fish and cooking method on heme and non-heme iron content - Catfish /Tilapia / Haruan / Siakap - Proximate composition/ heme iron/ non-heme iron

2.2. FRESHWATER FISH NUTRIENT

For decades, fish including marine and freshwater fish has becoming great sources of protein and other nutrients including macronutrient and micronutrient. It also has a good nutritional values than meat and dairy products (Pathak, 1989). Hossain (as cited in Ayeloja et al., 2013) and Aberoumand (2014) stated that fish provided superior quality of protein to that of meat, milk and eggs and well balanced essential amino acid profile and it also contain a necessary mineral and fatty acid. Based on the review carried out by Marimuthu et al. (2014), Aberoumand (2014) and Sub-Committee on Fish Trade (2014), fish contain high level of omega-3 fatty acid, eicosapentaenoic acid (EPA) and decosahexaenoic acid (DHA) that bring positive effects on the neural development and eye function in infants and young children. Although fish are usually linked with the nutritional concern through it contribution to increased protein supply and poly-unsaturated fatty acid, it is also one of the important sources of the micronutrient where it contribute to the food security to more than two billion people in the world that are undernourished due to the deficiency of the essential vitamin and mineral especially in vitamin A, iron and zinc (The World Fish Center, 2011). In addition, based on Pathak (1989), fish are important in Asia region where they contribute to the human need for food especially in the densely populated countries at risk of under-nutrient and malnutrition. Similar evidence has been reported by Roos et al. (2007) that focused on rural Bangladesh and Cambodia where the fish and fisheries play an important role in the diets, livelihoods and incomes of many poor population groups who suffer from vitamin and mineral deficiency. Due to its great nutritional value, it contributes a great benefit to the human health and it becomes one of the great sources of iron in

Australia and New Zealand (Australian National Health and Medical Research Council (NHMRC), 2006). It is noted that, even though, red meat has higher iron content compared to fish but due to its affordability, availability and cultural acceptability, fish has becoming one of the best alternative to combat micronutrient deficiency especially in rural area and densely-populated countries (Kawarazuka & Béné, 2010).

2.3. IRON

Iron is one of the essential elements needed for the metabolism of almost all living organism. According to Choi (n.d.), iron is important to maintain good health and to strengthen the immune system and are also constituents of many important protein and enzymes. Body iron can be categorized into four classes according to its function. First, it exists as a component of a number of proteins including haemoglobin, myoglobin, cytochromes and enzyme that involved in redox reaction (Australian National Health and Medical Research Council (NHMRC), 2006). The other three classes of body iron include sulphur enzyme which involved primarily in energy metabolism, exists as iron storage and transport protein that is responsible to participate in iron uptake, transport and storage in the body and the last classes are exist as iron-containing or activated enzyme ("Iron," n.d.).

In addition, there are two iron classes that responsible to detect any abnormalities of iron in the human body. Haemoglobin, myoglobin and enzyme are one of the iron major pools in adult human. Then iron storage and transport protein including ferritin, hemosiderin and transferrin is another major iron pools and both of the iron pools are responsible to detect the abnormalities of iron in human body. At the same word, both pools are used as biochemical measures that are used as the key to indicate the iron requirement in the body. The iron requirements are different based on gender, age and the person condition. Based on Swanson (2003), the iron requirement are generally increased in man and in women during adulthood and the average iron body stores are about 3.8 g in man and 2.3 g in women. Food and Nutrition Board, Institute of Medicine stated that the iron requirement are generally higher in older infants, toddlers, teenage girl, women of childbearing age, pregnant women and women who breastfeeding (Swanson, 2003). Iron exists in two form of group that are heme iron and non-heme iron ("Iron," n.d.). According to Barris (2012), both form of iron have their own unique chemical structure that result in different mechanism of absorption and metabolism in the human body. Hunt (2003) in his article review that less than 40% of the iron content in meat, poultry and fish is in heme iron form while the rest is are non-heme iron form.

Barris (2012) also stated that heme iron consists of protoporphyrin IX molecule containing a central divalent iron (Fe II) atom attached to the four nitrogen atoms of the porphyrin ring and Voet et al. (as cited in Barris, 2012) stated that the porphyrin complex of heme iron allows the molecule to prevent oxidation to ferric iron (Fe III), its insoluble form which can allows heme iron to be absorbed at the duodenum in human body. Heme iron are mostly found in meats, fish and poultry that consists primarily of haemoglobin and myoglobin (Benito & Miller, 1998; Hallberg, 1981; Swanson, 2003). It is normally represents a small portion of iron intake but it is well absorbed because other dietary factor like phytates do not interfere with its absorption and the only condition for heme iron to be absorbed into the body is the present of meat and blood-derived foods and also its catalyst that is calcium that can increase it absorption (López & Martos, 2004). Normally these types of food such as meat, poultry and fish not only contain primarily heme iron but also contain non-heme iron (Turhan et al., 2004).

On the other hand, non-heme iron that can be obtain from cereals, grain, fruits and vegetables is a main part of the dietary iron that providing about 90% of total dietary iron intake in developed countries (Hallberg et al. as cited in Barris, 2012; Hurrell & Egli, 2010). It present in food either ferric or ferrous salts or ferric iron is insoluble in a pH greater than three. Non-heme iron absorption normally will be affected by the dietary components that act as chelated whether it can enhance the absorption or decreased the absorption of the non-heme iron in body.

2.3.1. BIOAVAILABILITY OF THE IRON

The absorption of iron usually will occur in stomach, ileum and colon with slightly extent in comparison with the duodenum and upper jejunum but its concentration will be varied based on its chemical form whether that form will be absorbed and passed through the proximal part of the gastrointestinal tract or not (Charlton & Bothwell, 1983). Based on Craig (1994), the iron absorption under normal condition is 10%-15% but it can be varied from as little as 1% until greatly as 40% because its bioavailability are mainly affected by its chemical form whether heme iron or non-heme iron content in the meal, the iron status of the individual and the composition of the ingested food. Heme iron is an important form of iron because it is easily absorbed by human body compare to non-heme iron (Kongkachuichai et al., 2002).

Craig (1994) and Monsen and Balintfy (as cited in Singla, 2011) stated that heme iron that mostly derived from animal sources has a much higher bioavailability that is 15% to 35% compare to non-heme iron with the bioavailability varying from 2% to 20%. In addition, Monsen et al. (1978) and Hunt (2003) stated in their articles that the absorption of the iron in the body are also affected by body iron stores. Monsen et al. (1978) mention that the proportion of heme iron which human usually absorb from their diet is high compare to non-heme iron where the individual with no iron stores may be expected to absorb approximately 35% of heme iron while the individual with adequate iron store of 500 mg may be expected to absorb approximately 25% of heme iron. This absorption will be varied based on the iron sources where normal diet with substantial amount of red meat will supply about 2mg/d or 10% to 12% of the heme iron from the total iron while compared with diet based on poultry or fish, the heme iron absorbed will be lesser and the vegetarian diet will consume no-heme iron (Hunt, 2003).

While Hallberg et al. (as cited in Barris, 2012) explain that the average daily intake of heme iron in developed countries is around 10% of total iron intake with approximately 25% of this being absorbed. Based on Zimmermann & Hurrell (2007), in meat product, 30% to 70% of iron is heme iron and almost 15% to 35% is absorbed but in developed countries most of the diet is plant based diet where consists of high non-heme iron and its absorption is often less than 10%. Hunt (2003) reported that many researcher believe that they who immerse in vegetarian diet get enough total dietary iron as in mixed diet containing animal flash but it is less available for absorption due to the differences in the chemical form of the iron. According to Hurrell & Egli (2010), heme iron is estimated to contribute 10% to

15% of total iron intake in meat-eating population and could contribute to higher than 40% of total iron absorbed while non heme iron is much less absorbed than heme iron because non-heme iron depends on the balance between the absorption inhibitors and enhancers and also the iron status of individual. There are a few factors that actually contributing to the bioavailability of the heme and non-heme iron in the body and one of it is a specific binding site in the intestinal tract. Heme iron has a greater bioavailability because the specific heme-binding site for heme iron absorption located in the intestinal tract that make it easier to be absorb into body compare to non-heme iron (Craig, 1994). In the other hand, non-heme iron needs rely on other substances to enhance its absorption. To support this, Hallberg et al. (as cited in Barris, 2012) has mention that many factor will affect non-heme iron bioavailability resulting only 17% of the 90% of non-heme iron consume in the diet will be absorbed, thus make it less bioavailable than heme iron.

According to Craig (1994) and Reddy et al. (2000), vitamin A and β-Carotene have also been shown to enhance non-heme iron absorption while phytic acid, polyphenols, phosphorus, and calcium have been identified to inhibit the non-heme iron absorption. In the study that has been conducted in Australia (Whilfield et al. as cited in Swanson, 2003), the low level of alcohol consumption that is 8 to 14 drinks per week can increased the serum ferritin level thus increase iron stores. The iron bioavailability in vegetarian diet also becomes one of the important issues because they have already eliminated an animal product from their daily diet. The Table 2.2 shows factors that will affect non-heme iron absorption in human body.

Table 2.2, Dietary Factor that determine the absorption of non-heme iron

Enhancers	Inhibitors
Meat, poultry, and fish (unidentified factors)	Phytic acid
Ascorbic acid	Polyphenol/tannins (tea and coffee)
Alcohol	Soy protein
Retinol and carotene	Egg
	Calcium and phosphate salts
	Antacids

(Data reference from Hunt, 2003)

2.3.2. THE DEFICIENCY OF IRON

Iron deficiency is one of the worldwide problem or probably known as epidemic problem with the statistic of its overall prevalence in developing countries and develop countries are around 50% and 10% respectively (Denic & Agarwal, 2007). They also believe that prevalence of iron deficiency still unchanged are due to an unrecognized genetic and cultural predisposition manifesting through diet and altered processing of food (Denic & Agarwal, 2007). Based on Craig (1994) also, due to very low absorption of non-heme iron, most of the vegetarians will facing a greater risk of iron deficiency.

2.4. COOKING METHOD

Scott (as cited in Brown, 2011) stated that Arno Schmidt said "Cooking is a craft which can rise on occasion, to an art". So to increasing the palatability, the fish usually will be cooked by using various type of cooking method and the most common are frying, baking, grilling and boiling (Marimuthu et al., 2012). Cooking involve applying heat to the food. Heating process of the food normally not only destroy the bacteria and microorganism that can cause food poisoning or food intoxication but it also an important process that can change the molecule of the food, altering their texture, taste, odor, and appearance (Brown, 2011; Weber et al., 2008). Based on Hallberg (2001), one of the factor that affect the absorption of the heme iron and non-heme iron is a food preparation that involve temperature and time. The heating process that occurs during preparation will cause a change in the nutrient content at the end of the preparation and it is involve the oxidation process.

2.4.1 OXIDATION IN COOKING

Oxidation of lipid is an important reaction that can affect the quality of meat, fish and other muscle foods and can affect the acceptability of the customer (Chaijan, 2008; Min & Ahn, 2005) and it usually will cause by both enzymatic and non-enzymatic mechanism (Sickler, 2000). Lipid oxidation is a free radical chain reaction that consists of three main phases that are initiation, propagation and termination reaction that happen in the present of oxygen. According to Min & Ahn, (2005), iron plays a critical role in lipid peroxidation process as major catalyst and also as a primary initiator in the development of lipid peroxidation in meat and meat product.

Singla (2011) in his master thesis explained that heme iron normally wills not affected by other dietary constituent but it can be affected when treated with different heat treatment and different processing method. Chen et al. (1984), Kalpalathika et al. (1991), Lombardi-Boccia et al. (2002), Kongkachuichai et al. (2002), Turhan et al. (2004), Mistura & Colli (2009) and Elder (as cited in Singla, 2011) explained that the process involved oxidation of the porphyrin ring followed by its cleavage that will result in the released of the iron due to the formation of the unstable complex. Garcia et al. (as cited in Singla, 2011) stated that the free iron that result from oxidation will contributed to the non-heme iron pool. Automatically, it will increase the non-heme iron content in the food (Turhan et al., 2004). Schricker and Miller (as cited in Min & Ahn, 2005) suggested that the heating or the addition of H_2O_2 will cause the released of heme iron due to oxidative cleavage of porphyrin ring of heme iron. Overall, heating process can promote the lipid peroxidation by disruption of muscle cell of muscle food structure, inactivation of antioxidant enzymes and release oxygen and iron from myoglobin (Min & Ahn, 2005).

They also reported that heating rate and final temperature will affect the released of non-heme iron (free iron from oxidation process) from heme pigment where slow heating will increased the released of non-heme iron than fast heating. This statement has be supported by Chen et al. (1984) in their study where the concentration of non-heme iron did not increased when undergoes short-time heating and it is related with the heme-iron stability toward temperature. Also, non-heme iron will increase resulting from oxidation process and it will act as a catalyst to stimulate further lipid peroxidation and off-flavour development (Min & Ahn, 2005; Turhan et al., 2004).

2.5. RELATION BETWEEN PROXIMATE COMPOSITION, TOTAL IRON, HEME, NON-HEME IRON AND COOKING METHOD

Even though the heating process can enhance the flavour, taste and increased it shelf life (Devi & Sarojnalini, 2012) but still it will affect the nutritive value especially in proximate and mineral composition of several fish that have been reported (Adeyemi et al., 2013; Devi & Sarojnalini, 2012; Hussein & Khaled, 2014; Marimuthu et al., 2012). In addition to that, The WorldFish Center (2011) in their working paper, "*Aquaculture , Fisheries , Poverty and Food Security* " stated that the nutritional benefits that can be obtain from the fish consumption are mostly affected by the processing methods and its eating pattern. Some research shows that the cooking process will cause the heme iron in foods decreased and the non heme increased (Kongkachuichai et al., 2002). It is important to take into consideration where the more intensive the heat treatment, the greater the degree of transformation of heme iron into non-heme iron occur (López & Martos, 2004). In Malaysia, the catfish, Siakap, Haruan and Tilapia normally will be prepared by using various methods to increase it palatability and it taste.

Kongkachuichai et al. (2002) in their study used acidified acetone extraction method by Hornsey (1956) and Clark et al. (1997) to determine heme iron content and they also using Rhee method from Rhee and Ziprin (1987) to determine non-heme iron content in food. Their result shows that steamed and uncooked fish (marine and freshwater) had similar heme iron content which is ranged from 0.2 mg to 0.9 mg/100 mg of sample while non-heme iron content in both raw and steamed fish (marine and freshwater) provided the moderated amount that range from 0.7 mg to 1.2 mg/100 mg for raw fish and from 0.9 mg to 1.9 mg/100 mg for steamed fish.

Gokoglu et al. (2004) has been conducted a study about the effect of cooking method on mineral content in rainbow trout. Their mineral analysis has been conducted by using Atomic Absorption Spectrophotometry (AAS) and the result on mineral iron shows that baked fish has higher iron content that is 2.91±0.52 mg/kg compare to raw fish that is 2.10±0.58mg/kg. The result of boiled fish shows a small different from raw fish that is 2.10±0.52 mg/kg. While the other cooking method including fried, grilled and microwave oven show a larger different from raw fish that are 1.76±0.19 mg/kg, 1.78±0.18 mg/kg and 1.40±0.07 mg/kg respectively.

Then the third review is on Turhan et al. (2004). The method used to determine the total iron is spectrophotometry method by using AAS. Then, the method used to determine the heme iron is acidified acetone extraction by Horney (1956) and Clark et al. (1997). The result shows that the effect of cooking method on total iron content in anchovy were significant ($p<0.05$). The total iron content in raw anchovy is 38.9±1.80 μg/g that is higher than baked, grilled, microwave oven and boiled anchovy that are 25.5±2.33μg/g, 18.4±0.55μg/g, 23.9±1.11 μg/g and 34.5±1.82 μg/g respectively. While the result for heme iron is higher in raw anchovy that is 16.5±0.64 μg/g followed by boiled anchovy (11.5±0.51μg/g), baked anchovy (7.6±0.76 μg/g), microwave anchovy (7.5±0.62 μg/g) and the lowest result for heme iron is in grilled microwave that is 4.9±0.59 μg/g. The higher total iron and heme iron loss is when anchovy grilled and the lowest total and heme iron lost is when anchovy was boiled.

Besides that, Devi & Sarojnalini (2012) has conducted a study on the impact of different cooking method on proximate and mineral composition of *Amblypharyngodon Mola* of Manipur. The mineral iron analysis has been conducted by using spectrophotometry method. The result of the study shows the decreased result in fried (1.83 μg/g) compare to fresh fish (4.82 μg/g). Meanwhile the steamed fish also shows a little decreased in iron content that is 3.27 μg/g.

The other study is conducted by Marimuthu et al. (2012) shows the result of mineral iron analysis by using spectrophotometry. The iron content of raw fish fillet was 6.4 mg/kg and the other cooking method shows the iron content range from 5.7 mg/kg to 7.4 mg/kg. But the result found from this study was not significant. The value of iron content in boiled fish fillet and grilled fish fillet was higher that the raw fish fillet that are 7.4 mg/kg and 7.2 mg/kg respectively. While the iron content in fried fillet and baked fillet are lower than raw fish fillet that are 6.1 mg/kg and 5.7 mg/kg respectively.

The study conducted by Badiani et al. (2013) was showed the result of iron content in raw European Sea Bass is 0.62 mg /100 g is higher compare to cooked fish fillet. The order of iron content is decreased from oven boiling, baking in aluminium foil and microwaving that are 0.57 mg/100 g, 0.57 mg/100 g and 0.56 mg/100 g respectively. Then, the study that conducted by Hosseini et al. (2014) shows the result of iron in raw fish s 8.8±0.28 mg/kg while the boiled fish shows the lowest iron content compare to baked fish, microwaved fish and fried fish that is 4.24±0.78 mg/kg. The result of baked fish, microwaved fish and fried fish are 4.32±0.65 mg/kg, 5.45±1.07 mg/kg and 6.20±0.14 mg/kg respectively.

In conclusion, most of the study shows that the boiling process is the best way to decrease the loss of total iron, heme iron and non-heme iron in cooked fish content. While the grilled fish shows the higher decreased of total iron, heme iron and non-heme iron content in cooked fish.

CHAPTER 3

METHODOLOGY

3.1. RESEARCH DESIGN

The research design for this experiment is an experimental study design. This experimental studied by focusing on four types of freshwater fish that commonly eaten by Malaysian; Catfish (*Clarius sp*), Tilapia (*Oreochromis sp.*), Haruan (*Channa sp.*) and Siakap (*Lates Calcarifer*) (Endinkeau & Kiew, 1993). Specifically, this experiment was carried out to determine the effect of common cooking method in Malaysia including frying, baking, steaming and boiling on the selected nutrient content in freshwater fish. So this experimental study was comparing the effect of cooking method on the proximate composition (moisture, lipid, protein and ash), total iron, heme iron and non-heme iron in four types of freshwater fish.

All selected freshwater fish has been bought at Pasar Siti Khadijah, Kota Bharu, Kelantan. The average weight and length of all fish is stated in Table 3.

Table 1.1: Average of length and weight of Haruan, Catfish, Siakap and Tilapia

Title	Average length (cm)	Average weight(g)
Haruan	24.9 cm	225.8 g
Catfish	31.3 cm*	201.8 g*
Siakap	32.8 cm*	511.7 g
Tilapia	24.58 cm*	381 g*

*n=5

All this freshwater fish has been weighed with external organ. Then, all fish has been divided into five group based on type of cooking such as control (fresh), frying, baking, boiling and steaming.

3.2. SAMPLE PREPARATION

All freshwater fish that has been bought at Pasar Siti Khadijah, Kota Bharu was processed at Dapur Pemakanan, Pusat Pengajian Sains Kesihatan, Universiti Sains Malaysia. Upon arriving at the kitchen, the sample's weight and length of the sample was measured by using top-pan scale and measuring ruler respectively.

Figure 3.1: Top pan scale

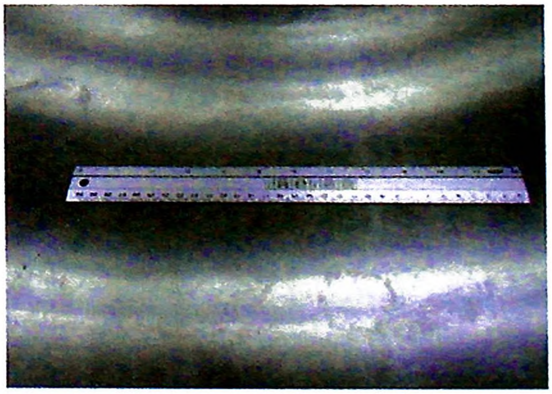


Figure 3.2: Measuring ruler