1. INTRODUCTION

1.1. Background

Marine and Fisheries Affair (2009) said that Indonesia is a maritime country which produces a lot of maritime product such as catfish 114,371 tons, snapper 4,371 tons, eastern little tuna 421,905 tons, and other marine fishes in the total amount of 243,376 tons which being exported and shared for domestic market mainly as fillets. Fillet production leads to an increase of fish by-products such as skin, bones, and others that so far have not been efficiently utilized and only converted into fish meal products (Rawdkuen *et al*, 2010). Global Business Guide Indonesia (2016) said that until the end of 2015, domestic fishery production in Indonesia reached 6.2 million tons, which made Indonesia become the world's second-largest capture fishery producer after China in the world. Fish is composed of 30% of by-products which are skin, bone, and scales (Regenstein, 2010). If the production of fish in Indonesia until the end of 2015 is 6.2 million tons so it's by-products are about 2 million tons. The limited uses of these fish by-products for added value products made these by-products become a pollutant to the environment and it will increase the costs to reduce impacts on environmental pollutants in line with fillet production (Abdi, 2011).The utilization of fish by-products is necessary to reduce its impacts as environmental pollutants and also can improve the quality of food products (AOAC, 1995). Because fish skin, bone, and scales are rich in collagen and amino acids, which can increase the value of food products if been added (Darmanto,1997).

Nowadays, a lot of people attempted to maintain their body to look younger than their age. So, the anti-aging product becomes precious and most wanted in the market. Unfortunately, because of their expensive ingredients, difficult processing, hype, and heavy marketing made anti-aging products so expensive. The making of an anti-aging product with less production cost is necessary.Many studies have been developed nutritious food using Fishbone flour as calcium fortification. But none of them discuss the utilization of fish bone to make an anti-aging product in food industry. This review will study the potential of three different types of fishbone flour as an anti-aging ingredient in food products. Three types of Fishbone flours that will discuss in this review are Fishbone flours from Walking Catfish (*Clarias batrachus*), Nile Tilapia (*Tilapia nilotica*), and Snakehead fish (*Channa striata*). Aging is a process characterized by a gradual loss of physiological integrity, leading to the death of almost all physiological functions and increased vulnerability to death (Lenart & Krejci, 2016). Aging can lead to some illnesses such as Osteoporosis, which is the condition when bones lose their density (Soltan, 2013). Beside Osteoporois, Wrinkles are also a factor to determine the degree of aging in people.

Even though marine fishes have higher nitrogen content than freshwater fishes. This study focused on using freshwater fishes than marine water fishes because freshwater fishes is more available and have cheaper price compared to marine fishes. Marine fishes also have a higher risk of a hazard than freshwater fishes. According to Tong Zhang (2010), marine water fishes like tuna, mackerel, and shark, which contaminated with mercury, even in lower concentrations than in freshwater fishes are dangerous for human health. Methyl mercury that contaminated marine fishes will latches onto chloride and become hard to degrade by heat. Meanwhile when methyl mercury contaminated freshwater fishes it will latches onto the dissolved organic matter that break easily by heat.

The reasons why this study focused to discuss about fish bone rather than other fish by-products are even though fish skin has higher collagen content than fish bone and has a soft form that makes the extraction process of collagen can be done easily without heat or enzyme. But fish skin development has been already common and effective. While the utilization of fish bone is still limited. Although the development of fish scale products is also limited, but the extraction process of collagen from fish scale is harder due to its hard form that needs hydrochloric acid (which is harmful to human health) and higher temperature (which can destroy collagen peptides) that make the extraction of collagen from fish scale needs higher manufacturing cost than the extraction process from fish bone.

This review focused on using fish collagen as anti-aging ingredients in food products than bovine collagen.The concern of not using bovine collagen in this review is because there is a chance of transmissible diseases such as mad cow disease, ovine and caprine scrapie, and other zoonoses (Pati *et al*, 2012). Besides, Muslims and Jews do not accept any pig-related food products, and Hindu does not consume cow-based products (Pranoto, 2007). Fish collagen is the best alternative as an anti-aging ingredient in food products because it has high availability, no risk disease transmission, and no religious barriers.

1.2. Literature Study

1.2.1 Snakehead Fish (*Channa striata*)

Snakehead fish (*Channa striata*) (Figure 1.) is a freshwater fish which has an abundant amount of albumin content that can accelerate the healing process for human (Asfar,2014). Snakehead fish can be easily found in various open waters in Indonesia, such as in Java, Sumatra, Kalimantan, Sulawesi, Bali, Lombok, Singkep, Flores, Ambon, and Maluku. The local names of snakehead in Java are Kutuk, Kocolan in Betawi, and Aruan or Haruan in Malaysia and Banjarmasin (Brotowijoyo,1995). Snakehead usually lives in estuaries or lakes and may inhabit dirty water, ditches, rice fields, ponds, and even abnormally can withstand the drought. (Qin & Fast,1996). Skin, bones, and scales are fish by-products which took 30% of parts in snakehead fish (Schrieber & Gareis,2007).

Figure 1. Snakehead Fish (*Channa striata*) (Private Collection)

There are 18 amino acids in snakehead fish skin and bones. The highest amino acid in those skin and bones is glycine and the second is proline. Glycine and proline content in the skin is a little bit higher in bones which are 238.11 (23.8%) glycines in the skin, 235.25 (23.5%) glycines in bone, 123.28 (12.3%) proline in the skin, and 121.81 (12.1%) proline in the bone. Moisture content in skin snakehead fish is 74.33% higher than in its bone which is 43.19%. Its protein content in the skin is slightly higher too which is 18.49% and 15.49% in the bone. Bone has higher lipid and ash content than skin which are 4.19% and 32.05%. Snakehead skin has 2.99% lipid and 0.20% ash.

The estimated collagen content in snakehead skin relatively higher which is 95.25% than in bone that has 89.01% (Rosmawati,2018). Ryo (2015) reported that snakehead fish bone powder contains 1.406 mg/100 g to 1.949 mg/100 g of calcium. Snakehead fish bones contain 33% of collagen, 39% of calcium, 0.2% of potassium, 0.7% of sodium, 0.5% of magnesium, 9.8% of carbonate, and 17% of phosphate (Liu *et al* , 2009). Fatty acids composition of *Channa striata* are 21.86% palmitic acid (16:0), 39.26% oleic acid (18:1(n-9)), 19.58% linoleic acid (18:2(n-6)), 0.54% linolenic acid - LNA (18:3n-3), 0.13% eicosapentaenoicacid - EPA (20:5n-3), 0.68% docosahexaenoic acid - DHA (22:6n-3). The content of total saturated fatty acid (SFA) were 29.68%, monounsaturated fatty acid (MUFA) were 45.45%, and polyunsaturated fatty acid (PUFA) were 24.95% (Ghassem,2009).

1.2.2 Catfish (*Clarias batrachus*)

In Indonesia, production of catfish is about 114.371 tonnes that have been exported to foreign countries and mainly sold as fillets in the domestic market (Marine & Fishes, 2009). Catfish fillets production is increasing each year and it lead to the increase of catfish by-products (bones, skin, and viscera of the fish). Unfortunately, these by-products have not been efficiently utilized so majorly it become wastes. Some of catfish by-products are converted into fishmeal products (Wang *et al*, 2008). Catfish skin also can be converted into gelatin products (Rawdkuen *et al*,2010). The study conducted by Darmanto (1997) and Kim & Park (2004) showed the potential use of fish waste as collagen products but this study is still limited.

Catfish (Figure 2.) is a common fish which easy to be cultivated but the utilization is still limited (Ferazuma *et al*, 2011). Catfish also known as warm-water fishes which has a superior amino acid than cold-water fishes. Nutrient content in catfish bone are 0.735% of calcium, 24.3% of protein, 3.84% of fat, 58.43% ash, and carbohydrates 6.02 % (Sa'adah, 2013).Catfish bones contain 21.2% of Glycine, 9% of Proline, 14.3% of Hydroxyproline + Proline, and 11.43% of water (Shyni,2014). Catfish frames have 6.33% of Calcium, 3.27% of Phosphorus, 0.27% of Sodium, 0.13% of Magnesium, 0.51% of Potassium, <0.2 ppm of Copper, 48.87 ppm of Zinc, 8.89 ppm of Manganase, <0.20 ppm of Nickel,and 6.16 ppm of Iron (Bechtel,2019). Fatty acids composition of *Clarias batrachus* are 24.34% palmitic acid (16:0), 38.18% oleic acid (18:1(n-9)), 18.86% linoleic acid (18:2(n-6)), 0.78% linolenic acid - LNA (18:3n-3), 0.05% eicosapentaenoicacid - EPA (20:5n-3), 0.27% docosahexaenoic acid - DHA (22:6n-3). The content of total saturated fatty acid (SFA) were 32.89%, monounsaturated fatty acid (MUFA) were 43.61%, and polyunsaturated fatty acid (PUFA) were 23.19% (Ghassem,2009).

Figure 2.Catfish (*Clarias batrachus*) (Private Collection)

Catfish is a native species that is found predominantly in the river of South East Asia which is a type of freshwater fish inhabiting freshwater, brackish water, muddy marshes, and stagnant water (Emenike *et al*,2012). It has a strong adaptive feature because it resists against various environmental challenges such as hypoxic, high environmental ammonia, and desiccation stress (Saha *et al*, 2011). So it has an abundance presence, cheaper, and is readily available fish collagen from a local source. Collagen of *catfish* can improve the adhesion of fibroblasts significantly (Li *et al*,2005). The effect of catfish collagen on the anchorage of fibroblast highlighted its potential to serve as a scaffold (Leong,2015). As a scaffold catfish collagen can improve cell anchorage and proliferation rate (Pati *et al*,2012). Because the presence of collagen improves the stability of the cytoskeleton and it will activate the signal transduction (Han,2011).

1.2.3 Nile Tilapia (*Tilapia nilotica* or *Oreochromis niloticus*)

Nile Tilapia (Figure 3.) is one of the most cultivated freshwater fish in the world (Chen *et al*, 2013). It is a species of African origin and is the most farmed fish in tropical regions all over the world. This type of fish is favored because of its rusticity, fast growth, adaption to diverse environments, good consumer acceptance and high quality, low-fat content, and absence of intramuscular “y” shaped bones (Maria *et al*,2008).

Figure 3. Nile Tilapia (*Tilapia nilotica* or *Oreochromis niloticus*) (Private Collection)

Nile tilapia can be found in most types of freshwater habitats like rivers, streams, canals, lakes, and ponds (Snoeks,2018). Nile Tilapia live in water in which temperature ranges between 8^0C to 42^0C, although typically above 13.5^0C, and usually at 39-40^0C will become lethal for some fishes (Froese,2015). Based on its temperature, habitat Nile tilapia can be classified as warm-water fishes. Even though Nile tilapia can survive in relatively cold temperatures, but the breeding process generally only occurs when the water at 24^0C (Nico,2019). Fishbone of Nile Tilapia consist of 14-20% moisture, 40-80% protein, 25.30% total lipid, 18.30% ash and mineral in 100 g fishbone it contain 2,715.90 mg calcium, 1,3 mg iron, and 1,1327 mg phosphorus.

Fishbone contains various minerals especially calcium and phosphor. The ash of the fishbone consists of 34-36% calcium, particularly calcium phosphate. In 100 g of Nile Tilapia Fisbone flour contain moisture (14.2%), protein (40.8%), total lipids (25.3%), ash (18.3%), and minerals (in 100 g) which are 2715.9 mg (calcium), 1.3 mg (iron), and 1132.7 mg (phosphor). There are 22 fatty acids detected in this fishbone which are 208.5 mg palmitic acid (16:0), 344.3 mg oleic acid (18:1(n-9)), 109.6 mg linoleic acid (18:2(n-6)), 29.9 mg linolenic acid - LNA (18:3n-3), 3.3 mg eicopentaenoicacid - EPA (20:5n-3), 12.9 mg docosahexaenoic acid - DHA (22:6n-3). The content of total saturated fatty acid (SFA) were 296.2 mg, monounsaturated fatty acid (MUFA) were 415.0 mg, and polyunsaturated fatty acid (PUFA) were 175.6 mg (Maria,2008). The collagen in Nile Tilapia composed of 33% glycine, 12% proline, and 10% hydroxiproline (Galves,2006). Haiying (2015) said that in warm-water Nile Tilapia fisbone collagen contain 337/1000 (33.7%) of Glycine, 110/1000 (11%) of Proline, and 76/1000 (7.6%) of Hydroxyproline. Fatty acids composition of *Oreochromis niloticus* are

20.80% palmitic acid (16:0), 28.45% oleic acid (18:1(n-9)), 20.55% linoleic acid (18:2(n-6)), 1.67% linolenic acid - LNA (18:3n-3), 0.35% eicosapentaenoicacid - EPA (20:5n-3), 4.66% docosahexaenoic acid - DHA (22:6n-3). The content of total saturated fatty acid (SFA) were 31.58%, monounsaturated fatty acid (MUFA) were 34.13%, and polyunsaturated fatty acid (PUFA) were 33.79% (Ghassem,2009).

1.2.4 Process Production of Fishbone Flour

There are a lot of methods that can be used to make fishbone flour which are by boiling the fishbone with water, alkaline, acid, or a combination of these solvents. This review focused on the process production of fishbone flour by water as boiling media because this method can hold nutrients such as protein especially collagen from being lost in the process. The use of alkaline or acid solvents as boiling media in the production of fishbone flour can remove organic matters such as protein and fat that make the produced fishbone flour will have a very low content of protein and fat (Amitha,2019). The process production of fishbone flour using water as boiling media are shown in Figure 4.

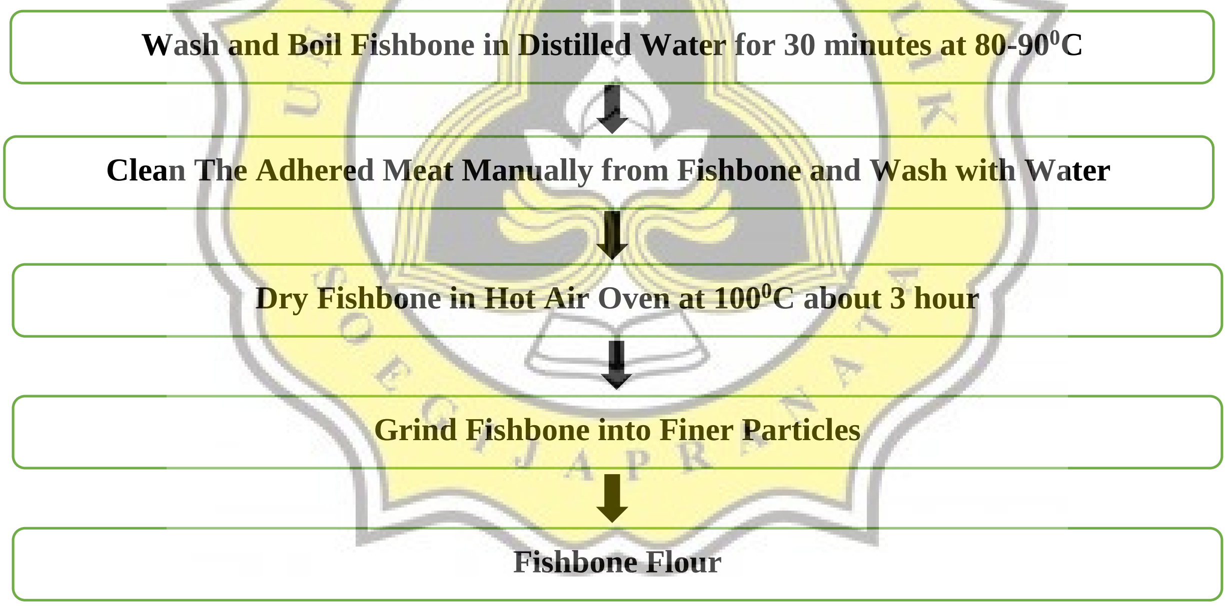

Figure 4.Process Production of Fishbone Flour by Water as Boiling Media (Amitha,2019)

The process production of fishbone flour in Figure 4 is perfect to maintain the collagen content in the fishbone because this process only uses the maximum heat of 100^0C. At 100^0C which is the normal boiling temperature, collagen will not undergo damage and it will begin to change to gelatin at 300^0C. According to Wulandari (2019), the yield of fishbone flour that uses water as boiling media is higher than using alkaline or acid solvent as boiling media. The process that uses water will produce a 67.57% yield from the total weight of fishbone. The process that uses alkaline will produce the lowest yield which is 39.67% of the total weight of fishbone. The acidic methods process also produces lower yield content than water methods which is 50.87%. Boiling fishbone in an alkaline or acid solvent can cause fishbone loss some compounds such as protein and fat that lowering the production of yield. Both alkaline and acid solvent can hydrolyze the crosslinking bonds between polypeptides in protein but alkaline methods cause a greater loss of protein and fat content than acidic methods that make the decrease of protein, fat, and yield more significant than acidic methods (Yang,2008). Generally, fishbone flour is composed of 25% calcium, 13% phosphor, 25% protein, 14% collagen, and other minerals (Wolber,2016).

1.2.5 Aging

Aging is a lifelong process that begins at conception and ends with death. Aging has different names in every stages of life. In babyhood aging is called as growth and development, in teenage or young adult years aging is called as maturation, and after the age of 30 aging is called as senescence. Senescence is the process when human physical body begins to wear out and its function declines gradually. There are five different types of aging which are biological, psychological, social, chronological, and functional aging. Biological aging sometimes referred to as Physical aging which is a process that involves the loss of cells over time. The function of tissues and organs becomes less efficient so the body's ability to repair itself becomes slower, the immune functions declining that makes the body more prone to infection.

A healthy diet and healthy lifestyle can make someone looks younger than someone who is the same age but does not maintain a healthy lifestyle. Psychological aging is a process that involves changes in memory, learning, intelligence, personality, and coping. Social Aging is a process when there are changes in roles and relationship as we age such as become grandparents in elderly years and from employee to a retiree. Chronological aging is the number of years a person has a life so far and may not match a person’s biological, psychological, or social age. Functional aging is related to how people compare psychologically to others to a similar age.

Due to aging, the human body will through several biological changes that can be classified into external, sensory, and organ system changes. External changes are the most obvious changes within aging it includes changes to the hair, nails, and skin. Hair will lose its pigment (turns gray or white) and become thinner during aging. Older people will have a receding hairline and some of them going bald. During the aging period, nails will thicken, become rigid, and more brittle along with skin that lose elasticity, become thin, and fragile. Skin will become dry and wrinkled (Figure 5.) due to the decrease of the blood flow and oil production in the skin. On the face and hands of older people often appear liver spots, their skin may look pale and translucent, their sweat glands decrease and there is an increased risk for heatstroke. Older persons will take a longer time for wound healing and at risk of getting decubitus ulcers (sores that are hard to heal).

Figure 5. Skin Become Dry and Wrinkled Due to Aging (Geriatric Nursing,2020)

Aging also leads to several sensory changes on taste, smell, sight, and hearing function. In later years the senses of taste, smell, sight, and hearing will decline. It will be hard for the elder to detect whether the food is spoiled or not because their sensory functions became weak. They will get vision loss because the lens in the eye becomes yellow and thickens. Their hearing ability will decline and lead to hearing loss. The organ system also will through several changes along with aging that include changes to the heart and cardiovascular system, the lungs and respiratory system, the gastrointestinal system, the urinary tract system, and the musculoskeletal system. The work of the heart and cardiovascular system will become slower because the heart pump with fewer forces and there is a decrease in cardiac output along with age.

Changes in the lungs and respiratory system will make breathing becomes difficult during aging. This difficulty was caused by the skeletal muscles become more rigid, the chest and the lungs become less elastic and will decrease in size. Changes to the musculoskeletal system during aging are shown with the declines of the width of the shoulders, muscles will weaken, loss of elasticity in ligaments, the cartilage between joints will become thin, and the decrease of lubricating fluid (Hom,2019). The changes in the musculoskeletal system are also shown with a decrease in bone density during aging. This loss may lead to a disease called osteoporosis which is related to the deficiency of bone solidity causing the bone to lose its power and becoming porous which makes it easy to become fractures. Osteoporosis usually occurs in women after menopause as the result of bone metabolism degradation. In menopause state, women will lose minerals in their bones rapidly because the bone loss it mass and ability to absorb minerals causing osteoporosis to happen (Masyitha,2006).

Aging also leads to changes in the gastrointestinal system which is shown in the decrease in the contraction of the muscle in the esophagus that makes food need more time to get to the stomach and give the sensation of being full before finishing a full meal. The urinary tract system also changing along with aging because kidneys will decrease in size and volume that affects renal function which makes older adults need to urinate more frequently. Muscles of the bladder will become weak and can lead to incontinence (the inability to control bladder functions) in the elderly (Hom,2019).

1.2.6 Collagen in Fish

Based on Rehn *et al* (2001), collagen constitutes 25%-35% of total proteins in vertebrates. Most collagen is derived from cow and pig skins but it causes outbreaks of certain animal diseases such as Bovine Spongiform Encephalopathy (BSE) and Foot and Mouth Disease (FMD) that are possible transmitted to human beings (Trevitt & Singh,2003). Collagen from fish has high biological value, high essential amino acid content, and low content of hydroxyproline. Collagen is the main structural component of connective tissue proteins. Thirty percent of total protein in the body tissue of vertebrates and invertebrates containing amino acids such as glutamine and asparagine (Balian &Bowes,1977), alanine, arginine, lysine, glycine, and proline (Poppe,1997). Collagen molecules composed of 3 α-chains intertwined in the so-called collagen triple helix (Te Nijenhuis, 1981). This triple helix is approximately 300 nm in length and 105 kDa in molecular weight (Papon *et al*,2007). Nitrogen content in bone collagen animals fed exclusively in the marine environment is 9% more than terrestrial environmental (Schoeninger, 1984).

Fishbone demineralization is a process that can be used in the fishbone flour production process with the purpose to remove calcium and other salts. Fishbone demineralization will make the quality of collagen in fishbone flour higher because during this process fishbone will release the collageneous producing substance so-called *ossein* (Ward & Courts, 1977). If calcium and phosphor are in high concentration in fishbone it will make the quality of collagen low. The fishbone demineralization process only occurs with the addition of an acid such as Hydrochloric Acid. In acidic solutions, collagen in fishbone will be swelled and does not dissolve so this demineralization process will not make fishbone lose its collagen content (Mardawati,2018). Fish collagen consists of zero fat, carbohydrate, and sugar. In 6.6 grams of fish, collagen contains 26 calories, 6.4 grams protein, and 51 mg sodium. Fish collagen peptides come from 60% of fish by-products which are thrown away as wastes. Fish skin contains a large portion of collagen and became the primary source for collagen extraction. Scales, fins, and bones also are sources for collagen extraction. Fish skin has soft formation so collagen can be easily removed without enzymes or heat. The formation of scales is hard so it needs to use hydrochloric acid which is harmful to our health for removing the collagen content in it. The process of collagen extraction from scales also uses heat which can destroy the collagen peptides (Chalamaiah, 2012).

1.2.7 Anti-Aging

Anti-Aging is a compound that treats the underlying causes of aging and aims at alleviating any age-related ailment to extend the healthy lifespan of humans having youthful characteristics (Balvant, 2008). To date, Gabriel (2020) said that skin aging is a natural and unavoidable process that involves oxidative activity during the metabolism of body tissue. This process results in the generation of free radicals with one or more unpaired electrons in a reactive state. The skin possesses its antioxidant defense against this oxidation process at subcellular compartments, organelles, and the extracellular space. Balavant (2008) said that antioxidant is a chain-breaking compound that can delay or inhibits oxidation of that substrate. The presence of free radicals can damage the cell membrane which is composed of lipids and proteins. This interaction results in the production of the chemical Melonialdehyde which is very harmful and contributes to other important aging processes which are glycation, methylation, and chronic inflammation. Glycation is a condition when glucose molecules and other sugars like fructose attach themselves to proteins and resulted in brownish discoloration of tissues. This condition will cause more damage if react with free radicals and other toxins because it will create Advanced Glycation End products (AGEs) that bind to cells at special attachment sites called RAGEs (Receptor of AGEs) which result in the production of several harmful chemicals damaging tissues.

The concentration of AGEs in most tissues in the body will increases after human past the age of 20. Glycation also interferes with DNA which makes DNA become a crossed linked molecule that lost its usefulness. Nutrients such as carnosine and other Anti-Aging supplements can help in breaking the glycation process. Methylation is a condition when methyl groups are being added to different constituents of proteins, DNA, and other molecules to keep them in a good active condition which is necessary for the normal maintenance of tissues and is usually kept at a healthy level naturally by the body. While chronic inflammation makes the body tissues are eaten away by toxin chemicals, resulting in dementia, thickening of the arteries, arthritis, diabetes, hormonal imbalance, and so on. Chronic inflammation also affects methylation because the immune system will gorges itself on methyl groups, leaving nothing for other tissues of the body. Low methylation is reflected in the increasing levels of homocysteine which is found in the chronic inflammatory process such as lupus, heart disease, and diabetes. A diet containing nutrients that contains anti-inflammatory chemicals, minerals, vitamins, and antioxidants can help reduces the risk of these diseases.

1.3. Problem Identification

Based on backgrounds and study literature that have been read, there are some problems which have been found which are:

1. Does Fishbone has the potential as an anti-aging ingredient in food products?
2. What is the impact of using Fishbone flour in food products?
3. Which Fishbone has the most potential for anti-aging ingredient in food product?

1.4. Purpose

To study the potential of three different types of fishbone flours from *Clarias batrachus, Tilapia nilotica,* and *Channa striata* as anti-aging ingredients in food products, the impact of using fishbone flour in food products, and the most potential fishbone flour as anti-aging ingredient.