EFFECTS OF COOKING ON THE STABILITY & ANALYSIS OF THE PHYSICO CHEMICAL CHARACTERISTICS OF COMMERCIAL CANNED FISH

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Roll No.: 0121/13
Registration No.: 982
Session: January-June 2021

A thesis submitted in the partial fulfilment of the requirements for the degree of Master of Science in Food Chemistry and Quality Assurance

Department of Applied Chemistry and Chemical Technology

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Chattogram-4225, Bangladesh

FEBRUARY,2023

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This is to certify that we have examined the above Master's thesis and have found that is complete and satisfactory in all respects, and that all revisions required by the thesis examination committee have been made

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PLAGIARISM VERIFICATION

Title of Thesis: Effects of Cooking on The Stability & Analysis of The Physico Chemical Characteristics of Commercial Canned Fish.

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List of Abbreviation

AOAC	Association of Official Analytical Chemists
ANOVA	One way analysis of variance
%	Percentage
°C	Degree Celsius
Mg	Milligram
M	Molar
Kg	Kilogram
mL	Milliliter
min	Minute
W.H.C	Water Holding Capacity
FAO	Food and Agricultural Organization
CVASU	Chattogram Veterinary and Animal Sciences University
N.A.	Not Applicable

Abstract

Canned fish is a widely consumed source of protein and omega-3 fatty acids, often reheated or cooked for convenience. However, little is known about how reheating affects its characteristics and nutritional value, potentially impacting overall quality. Additionally, canned fish may contain heavy metals like mercury and lead due to environmental pollution, posing potential health risks to consumers. Therefore, this study aims to investigate the effects of two commonly used reheating methods, sauteing and pan frying, on the physicochemical characteristics of commercially canned fish by measuring proximate composition, pH, water holding capacity, cook loss, calcium, phosphorus content. The presence of mercury and lead is also examined. In proximate analysis, the moisture (%) ranged from 65.87 ± 7.95 to 56.34 ± 2.72, ash (%) from 2.63 ± 1.12 to 1.82 ± 0.80, fat (%) from 20.23 ± 5.07 to 8.92 ± 4.37, protein (%) from 23.87 ± 2.27 to 21.85 ± 2.66. In physical test analysis, pH, W.H.C (cm^2), cook loss (%) ranged from 6.67 ± 0.12 to 6.27 ± 0.25, 3.94 ± 1.46 to 3.51 ± 1.18, 11.98 ± 7.22 to 5.49 ± 3.24. Calcium (%) and phosphorus (%) ranged from 0.73 ± 0.49 to 0.65 ± 0.48, 0.29 ± 0.16 to 0.14 ± 0.01. Mercury & lead was not detected in any of the samples. The results of this study showed that there were no significant changes in the protein, ash, water holding capacity, calcium, phosphorus content of canned fish after either sauteing or pan frying. These findings indicate that these commonly used cooking and reheating methods do not significantly affect the physicochemical properties of commercially canned fish. Therefore, these methods can be considered as appropriate methods for cooking and reheating canned fish.

Keywords: Canned Fish, Cooking, Reheating, Physicochemical Characteristics.

Chapter 1

Introduction

Fish is a valuable and significant part of the human diet, as it provides essential nutrients that are often lacking in diets high in cereal (Olomu, 1995). Fish contains an abundant amount of protein of excellent quality, characterized by a balanced distribution of essential amino acids, making it an important component of a healthy diet (Pigott and Tucker, 1990). With a digestibility rate of over 90%, the protein in fish is easily absorbed by the body. In addition to its protein content, fish is a good source of vitamins and minerals, such as calcium, iron, and zinc. Studies have shown that regular consumption of fish can have a positive impact on heart and cardiovascular health, as well as a variety of other health conditions (Damsgaard et al., 2006). Overall, the inclusion of fish in a balanced diet can offer numerous health benefits and contribute to overall well-being.

While fresh fish can be cooked using traditional and conventional methods, fresh fish is highly perishable with very limited shelf-life, which makes it challenging to store and transport (Huss, 1995). To overcome these challenges, fish are processed into canned fish for people's convenience. Canning is an effective way to preserve fish by heating it at high temperatures and sealing it in airtight containers, which extends its shelf life and makes it easier to transport and store. For its longer shelf life, it can be stored in a pantry for an extended period without the need for refrigeration, making it a convenient option for people with busy schedules or limited access to fresh fish. Canned fish is available year-round, regardless of the season or location, and it is not affected by fluctuations in supply and demand, which makes it a more reliable source of fish for consumers. Additionally, canned fish is more convenient to use since it does not require cleaning, filleting, or cooking, which saves time and effort. These advantages of canned fish have made it more popular than fresh fish and many consumers prefer it over fresh fish due to its convenience and availability (Aitken et al., 1979).

Fish in cans makes for a convenient and quick choice because it is often fully cooked and can be eaten cold right out of the can. However, some consumers prefer to cook or reheat their canned fish beforehand to enhance its texture or flavor, or to incorporate it

into other dishes like pasta, soup or salad. During cooking or reheating, chemical and physical reactions take place that can affect the nutritional value of the food, such as increased protein digestibility but reduced levels of fat-soluble vitamins and polyunsaturated fatty acids (Bognar, 1998; Finot and P. A, 2017). Due to reheating or further cooking fish from cans can undergo a number of chemical changes. The breakdown of proteins and lipids is one of the biggest alterations since it can result in the formation of chemicals that can affect taste and odor. When exposed to prolonged high temperatures, the quality of the product may be compromised in various ways. For instance, its sensory attributes may be altered, and some essential nutrients like vitamins and amino acids may be lost (Venugopal and Vazhiyil, 2006). Reheating canned fish can modify its physical qualities such as pH, water holding capacity and cooking loss. Due to modification in physical qualities, it can bring changes in dryness & tenderness of the product (Gall et al., 1983; Ohta et al., 1988).

Despite the convenience and popularity of canned fish, it is crucial to examine the heavy metal content in such products, particularly those fishes that are predominantly caught from oceans and seas, as these contaminants can accumulate in fish and potentially harm human health. Aquatic pollution in the form of heavy metals is of utmost concern due to their tendency to accumulate within the tissues of aquatic organisms. Fish, in particular, has been found to accumulate significant amounts of these metals, especially in their muscles. This poses a serious risk to human health (Ashraf et al., 2006; Kalay et al., 1999; Rose et al., 1999; Tariq et al., 1993).

Considering these facts, a study was conducted to investigate the impact of cooking and reheating on the physicochemical characteristics of canned fish and to assess the presence of heavy metals in the samples.

Specific objective:

The study aims to analyze:

I. The proximate composition, physical parameters such as pH, water holding capacity, and cook loss of canned tuna and sardines before and after reheating.
II. Mineral content (calcium, phosphorus) in canned tuna and sardine before and after reheating.
III. Heavy metal presence (mercury, lead) in canned tuna and sardines.

Chapter 02
Review and Literature

2.1. Fish as food

Fish has been generally eaten as a superb source of animal protein and various nutrients. It has the function to keep people away from many illnesses around the world. Through various handling techniques, accomplishing the keeping of value and getting fish accessibility round the year is conceivable. Proteins, fat (poly-unsaturated fatty acids), vitamin (including vitamin A, vitamin B2, and vitamin B6), minerals (iron, calcium, iodine, potassium, and other minerals), and carbohydrates are all abundant in fish (Boulenger, 1896; Longwe et al., 2016; Trewavas, 1941). A good source of nourishment and a staple in many cultures around the world, fish is used as the major food item. Compared to other animals, fish is a more significant supplier of protein **(**Akinneye et al., 2010; Farid et al., 2014; Holma et al., 2013; Reza et al., 2015). Due to its very high quality and important amino acid composition, fish's increased crude protein level is essential from a dietary perspective. Furthermore, according to research, fish muscle has less connective structure than other animal proteins, making it easier to digest. It has health advantages, especially for blood pressure, heart disease, and a reduced chance of prostate cancer and alzheimer's disease (Mazrouh et al., 2015; Mesias et al., 2015; Oduro et al., 2011; Suganthi et al., 2015; Tidwell et al., 2001).

2.2. Chemical composition of fish

Fish is commonly composed of approximately 70-84 percent water, 15-24 percent protein, 0.1-22 percent fat, 1-2 percent minerals, and 0.1-1 percent carbohydrates. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), both of which are omega-3 fatty acids, are found in the fats from fatty fish species and are crucial for children's healthy growth as well as the prevention of cardiovascular disorders including coronary heart disease (Hantoush et al., 2014; Holma et al., 2013; Marta et al., 2015; Suganthi et al., 2015; Tidwell et al., 2001)**.** Fish used as food has a chemical makeup that varies greatly between species and even within a single species, which determines how nutritious it is. Fish chemical composition is mostly influenced by eating habits, sex, and seasonal fluctuations. Understanding the chemical composition of fish is crucial for developing processing methods for fish and fish products, both in

commercial and industrial settings. This awareness facilitates the evaluation of the protein value of fish in relation to other food items, the possibility of enhancing it during product development, and the enrichment of its nutritional composition to align with dietary requirements, industry standards, and optimal utilization. By doing so, the loss of nutritional content can be minimized (Akinneye et al., 2010; Hantoush et al., 2014; Mohamed, 2013).

2.3. Canning of fish

In the early nineteenth century, the technique of preserving food in cans was invented by a Frenchman named Nicolas Appert, who won a competition initiated by the famous French leader Napoleon Bonaparte. Despite being remembered primarily for his achievements as a conquering General, Napoleon also provided the impetus for the development of this food preservation method, which ultimately marked the beginning of the canned food industry. Nicolas Appert was awarded 12,000 francs for proving that meals held in airtight metal cans, after being heated, could remain fresh even without refrigeration. This breakthrough allowed for the development of markets for shelf-stable canned products, which had not been explored previously due to reliance on the refrigerated and frozen food chain. While the fundamentals of canning have not changed significantly since Appert's invention, technological advancements have led to improvements in the process. Proper adherence to the guidelines is crucial for the success of the global fish canning industry (Warne, 1998).

2.4. Process Description of Canning fish

Both the production of fish byproducts like meal and oil as well as the canning of fish for human consumption are considered to be fish processing. When canning, either a precooking method or a raw pack method can be applied. The raw fish is cleaned and cooked in the precooking method prior to canning. The raw fish is cleaned and packed using the raw pack technique before being cooked. Typically, the precooking technique is used for larger fish like tuna, whereas the raw pack technique is utilized for smaller fish like sardines. Thawing the fish, if necessary, is the first step in the precooking technique of canning (Figure 2.4). The fish is gutted, cleaned, and then cooked. Depending on the size of the fish, cooking is done using steam, oil, hot air, or smoke for 1.5 to 10 hours. Fish flesh is made more tender by pre-cooking because the fish protein coagulates, and the fish oils are removed. The next step is cooling the fish,

which might take many hours. Refrigeration may speed up the cooling process. The head, fins, bones, and unwanted flesh are removed after cooling, and the remaining fish is then chopped or sliced into smaller pieces for canning. The cans are filled with oil, brine and/or water before being sealed and pressure baked for shipping.

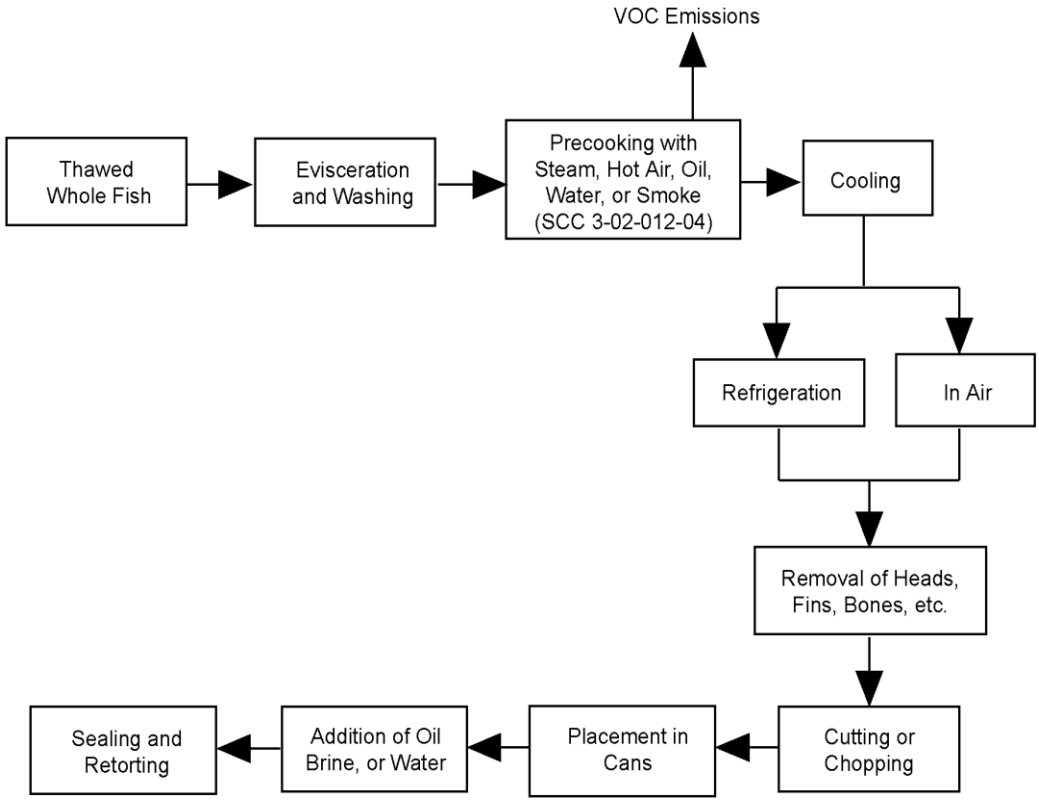

Figure 2.4.: Flowchart for the precooking process.

Thawing and weighing the fish are the first steps in the raw pack method of canning (Figure 2.4.1). Then the heads, viscera, and tails are removed, after being cleaned and perhaps brined. After being canned, the fish is boiled, drained, and dried. Oil, brine, water, sauce, or other liquids may be added to the cans after drying. The cans are then sealed, cleaned, and disinfected using steam or hot water (Warne, 1988; Wheaton et al., 1985; Gillies, 1971; Windsor et al., 1981; Prokop, 1992; Summer, 1963)

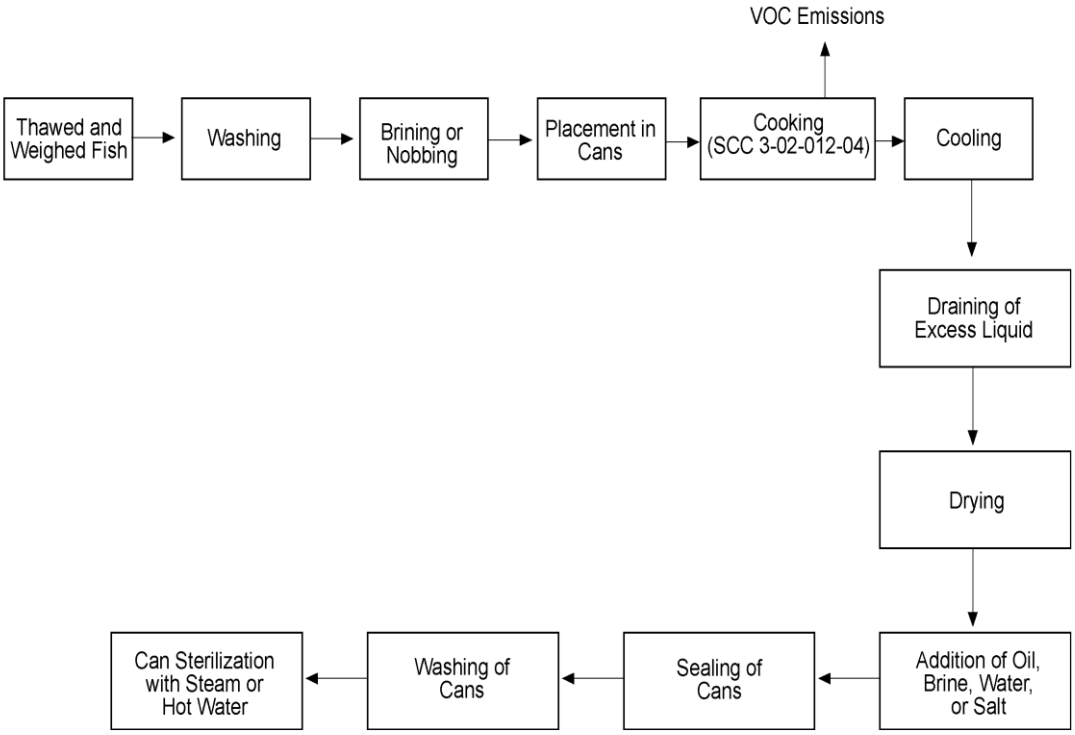

Figure 2.4.1: Flowchart for the raw packing process.

2.5. Changes in fish due to reheating

Depending on personal taste, culture, and culinary customs, people may choose different ways to reheat fish. Reheating fish is a popular practice in certain cultures but less so or even frowned upon in others. Reheating fish may be an easy way to consume leftovers or make a quick supper. Fish's proximate composition, or the primary nutritional elements of a food product including moisture, protein, fat, and carbs, may change when it is heated again (Creed, 2001). Fish quality may be impacted by physicochemical changes that can occur when it is heated again. The particular reheating conditions, such as the temperature, length, and manner of reheating, determine the precise changes in proximate composition that can occur. Fish can lose moisture when heated again, making the final product drier and less tender. Due to the denaturation of its proteins, even it may make it rough and rubbery. Fish can have a "fishy" or off-flavor, changing its flavor (Gall et al., 1983; Ohta et al., 1988).

2.6. Changes in quality of canned fish during storage

Food quality changes during storage are the consequence of a wide range of physicochemical reactions that reduce the nutritional content of the foodstuff, palatability, and safety. Various categories of procedures impacting the quality should be taken into account when it comes to preserved food products: (1) migration of metal ions from the inner surface of the can into the product, and (2) hydrolytic and oxidative processes in protein and lipid components of the product (Riera Valls, 1987). Proteins, lipids, nutrients, minerals, and sensory qualities including color, flavor, texture, and overall appearance are the components of fish that can most significantly change in composition as a result of these factors. The most significant real alterations that can occur to fish are texture changes (becomes hard and rigid), color changes and yields. The degree of these progressions relies upon the temperature, storage conditions and season of treatment. This multitude of changes influence the consumer acceptability of the product.

2.7. Heavy metal aspect in fish

Heavy metals are present throughout the environment due to both natural and human activities, resulting in heightened exposure of human communities to their impacts via diverse routes (Poty et al., 2012; Wilson et al., 2007). Increased levels of specific trace elements, particularly their readily mobile variants, can give rise to notable environmental apprehensions concerning pollution and buildup in soil, plants, animals, surface water, and groundwater (Chopin et al., 2007)**.** Metals, primarily lead, zinc or copper, as well as antimony, arsenic, mercury, cadmium and other metals, are the main cause of environmental contamination. Both the production of metals and the combustion of fossil fuels, particularly coal, are significant sources of metal pollution of the environment. Fly ash produced by the combustion of fossil fuels passes through contaminated soil and has become an increasingly significant source of environmental pollution due to its heavy metal content. Additionally, burning municipal waste streams and pollution effluents that contain high levels of toxic metals have also become a major contributor to this type of pollution (Bencko et al., 1995)**.** The top soil layer is where the majority of pollutants from the atmosphere (heavy metals) deposits collect and are concentrated**.** Assessing the quantity of heavy metals absorbed by the root system and leaf surface, especially in the case of lead, can present significant difficulties (Hovmand et al., 2009; Steinnes et al., 2005). Surface water and groundwater serve as natural

reservoirs for nearly all metallic elements, many of which are necessary in trace quantities but can present hazards when present in elevated levels. At present, the presence of certain metals like Cd, Pb, Hg, and Cr poses the highest level of concern, and their influence on all ecosystems is considerable. Heavy industry, metallurgy, and agriculture are the main contributors to environmental contamination, with the agricultural sector having a particularly significant impact on aquatic ecosystems (Dercová et al., 2005). Heavy metal pollution in fish encompasses two primary dimensions. The initial aspect pertains to the health and hygiene considerations associated with consuming fish as a food source. The second dimension involves bioindication, which stems from the role and integration of fish within the ecological and energy transfer networks of aquatic ecosystems. Heavy metals tend to accumulate in different organs of fish to varying extents, with the kidneys, liver, and gills showing the highest concentrations. Among these organs, the muscle presents the most significant concern in terms of fish consumption as food. (Árvay et al., 2014).

2.8. Canned fish prospect in Bangladesh

With the largest flooded wetland in the world and Asia's third-largest aquatic biodiversity after China and India, Bangladesh is regarded as one of the world's most appropriate fisheries regions (Islam et al., 2004). Bangladesh is endowed with extensive inland lakes and river systems, which provide vast opportunities for catch fishery and aquaculture due to their abundance of fish resources. According to annual report of department of fisheries, ministry of fisheries and livestock in the fiscal year 2019–20, Bangladesh produced 45.03 million tonnes of fish, ranking third in the world, of which 6.71 million tonnes of marine fish were taken from the Bay. Salmon, tuna, and herrings which include sardines and anchovies—are high-value species that are typically canned. Bangladesh now has complete sovereignty over a sizable maritime area thanks to the simultaneous decisions of the Permanent Court of Arbitration (PCA) on July 7, 2014, and the International Tribunal for the Law of the Seas (ITLOS) in Hamburg, Germany, regarding the dispute over the maritime border between Bangladesh and Myanmar on March 14, 2012. Overall, 111,631 square kilometers of the pertinent area with Myanmar (about 171,832 square kilometers to Myanmar) and 19,467 square kilometers of 25,602 square kilometers with India were allotted to Bangladesh. By acquiring this huge sea land Bangladesh has got the chance to explore more sea fish like tuna, sardine which are available in Indian ocean. Bangladesh also joined the Indian

Ocean Tuna Commission to investigate tuna populations beyond its 200 nautical mile limit. Deep sea tuna is available in at least six more varieties, including yellowfin, albacore, bigeye, and skipjack. In 2017, the worldwide tuna market was valued at $11.38 billion. Those sea fishes are marketed in fresh, frozen & canned form. The market for canned seafood was predicted to reach USD 31.37 billion in 2022 from an estimated USD 30.9 billion in 2021. Bangladesh is not well established in the field of canned fish industry due to its low infrastructure. If the government takes necessary steps in this regard, it will not only make jobs but also achieve foreign currency which is highly valuable for a country like us (Haque et al., 2019).

Chapter 03

Materials and Methods

3.1. Site of the study

The study was conducted in the Department of Applied Chemistry and Chemical Technology, Department of Animal Science and Nutrition of Chattogram Veterinary and Animal Sciences University.

3.2. Collection of samples

To measure the proximate analysis & other physical property, four different brands of canned fish samples were collected from the local supermarkets. Among four brands, two are tuna & other two are sardine fish. Those samples are already two years old from their processed date.

3.3. Preparation for reheating canned samples

For reheating of 100gm sample of canned tuna, sardine, first the can is opened, filling oil in can is drained & separated. Then the canned fish flesh was taken in a pan & fried with soyabean oil in 120 °C for 3 minute & 150 °C for 5 minutes in an electric induction cooker. 15 ml of soybean oil was used for frying at 120°C, while 40 ml of soybean oil was used for frying at 150°C.

3.4 Proximate composition of canned fish samples

The proximate composition of the canned tuna and sardine before and after reheating was examined, including moisture, crude protein, crude fat, ash content and the results were expressed as percentages. The procedure was done in accordance with AOAC guideline.

3.4.1 Determination of moisture content

Food staffs always contain moisture. To estimate moisture, Initially, the vacant crucible is subjected to a drying process in the oven at a temperature of 105°C for a duration of 3 hours, after which it is transferred to a desiccator to cool down. After weighing the crucible, 10g of sample is taken to the crucible and weighted the crucible with sample. Then the crucible is placed in the hot air oven for drying at 105°C. Reading was taken

at 1 hour interval up to constant weight. After drying, crucible is taken to the desiccator to cool followed by reweighing the crucible and its dried sample (Horwitz, 1980).

Calculation: moisture (%) = ((w1-w2)/w1) *100

Here, w1= weight(g) of sample before drying, w2= weight(g) of sample after drying

3.4.2 Determination of crude protein content

The kjeldhal technique was used here. In this technique, A measured quantity of the sample is digested using sulfuric acid (H_2SO_4) in the presence of a digestion mixture containing copper sulfate ($CuSO_4$) and potassium sulfate (K_2SO_4) in a 1:20 ratio. The digested sample is then distilled, and any excess acid is neutralized using a 40% sodium hydroxide (NaOH) solution (w/v). The ammonia that is liberated during this process is captured in a 2% boric acid solution. To initiate determining of protein content, 1g of the sample containing protein, added with 12–15 ml of strong sulfuric acid to a digestion flask with the sample. After adding a catalyst, typically copper, and seven grams of potassium sulfate, the digestion tube or flask containing the mixture was heated using a heating block until it reached a "rolling boil" at around 370°C to 400°C. The heating was then continued for 60-90 minutes. By employing a solution of sodium hydroxide (40% NaOH), the mixture's pH was raised. As a result, the ammonium (NH_4^+) ions that were previously in solution underwent a transformation into ammonia (NH_3), a gaseous form. To eliminate the nitrogen present in the digestion mixture, the ammonia was subjected to a distillation process, where the resulting vapors were collected in a specialized trapping solution containing approximately 15 ml of hydrochloric acid (HCl) dissolved in 70 ml of water. The acid/ammonia trapping solution was supplemented with an indicator dye to detect the presence of a substantial amount of the original trapping acid. A standardized solution of sodium hydroxide (NaOH) is gradually introduced to the acidic solution containing the dye until the solution turns orange, indicating that the "endpoint" has been achieved. The volume of the sodium hydroxide solution needed to reach the endpoint is measured, and calculations are carried out (Horwitz, 1980).

Calculation: crude protein (%) = ((a*b*0.014)/w) *6.25*100

Here, a = volume of standard HCL, b = normality of standard HCL, w = weight of the sample.

3.4.3 Determination of crude fat content

To determine the fat content of food samples, they are typically placed in organic solvents such as chloroform or methanol and then filtered to separate the extract. The resulting mixture is separated into two funnels, dried, and the extract is measured to estimate the fat content. To begin the process of measuring the fat content, the glass apparatus was initially washed with petroleum ether and subsequently dried in an oven at 102°C. After removing them, they were kept in a desiccator. Five grams of pulverized and dehydrated sample were measured and inserted into a thimble, which was subsequently positioned within the Soxhlet extractor. A 150 ml round bottom flask was filled with 90 ml of petroleum ether and placed on a heating mantle to boil. The extraction process continued for approximately 6 hours before removing the condensing unit and allowing the sample to cool down. The lipid was then removed, and the solvent was collected after distillation. The sample was dried in an oven, and the weight was recorded to obtain a defatted sample (Horwitz, 1980).

Calculation: crude fat (%) = ((w2-w1)/p) *100

Here, empty thimble weight = w1, thimble with sample weight = w2, weight of sample= p

3.4.4 Determination of ash content

To ascertain the ash content, a 5g sample was measured and transferred into a crucible that had been pre-weighed. Subsequently, the crucibles were carefully positioned inside a cooled muffle furnace. Protective equipment such as tongs, gloves, and safety goggles were employed due to the elevated temperature of the muffle furnace. It was Ignited for 12–18 hour (or overnight) at about 550°C. Once the muffle furnace was turned off, it was left to cool down to a temperature of at least 250°C, preferably lower. The door was then carefully opened to prevent the loss of any fluffy ash. The crucibles were promptly moved to a desiccator equipped with a porcelain plate and drying agent, utilizing appropriate tongs for safety. The desiccator was then closed, and the crucibles were covered and left to cool down before weighing (Horwitz, 1980).

Calculation: ash (%) = ((w1-w2)/(w1-w)) *100

Here, w1= weight of crucible + sample (before ashing), w2= weight of empty crucible w= weight of crucible + dry matter

3.5 Physical test of canned fish samples.

For the physical test of canned tuna, sardine & its reheated sample pH, water holding capacity, cooking loss was measured.

3.5.1 Determination of pH

To evaluate pH, 10 gm of fish sample was taken, grinded it to 3-4 times. Then, ground meat placed into a blender, 40mL of distil water is added there. The mixture was homogenized using high-speed blending for 10 to 20 seconds until a smooth slurry was obtained. The pH of the slurry was determined using a pH meter that had been calibrated using standard buffer solutions (Horwitz et al., 2016).

3.5.2 Determination of water holding capacity

To commence the procedure for evaluating water holding capacity, a quantity of 3 mg of the provided meat sample was deposited onto a filter paper of type Whatman no. 41. The filter paper was placed between two glass slides and a 100g weight was placed on top of the upper slide. This assembly was then placed on a hard plate for 3 minutes, during which the water released from the meat sample was absorbed by the filter paper and left an impression. The boundary of the impression was carefully delineated using a sharp pencil, and the area was measured using a compensating polar planimeter (Parrish and Boles, 2009)**.**

3.5.3 Determination of cooking loss

Cooking loss determination is based on determining the loss of water and soluble matter from fish during cooking. First the sample was weighed then it was heated again, after cooling its weight measured again to determine cooking loss. Cooking losses were calculated as a percentage weight difference between the without heated sample and heated sample relative to the weight of the without heated sample (Horwitz et al., 2016).

Calculation: cooking loss (%) = ((w1-w2)/w1) *100

Here, w1= sample weight without heating or cooking, w2= Reheated sample weight

3.6 Determination of mineral content

For determining mineral content calcium, phosphorus was measured in canned tuna, sardine fish and its fried sample.

3.6.1 Determination of calcium

For determination of calcium, 2g of finely powdered sample are weighed into a porcelain or SiO_2 dish, then ignited in a furnace to produce carbon-free ash. With 40 mL of HCL (1 + 3) and a few drops of HNO_3, the residue was heated, transferred, cooled, diluted to volume, and well mixed into a 250 mL volumetric flask. Next, 25 mL of a clear liquid were pipetted into a beaker, diluted to 100 mL and 2 drops of methyl red were added. NH_4OH (1 + 1) was added dropwise to pH 5.6, as indicated by intermediate brownish orange. HCL (1 + 3) is increased by 2 more drops. Now, the color should be pink (pH 2.5-3.0), not orange. It was diluted to 150 mL, heated to a boil, and slowly stirred while a 10 mL hot saturated (4.2%) solution of $(NH_4)_2C_2O_4$ solution was added. Waited all night for the precipitation to settle, filtered supernate using a fine pyrex filter, then thoroughly washed the precipitate with NH_4OH (1 + 50). A mixture of 125 mL of water and 5 mL of H_2SO_4 was added to the paper or crucible containing the precipitate in the original beaker. Titrated with 0.1 N $KMnO_4$ and heated to 70°C. The little pink color faded quickly due to the paper's presence. Then, it was adjusted for blanks and the percentage of calcium was calculated (Horwitz, 1980).

3.6.2 Determination of phosphorus

First sample solution was prepared & pipetted into beaker, aliquoted corresponding to 0.4 g sample. Added 5 to 10 mL of HNO_3, then NH_4OH was added, and the mixture was vigorously stirred until the precipitate it formed slowly dissolved. The solution was diluted to a volume of 75 mL and maintained at a temperature range of 25-30°C. To facilitate the precipitation process, NH_4OH was used as a neutralization test, ensuring that the solution became slightly alkaline according to litmus paper when NH_4OH was added, and slightly acidic when HNO_3 (1+3) was added. For samples with a P_20_5 content of less than 5%, 20-25 mL of acidified molybdate solution was added. For samples with a P_20_5 content between 5-20%, 30-35 mL of acidified molybdate solution was added. For samples with a P_20_5 content greater than 20%, enough acidified molybdate solution was added to ensure complete precipitation. After stirring mechanically for 30 minutes at room temperature, the precipitate was washed twice by decanting amounts of water of 25 to 30 mL each, thoroughly agitating it and allowing it to settle. The precipitate was transferred onto a filter and washed with cold water until the filtrate from two filter fills produced a pink color when one drop of standard alkali

and phenolphthalein were added. The precipitate and filter were then transferred to a beaker or other precipitating vessel, where they were dissolved in a tiny amount of standard alkali, phenolphthalein was added, and then the solution was titrated with standard acid. Reported as percentage of phosphorus (Helrich, 1990).

3.7 Determination of heavy metal presence

Heavy metal such as mercury, lead was measured for canned tuna and sardine fish.

3.7.1 Determination of mercury

Direct Mercury Analyzer (DMA 80) was used for mercury concentration in the sample. The DMA 80 analyzer's operation is based on the principles of thermal decomposition, amalgamation and atomic spectrometry detection. Without the need for sample preparation, we can do direct determination of total mercury using the DMA-80 using acid digestion. As a result, there are no hazardous chemicals to buy, handle, or dispose of. An analysis typically takes five minutes to complete. To determine mercury content, a 100 mg sample was weighed and placed into a metal or quartz boat, which was then transferred from the analytical balance to the DMA-80. The sample boats were subsequently loaded onto the instrument's autosampler. Samples were thermally decomposed in an oxygen-rich furnace after being initially dried. The sample releases mercury and other combustion products, which were then transported to the catalyst area of the furnace where halogens, nitrogen, and sulfur oxides, as well as other interfering chemicals removed. Through gold amalgamation, mercury was selectively trapped in a separate furnace. By-products of combustion washed away. Then the amalgamation furnace was heated, and mercury was quickly emitted. Emitted mercury transported by a carrier gas into a special block with a dual- or tri-cell configuration that was placed along the spectrophotometer's optical path, where it was quantitatively quantified by atomic absorption at 253,65 nm (Milestone, 2013).

3.7.2 Determination of lead

Lead concentration in samples was measured by atomic absorption spectrophotometric. Its principle is based on digestion of organic matter and there released Pb coprecipitates with $SrSO_4$. The soluble sulfate salts were removed by pouring off, and the remaining precipitate was converted to a carbonate salt. The salt was then dissolved in acid and measured using atomic absorption spectrophotometry at either 217.0 or 283.3 nm. A 10

g dried sample and 3 g of Pb were weighed and placed in a 500 mL boiling or Kjeldahl flask. To this, 1 mL of a 2% Sr solution and several glass beads were added. A reagent blank was also prepared and treated in the same way as the sample. For every gram of dry sample, 15 mL of ternary acid mixture was added, and the mixture was allowed to stand for 2 hours. The flask was then heated either under a hood or in a H_2O vacuum manifold system until only H_2SO_4 and inorganic salts remained. Then it was kept for some minute to become cool and washed while still hot into 40-50 ml tapered-bottom centrifuge tube and swirled. After allowing it to cool, the mixture was centrifuged for 10 minutes, and the liquid was poured into a waste beaker. The precipitate was then removed by stirring it vigorously using an eccentric-coupled stirring motor. To ensure complete transfer, 20 ml of water and 1 ml of 0.5 M H_2SO_4 were added to the original flask and heated. To dislodge precipitate continue stirring vigorously with added 25 ml saturated $(NH_4)_2CO_3$ solution and stir until all precipitate is dispersed. It was kept on stand for 1h, then centrifuged and liquid decanted into waste beaker. $(NH_4)_2CO_3$ treatment repeated. After decanting, centrifuge tube inverted on paper towel and drained all liquid. 5 ml 1M HNO_3 added, stirred vigorously to expel CO_2 for 2-3 minute, stood for 30 minutes. Instrument set up under previously stated ideal circumstances, employing a 217 or 283.3 nm resonant wave length and an air C_2H_2 oxidizing flame. Standards within the ideal working range (10-80% T) were determined for test and blank solutions, and before-and-after sample readings were taken. Pb determined from standard curve of A against $\propto$ g Pb/ml (Horwitz, 1980).

3.8. Statistical analysis

The statistical software SPSS (Version 26) was utilized for conducting all data analysis. One-way ANOVA tests were performed to determine the mean and standard deviation of the data, followed by a LSD post hoc test for comparing the values. A significance level of $p<0.05$ was used for all statistical comparisons.

Chapter 4

Result

The results & findings of the studies on commercial canned fish are reported in this part, including information on the proximate composition, mineral content, physical test and heavy metal analysis of canned tuna, sardines and comparison done with its reheated sample.

4.1 Proximate composition of canned tuna and sardine before and after reheating

The proximate composition for four different brands of canned fish T1, T2, S1, S2(twos are tuna & other twos are sardine fish) & it's eight reheated sample T3, T4, T5, T6, S3, S4, S5, S6 (fours were fried in 120°C & other fours were fried in 150°C) are presented in table 4.1, 4.1.1. Reheating of same brand's fish was done at 120 °C for 3 minute & 150 °C for 5 minutes. They are denoted as sauteed & panfryed. T3 (sauteed) and T5 (panfryed) are reheated samples of the T1 brand, while T4 and T6 are for the T2 brand. S3 and S5 are for the S1 brand, and S4 and S6 are for the S2 brand.

The results from the Table 4.1, 4,1.1 showed that between sauteing & panfrying, panfryed canned fish had the lowest moisture content (56.34%) compared to sauteed canned fish (64.35%). The ash content was higher in panfryed canned fish (2.63%) than in sauteed canned fish (1.74%). The fat content was found to be highest in panfryed canned fish (20.23%) as compared to sauteed canned fish (8.92%). The protein content remained close in both panfryed and sauteed canned fish (22.58% and 23.87% respectively). The results of the data analysis revealed that, there was no significant difference found between canned and sauteed fish. However, a significant difference ($p<0.05$) was observed between canned and panfryed fish, except for protein and ash content.

Table 4.1: Proximate composition of canned tuna and sardine before and after reheating.

Sample	Moisture (%)	Ash (%)	Crude fat (%)	Crude protein (%)
Canned sardine fish (S1, S2)	59.37 ± 0.90 [a]	2.48 ± 0.22 [a]	16.11 ± 2.08 [a]	23.19 ± 3.71[a]
Canned tuna fish (T1, T2)	72.37 ± 0.42 [b]	1.16 ± 0.37 [b]	6.66 ± 5.39 [b]	20.52 ± 0.55 [a]
Sauteed sardine fish (S3, S4)	61.52 ± 1.61 [a]	2.49 ± 0.13 [a]	12.48 ± 0.68[a]	24.98 ± 0.43 [a]
Sauteed tuna fish (T3, T4)	67.19 ± 0.27 [c]	0.99 ± 0.06 [c]	5.36 ± 2.44 [c]	22.76 ± 3.22 [a]
Panfryed sardine fish (S5, S6)	58.03 ± 2.02 [a]	3.59 ± 0.22 [d]	16.15 ± 0.47[a]	22.70 ± 0.42 [a]
Panfryed tuna fish (T5, T6)	54.66 ± 2.61 [a]	1.68 ± 0.29 [e]	24.32 ± 3.16 [d]	22.46 ± 1.40 [a]

The results are reported as means ± standard deviations. Values in the same column having the same superscript letters are not significantly different ($p>0.05$). Values in the same column having different superscript letters differ significantly. ($p<0.05$).

Table 4.1.1: Comparison among different reheating method about proximate composition of canned tuna, sardine.

Sample	Moisture (%)	Ash (%)	Crude fat (%)	Crude protein (%)
Canned fish (S1, S2, T1, T2)	65.87 ± 7.95 [a]	1.82 ± 0 .80 [a]	11.39 ± 6.40 [a]	21.85 ± 2.66 [a]
Sauteed canned fish (S3, S4, T3, T4)	64.35 ± 3.41 [a]	1.74 ± 0.87 [a]	8.92 ± 4.37 [a]	23.87 ± 2.27 [a]
Panfryed canned fish (S5, S6, T5, T6)	56.34 ± 2.72 [b]	2.63 ± 1.12 [a]	20.23 ± 5.07 [b]	22.58 ± 0.86 [a]

The results are reported as means ± standard deviations. Values in the same column having the same superscript letters are not significantly different ($p>0.05$). Values in the same column having different superscript letters differ significantly. ($p<0.05$)**.**

4.2 Physical test of canned tuna and sardine before and after reheating

In addition to proximate analysis, several physical tests were performed to evaluate the effects of panfrying and sauteing on the canned fish and the result has been presented in Table 4.2, 4.2.1. It was observed that the pH of sauteed canned fish (6.67) was higher than that of panfryed canned fish (pH 6.27). The water holding capacity of sauteed canned fish was found to be higher compared to that of panfryed canned fish, their values were 3.94 cm^2, 3.51 cm^2. In comparison to sauteed canned fish, it was shown that panfryed canned fish had a larger cook loss which was 11.98% compared to 5.49%. The data analysis revealed that the p-value for pH, cook loss was found to be less than 0.05, for the comparison between canned fishes that were panfryed and sauteed. This indicated that they were significantly different from each other based on their pH, cooking loss. However, no significant difference was observed for other parameter that were analyzed.

Table 4.2: Physical test of canned tuna and sardine before and after reheating.

Sample	PH	W.H.C (cm^2)	Cook loss (%)
Canned sardine fish (S1, S2)	6.38 ± 0.035^{a}	4.18 ± 1.02^{a}	N.A.
Canned tuna fish (T1, T2)	6.25 ± 0.07^{b}	2.71 ± 0.62^{c}	N.A.
Sauteed sardine fish (S3, S4)	6.63 ± 0.18^{a}	4.64 ± 0.38^{a}	4.34 ± 0.63^{a}
Sauteed tuna fish (T3, T4)	6.71 ± 0.07^{c}	2.96 ± 1.12^{d}	6.64 ± 5.08^{c}
Panfryed sardine fish (S5, S6)	6.40 ± 0.35^{a}	4.91 ± 0.55^{b}	5.76 ± 1.33^{b}
Panfryed tuna fish (T5, T6)	6.14 ± 0.03^{b}	2.74 ± 1.03^{c}	18.20 ± 0.13^{d}

The results are reported as means ± standard deviations. Values in the same column having the same superscript letters are not significantly different ($p>0.05$). Values in the same column having different superscript letters differ significantly. ($p<0.05$)

Table 4.2.1 Comparison among different reheating method about physical test of canned tuna, sardine.

Sample	pH	W.H.C (cm^2)	Cook loss (%)
Canned fish (S1, S2, T1, T2)	6.31 ± 0.09^{a}	3.60 ± 1.08^{a}	N.A.
Sauteed canned fish (S3, S4, T3, T4)	6.67 ± 0.12^{b}	3.94 ± 1.46^{a}	5.49 ± 3.24^{a}
Panfryed canned fish (S5, S6, T5, T6)	6.27 ± 0.25^{a}	3.51 ± 1.18^{a}	11.98 ± 7.22^{b}

The results are reported as means ± standard deviations. Values in the same column having the same superscript letters are not significantly different ($p>0.05$). Values in the same column having different superscript letters differ significantly. ($p<0.05$)**.**

4.3 Changes of mineral content in canned tuna and sardine before and after reheating

Mineral content analysis shows that the panfryed sample had lower levels of calcium (0.65%) and phosphorus (0.28%) than the non-reheated canned sample (0.73% for calcium and 0.29% for phosphorus). The results of the data analysis indicated that the p-values for the reheated (panfryed and sauteed) and canned fish samples were not less than 0.05 ($p<0.05$). This suggested that they were not significantly different to each other.

Table 4.3: Changes of mineral content in canned tuna and sardine before and after reheating.

Sample	Calcium (%)	Phosphorus (%)
Canned sardine fish (S1, S2)	1.15 ± 0.07^{a}	0.44 ± 0.014^{a}
Canned tuna fish (T1, T2)	0.30 ± 0.01^{b}	0.14 ± 0.014^{b}
Sauteed sardine fish (S3, S4)	1.07 ± 0.16^{a}	0.44 ± 0.01^{a}
Sauteed tuna fish (T3, T4)	0.28 ± 0.04^{b}	0.15 ± 0.01^{b}
Panfryed sardine fish (S5, S6)	1.05 ± 0.21^{a}	0.43 ± 0.04^{a}
Panfryed tuna fish (T5, T6)	0.25 ± 0.07^{b}	0.14 ± 0.01^{b}

The results are reported as means ± standard deviations. Values in the same column having the same superscript letters are not significantly different (p>0.05). Values in the same column having different superscript letters differ significantly. (p<0.05)**.**

Table 4.3.1: Comparison among different reheating method about mineral content of canned tuna, sardine.

Sample	Calcium (%)	Phosphorus (%)
Canned fish (S1, S2, T1, T2)	0.73 ± 0.49 [a]	0.14 ± 0.01 [a]
Sauteed canned Fish (S3, S4, T3, T4)	0.67 ± 0.47 [a]	0.29 ± 0.16 [a]
Panfryed canned fish (S5, S6, T5, T6)	0.65 ± 0.48 [a]	0.28 ± 0.20 [a]

The results are reported as means ± standard deviations. Values in the same column having the same superscript letters are not significantly different (p>0.05). Values in the same column having different superscript letters differ significantly. (p<0.05)**.**

4.4 Heavy metal presence in canned tuna and sardine

In addition to the previous tests, the canned tuna and sardine fish samples were also analyzed for the presence of lead and mercury, which are heavy metals. The findings of this analysis can be found in Table 4.4. Samples of canned tuna and sardine fish were both confirmed to be free of mercury and lead contamination. The levels of these heavy metals were below the limit of detection.

Table 4.4: Heavy metal presence in canned tuna and sardine

Sample	Mercury	Lead
Canned sardine (S1, S2)	Not detected	Not detected
Canned tuna (T1, T2)	Not detected	Not detected

Chapter 5

Discussion

The current research assessed the effects of cooking or reheating on the physicochemical properties of canned fish. This study results suggest that the stability and physicochemical properties of canned fish may be impacted by cooking and reheating methods, but not to a significant extent. The differences observed in the moisture content, fat content, pH, water holding capacity, cook loss, mineral content between the canned, sauteed, and panfryed samples indicate that the choice of cooking method can affect the nutritional quality of the fish (Garcıa-Arias et al., 2003)**.**

The panfryed sample's increased fat content and decreased moisture content may have resulted from the high temperature utilized in this cooking technique, which can accelerate lipid oxidation and cause water to evaporate. Lipid oxidation, a common occurrence in cooking processes, can result in the breakdown of fats and the formation of undesirable compounds, such as free radicals and peroxides. This oxidation process can contribute to the increased fat content observed in the panfryed sample. Moreover, the evaporation of water during panfrying can lead to a concentration of other components in the fish, including fats. This finding is consistent & supported by previous research on the effect of cooking on fish which has shown that frying can significantly reduce the moisture content and increase the fat content of fish (Gall et al., 1983; Ohta et al., 1988). On the other hand, the sauteed samples showed a lower fat content and a higher protein content compared to the canned samples. This could be due to the use of lower temperatures and shorter cooking times in sauteing, which can preserve the protein content of the fish. Proteins are sensitive to heat and can undergo denaturation, which involves changes in their structure and functionality. The lower cooking temperatures used in sauteing may have minimized the extent of protein denaturation, thus preserving a higher protein content in the cooked samples. (Chalamaiah et al., 2012).

One possible explanation for the differences observed in the physicochemical properties of the canned fish samples after cooking or reheating could be due to the thermal degradation of various components. For instance, the reduction in water

holding capacity and increase in cook loss in the panfryed samples may be attributed to the denaturation of proteins, resulting in a loss of their ability to retain water. During the cooking process, the application of high temperatures can cause the denaturation of proteins present in the fish. Denaturation involves the disruption of the protein's native structure, leading to changes in its physical and functional properties. In the case of panfrying, the high heat can induce protein denaturation, resulting in a loss of their ability to retain water (Botta, 1994)**.** The increase in fat content in the panfryed samples may be due to the release of fat from the fish during the frying process. When fish is subjected to high temperatures during frying, the heat causes the fish's fat to melt and separate from the fish tissue. As a result, the fat is released and absorbed by the fish, leading to an increase in fat content. Similarly, the increase in ash content may be due to the concentration of minerals as water is lost during frying. As the moisture evaporates, the concentration of minerals presents in the fish, such as calcium and phosphorus, becomes more concentrated. This concentration effect leads to an increase in ash content (Talab et al., 2014)**.**

Furthermore, the physical tests showed that sauteed canned fish had a higher pH and water holding capacity compared to the canned and panfryed samples. This may be due to the different cooking methods and the resulting changes in the protein structure. Sauteing involves cooking the fish in a small amount of oil or fat at moderate temperatures for a shorter duration. The addition of oil or other components that can have an alkalizing effect on the fish may be to blame for this. In addition, the sauteed sample’s higher pH may be responsible for their greater water holding capacity and lower cook loss when compared to the panfryed samples (Zapata et al., 1982)**.**

The changes in water holding capacity and cook loss between the canned, sauteed, and panfryed samples may also be related to the cooking technique. The filter press method used in this investigation allowed for the measurement of water holding capacity, providing insights into the moisture retention capabilities of the cooked fish. The filter press method revealed that the water holding capacity was highest in the sauteed samples and lowest in the panfryed samples, which is consistent with the larger cook loss found in the panfryed samples. The gentle cooking process of sauteing allows the fish to retain more of its natural moisture, resulting in a higher water holding capacity. Panfrying involves cooking the fish in a larger amount of oil or fat at higher temperatures and for a longer duration. The higher temperatures and prolonged

exposure to heat during panfrying can cause the proteins in the fish to denature and lose their ability to retain moisture effectively. The variations in each cooking method's use of oil, cooking temperature, and cooking time may be to blame for the variations in water holding capacity and cook loss (Aryee et al., 2018).

The mineral content analysis showed a slight decrease in calcium and phosphorus content in the panfryed samples compared to the canned samples, but the difference was not significant. This shows that the amount of minerals in canned fish may not be significantly affected by the cooking and reheating processes. This may be due to the fact that the thermal degradation of minerals is less significant compared to other components such as proteins and fats. The stability of minerals during cooking can be attributed to their relatively low susceptibility to thermal degradation compared to other components such as proteins and fats. Calcium and phosphorus, being essential minerals present in fish tissue, are less prone to significant changes under cooking conditions (Tadesse et al., 2020). To confirm this discovery and assess the impact of various cooking techniques on other minerals, additional study is required.

The heavy metal presence test revealed that there were no detectable amounts of lead or mercury in the canned samples. The absence of lead and mercury in the canned samples is particularly significant as these heavy metals can pose serious health risks when consumed in excessive amounts. Lead is known to cause neurological and developmental disorders, while mercury is associated with neurological and reproductive impairments. The fact that these harmful substances were undetectable in the canned fish samples reinforces the notion that canned fish can be considered a secure food option. This supports earlier research that found that canned fish often contains minimal amounts of heavy metals. Research suggests that fish in cans is a secure and healthy food option (Lourenço et al., 2014).

Chapter 6

Conclusion

From this research, it can be concluded that cooking and reheating methods have a minimal impact on the stability and physicochemical properties of canned fish. According to this research, sauteing is better than panfrying for preserving canned fish quality. Sauteing and panfrying caused changes in moisture, ash, fat, and protein contents of the fish, but the changes in protein and ash contents were not significant. The fat content was highest in panfryed samples, while the protein content remained relatively stable across all cooking methods. This study found variations in pH, water holding capacity, and cook loss across different types of canned fish, but changes in water holding capacity were not significant. Sauteed samples had the highest pH and water holding capacity, while panfryed samples had the highest cook loss, indicating greater moisture loss during cooking. The study showed a slight decrease in calcium and phosphorus in panfryed canned fish, but the difference was not significant. Heavy metal presence examination revealed that canned tuna and sardine fish products are safe for consumption as they did not contain detectable levels of mercury or lead. In conclusion, this study highlights the importance of considering the cooking or reheating methods for canned fish, which may impact its nutritional value and physicochemical characteristics. These findings can help consumers choose the best cooking methods to preserve nutritional quality.

Chapter 7

Recommendations and Future Aspect

Based on the findings of this study, some recommendations and suggestions can be made for future research :

1. More research is required to determine how different cooking techniques affect the levels of other nutrients, such as vitamins and minerals, in canned fish.
2. In order to learn more about the general quality and acceptance of these items, it would be worthwhile to look into the sensory qualities of canned fish after cooking and reheating. In this, sensory evaluation methods like descriptive analysis or hedonic scaling may be used to assess the fish's scent, flavor, and texture.
3. Further investigation can be done on the possibility of adding stabilizers or other additives to canned fish to improve its stability while reheating without sacrificing the fish's flavor or nutritional content.
4. To investigate the effects of various storage conditions on the stability of canned fish, more research might be done. For instance, the ideal storage conditions for maintaining the quality and safety of canned fish might be determined by looking into the effects of temperature, light exposure, and oxygen levels.

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Brief Biography

Akash Dey achieved a grade point average (GPA) of 5.00 on the Secondary School Certificate (SSC) Exams in 2013 and a GPA of 4.67 on the Higher Secondary Certificate (HSC) Examinations in 2015. He graduated from Chittagong Veterinary and Animal Sciences University (CVASU), Bangladesh, with a B.Sc. (Hons) in Food Science and Technology in 2019 (held in 2020). He is currently a candidate for the MS in Food Chemistry and Quality Assurance in the Department of Applied Chemistry and Chemical Technology within the Faculty of Food Science and Technology at CVASU. He finds his interest & career objective is to work in research and development. He has a strong desire to work in a demanding setting where his capacity for creative problem-solving may be put to good use.