# The Study of Physicochemical Properties of Edible Oil and Heated Oil and Evaluation of The Changes of Oil Characteristics After Reheating


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Roll No.: 0121/18

Registration No.: 1022

Session: January-June, 2021


**A thesis submitted in the partial fulfillment of the requirements for the degree of Masters in Public Health (MPH)**


**One Health Institute**

**Chattogram Veterinary and Animal Sciences University**

**Chattogram-4225, Bangladesh**


**February, 2023**

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**Sushmita Roy Chowdhury**

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# PLAGIARISM VERIFICATION

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**Dr. Md Abdul Alim**

Professor

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**The Author**

**February, 2023**

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# LIST OF ABBREVIATIONS

| Abbreviations | Elaboration |
|---|---|
| % | Percentage |
| $^{0}C$ | Degree Celsius |
| AOAC | Association of Official Analytical Chemists |
| CHD | Coronary Heart Disease |
| CVD | Cardio Vascular Disease |
| MUFA | Mono Unsaturated Fatty Acid |
| PUFA | Poly Unsaturated Fatty Acid |
| ppm | parts per million |
| SFA | Saturated Fatty Acid |
| SFO | Sunflower Oil |

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# Abstract

The physicochemical properties of edible oils are crucial elements of the human diet and play a vital role in protecting health and preventing disease. The study focused on the effects of heating and reheating on oil quality, which is of great interest to both the consumers and food industry.

In this investigation, we considered analyzing samples of sunflower oil, soybean oil, mustard oil, olive oil and palm oil which are commonly used in food preparation. The samples were heated and reheated at specific temperatures and times, and their physical and chemical properties such as specific gravity, moisture content, saponification value, peroxide value, acid value and iodine value were measured using various analytical techniques.

This thesis provides valuable insights into the physicochemical properties of edible oils and changes that occur when oil is reheated. Changes in the physicochemical properties of edible oils heated and reheated condition can lead to a loss of nutritional value and safety concerns, such as increased oxidative stress and inflammation, which have been linked to life threatening diseases like cancer and cardiovascular disease.

Our findings indicated the importance of using fresh oil whenever possible and avoiding reheating whenever feasible, as this can help maintain the quality and safety of the food. Moreover, the development of new, more stable varieties of edible oil may be necessary to reduce the risks associated with oil degradation and rancidity.

We also recommended further extensive research to explore the effects of different types of oils and cooking methods on oil quality, as well as the long-term health effects of consuming degraded or rancid oils.

Keywords: Edible oils, fresh oils, heated oils, reheated oils, physicochemical properties.

# Chapter 01

# Introduction

Oil is generally a triglyceride that remains in liquid form in room temperature. Oil is called triglycerides as they are esters composed of fatty acid and glycerol (a trihydroxy alcohol). A typical mixed triglyceride is one made from naturally occurring fats and oils that has two or three separate fatty acid components. A triglyceride is referred to as an oil if it is a liquid at 25 °C and a fat if it is a solid at that temperature. These variations in melting points are a result of variations in the amount of unsaturation and carbon atoms present in the constituent fatty acids. Triglycerides derived from animals are often solids, but those derived from plants are typically oils. As a result, we frequently refer to vegetable oils and animal fats. Edible vegetable oils are foodstuffs which are composed primarily of glycerides of fatty acids being obtained only from vegetable sources. They may contain small amounts of other lipids such as phosphatides, of unsaponifiable constituents and of free fatty acids naturally present in the fat or oil (Codex Alimentarius, 1999).

Bangladesh, a rapid developing country of south Asia, is one of the most densely populated country in the world. At present Bangladesh has about 165 million people with a population density of 1,156.84 people per square kilometer (Population and Housing Census,2022). Due to rapid industrialization in this country people are moving towards more hectic lifestyle day by day. For this very reason fast foods and fried foods are becoming more and more popular in this country. Many small stalls are situated almost in every corner of Bangladesh which serves fried foods such as Singara, Samosa, Pakora etc. These snack items are very popular among the peoples of Bangladesh specially among the students and office people. But the stall owners and workers have little knowledge about the effect of oil on human health. Moreover, to save money and time they use the same oil to fry food again and again. Recent Russia-Ukraine war and Covid 19 pandemic situation has increased the price of edible oil rapidly and it is increasing more day by day (Ben Hassen et al.,2022; Jagtap et al.,2022; Allam et al.,2022). Hence, in order to save money, the usage of warmed oil for frying food has expanded more than ever in recent years. Again, they keep this oil in an open place after use. The immediate reaction of atmospheric oxygen with the lipids and other organic molecules in these oils results in structural breakdown in the oil, which can lower food quality and be very dangerous for human health (Bhattacharya et al., 2008).

Nonetheless, vegetable oils are crucial for world nutrition, and different types of edible oils are produced in various grades in response to regional need (Tesfaye & Abebaw, 2016). A study conducted by Jambunathan et al. in 2008 shows that, the value of the oil and its ability to maintain its consistency are extremely important for the consumer's preferred use, which is typically as an ingredient in culinary preparation (Bou et al., 2012). All types of oil have some physicochemical characteristics which can be used to determine both its nutritional and physical qualities. These characteristics include iodine value, peroxide value, saponification value, free fatty acid, color appearance, etc.

When vegetable oils are used for an extended period of time, these oils should be handled carefully as they are susceptible to oxidative deterioration, which can reduce their shelf life. Oxygen reacts with unsaturated fatty acids and creates hydro per-oxides which are considered the main product of oxidation reaction. This produced hydro per-oxides amount is used to determine peroxide value of oil (Ahmad Tarmizi & Ismail, 2008). The amount of oil damage is assessed using the peroxide value. Oils are regarded as being in a low oxidation condition if their peroxide levels are between 1 and 5 mEq/Kg. Whereas oils with per-oxide values between 5 and 10 mEq/Kg are regarded as being in an average oxidation condition (O'Brien, 2008). The acid value is often used as an indicator of the general condition and helps to evaluate whether it is safe to consume or not (Pardeshi, 2020). Iodine value is used to determine the degree of unsaturation of vegetable oils (Kyriakidis & Katsiloulis, 2000). The higher degree of unsaturation of oil means it will be easily absorbed into our body. Saponification value is very important for estimation of average molecular weight of edible oil sample (Luzzi & Gerraughty, 1964). Moisture content and Specific gravity helps us to determine the rate of oil decomposition and degree of unsaturation of oil, respectively (Mengistie et al., 2018a).

Frying of oil can create new compounds and these compounds can change the physical and chemical parameters of frying oil (Choe & Min, 2007; Dobarganes et al., 2000; Gertz & Matthäus, 2008). Because most of the fried foods' lipid content is derived from the frying oil, which is absorbed during the process. The main effect is a change in the frying oil's quality, which has an impact on the quality of the fried food. So, the overall nutritional characteristics of the fried products can be modified according to the type of frying oil selected (Gertz & Matthäus, 2008). Therefore, considering above findings, the current investigation has been undertaken to determine the physical and chemical properties of five edible oils (sunflower oil, soyabean oil, mustard oil, olive oil and palm oil), heated oils and reheated oils of the same and effect of heating upon these oil samples. The specific objectives of this investigation included as follows:

1. To determine the changes of physicochemical characteristics (e.g. specific gravity, moisture content, saponification value, peroxide value, acid value and iodine value)

2. To make a comparison of different types of oil after heating and reheating.

# Chapter 02

# Review of Literature

## 2.1 Oils and Fats

Oils and fats are basically subsection of lipids which differs from each other from being liquid or solid at ambient room temperature. The physical properties of oil and fat mainly depends upon the number of fatty acids that they contain. Fatty acids, triacylglycerols and lipid soluble compounds are the component of natural oils and fats (Gunstone, 2009). Highest quality oils and fats are generally tasteless, odorless, pure, and resistant to oxidation. However, during preparation, storage, and usage oxidation can happen to oils and fats. Despite the fact that oil processing is partially meant to eliminate or destroy oxidized products or elements that may trigger or enhance oxidative processes, some processing procedures, such as caustic refining and bleaching, may encourage oxidation. Oxidative reactions can decompose and form compounds during storage which are responsible for a rancid odor and taste (Chow & Gupta, 1994). Fatty acids, which are found in oils and fats, are vulnerable to attack from a variety of sources, including light and oxygen (Choe & Min, 2007). Specifically, the binding of fat with proteins and carbohydrates can affect the overall textural and other aspects of food quality. For food formulations, proteins' capacity to absorb and retain fat as well as interact with lipids in emulsions and other food systems is crucial (Zayas & Zayas, 1997). At the time the oil is being fried, the presence of oxygen, moisture, trace elements, and free radicals can cause a variety of chemical reactions, including thermoxidation, hydrolysis, polymerization, isomerization, and cyclization. These reactions ultimately cause the frying oil to break down and produce monomeric, polymeric, primary, and secondary oxidative products. These products affect the quality of oil and fried products (Andrikopoulos et al., 2002). The oxidized product of fatty acids brings off-flavors and odors (hydrolytic rancidity) to the fried food items (Lin et al., 2001).

## 2.2 Edible Oils and its health impact

Edible oils which are basically vegetable oils are used in cooking as well as in conventional medicine to treat different disease conditions such as colds, coughs, bronchitis, edema, and burns. Additionally, these oils are necessary for the production of prostaglandins, which serve a variety of important roles in the body (Ilyas, 2016). Vegetable oils are crucial to human nutrition because they supply necessary fatty acids, energy, and help the body absorb fat-soluble vitamins (Grace et al., 2008). Although it's a common misconception that fat should be avoided, certain edible oils have a significant positive impact on cardiovascular health when consumed as a dietary supplement (Bester et al., 2010a).

According to some experts, a person's health largely depends on the type of lipids they consume because larger intakes of trans and saturated fats have been linked to negative health outcomes like inflammation and insulin resistance and increase in LDL (low-density lipoprotein) cholesterol, develop risk of diabetes and cardiac death (Bendsen et al., 2011; Mozaffarian et al., 2006). Edible oils are important source of natural antioxidant which reduce the oxidative stress in our cells. Some major antioxidant found in edible oils are tocopherols, tocotrienols and carotenoids (Chow, 2007). By regulating the generation of eicosanoid, dietary lipids also play a significant part in maintaining the body's essential processes (Lands, 2019; Lees, 2020). Oils have diverse biological effects in various circumstances, particularly in people with dyslipidemia and type 2 diabetes (Dineshkumar et al., 2009). Vegetable oil contains omega-6 and omega-3 polyunsaturated fatty acids (PUFAs), both of which are crucial for maintaining good health since they serve as building blocks for various molecules like prostaglandins, leukotrienes, thromboxanes, and lipoxins (Das, 2006). Despite the fact that essential fatty acid deficiency has rarely been observed in people, it is believed to be a factor in dermatitis, renal hypertension, issues with mitochondrial activity, CVDs, type 2 diabetes, delayed brain development, arthritis, melancholy, and a weakened immune system (Simopoulos, 1999).

### 2.3 Sunflower seed oil

This refinery method of sunflower seed oil produces yellow oil that is high in PUFA, the most abundant of which is linoleic acid (60-70%). The primary MUFA and SFA are oleic acid and stearic acid, respectively. Depending on the desired product, SFO can have different amounts of MUFA and SFA (AbuGhazaleh et al., 2007; Aguilera et al., 2004; Eder et al., 2006a; Larsen et al., 1999; Navarro et al., 1992). Commercially there are three main forms of SFO available. The first is a high-PUFA SFO which consists of up to 75 % PUFA. The second type is a high-MUFA SFO that contains up to 45% MUFA, and the third type is a high-stearic acid SFO that may contain up to 14% stearic acid. The most prevalent type of SFO is high-PUFA SFO. While high-stearic acid SFO is mostly employed in industrial processes, high-MUFA SFO is commonly used in cooking (Bester et al., 2010b). SFO contains naturally occurring vitamin E in the form of tocopherol (AbuGhazaleh et al., 2007; Aguilera et al., 2004; Eder et al., 2006a). SFO-provided omega-6 PUFA may have an important function in inflammation management since it is used to build pro-inflammatory prostaglandins (Das et al., 2008; Navarro et al., 1992; Simopoulos, 1991). Some research shows that SFO has been demonstrated to modify the serum lipid profile, with the main finding being a reduction in total cholesterol and LDL-cholesterol (Girardet et al., 1977; Lambert et al., 2007; Nydahl et al., 1994). Since SFO is deficient in micronutrients, hydroxytyrosol is sometimes added to commercial mixes to boost antioxidant content. Hydroxytyrosol is a polyphenol that has been found in this study to boost immunity by enhancing leucocyte glutathione peroxidase activity (Baeza et al., 2008; Díaz et al., 2008). Linoleic acid is required for optimal immunological response, and a lack of EFA inhibits B and T cell-mediated responses. SFO can supply enough linoleic acid for immune response maintenance (Meydani et al., 1991).

## 2.4 Olive oil

Olive oil is a yellowish oil that contains a lot of monounsaturated fatty acids (MUFAs), with oleic acid making up 72–79% of the total. ((Newmark, 1997; Owen et al., 2000; Quiles et al., 2004). As compared to PUFA, MUFA are less prone to oxidation. This, in turn, increases the availability of antioxidants in their active form and improves the stability of olive oil (Diniz et al., 2004; Eder et al., 2006b; Newaz et al., 2003; O'Farrell & Jackson, 1997; Owen et al., 2000; Turpeinen et al., 1998; Wahle et al., 2004). Olive oil contains two antioxidant micronutrients called polyphenols and squalene (Gómez Caravaca et al., 2005; Lercker & Rodriguez-Estrada, 2000; Newmark, 1997; Owen et al., 2000; Quiles et al., 2004). The phenolic component concentration of virgin olive oil is greater than that of ordinary olive oil (approximately 230 mg/kg, usual range 130-350 mg/kg) (Owen et al., 2000; Tuck & Hayball, 2002). Tyrosol, hydroxytyrosol, oleuropein, and ligstroside are the most important phenols present in olive oil (Gómez Caravaca et al., 2005; Owen et al., 2000; Perona et al., 2006). Tyrosol and hydroxytyrosol, which make up 30% of the total amount of polyphenols in olive oil, are the most common polyphenols (Owen et al., 2000). Oleuropein and ligstroside are hydrolyzed during the preservation of the olive oil to produce tyrosol and hydroxytyrosol (Romero et al., 2007). The catechol group found in tyrosol, hydroxytyrosol, and oleuropein has been demonstrated to have antioxidant action (Gómez Caravaca et al., 2005; Montedoro et al., 1993; Owen et al., 2000; Visioli et al., 2002). According to studies, the most powerful antioxidants in olive oil are hydroxytyrosol and oleuropein, which may have greater antioxidant effects than vitamin E (Owen et al., 2000; Perona et al., 2006). Squalene is a hydrocarbon that is thought to be particularly abundant in olive oil; it makes up around 0.7 percent of its composition (Newmark, 1997; Owen et al., 2000). It may be present in other foods and oils in amounts ranging from 0.002 to 0.03%. Squalene reduces cholesterol formation by inhibiting 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase and acts as an antioxidant by quenching singlet oxygen radicals (Newmark, 1997; Relas et al., 2000). Secoiridoids, phenyl-ethyl alcohols, and lignans are some of the other micronutrients found in olive oil. Several micronutrients have been linked to antioxidant activity as well, though not to the same extent as oleuropein and hydroxytyrosol (Carrasco-Pancorbo et al., 2005). Consuming olive oil may lower your risk of developing degenerative illnesses including cancer and coronary heart disease, according to research (Keys et al., 1986). The prevailing consensus is that olive oil reduces oxidative stress (Diniz et al., 2004; Kritchevsky, 2000; Quiles et al., 2004; Theriault et al., 1999). Studies have showed that using more olive oil lowers blood pressure in people (Ferrara et al., 2000; Hassan Gilani et al., 2005; Keys et al., 1986; Manna et al., 2004).

### 2.5 Soyabean Oil

Only palm oil is produced in greater quantities and is sold more often than soybean oil. The commerce in palm fruits, which are harvested as soon as possible at the place of collection, is not analogous to the trade in soybeans, which are also widely traded. According to Cartter and Hopper's (1942) research, the soybean seed's oil content is primarily a varietal trait, and environment and variety have a relatively similar impact on the oil's iodine level. The amount of calcium present in the seed produced by a particular variety was considerably impacted by temperature levels. When soybeans were cultivated at high temperatures, there was almost always a high calcium content. The seed's total ash, phosphorus, and potassium contents appeared to be affected by soil fertility and type more so than seed variety or climatic variations (Cartter & Hopper, 1942). Small, stable oil bodies compose from 18% to 22% of the total mass of soybeans. These structured triacylglycerol droplets, which are rich in small bioactive components, are what these oil bodies are. These are encircled by a phospholipid monolayer composed of seven oleosins, two caleosins, and one steroleosin. (Zhao et al., 2022). A study by shows that reduced risk of coronary heart disease and improved general health are two major benefits of soybean oil (Messina et al., 2021).

### 2.6 Mustard Oil

One of the most significant vegetable oils used for deep-frying is mustard oil. Black mustard (Brassica nigra), brown Indian mustard (Brassica juncea), and white mustard can all be used to make mustard oil (Brassica hirta). Since ally isothiocyanate is present, it has a strong smell (Nayak et al., 2016) . The Brassica nigra plant, which is used to make mustard oil, has a high amount of protein (30%), calcium (5%), phytins (5%), phenolics (5%), and natural antioxidants (5%). Mustard oil is good for the heart because of its high monounsaturated fatty acid content and balanced polyunsaturated fatty acid ratio. Since mustard oil has the fewest saturated fatty acids, it is safe for people with heart issues. Furthermore, glucosinolate, which possesses antibacterial, antifungal, and anticarcinogenic properties, is a component of mustard oil. These characteristics are the cause of many of the mustard oil's therapeutic advantages (Yadav & Kumari, 2013). Its medical benefits include preventing the development of cancer in the body, lowering body temperature, treating fungal and bacterial skin infections, making a tasty appetizer, and acting as a body toner. Also, reducing the adhesive impulses in blood platelets lowers the risk of heart failure, protects children from allergies, asthma, and eczema, guards against eye and throat irritation, and strengthens our RBC by lowering cholesterol and enhancing the structure of the RBC membrane (Kaur et al., 2019).

### 2.7 Palm oil

In palm oil, both saturated and unsaturated fatty acids are present in equal proportions. These fats are made up of 51% SFA, 39% MUFA, and 10% PUFA (mostly linoleic acid) (Ong & Goh, 2002; Wilson et al., 2005). The main SFA in palm oil is palmitic acid, which accounts for 44% of the total fatty acids. Myristic acid and stearic acid make up the remaining 7% of the SFA. Oleic acid makes up the bulk of the MUFA in palm oil. Palm oil contains about 62 mg/g of vitamin E, of which 16 mg/g is made up of -tocopherol and the remaining 70 mg/g is primarily made up of tocotrienols (Rossi et al., 2001; Sundram et al., 2003; Wilson et al., 2005). According to research, palm oil may possess some anti-arrhythmogenic qualities (Charnock et al., 1991). Palm oil may lessen oxidative stress and have a neutral or beneficial effect on the serum lipid profile as a result of the effects of its fatty acid content and tocotrienols (Hornstra, 1988; Kritchevsky et al., 1999; Ng et al., 1991; Qureshi et al., 1995; Sundram et al., 1997).

## 2.8 Frying oil effect on human health

Foods taste better after being fried. The use of a lot of oil is required while frying food. Increasing oil intake is not seen to be beneficial for people's health. Fried meals are widely regarded as harmless, despite the fact that several potentially hazardous chemicals are created during frying (such as polar compounds or polymers) (Chao et al., 2004; Frankel, 1983; Wai, 2007). Only after frequent usage can frying oil turn harmful for human consumption (Soriguer et al., 2003). Repeated heating of the oil accelerates the oxidative breakdown of lipids, producing harmful reactive oxygen species and decreasing the natural antioxidant content of the cooking oil. Consuming meals cooked with warmed oil over an extended period of time may seriously weaken one's antioxidant defense system, resulting in diseases including hypertension, diabetes, and vascular inflammation (Clemente & Cahoon, 2009; Edem, 2002; Kamisah et al., 2005). Coronary heart disorders are most likely to occur as a result of lipid oxidation. The imbalance between ant oxidative protection mechanisms and the generation of potent oxidizing chemicals, particularly free radicals, subject the human body to considerable oxidative stress on a daily basis. This stress may impair intracellular signal transmission and harm DNA, proteins, lipids, and carbohydrates (Goswami et al., 2015).

# CHAPTER 3

# MATERIALS AND METHODS

This experiment was conducted in Department of Applied Chemistry and Chemical Technology; Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh.

## 3.1 Collection of Oil Samples

Total 5 oil samples of different brand were collected from different shops, local vendor and super market of Chattogram Metropolitan Area, Bangladesh. These oil sample are Sunflower seed oil, Olive oil, Palm oil, Mustard oil and Soyabean oil.

## 3.2 Sample Preparation

Each oil bottle was kept in a dry, cold, and dark location. All the bottles were covered with carbon paper to prevent photo-oxidation. Each and every reagent used was specified and of analytical grade. All the oil samples were prepared for analysis as the method described by Birnin-Yauri and Garba (Birnin-Yauri et al., 2011). Oil Samples were taken directly from the container after inverting it several times.

For the preparation of heated and reheated oil sample, at first potatoes were fried in each type of fresh oil for 5 minutes. Then it was cooled and heated oil sample was separated from fried potatoes. From these heated oil samples necessary amount was separated to check heated oil samples physicochemical properties. Remaining heated oil samples were used again for frying fresh potatoes for 5 minutes. The reheated oil samples were separated and cooled for further testing like heated oil samples.

## 3.3 Determination of Physicochemical Properties of Oils

All the samples were tested according to AOAC official methods of analysis (21st edition) (AOAC,2019).

### 3.3.1 Moisture Content

**Procedure:**

Weighed a previously dried and tared dish about 5-10g of oil or fat which has been thoroughly mixed by stirring. Then, the lid of the dish was loosened and heat was applied, in an oven at 105°C for 1 hour. Removed the dish from the oven and the lid was closed. Cooled it in a desiccator containing phosphorus pentoxide or equivalent desiccant and then weighed. Heated in the oven for a further period of 1 hour, cooled and weighed. We repeated this process until change in weight between two successive observations does not exceed 1 mg. Carried out the determination in duplicate (AOAC,2019).

Moisture and volatile matter = $\frac{W1 \times 100}{W}$

Where,

W1 = Loss in gm of the material on drying

W= Weight in gm of the material taken for test

### 3.3.2 Specific Gravity

**Standardization of Pycnometer:**

A cleaning solution containing chromic acid was added to the pycnometer, and it was then allowed to sit for a period of hours. The pycnometer was put in a water bath that was kept at a constant temperature of 30℃ after being filled with newly boiled water and cooled to around 20℃. The item was removed, cleaned off using a towel, and weighed after 30 minutes of soaking.

**Procedure:**

Before applying the prepared oil sample to the side arm of the pycnometer, the cap was removed. The instrument was then filled without any air bubbles being trapped. The container was sealed, and it was left to sit for 30 minutes in a water bath that had been heated to 30℃. Properly cleaned the capillary hole to get rid of any possible oil leaks. Removed the bottle from the water, gave it a thorough rinse, and dried it entirely. When the temperature falls below 30℃, quickly removed the cap from the side arm and weighed quickly (AOAC, 2019).

**Calculation:**

Specific Gravity, C = $\frac{A-B}{C-B}$

Where,

A = wt. in g of specific gravity bottle filled with oil at 30°C

B = wt. in g of specific gravity bottle at 30°C

C = wt. in g of specific gravity bottle filled with water at 30°C

### 3.3.3 Saponification Value

**Procedure:**

To measure saponification value of oil samples, at first 2-3 ml of oil was taken in a round bottom flask. After that, 25 ml of alcoholic potassium hydro-oxide (KOH) was added in round bottom flask. Then one (1) hour of heating of this mixture was done in reflux condenser at 100℃. After cooling it 0.5 ml phenolphthalein indicator was added with this solution. Then it was titrated with 0.5 N hydrochloric acid (HCl). Blank titration was also done in similar ways but without sample (AOAC,2019).

**Calculation:**

Saponification Value = $\frac{56.1 \times N \times (B-A)}{\text{Weight of sample taken}}$

Here,

B = ml of HCl required by blank

A = ml of HCl required by oil sample

N = Normality of HCl

### 3.3.4 Acid Value

**Procedure:**

At first, 5 ml of oil sample taken in a round bottom flask. 50 ml ethanol was added with it and it was heated for 5 minutes. After cooling this solution 4-5 drops of phenolphthalein indicator were added with it. Then titration was done with 0.1N KOH. Blank titration without sample was also done in the similar way (AOAC,2019).

**Calculation:**

Acid Value = (56.1*(sample-Blank) *Normality)/ (Weight of sample)

### 3.3.5 Per-oxide value (PV)

**Procedure:**

At first, 5 ml of sample was taken in a stoppered conical flask.10 ml chloroform, 15 ml acetic acid and 1 ml of 15% potassium iodide (KI) was added with the sample one by one. Then the stoppered conical flask was placed in a dark place for 5-10 minutes. Then 30 ml distill water and 1-2 drops of slightly boiled starch indicator were added with it. Lastly, titration of this solution was done with 0.1N sodium thiosulphate ($Na_2S_20_3$). Similarly blank titration without sample was also done (AOAC,2019).

**Standardization of $Na_2S_2O_3$:**

For standardization of sodium thiosulphate ($Na_2S_2O_3$) 10 ml 0.01N $K_2Cr_2O_7$ was taken in a stoppered conical flask. 1 ml of concentrated HCl and 1 ml of 15%potassium iodide (KI) was added with it. It was kept in a dark place for 10 minutes. 20 ml distill water and 1-2 drops of starch indicator were added with it. Then the solution was titrated with 0.1N $Na_2S_2O_3$ (AOAC, 2019).

**Calculation:**

Peroxide Value = ((Titer value of sample-Titer value of blank) *N*100)/W

Here,

N= Normality of Sodium thiosulphate

W= Weight of the sample

### 3.3.6 Iodine Value

**Procedure:**

At first 1-2 ml of sample was taken in a stoppered conical flask. Then, 10 ml of chloroform and 25 ml of Henus solution was added with it. After that the solution was kept in a dark place for 30 minutes. At the end of this time, 100 ml of distill water and few drops of starch indicator was added with the solution. Then titration with 0.1N sodium thiosulphate ($Na_2S_2O_3$) was done. Similarly blank titration was done without sample (AOAC, 2019).

**Preparation of Hanus Solution:**

13.2 gm pure resublime iodine taken in a 500ml volumetric flask. 100 ml acetic acid and 1.5 ml pure bromine (sulfur free) were added with it. After that acetic acid was added with this solution up to 500 ml (AOAC, 2019).

**Calculation:**

Iodine Value = ((B-S) *Normality*12.69)/ (Weight of sample taken)

Here,

B = ml of 0.1N $Na_2S_2O_3$ required by blank

S = ml of 0.1N Na2S2O3 required by oil sample

### 3.4 Statistical Analysis

Statistical data were at first stored in Microsoft excel 2007. Later analyses of these data were carried out using IBM SPSS Statistics Version 26 (IBM Corporation, USA). The results of the experiment are presented as mean ± SD of measurements. One way analysis of variance (ANOVA) was done and their statistical significance ($p < 0.05$) was carried out by Tukey's pairwise comparison analysis.

# CHAPTER 4

# RESULTS

Proper analysis of physico-chemical characteristics of oil is very important for measuring their authenticity, quality, purity and designing an advanced technological process. For proper checkup of physico-chemical characteristics specific gravity, moisture content, saponification value, per-oxide value, acid value and iodine value were tested. These parameters were tested for 5 oil samples-Soyabean oil, Mustard oil, Olive oil, Palm oil and Sunflower oil. Same oil sample was given heat at $180^0$C for 10 minutes for two times and these parameters was also tested for heated oil samples too. The result for each type of oil is shown in table 1-table 5.

### 4.1 Soyabean oil

Table 1 shows that the physicochemical characteristics of soyabean oil changes significantly in heated and reheated condition. Among these physicochemical characteristics of soyabean oil it is found that saponification value and per-oxide value change the most.

**Table 1 : Physicochemical properties of fresh, heated and reheated Soyabean oil sample**

| Parameters | Fresh Oil | Heated Oil | Reheated Oil |
|---|---|---|---|
| **Density** | 0.9020±0.0003$^a$ | 0.9006±0.0021$^b$ | 0.8744±0.0006$^c$ |
| **MC** | 0.5333±0.0451$^a$ | 0.4467±0.2517$^b$ | 0.3633±0.0208$^c$ |
| **SV** | 182.0233±2.2972$^c$ | 235.9733±3.4460$^b$ | 267.7533±2.4601$^a$ |
| **PV** | 1.6333±0.0551$^c$ | 3.4733±0.3729$^b$ | 5.4300±0.2406$^a$ |
| **Acid value** | 0.3567±0.0153$^c$ | 0.4267±0.0160$^b$ | 0.4700±0.0200$^a$ |
| **Iodine Value** | 86.3167±0.7637$^a$ | 65.3500±1.3457$^b$ | 48.3967±0.7467$^c$ |

Legends: Values in the same row with the same superscripts but different Means ± SD are not statistically significant (P<0.05).

a= Highest value, b=medium value, c= lowest value, MC= Moisture Content, SV= Saponification Value, PV= Per-oxide Value.

### 4.2 Mustard oil

In case of mustard oil sample this investigation showed all the parameters changed significantly due to heating and reheating.

**Table 2: Physico-chemical properties of fresh, heated and reheated Mustard oil sample**

| Parameters | Fresh Oil | Heated Oil | Reheated Oil |
|---|---|---|---|
| **Density** | 0.9683±0.0101[a] | 0.9355±0.0105[b] | 0.89±0.01446[c] |
| **MC** | 0.2267±0.03215[a] | 0.2233±0.0208[b] | 0.1400±0.0200[c] |
| **SV** | 165.8800±6.4254[c] | 202.0100±8.2267[b] | 228.6267±5.9271[a] |
| **PV** | 8.9500±0.1500[c] | 9.6733±0.17898[b] | 11.6433±0.2909[a] |
| **Acid value** | 0.5867±0.0802[c] | 0.7333±0.05132[b] | 0.8767±0.0305[a] |
| **Iodine Value** | 77.2000±5.9419[a] | 66.6667±3.9287[b] | 45.8300±3.3704[c] |

Legends: Values in the same row with the same superscripts but different Means ± SD are not statistically significant (P<0.05).

a= Highest value, b=medium value and c= lowest value, MC= Moisture Content, SV= Saponification Value, PV= Per-oxide Value.

### 4.3 Olive oil

The investigation result on physicochemical properties of olive oil is shown in table 3. This table shows that all the physicochemical properties of olive oil changes significantly both in heated and reheated conditions. It also shows that the overall change in physicochemical properties of olive oil due to heat treatment is very small in amount.

**Table 3: Physico-chemical properties of fresh, heated and reheated Olive oil**

| Parameters | Fresh Oil | Heated Oil | Reheated Oil |
|---|---|---|---|
| **Density** | 0.9096±0.0027$^{a}$ | 0.9038±0.0006$^{b}$ | 0.8938±0.0006$^{c}$ |
| **MC** | 0.1700±0.0100$^{b}$ | 0.1900±0.0153$^{a}$ | 0.1367±0.01528$^{c}$ |
| **SV** | 186.5467±1.6590$^{c}$ | 199.2467±3.0425$^{b}$ | 207.2233±1.7221$^{a}$ |
| **PV** | 11.8600±0.6355$^{c}$ | 13.4567±0.20257$^{b}$ | 14.6667±0.16503$^{a}$ |
| **Acid value** | 4.3433±0.1301$^{c}$ | 4.5800±0.0100$^{b}$ | 4.6500±0.0100$^{a}$ |
| **Iodine Value** | 78.4667±0.9604$^{a}$ | 66.2567±0.3525$^{b}$ | 58.5000±1.2919$^{c}$ |

Legends: Values in the same row with the same superscripts but different Means ± SD are not statistically significant (P<0.05).

a= Highest value, b=medium value and c= lowest value, MC= Moisture Content, SV= Saponification Value, PV= Per-oxide Value.

### 4.4 Palm oil

Table 4 presents the findings of the research that was conducted on the physicochemical characteristics of palm oil. The following table demonstrates that considerable changes take place in all of the physicochemical parameters of palm oil when it is either heated or reheated.

**Table 4: Physico-chemical properties of fresh, heated and reheated Palm oil**

| Parameters | Fresh Oil | Heated Oil | Reheated Oil |
|---|---|---|---|
| **Density** | 0.9125±0.0010$^{a}$ | 0.9091±0.0005$^{b}$ | 0.8527±0.0006$^{c}$ |
| **MC** | 0.2300±0.0200$^{a}$ | 0.1867±0.0153$^{b}$ | 0.1367±0.0153$^{c}$ |
| **SV** | 200.0000±1.5853$^{c}$ | 245.5233±1.8045$^{b}$ | 272.4067±0.6967$^{a}$ |
| **PV** | 1.0667±0.0252$^{c}$ | 2.7967±0.0306$^{b}$ | 4.7933±0.07506$^{a}$ |
| **Acid value** | 0.9343±0.1528$^{c}$ | 0.9867±0.0208$^{b}$ | 1.0733±0.0153$^{a}$ |
| **Iodine Value** | 44.9233±0.2515$^{a}$ | 28.4200±0.1900$^{b}$ | 17.1267±0.4441$^{c}$ |

Legends: Values in the same row with the same superscripts but different Means ± SD are not statistically significant (P<0.05).

a= Highest value, b=medium value and c= lowest value, MC= Moisture Content, SV= Saponification Value, PV= Per-oxide Value.

### 4.5 Sunflower Oil

The results of the study that was carried out on the physicochemical properties of sunflower oil are outlined in Table 5, which can be seen here. The data shown in the accompanying table reveals that when palm oil is subjected to heat treatment, significant changes occur in all of its physicochemical properties.

**Table 5: Physico-chemical properties of fresh, heated and reheated Sunflower oil**

| Parameters | Fresh Oil | Heated Oil | Reheated Oil |
|---|---|---|---|
| **Density** | $0.9241 \pm 0.0019^a$ | $0.9125 \pm 0.0005^b$ | $0.9086 \pm 0.0002^c$ |
| **MC** | $0.2067 \pm 0.0058^a$ | $0.1867 \pm 0.0153^b$ | $0.1400 \pm 0.0100^c$ |
| **SV** | $191.6967 \pm 1.162^c$ | $209.1333 \pm 0.3677^b$ | $219.5000 \pm 1.0157^a$ |
| **PV** | $6.3833 \pm 0.0603^c$ | $7.5900 \pm 0.0400^b$ | $8.4867 \pm 0.0351^a$ |
| **Acid value** | $0.9333 \pm 0.0200^c$ | $0.9867 \pm 0.0208^b$ | $1.0667 \pm 0.0252^a$ |
| **Iodine Value** | $135.8433 \pm 0.3275^a$ | $122.8467 \pm 0.2854^b$ | $115.4667 \pm 0.3302^c$ |

Legends: Values in the same row with the same superscripts but different Means ± SD are not statistically significant ($P<0.05$).

a= Highest value, b=medium value and c= lowest value, MC= Moisture Content, SV= Saponification Value, PV= Per-oxide Value.

Analyzed data showed that the p-values for density, saponification value, peroxide value, acid value and iodine value of all types of oil sample were found lower than 0.05 ($p < 0.05$). This means that these parameters were significantly different among fresh, heated and reheated condition. But in case of moisture content of mustard oil and sunflower oil the p value was greater than 0.05 ($p > 0.05$) between fresh and heated condition. So, it meant the moisture content did not have any significant change after 1st time heating for mustard and sunflower oil. As other oils p-values for moisture content in fresh, heated and reheated condition was less than 0.05, it can be told that there was a significant difference of moisture content in fresh, heated and reheated condition of these oils.

# CHAPTER 5

# DISCUSSION

## 5.1 Specific Gravity

The density of a liquid was determined by analysis of its specific gravity. Oils with lower density levels are highly valued by consumers. From the resulted tabulated in table 4.1-4.5 shows that in fresh condition soyabean oil and olive oil had lowest density while mustard oil had the highest density. That was due to the bonds that stiffen the bonding and make the rotation between C-C bonds more difficult (Coupland et al.,1997). The table of 4.1-4.5 also shows us that in every type of oil the specific gravity became lower with heating and these heated oil shows further degradation in specific gravity when those were reheated. Oil suffers thermal breakdown when heated, which could cause modifications to its chemical structure and physical characteristics. Oil can vary in density as a result of heating, which could cause the oil to oxidize and produce polar chemicals. Vegetable oil could potentially undergo additional thermal oxidation and breakdown upon heating, altering the density of the oil further. The length and temperature of heating, the kind of cooking method employed, and the original oil quality are all likely to have an impact on the magnitude of these alterations. The oil's composition deteriorated during frying as a result of hydrolysis, oxidation, and polymerization reactions, which reduces the compounds' stability (Gloria & Aguilera, 1998). The results showed that the specific gravity of sunflower shows lowest change both after heating and reheating followed by olive oil and then soyabean oil, palm oil, mustard oil sequentially. That means heating and reheating causes less thermal oxidation in sunflower oil and olive oil when compared with soyabean oil, palm oil and mustard oil which indicates superior quality and positive health impact of sunflower and olive oil.

## 5.2 Moisture Content

Moisture content helps to determine the decomposition of edible oils (Mengistie et al., 2018a). Because the presence of water in oil causes the triglycerides to hydrolyze, which in turn causes rancidity. Thus, oils must have minimal moisture content to maintain their quality over time (Negash et al., 2019). The higher the moisture content of the oil, the more it is used for food texturing, baking, and frying, as well as in the industrial production of soaps, detergents, cosmetics, and oil paints (Birnin-Yauri & Garba, 2011). In this sense soyabean oil was the best oil according to the results shown in table 1-5. However, according to the Australian Oil Seed Federation Quality Standards of Technical Information and Typical Analysis, edible oils may contain no more than 0.2% moisture (Federation, 2011). This was only found in the result of sunflower oil and olive oil. Again, sunflower oil and olive showed lowest change in case of moisture content after heating and reheating. So, in case of these two oils quality deteriorate was not that much at heating and reheating condition when compared with other three (3) oils. That made sunflower oil and olive oil more acceptable for human consumption.

### 5.3 Saponification value

Saponification value (S.V.) is a measure of the molecular weights of the oil's triglycerides. It is inversely related to the fatty acids' average molecular weight or chain length (Mengistie et al., 2018b). Therefore, the shorter the chain length, the higher the S.V. Lower saponification values imply a lower mean molecular weight of fatty acids or a smaller number of ester linkages. This might indicate that the fat molecules did not interact with one another (Firestone, 1994).From the table 1-5 it can be clearly seen that palm oil has highest saponification value followed by sunflower oil, olive oil, soyabean oil and mustard oil sequentially at fresh condition. However, due to heating and reheating the saponification value of palm showed the second most value increase after soyabean oil and olive oil showed the lowest change followed by sunflower oil and mustard oil. That made olive oil and sunflower oil more acceptable to use in frying purpose when compared with mustard oil, palm oil and soyabean oil.

### 5.4 Peroxide Value

The peroxide value is used to assess how much rancidity reactions took place during storage. Fats and oils can be evaluated for quality and stability using their peroxide value (Gharby et al., 2015). In case of fresh sample of oils, from table 1-table 5 we can see that Olive oil showed the highest peroxide value, followed by mustard oil, sunflower oil, soyabean oil and palm oil one by one. However, due to heating and reheating soyabean oil showed the highest amount of increase in saponification value and then comes palm oil, olive oil, mustard oil and sunflower oil sequentially. That means sunflower oil showed lowest rancidity due to heating and reheating.

### 5.5 Acid Value

The amount of free fatty acids in an oil is indicated by its acid value. It assesses the quality of oils. The higher the levels, the less likely the oils are to be utilized for cooking. Higher levels suggest that oil triglycerides are being transformed into fatty acids and glycerol, which induce rancidity (Zahir et al., 2017). From the analyses of the data showed in table 1-5, in fresh condition soyabean has lowest A.V followed by mustard oil, sunflower oil, palm oil and olive oil. That makes olive oil less acceptable for cooking. This has been found also after heating and reheating of all oil types.

### 5.6 Iodine value

When evaluating the health benefits of oils, the quantity of unsaturated fatty acids is frequently measured in terms of iodine levels. Oils with a greater proportion of saturated fatty acids are less healthful than oils containing a larger percentage of unsaturated fatty acids (Negash et al., 2019). It assesses the oxidation stability of oils and enables for the qualitative determination of the fat's total unsaturation (He et al., 2010). The stronger the oxidative storage stability, the lower the values. Oils undergo oxidative and chemical changes during storage, which result in an increase in free fatty acid content and a decrease in total unsaturation (Perkins, 1992). In fresh condition sunflower oil showed highest value of iodine value which was followed by soyabean oil, olive oil, mustard oil and palm oil. On the other hand, olive oil after heating and reheating shows lowest change in iodine values followed by sunflower oil, palm oil, mustard oil and soyabean oil. So, olive oil and sunflower oil are the healthiest oil even after reheating if iodine value is considered.

### 5.7 Possible Impact on Public Health

In this investigation all fresh oil showed highest amount of density. The higher density means lesser rate of absorption in human body which is good for heart health (Sahasrabudhe et al.,2017). But all reheated oil showed lowest amount of density. Such results indicate that using reheated oil can increase blood cholesterol and heart diseases. In case of moisture content, all reheated oil sample showed lowest moisture content compared to fresh and heated samples. Moisture content determines decomposition of edible oils. Lower moisture content means the oil is hydrolyzed and become rancid (Robards et al.,1988). Higher saponification value indicates the oil sample has degraded and should be dumped or use for making soap (Te Morenga et al.,2017). All reheated sample showed highest saponification value proving that reheated oil samples can create adverse health impact on our health. In case of per-oxide value, higher value indicates high amount of aldehyde and ketone which makes oil rancid. Rancidity of oils can produce potentially toxic compounds associated with long term health effects like neurological disorders, heart diseases and cancer (Kaleem et al.,2015). All the reheated oil sample showed highest per-oxide value indicating these oils are not suitable for human consumption. Acid value is taken an important indicator of oxidation of oil. Acid values are dependent on free fatty acids, acid phosphate and amino acids. The higher the free fatty acid content the higher the acid value and vice-versa. High free fatty acid content in the oil may cause further oxidation and lead to development of offensive taste and flavor in the oil. Free fatty acids may have toxic effects by increasing reactive oxygen species leading to cell death and necrosis (Arabi et al., 2019; Drako et al., 2014). In this investigation all the reheated oil samples showed highest acid value. This proves that if foods are fried in this oil, it will be very harmful for public health. Iodine value, another important parameter of oil fitness for consumption, is used for measuring degree of unsaturation present in oil. Higher iodine value oils are likely to be healthier for consumption because the risk of cardiovascular disease associated with high consumption of saturated fatty acids which is present in low iodine value oils (Negash et al., 2019). The results of this investigation showed that iodine values of reheated oils were the lowest among fresh, heated and reheated oils. So, frying food items in reheated oil can enhance the risk of cardiovascular diseases.

# CHAPTER 6

# CONCLUSION

In conclusion, this study has provided valuable insights into the physicochemical properties of edible oil and heated oil, and the changes that occur when oil is reheated. The results show that reheating oil can lead to significant changes in its chemical composition and physical properties, including increased levels of saponification value, peroxide value, acid value and degradation of density, moisture content and iodine value.

The results of this study make it abundantly evident that how edible oils are handled, stored, and heated—both often and for long periods of time—can have a significant impact on their quality. Therefore, it is essential to educate consumers and food service personnel about the importance of using fresh oil and avoiding reheating whenever possible, in order to maintain the nutritional value and safety of the food. The results of this study also have important ramifications for public health because eating rancid or degraded oils has been associated with a number of negative health outcomes, such as oxidative stress, inflammation, and an increased risk of chronic illnesses like cancer and cardiovascular disease. Additionally, the production and disposal of waste oil is a major environmental concern, further underscoring the importance of minimizing oil degradation through proper handling and storage.

One of the limitations of this study is that it focused on a limited number of heating conditions, and further research is needed to explore the effects of different types of oils and cooking methods. Moreover, future studies should investigate the impact of using alternative methods of reheating, such as microwaving or sous vide cooking, which may be less detrimental to oil quality compared to traditional frying.

Despite these limitations, the findings of this study have important implications for the food industry, food service providers, and consumers, highlighting the need for greater awareness of the risks associated with reheating oil and the importance of using fresh oil whenever possible. By taking steps to improve oil quality and reduce waste, we can ensure a safer and more sustainable food supply for ourselves and future generations.

# CHAPTER 7

## Recommendation and Future aspect

Based on the findings of this study, there are several recommendations that can be made to improve the quality and safety of edible oils:

1. Educating consumers and food service personnel about the importance of using fresh oil and avoiding reheating whenever possible.
2. Implementing more effective methods for monitoring and controlling the quality of oil in food service settings, such as routine testing for polar compounds.
3. Exploring alternative methods of reheating, such as microwaving or sous vide cooking, which may be less detrimental to oil quality compared to traditional frying or sautéing.
4. Encouraging the development of new, more stable varieties of edible oil that are less prone to degradation and rancidity.
5. Improving regulations and guidelines for the storage and handling of edible oils, both in the food industry and in the home.

In terms of future research, there are several areas that could be explored to build upon the findings of this study:

1. Investigating the effects of different types of oils and cooking methods on oil quality, including the use of unconventional oils such as algae or insect oils.
2. Developing more accurate and reliable methods for measuring the levels of polar compounds and other markers of oil degradation.
3. Studying the long-term effects of consuming degraded or rancid oils on human health, including the role of oxidative stress and inflammation in disease progression.
4. Examining the environmental impact of waste oil disposal and exploring new methods for recycling and repurposing used oils.

By pursuing these research directions, we can deepen our understanding of the physicochemical properties of edible oils and work towards a more sustainable and healthy food supply for all.

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# Apendices

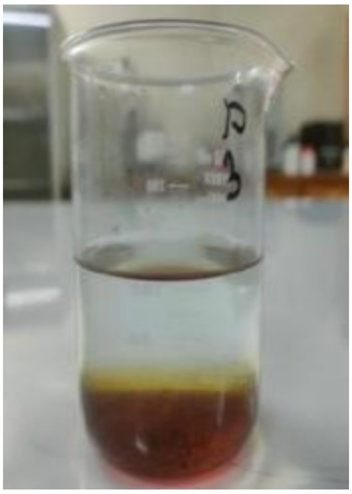
## Appendix-I: Photo Gallery

a) Sample Preparation

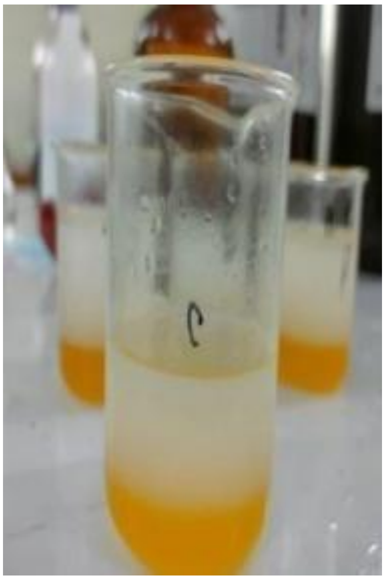

1=Olive oil, 2= Soyabean oil, 3= Sunflower oil, 4= Palm oil, 5= Mustard Oil

b) Experiments

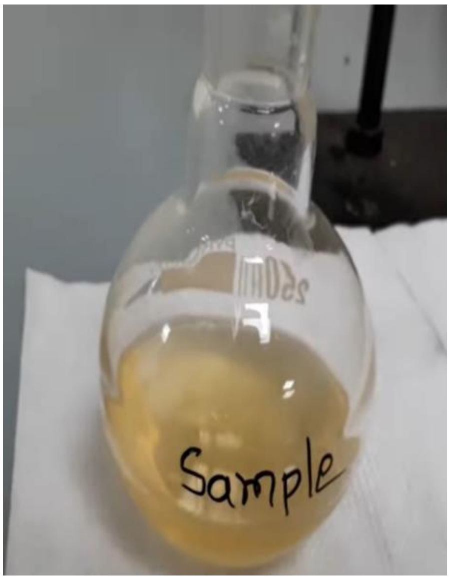
Acid Value Test Endpoint



Iodine Value Test Endpoint




Peroxide Value Endpoint




Saponification test Endpoint

## Brief Biography

Sushmita Roy Chowdhury achieved a grade point average (GPA) of 5.00 on the Secondary School Certificate (SSC) Exams in 2012 and a GPA of 5.00 on the Higher Secondary Certificate (HSC) Examinations in 2014. She graduated from Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh, with a B.Sc. (Hons) in Food Science and Technology in 2019 (held in 2020). She is currently a candidate for the MS in Masters of Public Health under the One Health Institute at CVASU. She finds her interest & career objective is to work in research and development. She has a strong desire to work in a demanding setting where her capacity for creative problem-solving may be put to good use.