# IDENTIFICATION AND QUANTIFICATION OF TRACES OF MICROPLASTIC CONTAMINATION IN BRANDED AND NON-BRANDED FLOUR IN DHAKA CITY, BANGLADESH

**A Thesis Submitted to the Department of Environmental Science, Faculty of Science and Technology, Bangladesh University of Professionals for Partial Fulfillment of the Requirements for the Degree of BSc in Environmental Science**


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# DEDICATION

I dedicate this thesis to my parents for supporting me with unwavering love and compassion throughout all the achievements of my life.

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# DECLARATION

I hereby declare that the research work "Identification and Quantification of Traces of Microplastic Contamination in Branded and Non-Branded Flour in Dhaka City, Bangladesh" has been carried out under the Department of Environmental Science, Faculty of Science and Technology, Bangladesh University of Professionals in fulfillment of the requirement for the Degree of BSc. in Environmental Science. I have composed this thesis based on the original research findings from " Identification and Quantification of Traces of Microplastic Contamination in Branded and Non-Branded Flour in Dhaka City, Bangladesh" acquired by me along with the references from published literature. This work has not been submitted in part or full to any other institution for any other degree or diploma.

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# CERTIFICATE OF THE SUPERVISOR

This is to certify that Sifat Aysha carried out her thesis under my guidelines and supervision, and hence prepared the thesis entitled "Identification and Quantification of Traces of Microplastic Contamination in Branded and Non-Branded Flour in Dhaka City, Bangladesh". As far as I am aware, the researcher duly acknowledged the other researchers' materials and sources used in this work. Further, the thesis was not submitted to any other universities or institutions for any other degree or diplomas.

It is thus recommended that the thesis be submitted to the Department of Environmental Science, Faculty of Science and Technology, Bangladesh University of Professionals, in fulfilment of the requirements for the award of the degree of BSc. in Environmental Science.

22 December 2023

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# ABSTRACT

In recent years, the ubiquity or microplastic (MPs) contamination in global food supply has been escalating with the increasing use of plastics in all sectors. This issue has emerged as a critical concern and is now receiving significant scrutiny by the researchers. From this perspective, this study has investigated the abundance and characteristics of microplastics in branded and non-branded flour in Dhaka City, Bangladesh. For this purpose, three branded and three non-branded flour samples were collected from various locations of Dhaka City. The branded flour samples were collected from different super-shops and the non-branded flour samples were collected from the open markets of Kawranbazar, Mirpur and Old Dhaka. The flour samples contained three replicates each and the microplastics were separated through density separation and digestion with $H_2O_2$. The visual and chemical identification of the microplastics were performed using a stereomicroscope and FTIR spectroscopy respectively. The results of the study revealed the presence of 12 different polymer types through FTIR analysis, which included ABS, EVA, HDPE, LDPE, Latex, Nitrile, Nylon, PC, PMMA, PP, PS and PU. Through visual observation with stereo microscope, the average abundance of identified microplastics was found to be 4578 ± 1984 (mean ± SD) particles/kg with branded flour having an average of 2747 ± 654 (mean ± SD) particles/kg and non-branded flour having an average of 6409 ± 625 (mean ± SD) particles/kg. Moreover, five morphotypes of microplastics including fiber, fragment, bead, foam and film were detected with fiber having the highest percent composition of 98.48%. From the analysis of color and size composition of microplastics, high frequency of transparent microplastics and microplastics >600μm have been found. This study has also revealed significant ($p<0.05$) higher abundance of microplastics in non-branded flour compared to branded flour through Student's T test. Furthermore, an estimation of health risk through microplastic exposure has determined that in Dhaka City, a person on average consumes 246,375 MPs particles/year from the ingestion of flour indicating high health risk of the flour consumers. The results of this study provide a comprehensive idea of microplastic contamination in flour and provide a foundation for further research and investigation to reduce microplastic contamination in food chain.


**Keywords:** Microplastic, Contamination, Polymer, FTIR, Fiber

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| | |
|---|---|
| MPs | Microplastics |
| FAO | Food and Agriculture Organization |
| FTIR | Fourier Transform Infrared |
| ABS | Acrylonitrile Butadiene Styrene |
| EVA | Ethylene Vinyl Acetate |
| HDPE | High Density Polyethylene |
| LDPE | Low Density Polyethylene |
| PC | Polycarbonate |
| PMMA | Polymethyl Methacrylate |
| PP | Polypropylene |
| PS | Polystyrene |
| PU | Polyurethane |
| NOM | Natural Organic Materials |
| ROS | Reactive Oxygen Species |
| TMP | Total Microplastics |

# CHAPTER ONE

# INTRODUCTION

## 1.1 Background of the Study

Plastics are a widely used material due to its versatility, high durability, lightweight and cost-effectiveness. Because plastic materials are low in density, have low thermal and electric conductivity, and are resistant to corrosion, they can act as an oxygen and water barrier and are very flexible (Alimba & Faggio, 2019; Strungaru et al., 2019). Their affordability also makes them simple to manufacture and widely used in a variety of applications, from food packaging to technical and medical (Chaudhry & Sachdeva, 2021). While John Wesley Hyatt created the first synthetic polymer in 1869, Leo Baekeland invented Bakelite, the first synthetic plastic, in 1907. In 1950, there were only 2 million tons of plastic produced industrially annually; today, there are about 400 million tons produced per year. However, because they are not adequately handled, these plastics wind up in our environment.

Depending on their intended purpose, plastic products can have a shelf life of anywhere from one to more than fifty years before being disposed of as plastic garbage. This meant that 71% of the recycled energy was lost to the environment, 9% was collected by 12%, and 8% was dumped on land. The majority of plastic products break down because of age and weathering; specifically, UV light causes plastic photo-oxidation. The environment experiences an accelerated phase of plastic deterioration, which should eventually lead to the formation of microparticles. They are the primary cause of the toxicity of plastic debris (Bergmann et al. 2015) and microplastic contamination. They have accumulated and endured for years to millennia in aquatic ecosystems (Jeong et al. 2016). Approximately, 14 million tons of plastic wastes are thought to be discarded into our oceans annually (Marine Plastic Pollution, 2021).

The two types of microplastics differ significantly in how they infiltrate the environment. While secondary microplastics are created by the weathering and wear of larger plastics into smaller particles directly in the environment, primary microplastics are released into the environment in their final form (Boucher & Friot, 2017). Primary microplastics include minuscule particles produced for commercial use, including those found in cosmetics, and microfibers shed from garments and other materials, like fishing nets. 53 million tons of these particles were used by the EU overall in 2013 (Lassen et al., 2015; Geyer, 2020). Twenty percent of the plastics generated went toward construction, but the majority, forty percent, went toward the production of packaging materials (Lassen et al., 2015; Geyer, 2020). When larger plastic products, such water bottles and polybags, degrade due to weathering, wind abrasion, ocean current dynamics, and exposure to ultraviolet radiation from sunlight (a process known as photodegradation), particles known as secondary microplastics are created.

Primary and secondary microplastics might differ in terms of size, shape, and color. Secondary microplastics are the byproducts of weathering and erosion and typically have an erratic shape. Primary microplastics are manufactured particles with a regular, typically spherical, or fibrous shape and a homogeneous surface. It is true; nonetheless, that weathering can cause considerable changes to both kinds of microplastics (Crawford & Quinn, 2017). Pellets, shards, and fibers, films, ropes, filaments, sponges, foams, rubber, microbeads, etc. are the most often observed forms of microplastics (Shim et al., 2018). The shape of the microplastics is important, as it can be an indication of their origin. For example, fragments are generally formed by the breaking of fibers or other plastics (Cverenkárová et al., 2021) and fibers, and fragments are mostly found in seawater (Shim et al., 2018). The sample preparation and sampling techniques determine the size of the microplastics recovered from sediment or water. In a review, Hidalgo-Ruz et al. (2012) compared the methodology used in 68 studies to identify and quantify microplastics from the marine environment. Particles larger than 500 µm are retained in standard sieves and can be sorted with a dissecting stereomicroscope, while studies utilizing density separation and filtration are typically the only ways to obtain particles smaller than 500 µm. It was suggested by the authors to separate the obtained microplastics into two size categories: <500 µm and 5 mm–500 µm. Microplastics come in an extensive range of colors in addition to sizes and shapes. According to Cverenkárová et al. (2021), the most popular hues are translucent, red, green, blue, white, and black. Studies involving aquatic organisms are expected to benefit from an understanding of color because certain species may be more likely to consume microplastics if they have a predilection for a certain color. Furthermore, color can also reveal how much pollution has polluted microplastics. Transparent and white microplastics are most frequently ingested by marine creatures, but yellow and black microplastics are the most contaminated by persistent organic pollutants (Frias et al., 2010). Microplastics also have a wide range of chemical types that include polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), polystyrene (PS), polyamide (PA), and polyvinyl chloride (PVC).

Recent studies have found the presence of microplastic in human food (Shruti et al., 2020). Microplastics have been found in a variety of food product that include freshwater fishes (Mercy et al., 2023), saltwater fishes (Mistri et al., 2022), shrimps (Severini et al., 2020), salts (Kapukotuwa et al., 2022), sugar (Afrin et al., 2022), flours (Apaza et al., 2014), fruit and vegetables (Conti et al., 2020) etc. Microplastics have also been found in beer, honey, milk, and refreshments (Diaz-Basantes et al., 2020). As discussed by Garrido Gamarro and Costanzo (2022), there are three basic ways that MPs can contaminate food goods. Due to their small size, MPs are first absorbed by plants and easily eaten by marine and terrestrial creatures where they accumulate inside the organs including kidney, intestine etc. Then the MPs easily enter the human bodies via the food chain (Bradney et al., 2019; Horton et al., 2017). Second, plastic is a material that is frequently used to package food and that, when in contact with food, may unintentionally release plastic particles (Kedzierski et al., 2020). An earlier study showed that a single plastic tea bag may discharge over 11.6 billion MPs and 3.1 billion nanoplastics (NPs) into a single cup of liquid when it was brewed (Hernandez et al., 2019). Sugar, salt, honey, etc. have all already been confirmed to be contaminated. A third possibility is contaminated raw materials.

One of the earliest crops to be cultivated, wheat has been the mainstay diet of the major civilizations in Europe, West Asia, and North Africa for 8,000 years. Wheat is still the most important source of grains for human use and is farmed on more acreage than any other commercial crop today. Among all crops, including rice, corn, and potatoes, it is the most produced. The process of making flour involves taking a wheat berry, removing the bran or outer shell, and grinding the seed until it resembles flour (Furner, 2020). We refer to this kind of flour as refined or white flour. Grinding the entire wheat berry, including the bran and seed, produces whole-wheat flour (Furner, 2020). In Bangladesh, flour is second in terms of importance only to rice, making up the majority of our diet (Hasan, 2017). According to researchers, this was the first domesticated crop, which led to the spread of agriculture and the quick rise in human population (Hasan, 2017). The top six producers of wheat worldwide are the US, China, Russia, India, and France (Hasan, 2017). To manufacture flour, wheat is normally dried and ground. Pastries, spaghetti, breakfast cereals, crackers, and bread are all made with this flour. The demand for wheat in Bangladesh has grown over time because of changing lifestyles and increased urbanization. Currently, domestic demand for wheat exceeds 8 million MT per year (Hasan, 2017). Modern technology has caused a dramatic change in the way that flour is milled. The sector is expanding quickly, facing several challenges with major market players.

However, different plastic products like polybags, plastic trays, pallets, containers, crates, carriers are used during the production, processing, storage and transportation of flour. This makes flour susceptible to microplastic contamination. The objective of this study is to determine whether flour in Dhaka City, Bangladesh is contaminated with microplastics and analyze their quantities and characteristics.

## 1.2 Problem Statement

The extensive use of plastics in daily life and the mismanagement of plastic waste have made microplastic contamination a growing phenomenon. It is now a global concern due to its significant impact on the ecosystem, food safety and human health. Microplastics are now present in terrestrial, aquatic and even atmospheric environments making their way up the food chain. Plastics enter the environment, accumulate in water, soil making its way to the bodies of floras and faunas and accumulate. Thus, they are entering the human bodies as well. On the other hand, plastics are widely used in packaging, which helps in storage, transportation, protection, and preservation of food while reducing food waste in the industry (Kedzierski et al., 2020). Regular people also use plastic containers to prepare, store, transport and consume food. Microplastic contamination can easily occur from these plastic products due to weathering, oxidative damage and mechanical stress (Fadare et al., 2020). Additionally, in a developing country like Bangladesh, it is economically inefficient to maintain all the protocols of food safety both industrially and domestically. Plastic products are widely used in the processing of different food products and flour is one of them. In addition, flour is processed, stored and sold in plastic packaging as well making them highly susceptible to microplastic contamination through temperature fluctuations, light exposure, mechanical actions, chemical reactions etc. (Kadac-Czapska et al., 2023). Vacuum plastic bags, Kraft paper bags, non-woven cloth bags, woven plastic bags etc. type of bags are used for the storage of flour to keep them free from infestation. However, these bags pose a potential threat of microplastic contamination.

### 1.3 Rationale of the Study

Plastic products are widely used in every step of food production, storage and transportation due to its availability, low cost and easy use. We have not yet discovered a more practical and affordable plastic substitute. This increases the risk of microplastic contamination in our diet. They have the potential to be extremely harmful, even though the overall effects on human health upon ingestion have not yet been determined (Afrin et al., 2022). Prior studies on a variety of animals, such as frogs and birds, have shown that microplastics both operate as a secondary vector and support other contaminants (Afrin et al., 2022). Due to its widespread consumption and the large number of plastic products it contains, flour is vulnerable to pollution. It is crucial to ascertain whether flour in Dhaka, Bangladesh, contains microplastic particles in the same way as some other comparable foods like sugar, salt, honey, fish, etc. Due to their longevity, resistance to moisture, and affordability, many brands of packaged flour are marketed in Bangladeshi markets. The flour is packaged in polyethylene or polypropylene bags inside the flourmill (Okedara, 2023) and then transported and sold in those same bags (Okedara, 2023). Instead of being packaged in these types of bags at the mill, non-branded flours are carried in woven polypropylene bags intended for bulk packing (Okedara, 2023). These generic flours are sold in paper or plastic bags and are kept and exhibited publicly in neighborhood markets, leaving them vulnerable to airborne contamination. Therefore, it is important to find out the contamination level in both branded and non-branded flour and understand if they show any significant difference.

### 1.4 Research Gap

Numerous researches have been carried out identifying the presence of microplastics in different food products all around the world including fish, sugar, salt, milk, honey, beer etc. However, to the best of the researcher's knowledge, a limited number of studies have been found to be conducted on the contamination of microplastic in flour. Moreover, it is mentioned in several articles that plastic is being used in the processing of flour but none of them mentions if these plastics contaminate the flour with its micro particles or not. From Bangladesh perspective, since flour is consumed on a daily basis in our everyday life, it requires a thorough investigation from the context of microplastic contamination.

### 1.5 Research Hypothesis

Flour in Dhaka City, Bangladesh (both brand and non-brand) is contaminated with microplastics.

### 1.6 Research Question

- Is flour (both brand and non-brand) in Dhaka City, Bangladesh contaminated with microplastics? If yes, then what are their characteristics and quantity?
- Is there any significant difference between branded flour and non-branded flour in the case of microplastic contamination?

### 1.7 Research Objective

#### Broad Objective

The broad objective of the study is the identification, quantification, classification and characterization of microplastic particles present in the branded and non-branded flour in Dhaka city, Bangladesh.

**Specific Objectives**

- To determine the abundance of microplastics in flour in Dhaka city, Bangladesh
- To assess the physical and chemical characteristics of the microplastic components
- To compare between the microplastic contamination of branded and non-branded flour and determine probable health impacts

## 1.8 Limitations of the Study

Limited number of sophisticated instruments and technologies (i.e. Raman Spectroscopy etc.) to apply modern methodologies of microplastic identification and characterization.

## 1.9 Definitions of Terms Used in Thesis

**MPs:** Microplastics (MPs) are defined as plastic pieces with size ranging from 1 μm to 5 mm (Frias & Nash, 2019) originating from primary and secondary sources.

**Contamination:** Contamination is the presence of any foreign substance that can cause impurity or pollution above background level.

**Branded and Non-Branded Flour:** The branded flour are the flour that is commercially packaged (usually packets of 1kg or 2kg) and sold with a specific brand name on it and the non-branded flour are the ones that are unpacked and unlabeled with any specific brand name and are displayed openly in local shops stored in huge woven plastic bags.

**Density Separation:** Density separation is the process of separating substances or chemical components in layers based on their density to make them easy to separate and analyze.

**Digestion:** Digestion is the process to breakdown the organic materials present in a solution.

**FTIR (Fourier-Transform Infrared Spectroscopy):** FTIR (Fourier Transform Infrared Spectroscopy) is technique used to identify the functional groups present in organic and inorganic compounds by measuring their absorption of infrared radiation over a range of wavelengths.

**Student's T Test:** A Student's T test is a type of statistical analysis used to compare the means of two groups and determine whether the differences are more likely caused by random chances and whether the associated means are significantly different.

**One Way ANOVA Test:** One Way ANOVA Test is a type of statistical analysis used to compare the mean of three or more groups and determine whether the differences are more likely caused by random chance and whether the associated means are significantly different.

### 1.10 Outline of the Thesis

1) **Chapter one** provides a brief introduction on the background, problem statement, and research gap, rationale of the study, research hypothesis, research questions and objectives of the study.
2) **Chapter two** discusses related works of literature on the microplastic contamination in food and discusses the domestic and international scenario.
3) **Chapter three** discusses the research methodology of the study that includes data source, research design, instruments, sampling technique, sample preparation and sample analysis.
4) **Chapter four** discusses the results of the study and its explanations.
5) **Chapter five** summarizes the study as a concluding remark and provides recommendations for future prospects.

The references and the appendices are attached at the end.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Microplastics and its Sources

According to Thompson et al. (2009), synthetic organic polymers, or plastics, are produced by polymerizing monomers that are taken from gas or oil. Despite plastic's many positive social effects, environmental concerns about this precious resource are growing. The world's fast rate of plastic consumption has resulted in a growing waste generation, which raises the total amount of waste that needs to be processed and/or disposed of. This is because plastic items have a very limited useful life; according to Achilias et al. (2012), almost 40% of plastic products have a useful life of less than one month. First, plastic is a durable substance that is very resistant to deterioration, which makes it a troublesome material to dispose of plastic trash (Sivan, 2011). Furthermore, a large portion of the plastic garbage produced today is obviously improperly disposed of, recovered, or collected, which adds to the growing amount of plastic debris that ends up in rivers and oceans where animals can easily consume it (Ghosh & Agamuthu, 2019). About 9.5 million tons of additional plastic wastes enter the oceans per year that also endangers the health of humans and the ecosystems (Ball, 2019). Micro-sized Plastic waste may directly enter the ocean and terrestrial environment as primary microplastics and add to the pollution or large sized plastics may be degraded into secondary microplastics through weathering or photo-oxidation.

Microplastics are invisible to the human eye and range in size from 1 µm to 5 mm (Frias & Nash, 2019). Because of their longevity, common occurrence, and potential toxicity in aquatic environments, their persistent concentration has just become known as a serious environmental concern, endangering biodiversity and human health. (Obolewski & Szymańska, 2020). Because of their small size, microplastics can travel large distances and have been found in remote locations. Microplastics have the potential to attract and spread other contaminants over a large region, including antibiotics, pathogenic microorganisms, and hydrophobic persistent organic pollutants (Xu et al., 2021).

Microplastics can be of different types of chemical composition, which include, Polyethylene Terephthalate (PET), Polyethylene Terephthalate (LDPE), Polyvinyl chloride (PVC), Low Density Polyethylene (LDPE), Polystyrene (PS), and Polypropylene (PP) etc. Microplastics may be of a variety of colors and sizes as well (Xu et al., 2021). The different morphologies of Microplastics include fibers, beads, fragments, foams and films that determine their origin and accumulation and transportation pattern. The analytical methods to determine the microplastic contamination in the environment include visual analysis, laser diffraction particle, dynamic light scattering, scanning electron microscopy, Fourier-transform infrared spectroscopy, Raman spectroscopy, thermal analysis, mass spectrometry, aptamer and in vitro selection, and flow cytometry etc. (Huang et al., 2022).

## 2.2 Microplastics in Food

According to Peixoto et al. (2019), MPs are present everywhere in freshwater, marine, and terrestrial ecosystems—even in isolated areas. Different kinds of MPs have been discovered in human excrement by recent investigations, suggesting that MPs have penetrated the human body (Schwabl et al., 2019). Human food and water consumption are the main causes of MPs in humans (Shruti et al., 2020). Additionally, MPs are consumed or breathed by oral contact (Gasperi et al., 2018). MPs may enter the food chain as discussed by Gamarro and Costanzo (2022) through three main pathways.

a. Microplastics in the aquatic environment are absorbed by the plants. These plants are ingested by aquatic animals. This is how MPs are accumulating inside the bodies and organs including kidney, liver, and intestine of marine fishes and entering into the food chain. When humans are ingesting these fishes in bulk, microplastics are accumulating inside human bodies.
b. Plastic products are widely used industrially, commercially and domestically in production, processing, packaging, transporting, storing etc. This helps increase the shelf life of food and keeps it free from bacterial contamination as well. However, this process has a huge potential of microplastic contamination in food.
c. Lastly, the raw material used in making the food may already be contaminated by MPs. Water is an essential raw material for most foods and also used in the cleaning purpose of the food processing area. Microplastics are now prevalent in the

freshwater ecosystem, which is why it may be a huge source of MPs contamination in food. In addition, MPs may enter into the food from air in the processing, storage, transportation and packaging process.

The earliest reports of MPs in food date back to the early 2010s (Barboza et al., 2018). According to a study on the MPs contamination of sugar and honey from various countries, fibers and bits were present (Liebezeit & Liebezeit, 2013). It was demonstrated in 2014 (Cauwenberghe & Janssen, 2014) that the mussels that were cultivated for human consumption contained MPs. Several investigations have found plastic particles in fish and shellfish that are meant for human consumption (Barboza & Gimenez, 2015). Another probable contributing element to the source of microplastic contamination is the bottling and/or packing procedures.

Jin et al. (2021) reviewed 108 publications in Web of Science concerning abundances, sources, and analytical methods of microplastics in human daily intake including fish, salt, drinking water, beverages, package food, and other food. The findings showed that aquatic food items including bivalves and fish contain a wide variety of microplastics (0-10.5 items/g for bivalves and 0-20 items/individual for fish). Salt represented a concentration of 0–13,629 particles/kg. For tap water and bottled water, the concentration ranges were 0 to 61 particles/L and 0 to 6292 particles/L, respectively. They have also been found in beverages, packaged food, sugar, honey, vegetables, and fruits.

In 2016, the European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain studied the presence of microplastics and nanoplastics in food focusing on seafood. Although there is no literature on the subject, the researchers' analysis indicated that microplastics are likely to come from sources besides food, such as processing aids, water, air, or releases from machinery, equipment, and textiles. It is probable that because of processing, the amount of microplastics increases. They said that there is experimental data from marine organisms suggesting that microplastics may be able to move between trophic levels. Microplastics may be found in non-marine foods because fishmeal is used in pig and poultry production.

In a study conducted by Lee et al. (2019) on the microplastic contamination of table salts in Taiwan, 43 microplastic particles from 4.4kg of salt were detected which averaged to 9.77 microplastic particles/kg. They added that although most contamination was caused by environmental pollution, some contamination of salt products occurred during the processing and packaging stages. From their global review, they found that 94% of salt products tested worldwide, contained microplastics.

Makhdoumi et al. (2023) identified the presence of microplastic in table salt and sugar in Iran. The average amounts of microplastics in different brands of salt and sugar observed were 55.2 particles/kg and 57.7 particles/kg, respectively. Polyethylene and polypropylene were identified as the most likely polymers.

Kutralam-Muniasamy et al. (2020) identified the presence of microplastics in branded milks and 1–14 particles/L microplastics were detected. They said that there might be a chance that milk could become contaminated with microplastics due to poor cleaning tools, the environment, the water supply, and improper milk handling. Additionally, there were increasing indications that the use of plastic-based packaging materials contributed to the contamination of different foods and beverages with microplastics (Novotna et al., 2019).

In a study, Zhang et al. (2023) examined 13 distinct kinds of newborn milk powder with varying packaging, processing methods, and milk sources. Compared to canned milk powder (4 items/100 g), the boxed milk powder (7 items/100 g) had more MPs. Boxed milk powder may include a significant amount of microplastics due to the plastic and aluminum foil lamination of the inner container, which released 8–17 items of MPs per 100 g. Additionally, they discovered that while the exposure to microplastics from milk powder itself is minimal, that from feeding bottles is 6.8 times greater and that from milk powder preparation is 1.7 times higher.

In 2020, Fadare et al. conducted research on MPs pollution from consumer plastic food containers. They identified and located MP in consumer plastic food containers—often used for meal delivery—and disposable plastic cups—often used for daily drinking—using spectroscopic techniques. They found that the separated MP packets weighed, on average, 12 mg, 38 mg, and 3 mg. This study shows that new plastic containers might contribute significantly to people and the environment's direct exposure to microplastics.

Parvin et al. (2021) from Bangladesh conducted research on the abundance, characteristics and variation of microplastics in different freshwater fish species. The results of the study showed that bottom feeders presented a higher amount of microplastics than midwater fishes and surface water fishes, indicating the ingestion of plastics in fish may relate to the feeding habitat.

Afrin et al. (2022) studied the presence of MPs in the sugar of Bangladesh. The number of plastic particles/kg sugar was found to be 343.7 on average and a tendency of higher frequency of microplastics < 300μm was observed. The FT-IR analysis identified nine polymeric types, ABS and PVC being the most frequent.

In Bangladesh, different brands sell packaged flour in the market that are packaged in the flour mill with polyethylene or polypropylene bags and then are transported and sold in the same bags due to their durability, moisture resistance and cost-effectiveness (Okedara, 2023). Non-branded flours are not packaged in such bags in the mill and rather they are packaged in woven polypropylene bags for bulk packaging and are transported (Okedara, 2023). These non-branded flours are stored and displayed openly in local markets making them susceptible to air-borne contamination and are sold in polyethylene or paper bags.

Flour is widely consumed in Bangladesh and might be very much susceptible to microplastics contamination due to the current practice of using plastic products. In an article published in the newspaper 'The Independent Bangladesh', Mosharraf (2018) discussed that numerous forms of plastic food packaging are broadly used in the world such as polythene bags, wrapping, bottles, rigid containers, caps and lids. From airtight wraps to shelf stable bottles and containers, plastic packaging plays a key role in delivering a safe food supply, from farm to table and is a material of choice for freezing food for longer-term storage. In 2021, in an article published in a daily newspaper 'New Age Bangladesh', Shahidul Islam Chowdhury mentioned that wraps used for packing flour, pulse, grain and rice packs were the second most used and multilayer plastic, which includes all kinds of food and non-food packaging materials, were the third most used plastic items found in the landfills in Dhaka, Bangladesh. Also in Bangladesh, flour is packaged in polyethylene or polypropylene bags and then are transported and sold in the same bags due to their durability, moisture resistance and cost-effectiveness (Okedara, 2023). Flour can be contaminated with MPs in the production process due to contaminated air in the production facility or due to the use of contaminated water in the production arena. Flour can also be contaminated with microplastics present in the air when they are kept openly displayed in woven polypropylene bags in the market.

### 2.3 Microplastic Exposure to Human Health

An increasing amount of research indicates that microplastics may find their way into commonly consumed food products through production contamination (Karami et al., 2017), animal consumption of microplastics in the environment (Santillo et al., 2017), and plastic packaging contamination (Mason et al., 2018). Humans are primarily exposed to microplastics through their skin, food, and respiratory systems. According to Prata (2018), the main sources of inhaled airborne microplastics include synthetic fabrics, powdered synthetic rubber tires, and city dust. The main routes to the gastrointestinal tract are through contaminated seafood, other foods, and water. Compared to eviscerated organisms, fully consumed organisms have a greater danger (Carbery et al., 2018). While sweat ducts, open skin wounds, and hair follicles are among potential entrance points, the human skin itself blocks microplastics and other contaminants from entering directly (Schneider et al., 2009). The total amount of microplastics that humans are exposed to comes from all three sources, but the exposure from seafood and the environment may pose a greater risk because of weathering, leaching of plastic additives, residual monomers, and long-term interactions with other toxic pollutants (Brennecke et al., 2016; Camacho et al., 2019; Li et al., 2018; Rochman et al., 2014). Additionally, pathogenic microorganisms from the environment may also play a role in the exposure (Virsek et al., 2017).

While the effects of MP consumption on human health are still mostly unknown, certain possible routes of harm have been identified (Wright & Kelly, 2017). Once in the stomach, MPs can release absorbed poisons, additives, and component monomers that can lead to a variety of physiological harms, including carcinogenic behavior and oxidative stress (Wang et al., 2018). According to Wright and Kelly (2017), MPs can also enter the human body by paracellular transport in the gut and cellular absorption in the lungs or gut. Studies have indicated that additional chemicals found in plastics or attached to microplastics, such as polyvinyl chloride monomer, residual low molecular weight styrene, PAHs, PCBs, PBDEs, and pharmaceuticals, including their metabolites, may be absorbed and subsequently cause cancer, mutagenicity, and endocrine disruption. Bisphenol A (BPA) is a common ingredient in plastics that is used as a stabilizer or antioxidant and has the potential to alter hormones (Halden 2010). It can move out of polycarbonates and stick to food or beverages (Calafat et al., 2008), whereupon people can consume it. Research has shown that BPA can contaminate highly consumed foods, such as meat (Shao et al., 2007), tap water (Colin et al., 2014), and tuna fish (Munguía-López et al., 2005). Meeker et al. (2010) discovered an inverse relationship between the amounts of BPA in the urine of 167 men and the serum levels of inhibin B and the estradiol: testosterone ratio, indicating a deleterious impact on hormone levels. According to Michalowicz (2014), BPA may also have an adverse effect on fat tissue hormone levels, disrupt alpha and beta receptors in fat tissues, and interfere with the function of lipoprotein lipase, aromatase, and lipogenesis regulators (Vom Saal et al., 2012). It might cause prostate and breast cancer in mammals, thereby advancing the same cancers in people (Michalowicz, 2014). The shape, size, solubility, and surface chemistry of MPs will all affect the degree of absorption. Particles as small as a few micrometers may be directly absorbed by gut or lung cells, whereas specialized cells in the ileum's Peyer's patch may take up particles as large as 10 μm (Powell et al., 2010). Even though the rate of particle transfer to blood over a 24-hour period may be as low as 0.002%, particles as large as 130 μm can reach tissue by paracellular transport in the form of persorption (Steffens, 1995). It is currently unknown how much of our estimate of human consumption of MPs poses a risk to human health. An investigation into the cumulative human exposure to microplastics (MPs) has not been conducted, despite mounting evidence that these particles contaminate a wide range of foods and beverages, as well as outdoor and indoor environments, and may have harmful effects on human health after ingestion and/or inhalation (Dassin et al., 2015).

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 Conceptual Framework

The study's conceptual framework is given in **Figure 3.1.**

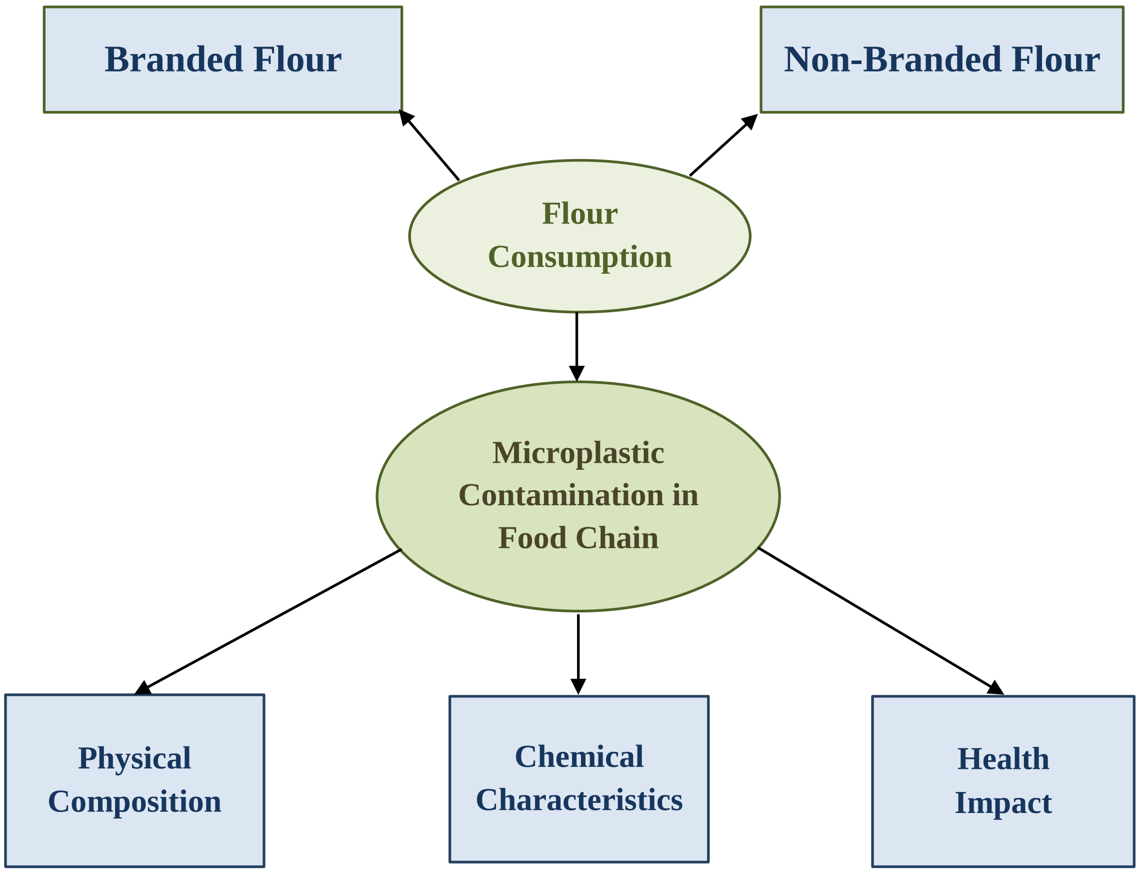


**Figure 3.1:** Conceptual framework of the study

## 3.2 Data Sources

- **Primary Data:** The primary data was collected from the laboratory experiment results of the collected samples.
- **Secondary Data:** The secondary data will be collected from different journals, books, news articles and websites.

### 3.3 Research Design

Both qualitative and quantitative approach was followed during the study.

The research design of the study is illustrated in **Figure 3.2.**

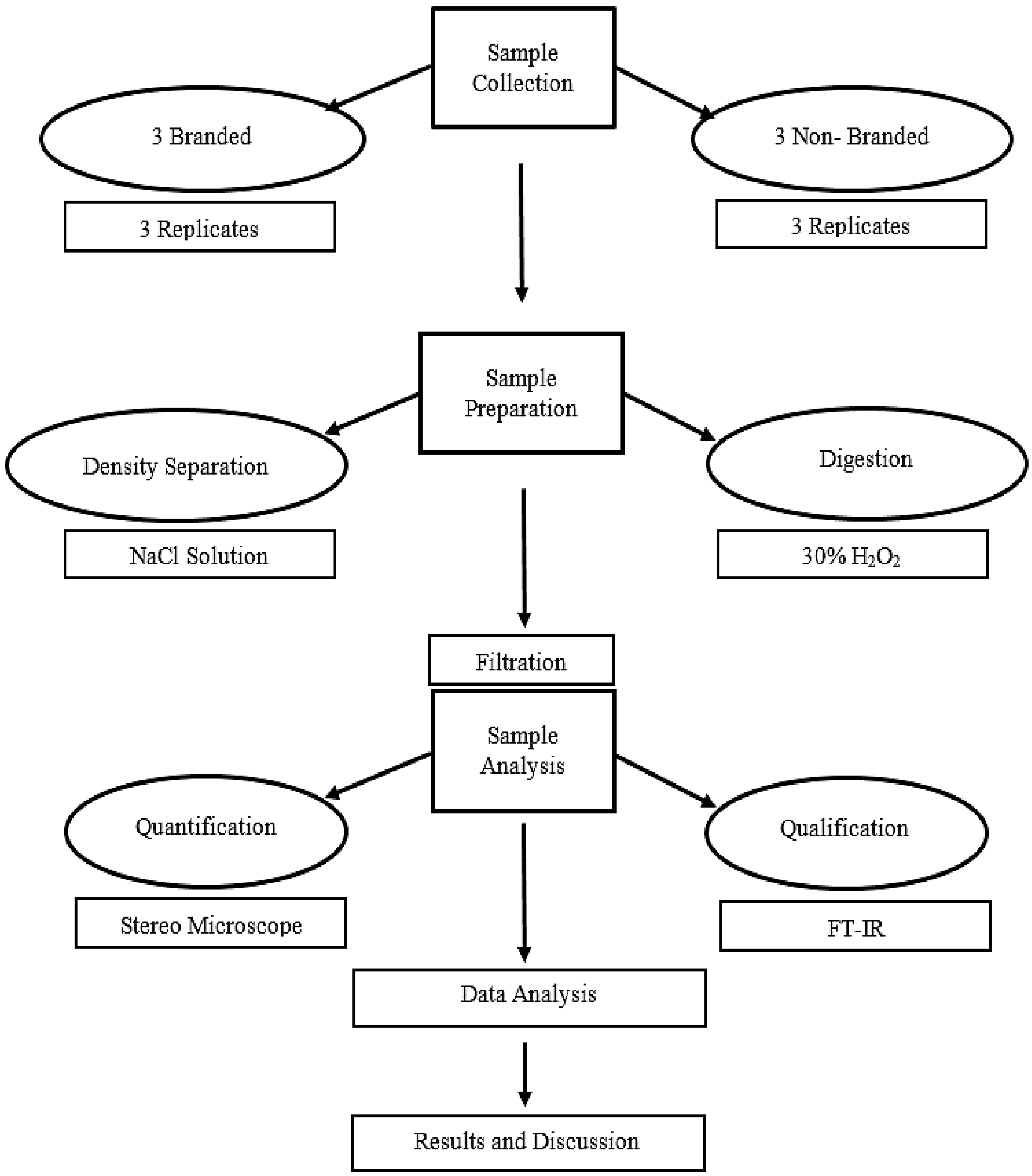


**Figure 3.2:** The research design of the study

### 3.4 Instruments

#### 3.4.1 Required materials and machineries in sample preparation

- Beaker (50ml, 100ml and 200ml)
- Measuring cylinder (1000ml)
- Spatula
- Forceps
- Dropper
- Glass rod
- Petri dishes
- Aluminum foil
- Digital balance (BSA224S-CW, Sartorius)
- Magnetic stirrer (HP630, Misung Scientific Co. Ltd., Korea)
- Pressure pump vacuum filter
- Drying oven (ED53, Binder)
- PALL nylon 6.6 membrane filter paper (0.45µm pore size, 47mm diameter)

#### 3.4.2 Required Chemical Reagents for Sample Preparation

- Distilled water
- Sodium Chloride (NaCl)
- 30% Hydrogen Peroxide ($H_2O_2$)

#### 3.4.3 Required Machineries and Software for Sample Analysis

- **Stereo Microscope Motic B410E and Motic Image Plus 3.0 ML Software:** The Motic B410E Stereo Microscope, made in Germany, features a resolution of 1360 1024 pixels on its sensor, together with a grade camera and fluorescent microscope. The Motic Image Plus 3.0 Software connects the microscope to the computer and helps observe on the bigger computer screen and capture necessary images. Microplastics on nylon filter paper were observed under this microscope at four times and ten times magnification. It was used to identify the microplastics, and their quantity, morph type, sizes and shapes were tallied and noted.

- **FT-IR (Frontier, PerkinElmer, Germany):** FTIR is used to analyze the chemical composition of microplastics (MPs). The infrared absorption spectrum generated by this instrument helps to determine the chemical bonds within a molecule. According to Yang et al. (2023), the vibrational frequencies of atomic bonds are represented by absorption peaks in an infrared spectrum. The unique atoms that make up each polymer prevent infrared spectra from being duplicated between molecules. For main vibration analysis, the mid-infrared (MIR; 4000-400 c/m) spectrum is employed. The most common choice for characterizing MPs is the mid-IR area of the infrared spectrum. The Fourier transform infrared spectroscopy used in this work was obtained using PerkinElmer Frontier FT-IR Spectroscopy.

#### 3.4.4 Software Used in This Study

- Microsoft Excel
- OriginPro v.8.5

### 3.5 Overview of the Sampling Method

#### 3.5.1 Sample Collection Area

The branded flour samples were collected from different super-shops and the non-branded flour samples were collected from the local markets of Kawran Bazar, Mirpur and Old Dhaka.

#### 3.5.2 Sample Collection Procedure

The samples were collected using simple random sampling Technique. Three 1kg-packets of each branded and non-branded flour samples were collected and mixed to make composite samples.

#### 3.5.3 Sampling Procedure

The sampling procedure of the research is shown in **Table 3.1.**

**Table 3.1:** Sampling procedure of the research

| Sample Type | Sample ID | Replicates | Quantity (gm) |
|---|---|---|---|
| **Branded Flour** | B1 | B1a | 50 |
| | | B1b | 50 |
| | | B1c | 50 |
| | B2 | B2a | 50 |
| | | B2b | 50 |
| | | B2c | 50 |
| | B3 | B3a | 50 |
| | | B3b | 50 |
| | | B3c | 50 |
| **Non-Branded Flour** | NB1 | NB1a | 50 |
| | | NB1b | 50 |
| | | NB1c | 50 |
| | NB2 | NB2a | 50 |
| | | NB2b | 50 |
| | | NB2c | 50 |
| | NB3 | NB3a | 50 |
| | | NB3b | 50 |
| | | NB3c | 50 |

### 3.6 Sample Preparation and Isolation of Microplastics

The methodology for sample preparation was developed by continuous and thorough pilot tests till the desirable result was reached. The sample preparation was conducted in two steps which include-

- **a)** Density separation (3.6.1)
- **b)** Chemical analysis (3.6.2)

### 3.6.1 Density Separation and Initial Filtration

Components heavier than water are typically separated from those lighter than water using density separation systems, also known as specific gravity or float-sink separation systems. Densities of plastics mostly range from 0.89 g/cm$^3$ (e.g. PP) to 1.58 g/cm$^3$ (e.g. PVC) regardless of various additives (Li et al., 2018). For the density gradient solutions, ethanol (0.8 g/cm$^3$), water (1.0 g/cm$^3$), and saturated NaI (1.8 g/cm3) are considered to be the best options (Li et al., 2018). However, several studies recommend using NaCl (1.2 g/cm$^3$) (Li et al., 2018). The majority of microplastics' densities may be determined using these density gradient solutions, which have a density range of 0.8–1.8 g/cm$^3$. NaCl solution has a density of 1.2g/cm$^3$, which is why the microplastics with lower density than NaCl solution tend to float at the surface and can be easily separated through the process of density separation.

1000ml distilled water was measured using a measuring cylinder and added to a labeled 1000ml beaker. NaCl (360g/l) was added to the water and stirred properly. Each sample was measured using a digital balance machine and 50g were taken into the beaker and added to the solution. Initially a glass rod was used to mix and stir the flour sample and the solution to remove any big clumps. Then the mixture was stirred for 30 minutes using a magnetic stirrer **(Figure 3.3)** (Mercy et al., 2023). After 30 minutes, the magnet was removed from the solution and the beaker was covered using aluminum foil immediately to prevent contamination and stored inside a cabinet for settling and separating.

**Figure 3.3:** Sample mixing in a magnetic stirrer

After 24 hours, the beaker was taken out and the aluminum cover was removed. It was observed that most of the flour particles had settled at the bottom and the water on top was almost clear containing the microplastics. The clear surface layer was manually separated into another beaker. The solution was then filtered using PALL Nylon 6.6 Membrane Filter Paper (0.45µm pore size, 47mm diameter) which entrapped all the MPs particles (Mercy et al., 2023). A vacuum pump **(Figure 3.4)** was used for this process and the filter papers were stored in petri dishes with covers keeping them contamination free.

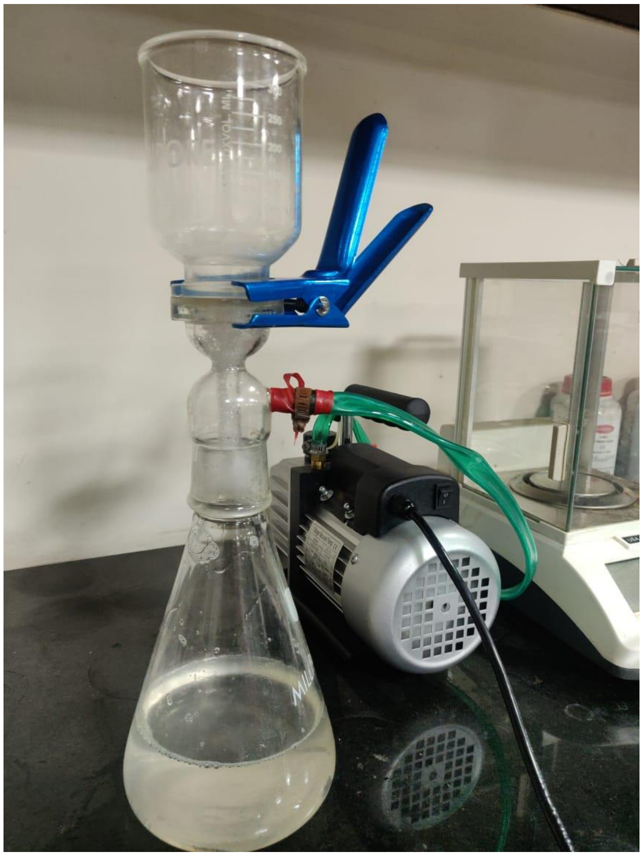

**Figure 3.4:** Separation of MPs through vacuum filtration process

### 3.6.2 Digestion and Final Filtration

The identification of microplastics (MPs) becomes more challenging since they are frequently coated in natural organic materials (NOM) from surroundings (Zhou et al., 2022). Now, hydrogen peroxide ($H_2O_2$) oxidative digestion is a widely used method for removing NOM from MPs (Zhou et al., 2022). Sodium hydroxide (NaOH), on the other hand, can damage and discolor MPs, while sulfuric acid ($H_2SO_4$) and nitric acid ($HNO_3$;) can disintegrate or degrade polymers, and hydrochloric acid (HCl) is ineffective in MPs assessment at ambient temperature (Cole et al., 2014).

The filter papers were picked by forceps and 30% $H_2O_2$ (v/v) solutions were dropped into it using a dropper **(Figure 3.5)**. The solution slowly picked all the microplastics from the filter paper surface and dropped them in a 200ml labeled beaker. About 150ml 30% $H_2O_2$ (v/v) was required to wash the filter papers of a single sample.


**Figure 3.5:** Collection of MPs residue with 30% $H_2O_2$

After the filter paper surface looked very clean through visual inspection, the beaker surface was covered immediately using aluminum foil to avoid contamination. The beakers were then placed in a drying oven **(Figure 3.6)** at 65°C for not more than 72 hours (Mercy et al., 2023). After 72 hours, the remaining organic material had been broken down and only the microplastic polymer remained. The solution was finally filtered using PALL nylon 6.6 membrane filter paper (0.45μm pore size, 47mm diameter) and a vacuum pump (Mercy et al., 2023). The microplastic particles then were stuck on the surface of the filter papers. The filter papers were then stored in petri dishes with covers and air-dried for 24 hours to keep them contamination free.

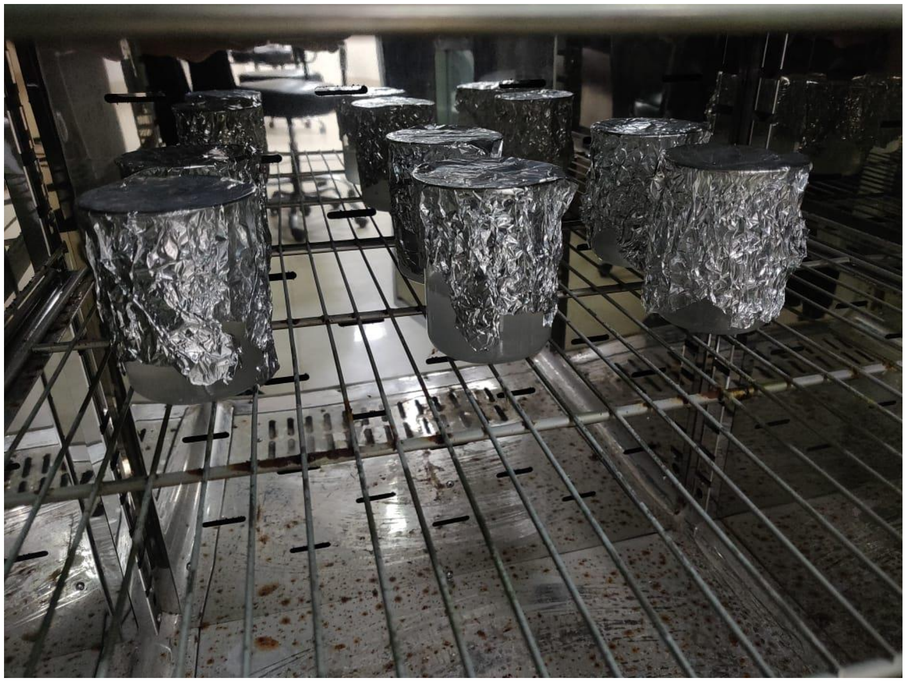

**Figure 3.6:** Digestion with 30% $H_2O_2$ in drying oven

### 3.7 Sample Analysis

The samples were analyzed in two steps which include-

- **a)** Visual observation (3.7.1)
- **b)** Chemical analysis (3.7.2)

#### 3.7.1 Visual Observation of MPs using Stereo Microscope

The visual observation with the naked eye could only observe some of the large MPs particles only. Therefore, the filter papers containing microplastics were examined using Stereo Microscope Motic B410E at 4X/10X **(Figure 3.7)**. This helped visually observe the MPs of all sizes and pictures were captured using moticams12 and displayed using Motic Image Plus 3.0 ML Software. Microplastics are thought to share several characteristics, such as glossy surfaces, vibrant colors, and sharp edge geometrical patterns. Artificial fibers, which are abundant because of human activity, are described as having vibrant colors, dull sides and string like appearance (Horton et al., 2017). In addition, the MPs were divided into categories of morphotype, color and size range. Based on their morphotype or physical properties or shape, they were divided into fibers, fragments, films, foams and beads. Several different colors were also used to classify the MPs that include black, white, red, blue, transparent, purple, pink, yellow, brown, orange, gray and green. The length of the MPs was also recorded and classified into ranges using the microscopic scale. While tallying the number of microplastics, certain criterion measures, such as the absence of branching, fibers with uniform thickness along their length, the absence of cellular formation, and particles devoid of metallic sheen were taken into consideration. The total number of MPs in each sample was recorded as well.

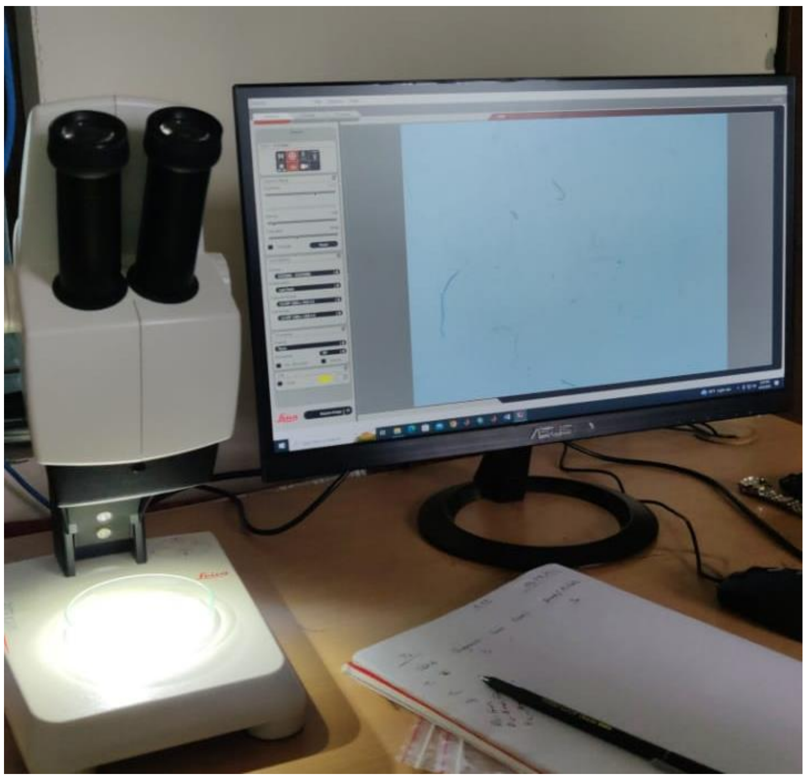

**Figure 3.7:** Visual observation of MPs using stereo microscope

### 3.7.2 Chemical Analysis of MPs using FT-IR Spectroscopy

ATR-FTIR (Frontier, PerkinElmer, Germany) **(Figure 3.8)** was used to evaluate the MPs extracted from the filter paper of each sample in order to determine the MP polymer types of microplastics (Browne et al., 2010). Fourier transform infrared spectroscopy (FTIR) is a technique, which is used to obtain an infrared spectrum of absorption, emission, and photoconductivity of solid, liquid, and gas. It is used to detect different functional groups in the MPs and the structure and origin of microplastics can be ascertained using the incredibly complex infrared spectra of plastic polymers, which have distinct band patterns (Van-der-Hal et al., 2017). Utilizing attenuated total reflection (ATR), spectra between 650 $cm^{-1}$ and 4000 $cm^{-1}$ were obtained in this study. After cleaning the diamond crystal with isopropyl alcohol, the MPs were scraped from the filter paper and the IR spectra of the sample containing peak values were shown. Using a peak height method in the Perkin Elmer application, the spectrum was measured. The spectrum was compared to a reference spectrum (Jung et al., 2018) after converting the transmittance value to absorbance value. The transmittance values were converted to absorbance values using **Equation (1).**

$$\textbf{Absorbance} = 2 - \log_{10} (\textbf{Transmittance}\ \%) \qquad \textbf{Eq. (1)}$$

**Figure 3.8:** Chemical analysis of MPs using FT-IR spectroscopy

### 3.8 Quality and Contamination Control

Quality and contamination control is an essential aspect of any research project. MPs contamination from air or water is very common which is why standard protocols are required to be maintained during microplastic analysis. Standard contamination control procedures were followed and precautionary measures were taken similar as Afrin et al. (2022) throughout the whole research and laboratory experimental activities to minimize errors and ensure the quality of the result. To avoid contamination, the lab coats used were made of 100% cotton and gloved hands were cleaned with purified water. Fresh samples directly from the packets were used every time and then sealed tightly. All the apparatus including beakers, glass rod, spatula, petri dishes etc. were rinsed with distilled water and wiped with paper towels before each use to avoid cross-contamination among samples. The beakers and petri dishes containing the samples were kept constantly covered with aluminum foil or glass lids to prevent contamination from air. The glass beakers of the suction pump were cleaned with distilled water after every use to remove the MPs residue. To reduce MPs contamination from outdoor air, the window frames and ventilation systems were sealed.

### 3.9 Probable Estimation of Microplastic Health Risk

This study aims to determine how much microplastics are entering into the human bodies in Dhaka, Bangladesh through the ingestion of one of the most common food items that are flour. Flour can be assumed to be ingested by everyone in a country as it is a strong source of carbohydrate and is used in making breads, wraps, pasta, noodles, biscuits, cakes and other bakery items etc. The minimum, average and maximum amount of microplastics in the flour per unit were determined to evaluate the microplastic exposure to humans through flour ingestion. The amount of flour consumed per day by an average person was assumed from the general eating habits of the people of Dhaka city, Bangladesh and the microplastic amount ingested was calculated. **Equation (2)** is a method adapted and modified from Afrin et al. (2022) in measuring the MPs emission through edible sugar, which was again adapted and modified from the calculation used by Praveena et al. (2018).

$$\textbf{EAI = DU-N x WF-SU x MPs-APU x D-Y} \qquad \textbf{Eq. (2)}$$

**EAI =** Estimated annual intake amount per person (particle/person/year)

**DU-N =** Number of daily usage of flour

**WF-SU =** Weight of flour used in single use (grams)

**MPs-NPU =** Number of MPs particles in per unit (grams)

**D-Y =** Total days in a year

### 3.10 Statistical Analysis

For statistical analysis, the Microsoft Excel 2016 program was used. One-way ANOVA was conducted to measure the difference of microplastic abundance among the six samples. Student's T test was conducted to determine the significance of difference among branded and non-branded flour samples regarding microplastic contamination. The threshold of significance was considered to be $p < 0.05$.

# CHAPTER FOUR
# RESULTS AND DISCUSSION

## 4.1 Results and Discussion

The overall results and discussions of this study were divided into five parts based on the objectives of this study. This includes-

**a)** Abundance and Distribution of MPs in Flour Samples (4.1.1)

**b)** Morphotype, Size and Color Composition of MPs in Flour Samples (4.1.2)

**c)** Comparative Analysis of Microplastic Contamination between Branded and Non-Branded Flour Samples (4.1.3)

**d)** Chemical Group Identification of MPs using FTIR Spectroscopy (4.1.4)

**e)** Health Impact Assessment of Microplastic Contamination (4.1.5)

### 4.1.1 Abundance and Distribution of MPs in Flour Samples

While visually observing the microplastics under a stereomicroscope, the microplastics were each characterized for their morphotype, size (length) and color and tallied. From the tally, the total number of microplastics for each sample was counted.

The presence of microplastics was successfully identified in every sample (5gm) including their replicates. They were divided into 5 categories, which are fiber, fragment, bead, foam and film. Their abundance and distribution based on their morphotypes are displayed in **Table 4.1.**

**Table 4.1:** Quantification of microplastics and their morphotypes identified in all collected flour samples including their experimental replicates.

| Sample | Sample ID | Fiber | Fragment | Bead | Foam | Film | TMPs (particles/5 gm) |
|---|---|---|---|---|---|---|---|
| **B1** | B1a | 133 | 0 | 0 | 1 | 0 | 134 |
| | B1b | 107 | 2 | 0 | 0 | 0 | 109 |
| | B1c | 108 | 0 | 1 | 0 | 0 | 109 |
| **B2** | B2a | 189 | 0 | 0 | 0 | 0 | 189 |
| | B2b | 136 | 1 | 0 | 1 | 0 | 138 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | B2c | 132 | 0 | 1 | 0 | 0 | 133 |
| **B3** | B3a | 118 | 0 | 0 | 1 | 0 | 119 |
| | B3b | 109 | 1 | 0 | 0 | 1 | 111 |
| | B3c | 193 | 1 | 0 | 0 | 0 | 194 |
| **NB1** | NB1a | 322 | 5 | 3 | 0 | 2 | 332 |
| | NB1b | 350 | 2 | 1 | 0 | 1 | 354 |
| | NB1c | 357 | 3 | 1 | 1 | 1 | 363 |
| **NB2** | NB2a | 290 | 1 | 0 | 0 | 0 | 291 |
| | NB2b | 302 | 1 | 2 | 0 | 0 | 305 |
| | NB2c | 278 | 2 | 4 | 0 | 0 | 284 |
| **NB3** | NB3a | 278 | 2 | 1 | 0 | 1 | 282 |
| | NB3b | 319 | 1 | 1 | 3 | 0 | 324 |
| | NB3c | 346 | 1 | 1 | 1 | 0 | 349 |

Even though microplastics were identified in all the samples, not all the samples contained all 5 morphotypes of microplastics. From the table we can observe that the most dominant microplastic type was fiber in every sample. The rest four types had pretty much similar distribution in all the samples.

The abundance of microplastics in each samples were the converted into their abundance in per kg of flour sample and the average abundance for each sample was counted. The total amount of MPs particles in each sample per kg were found to be 2347 ± 289 (mean±SD) in B1, 3067 ± 620 (mean±SD) in B2, 2827 ± 916 (mean±SD) in B3, 6993 ± 319 (mean±SD) in NB1, 5867 ± 214 (mean±SD) in NB2 and 6367 ± 677 (mean±SD) in NB3 where "B" represents branded samples and "NB" represents non-branded samples (Figure 4.1). The mean abundance for all 6 samples were found to be 4578 ± 1984 (mean±SD) particles/kg. Here we can observe the comparative abundance of microplastics in the flour samples to be NB1>NB3>NB2>B2>B3>B1 with NB1 which was collected from Kawranbazar having the highest amount of microplastics which is 6993 ± 319 (mean±SD) particles/kg and B1 having the lowest amount of microplastics which is 2347 ± 289 (mean±SD) particles/kg **(Figure 4.1).**

A one-way ANOVA was executed in Microsoft Excel to determine if there was a significant difference in the abundance of microplastics among the 6 samples at a 5% level of certainty. The result from the one-way ANOVA was found to be $p < 0.001$ which revealed significant difference of microplastic abundance among all the samples.

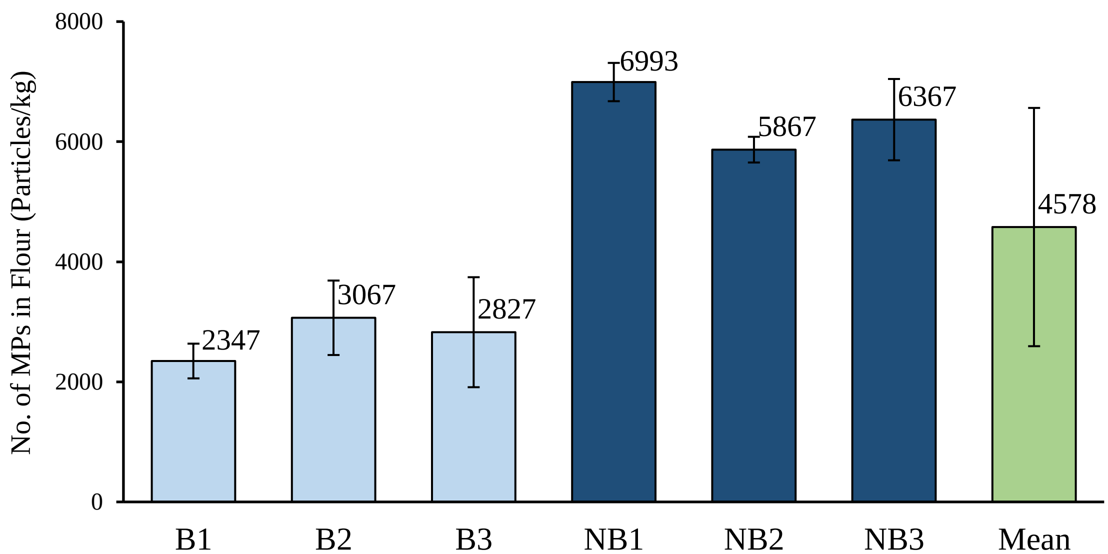


**Figure 4.1:** Number of microplastic particles/kg found in different branded and non-branded flour samples collected from Dhaka City, Bangladesh

Several studies have found the abundance of microplastics in different food items including Afrin et al. (2022) who identified the average abundance of MPs in branded and non-branded sugar in Bangladesh to be 343.7 ± 32.08 (mean ± SD) particles/kg. Similarly, the current study has identified the presence of microplastics in branded and non-branded flour of Bangladesh. Microplastics have also been identified in the raw and refined salts in Bangladesh. Zafar et al., (2020) identified the presence of 283 particles/kg MPs in refined salts and 2105 particles/kg MPs in raw salts in Bangladesh. Parvin et al., (2021) identified the presence of 9 MPs particles/individual freshwater fishes in Bangladesh. Similarly, Mercy et al., (2023) found 8.2 MPs particles/individual freshwater fishes. All of these studies indicate the ubiquity of microplastic contamination in the food chain of Bangladesh.

The abundances of microplastics in different branded and non-branded samples were identified to be significantly different. This indicates that the concentration of microplastics might be influenced by different production procedure, packaging or the marketing of different flour brands or flourmills. Different flourmills contain different technologies, which are contamination free at different degrees. Depending on the purity of raw materials and the anti-contamination facilities in the flourmills, concentration of microplastics might vary. However, flours are packaged using plastic materials in flourmills, which have high potential of microplastic contamination through surface degradation. From an interview with a representative from a flourmill, it has been found that packaged flour may stay in the production facility for about one week before they are supplied to the local market. Another representative informed that packaged flour might stay in production facility storage for up to a month depending on the market demand. In addition, packaged flour has a shelf life of three months on average. With time, microplastics may degrade more from the packaging material and contaminate the flour more. Therefore, the amount of time flour stays wrapped in its plastics packaging may have influence in the concentration of microplastics. Non-branded flours are again kept openly in the market in PP bags or HDPE bags and are often sold in LDPE bags, which contain potential of microplastic contamination from packaging and surrounding air. Therefore, several factors altogether contribute to the contamination of microplastics in branded and non-branded flour in Dhaka City, Bangladesh.

The pictures of different microplastic morphotypes observed under microscope are displayed in **Figure 4.2.**

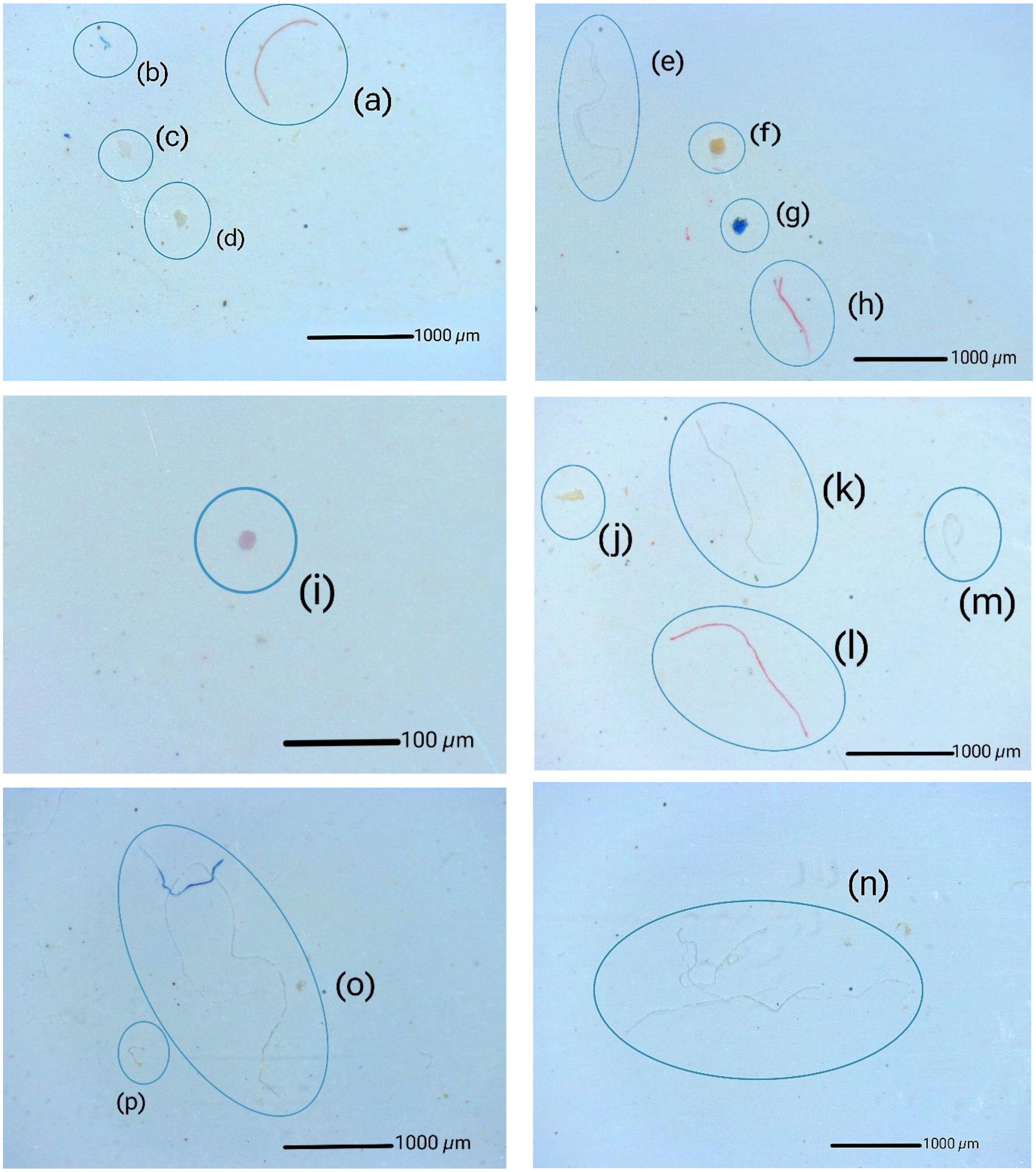


**Figure 4.2:** Visual identification of microplastics with different morphotypes, size and colors in branded and non-branded flour samples. Here, (a), (b), (e), (h), (k), (l), (m), (n), (o), (p) denotes fibers, (c), (d), (f), (g) denotes fragments, (i) denotes beads and (j) denotes films.

**Figure 4.3** shows the distribution of fibers, fragments, beads, foams and films in the branded samples. All the samples showed the dominance of fiber type of microplastic. The highest amount of Fiber was found in sample B2, which was 3047±636 (mean±SD) particles/kg. The second most abundant type of microplastic is fragment which was found in all the samples with B1 and B3 having the highest amount which was 13±23 (mean±SD) particles/kg and 13±12 (mean±SD) particles/kg respectively. It is also noteworthy that film was found in only sample B3, which was 7±12 (mean±SD) particles/kg. In addition, beads were not identified in sample B1 and B2. Overall, the sample B1 had the least amount of microplastic and the least type of microplastics among the branded ones.

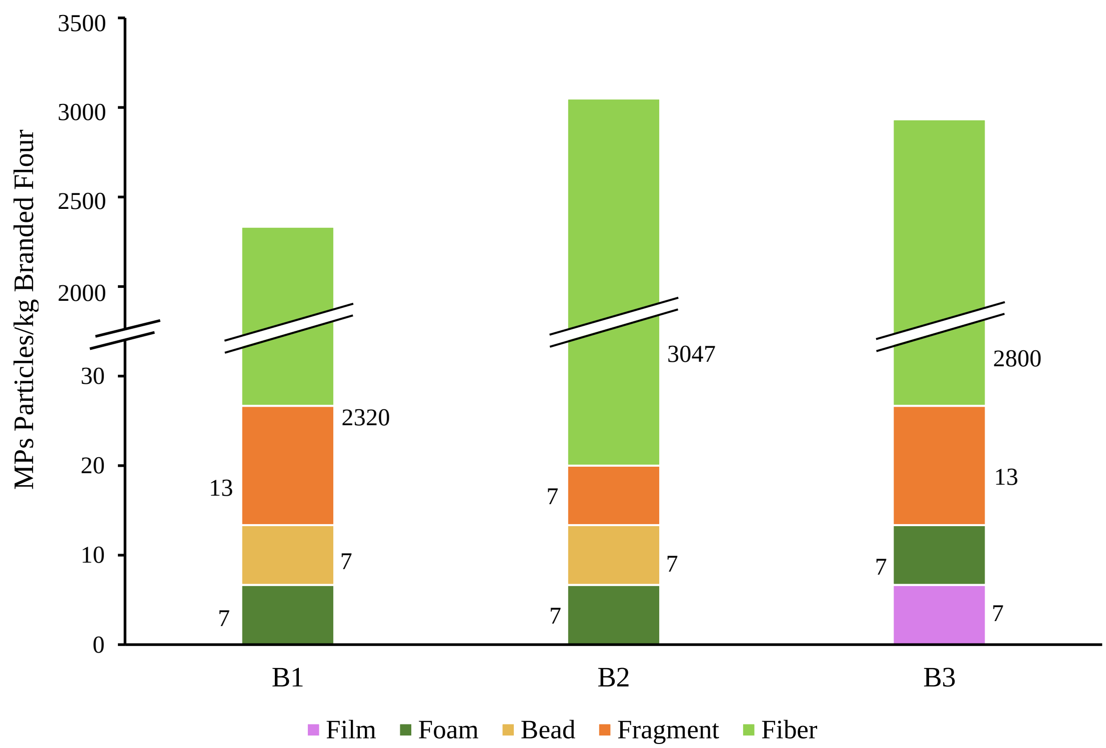


**Figure 4.3:** Composition of MPs morphotype (fiber, fragment, bead, foam, film) per 1kg branded flour in Dhaka City, Bangladesh

**Figure 4.4** shows the distribution of fibers, fragments, beads, foams and films in the non-branded samples. All the samples showed the dominance of Fiber in this case as well. The highest amount of fiber was found in sample NB1, which was 6860±370 (mean±SD) particles/kg. The second most abundant type of microplastic is fragment which was found in all the samples with NB1 having the highest amount which was 67±31 (mean±SD) particles/kg. It is important to mention that no foam and film was found in sample NB2 and had the least amount of microplastics altogether. However, sample NB2 has higher abundance of bead than sample NB1 and NB2, which was found to be 40±40 (mean±SD) particles/kg. Overall, the sample NB2 had the least amount of microplastic and the least type of microplastics as well. In addition, sample NB1 had all 5 types of microplastics with the highest amount of total abundance among the non-branded ones.

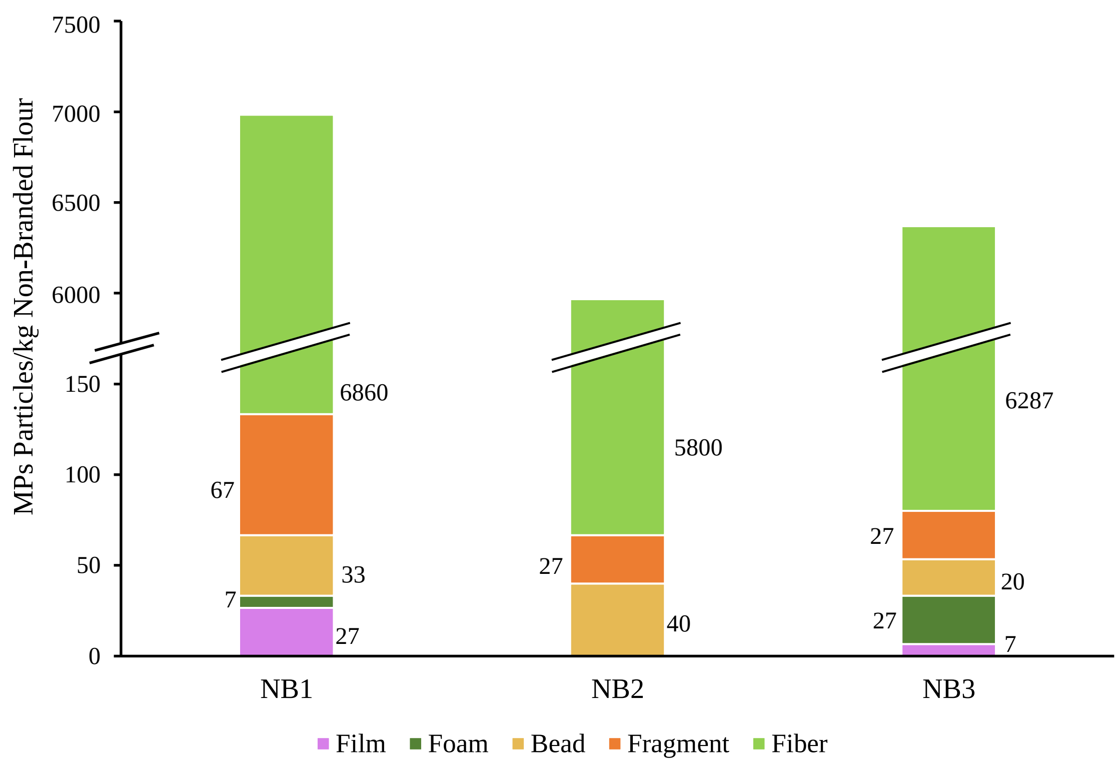


**Figure 4.4:** Composition of MPs morphotype (fiber, fragment, bead, foam, film) per 1kg non-branded Flour in Dhaka City, Bangladesh

### 4.1.2 Morphotype, Size and Color Composition of MPs in Flour Samples

The identified microplastics in the flour samples had different morphotypes, sizes and colors. The morphotypes of the microplastics were divided into 5 types that include fiber, fragment, bead, foam and film. The size ranges include < 100 µm, 100 – 200 µm, 200 – 400 µm, 400 – 600 µm, 600 – 800 µm, 800 – 1000 µm, 1000 – 1500 µm, 1500-2000 µm and > 2000 µm. MPs smaller than 600 µm were considered as small microplastics and larger than 600 µm were considered as larger microplastics to determine the composition of each type. Finally, the colors that were identified include red, blue, white, brown, transparent, purple, pink, gray, black, green, yellow and orange.

The percent compositions of each type of microplastics were determined to make a comparison among them and understand the occurrence or dominance of any particular type and identify the potential cause.

#### 4.1.2.1 Morphotype Composition

Five different types of morphotypes of microplastics were found to be present in the collected flour samples whose percent composition has been presented in the following pie chart **(Figure 4.5).** The percent compositions of these morphotypes have been found to be 98.84% for fibers, 0.69% for fragments, 0.42% for beads, 0.30% for foams and 0.12% for films; which concludes the comparative composition of morphotypes to be fiber>fragment>bead>foam>film. As we can see, the most prominent type of microplastic was found to be fibers. Although the exact source of these type of microplastics is unknown, it can be assumed that a plausible source of fiber contamination might be the polythene and polypropylene packaging bags used for the storage, transport, preservation and protection of flours. These bags can easily release microplastic to the flour they contain inside due to friction, or mechanical damage etc. Contamination from air and water during production in the flourmill might be another potential cause of different types of MPs contamination. In addition, the non-branded flours are sold openly in the market without any cover making them prone to microplastic contamination from air.

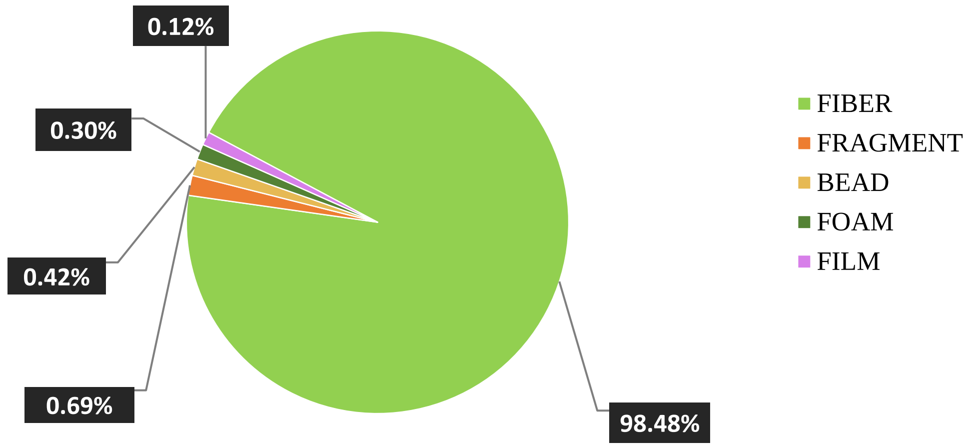


**Figure 4.5:** Morphotype composition of microplastics found in the branded and non-branded flour samples collected from Dhaka City, Bangladesh

#### 4.1.2.2 Size Composition

Among all the identified MPs particles in all branded and non-branded samples, 18% of the microplastic particles were found to be in 200-400 µm range, 15% in 1000-1500 µm range, 14% in 400-600 µm range, 12% were >2000 µm, 11% in 600-800 µm range, 10% in 800-100 µm range, 9% in 100-200 µm range, 9% in 1500-2000 µm range and 2% were <100 range **(Figure 4.6).**

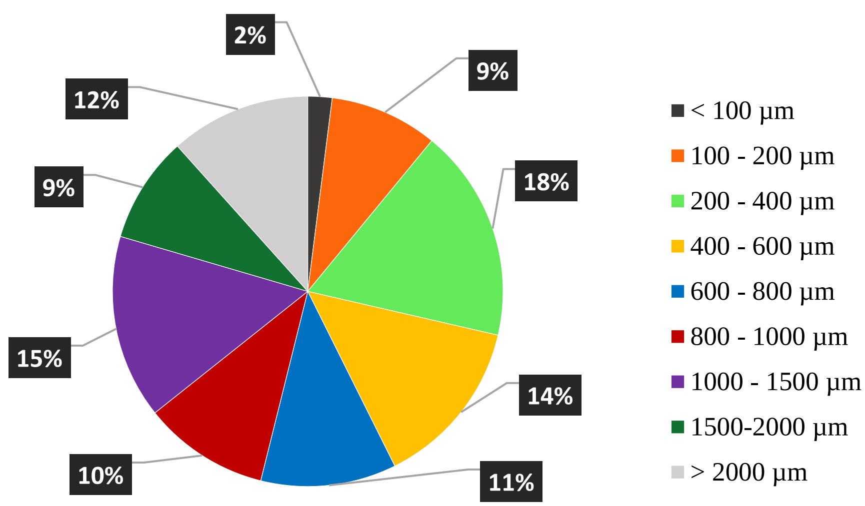


**Figure 4.6:** Size composition of microplastics found in the branded and non-branded flour samples collected from Dhaka City, Bangladesh

The size ranges of the microplastics identified in different flour samples were again divided into two categories considering microplastics <600μm to be small microplastics and microplastics >600μm to be large microplastics. The pie chart below **(Figure 4.7)** shows that 42% of large microplastics and 58% of small microplastics have been found indicating the predominance of larger MPs. A Student's T test has determined that the percentage difference of smaller and larger microplastics to be significant; $t\ (34) = -11.419$, $p < 0.001$. The smaller microplastics have more potential to transfer into different organs of the human body. However, the dominance of larger microplastics found in the flour samples might be due to the much higher abundance of fiber type of microplastics as fibers are usually larger in length than the other four types of microplastics.

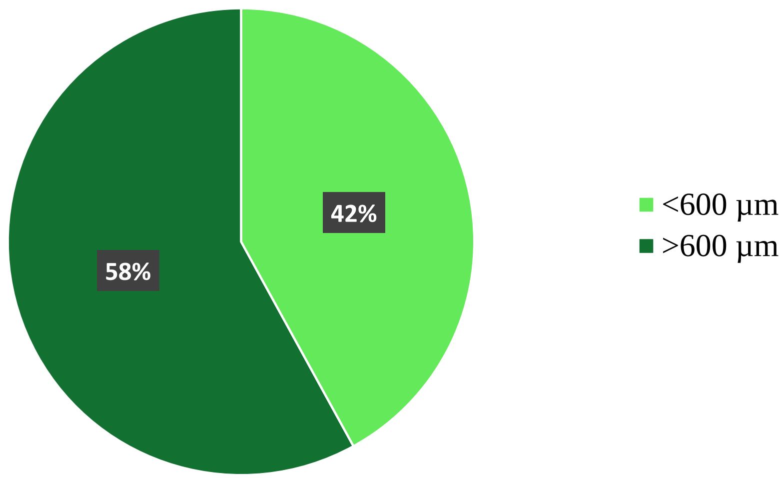


**Figure 4.7:** Comparison between the mean percentage of small MPs and large MPs found in the flour samples collected from Dhaka City, Bangladesh.

#### 4.1.2.3 Color Composition

12 different colors of microplastic particles were identified in the flour samples collected from Dhaka City, Bangladesh. 38% of the MPs were transparent, 11% pink, 10% blue, 7% red, 9% orange, 6% purple, 5% gray, 4% yellow, 3% brown, 3% green, 3% white and 1% black (Figure 4.8). The most prevalent color of microplastics was identified to be transparent which might be because of the transparent colored polythene and polypropylene bags used in the packaging of flour. These bags are often of pink, red, blue, orange and other attractive colors for marketing purposes. Many transparent or white packaging bags include colored writings as well which may contribute to the different colors of fibers and other type of microplastics discovered in the flour samples.

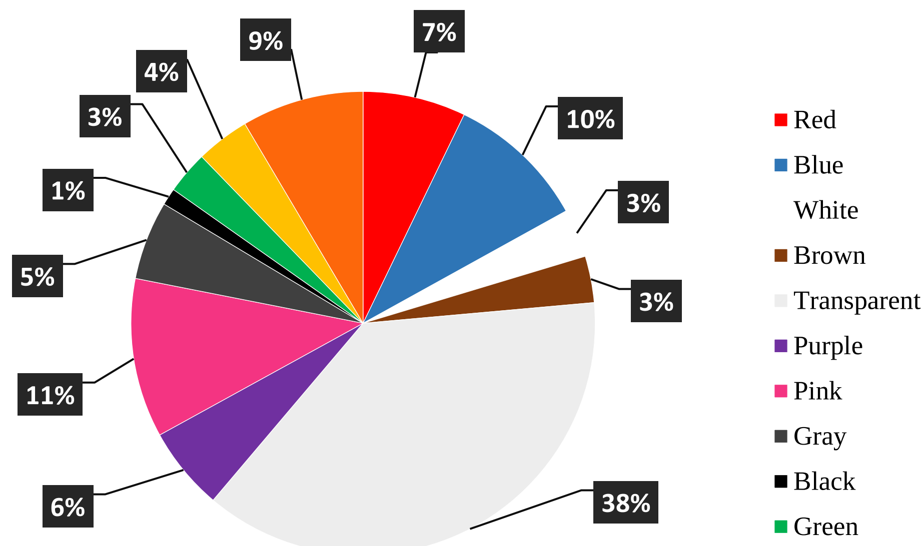


**Figure 4.8:** Color composition of microplastics found in the branded and non-branded flour samples collected from Dhaka City, Bangladesh

### 4.1.3 Comparative Analysis of Microplastic Contamination between Branded and Non-Branded Flour Samples

#### 4.1.3.1 Comparison of MPs Abundance

The mean abundance of microplastics have been found to be 2747 ± 654 (mean±SD) particles/kg in branded flour samples and 6409 ± 625 (mean±SD) in non-branded flour samples **(Figure 4.9).** The mean abundance of microplastics in non-branded flour samples is about 2.3x higher than the mean abundance of microplastics in branded flour samples. A student's T test has been performed to compare the mean abundance of branded and non-branded flour samples and a significant difference has been identified; $t\ (16) = -12.149$, $p < 0.001$.

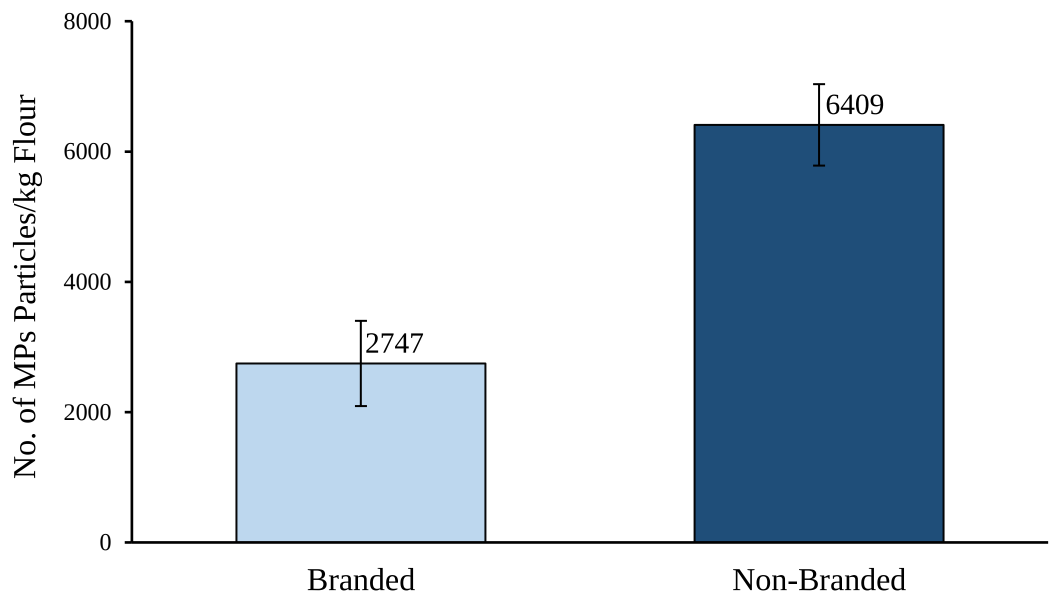


**Figure 4.9:** Comparison between the number of microplastic particles/kg found in different branded and non-branded flour samples collected from Dhaka City, Bangladesh

#### 4.1.3.2 Percent Comparison of MPs Morphotype

The percent composition of different morphotypes in branded and non-branded flour samples have been shown in the following graph **(Figure 4.10).** Both types of samples contained all 5 types of morphotypes at different percentages. Fiber has been found to be the most frequent (99.11% and 98.54% respectively) type of MPs in both branded and non-branded flour samples and film has been found to have the least frequency (0.08% and 0.17% respectively) for both types. Student's T test have been conducted to determine if there was any significant difference in the percentage of five different morphotypes of microplastics in branded and non-branded flour samples. However, no significant difference has been identified between the two in morphotype percentage composition.

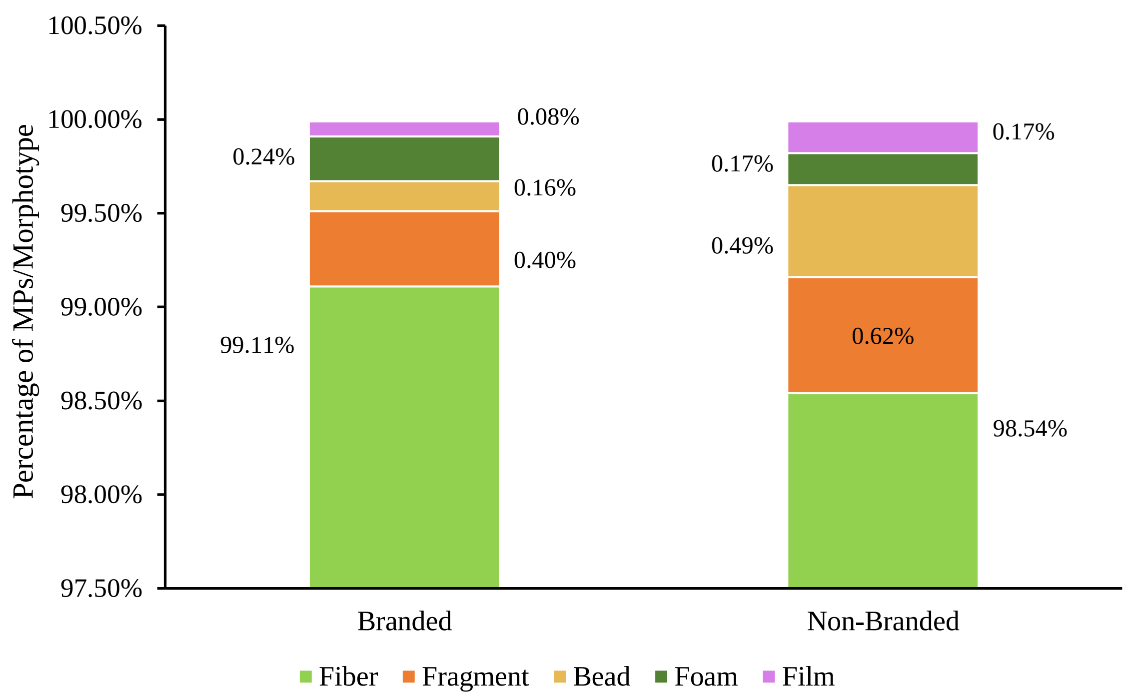


**Figure 4.10:** Comparison between the percentages of different morphotype of MPs found in the branded and non-branded flour samples collected from Dhaka City, Bangladesh.

#### 4.1.3.3 Percent Comparison of MPs Size

The percent composition of the size ranges of the microplastics identified in different branded and non-branded flour samples have been determined compared **(Figure 4.11).** Student's T test has been conducted to determine any observable difference in the percentage of MPs of different size ranges in branded and non-branded flour samples. Interestingly, no significant difference has been identified in any size range between branded and non-branded flour samples.

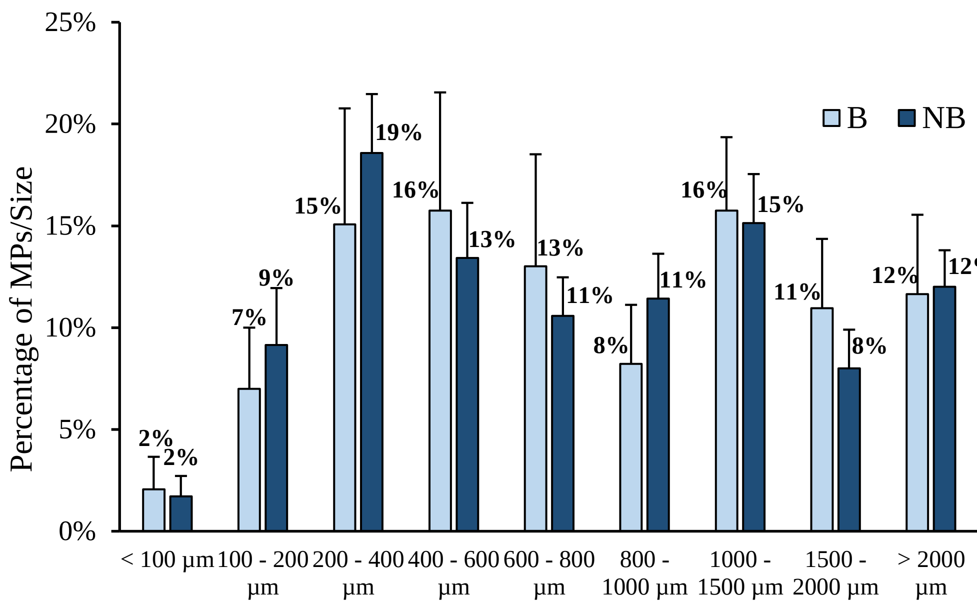


**Figure 4.11:** Percentage of microplastics/size and variation between the branded and non-branded flour samples collected from Dhaka City, Bangladesh

The microplastics found in the branded and non-branded flour samples have been divided into smaller microplastics (<600 µm) and larger microplastics (>600 µm). The percentage of smaller and larger microplastics in branded and non-branded flour samples have been compared. Both types of samples have been found to have higher percentage of larger microplastics (59% and 57% respectively) and lower percentage of smaller microplastics (41% and 43% respectively**) (Figure 4.12).** The student's T test has found this occurrence to be significant for both branded and non-branded samples; $t$ (14) = -7.474, $p < 0.001$ for branded samples, $t$ (16) = -7.161, $p < 0.001$ for non-branded samples.

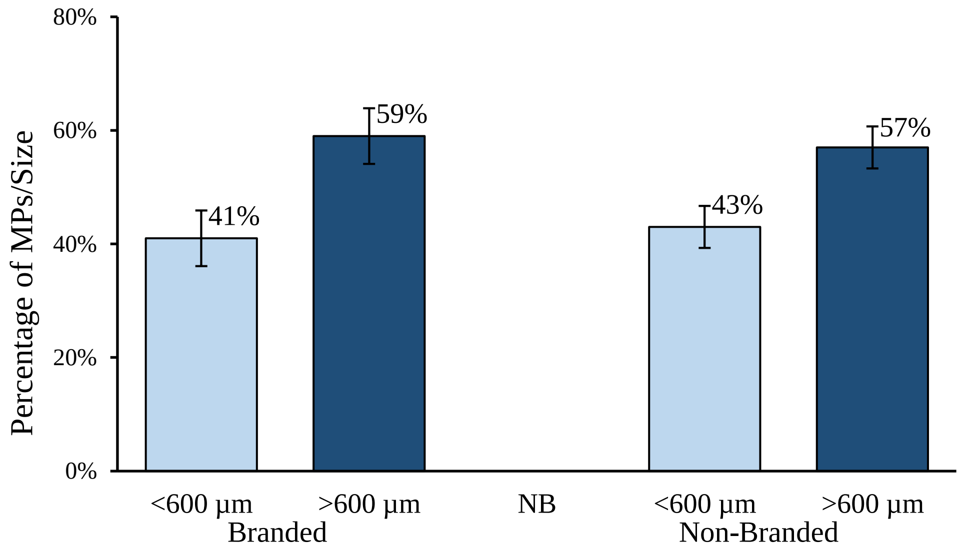


**Figure 4.12:** Comparison between the percentages of small MPs and large MPs found in the branded and non-branded flour samples collected from Dhaka City, Bangladesh.

#### 4.1.3.4 Percent Comparison of MPs Color

The percent composition of the different colors or microplastics found in the branded and non-branded flour samples have been compared **(Figure 4.13)** and the transparent microplastics have been found to be the most abundant (28% and 44% respectively) in both branded and non-branded samples. The black colored microplastics have been found to be the least abundant (2% and 1% respectively) in both branded and non-branded flour samples. Student's T test has been conducted to determine any significant difference in the percentage of MPs of different colors in branded and non-branded flour samples. Significant difference ($p$<0.001) has been observed in the percentage of red, white, transparent and pink colored microplastics of branded and non-branded flour samples. Higher percentage (11% and 7% respectively) of red and white MPs have been observed in branded flour samples and lower percentage (4% and 1% respectively) in non-branded flour samples. The probable cause might be the common use of attractive and colorful packaging in branded flour samples. On the other hand, lower percentage (28% and 8% respectively) of transparent and pink MPs have been observed in branded flour samples and higher percentage (44% and 13% respectively) in non-branded flour samples. Transparent polythene and polypropylene bags and pink plastic ropes are frequently used in packaging of non-branded local flour in Dhaka City, which might be a probable cause for this significant difference.

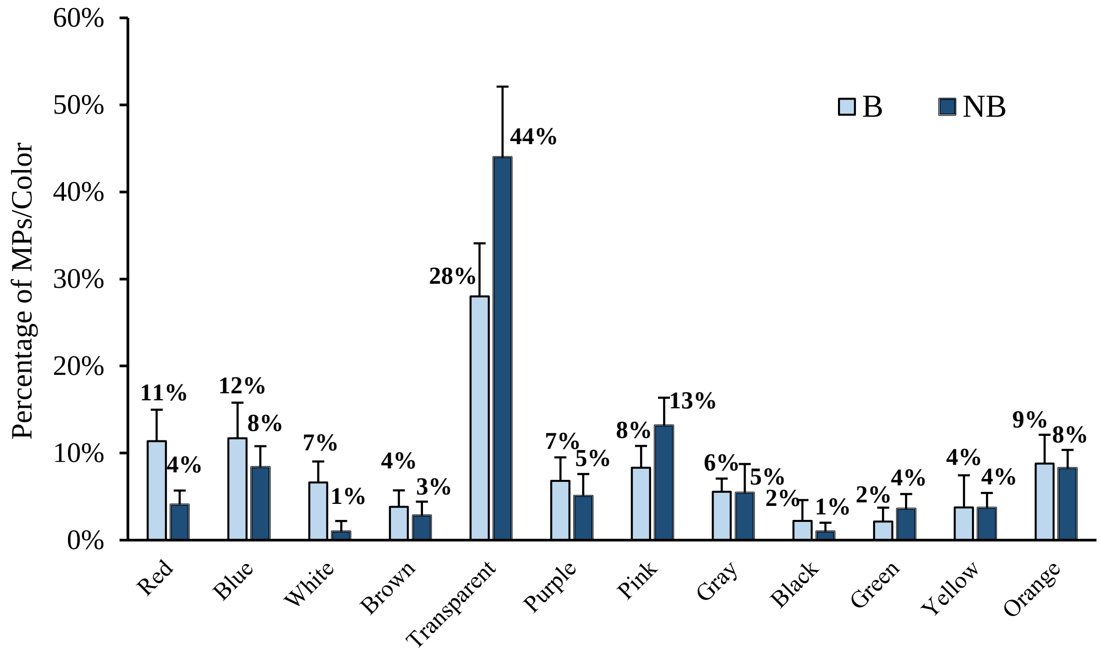


**Figure 4.13:** Percentage of Microplastics/Color and variation between the Branded and Non-Branded Flour samples collected from Dhaka City, Bangladesh

### 4.1.4 Chemical Group Identification of MPs using FTIR Spectroscopy

A total of 12 individual types of microplastic polymers were identified from the FTIR analysis of the microplastics identified in the flour samples. The presence of the microplastic polymers were identified by observing the peak values of the FTIR graph and comparing them with the absorption bands for polymer identification (Jung et al., 2018). The identified polymers include Acrylonitrile Butadiene Styrene (ABS), Ethylene Vinyl Acetate (EVA), High Density Polyethylene (HDPE), Low Density Polyethylene (LDPE), Latex, Nitrile, Nylon, Polycarbonate (PC), Polymethyl Methacrylate (PMMA), Polypropylene (PP), Polystyrene (PS) and Polyurethane (PU).

The obtained peak values in **Figure 4.14** were 3032, 2845, 2806, 2226, 1740, 1652, 1544, 1451, 1396, 1077, 895, 709 and 654. After comparing the peak values with the absorption bands for polymer identification (Jung et al., 2018), the microplastic polymers were identified to be ABS, EVA, HDPE, LDPE, Nitrile, Nylon, PC, PMMA, PP, PS and PU.

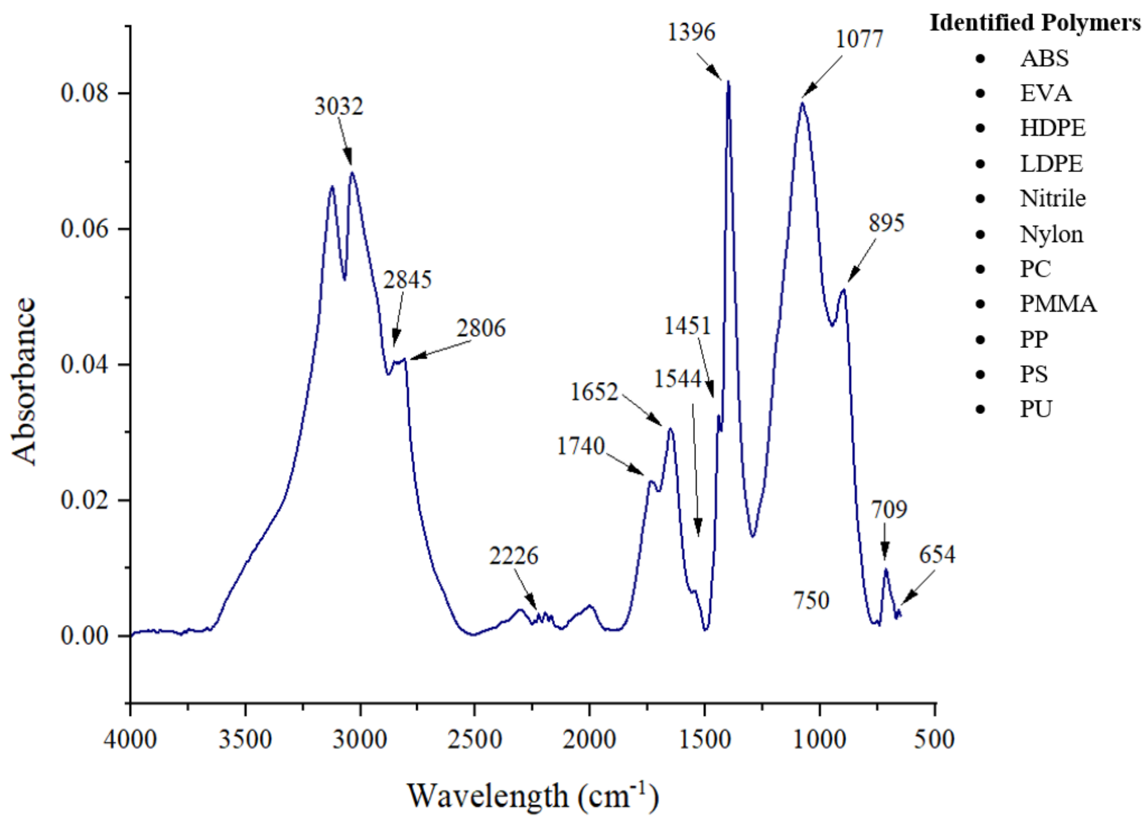


**Figure 4.14:** FTIR spectra of the MPs found in the flour samples. Probable polymer types include ABS, EVA, HDPE, LDPE, Nitrile, Nylon, PC, PMMA, PP, PS and PU.

The obtained peak values in **Figure 4.15** were 3028, 2830, 2222, 1734, 1656, 1396, 1057, 920, 685 and 655. After comparing the peak values with the absorption bands for polymer identification (Jung et al., 2018), the microplastic polymers were identified to be ABS, EVA, HDPE, LDPE, Nitrile, Nylon, PC, PMMA, PP, PS and PU.

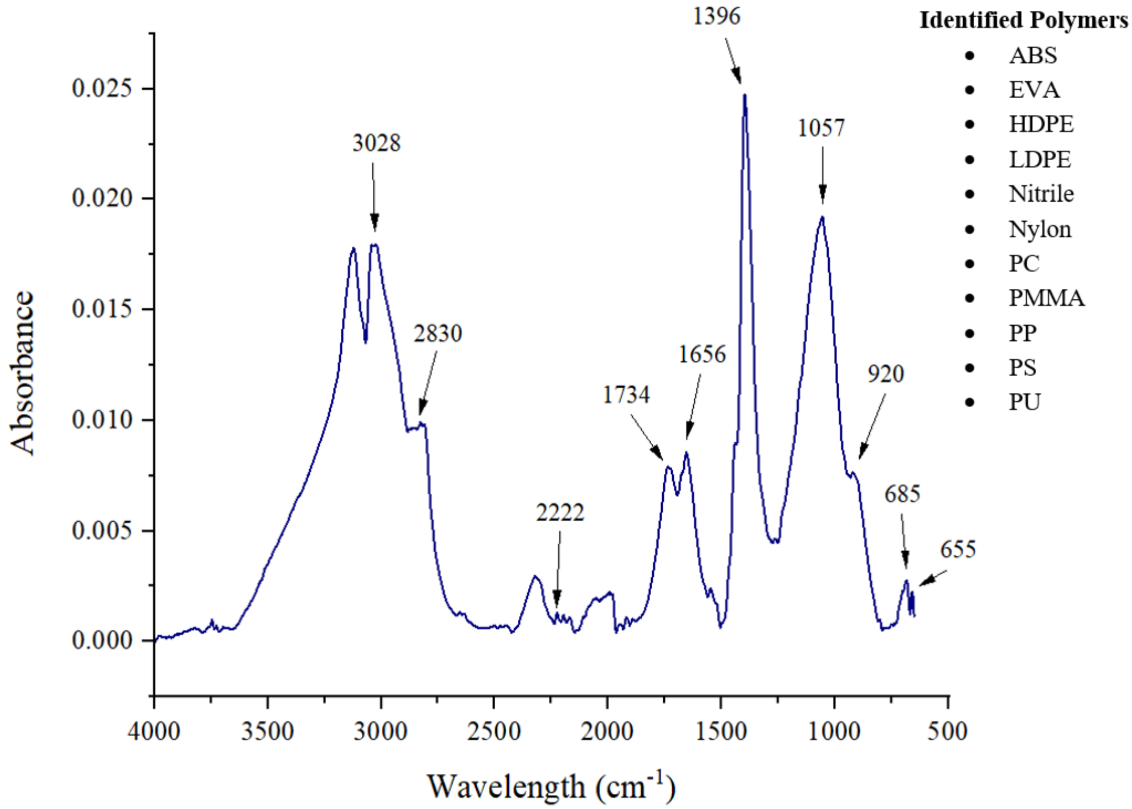


**Figure 4.15:** FTIR spectra of the MPs found in the flour samples. Probable polymer types include ABS, EVA, HDPE, LDPE, Nitrile, Nylon, PC, PMMA, PP, PS and PU.

The obtained peak values in **Figure 4.16** were 3016, 2972, 2854, 2825, 2221, 1911, 1739, 1209, 1061, 900, 747, 683 and 654. After comparing the peak values with the absorption bands for polymer identification (Jung et al., 2018), the microplastic polymers were identified to be ABS, EVA, HDPE, LDPE, Latex, Nitrile, Nylon, PC, PMMA, PP, PS and PU.

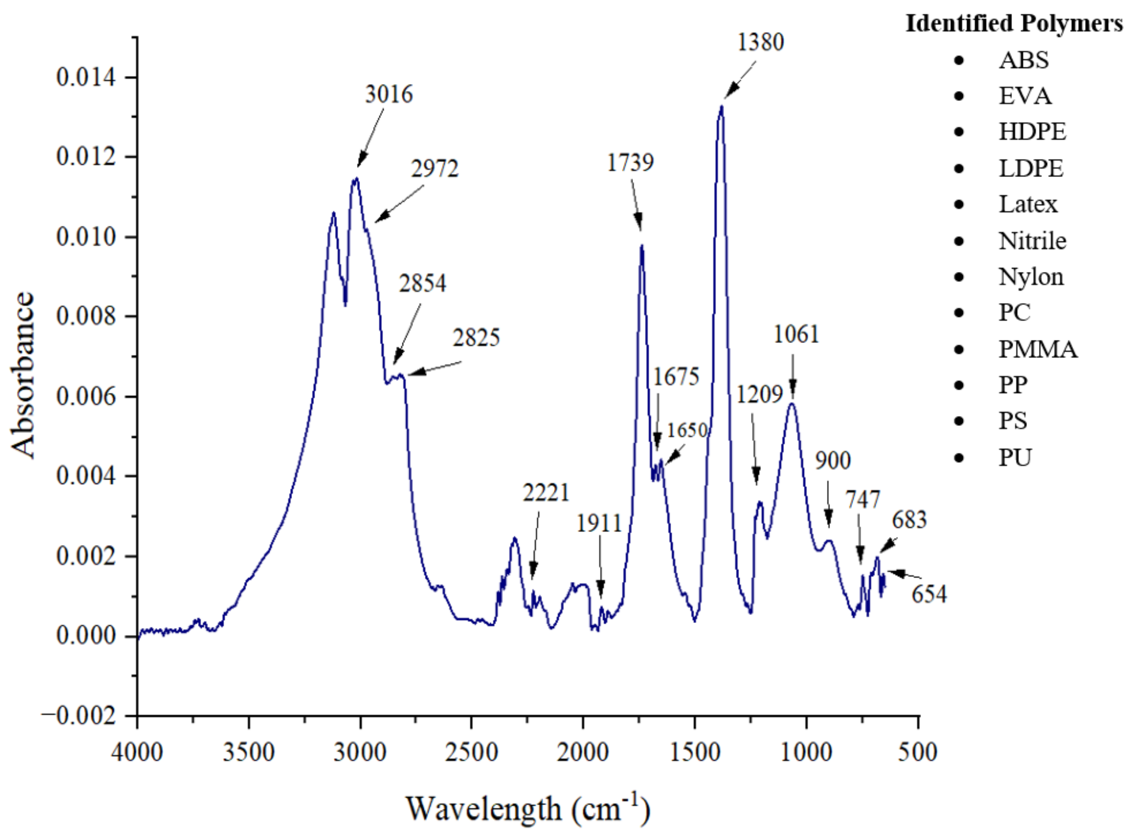


**Figure 4.16:** FTIR spectra of the MPs found in the flour samples. Probable polymer types include ABS, EVA, HDPE, LDPE, Latex, Nitrile, Nylon, PC, PMMA, PP, PS and PU.

The sources of HDPE, LDPE, PP, and PS can be traced back to the use of plastic bags used in the storage, transportation, packaging of flour. Sources of other types of microplastic contamination might include the use of plastic equipment in the flourmill and contamination from air. ABS and HDPE were shown to have the highest potential to endanger human health among all the identified polymers (Yuan et al., 2022). Significant immunotoxicity was generated by ABS exposure for peripheral blood mononuclear cells (Han et al., 2020). Analogous cytotoxic effects were also observed in various human cell types exposed to plastic PE (Choi et al., 2021) and Nylon (Sivagami et al., 2021). Furthermore, it has been discovered that exposure to high PS concentrations is harmful to human lung cells, raising the chance of developing pulmonary illnesses (Dong et al., 2020). Furthermore, it has been found that 44 nm PS nanoparticles significantly enhanced the expression of the IL-6 and IL-8 genes, which are important pro-inflammatory molecules in the body, in human gastric cancer cells (Forte et al., 2016). Once more, the T98G cell line was the only one in which treatment to PE microplastics increased the production of ROS (Reactive Oxygen Species), but exposure to PS microplastics increased the production of ROS in both human glioblastoma multiforme cells and human cervical cancer cells (Schirinzi et al., 2017). Accordingly, exposure to microplastics causes an increase in oxidative stress in colon and small intestine epithelial cells (Zhang et al., 2022) as well as lung epithelial cells (Dong et al., 2020). It also increases the generation of reactive oxygen species (ROS) in brain and epithelial cells. Thus, it can be concluded that microplastics have the potential to seriously endanger human health, depending on the type of polymer they are made of.

### 4.1.5 Probable Health Impact Estimation of Microplastic Contamination

In this investigation, human exposure to microplastics through the ingestion of flour has been evaluated **(Table 4.2).** This study estimates the annual intake of microplastic particles per person through the ingestion of Flour in Dhaka City, Bangladesh, which was calculated using **Equation (2)** mentioned in Chapter 3, Methodology.

**Table 4.2:** Estimated annual intake of microplastic particles per person through the ingestion of flour in Dhaka City, Bangladesh

| Parameters | Minimum Value | Maximum Value | Average Value | Reference |
|---|---|---|---|---|
| Number Of Daily Usage (DU-N) | 01 | 02 | 1.5 | Estimated |
| Weight Of Flour Required in Single Use (WF-SU), g | 60 | 120 | 90 | Estimated |
| Number Of MPs Particles Per Unit (MPs-NPU), g | 2.954 ≈ 3 | 6.562 ≈ 7 | 4.578 ≈ 5 | Author's Finding (Mean MPs Particles/kg±SD) |
| Total Days In A Year (D-Y) | 365 | 365 | 365 | |
| Estimated Annual Intake Of MPs Per Person (EAI), Particle/Person/Year | 65,700 | 613,200 | 246,375 | Author's Finding |

The maximum and minimum values number of daily usage of flour and the maximum and minimum weight of flour required in single use were estimated based on the consumption habit of local people in Dhaka City, Bangladesh. This study has found the mean amount of microplastics in per kg flour sample to be 4578 ± 1984 (mean±SD) particles/kg. Considering a Standard Deviation of 1984 particles/kg, the minimum and maximum value of MPs per kg flour was calculated to be 2954 particles/kg and 6562 particles/kg respectively. The value was mathematically converted to determine the amount of MPs present per grams of flour and used in the equation. As per the estimation in this study, the average annual intake of microplastic particles per person from the ingestion of flour has been found to be 246,375 particles/person/year with a minimum value of 65,700 particles/person/year and a maximum value of 613,200 particles/person/year.

Microplastics can affect the digestive, respiratory, endocrine, reproductive, and immunological systems in humans, according to the findings of cellular and animal studies (Lee et al., 2023). When microplastics are consumed, they first impact the digestive systems. Physical irritation of the gastrointestinal tract can lead to inflammation, which in turn causes a variety of gastrointestinal symptoms. In terms of the respiratory system, breathing in microplastics may result in oxidative stress in the lungs and airways, which can cause respiratory symptoms like fatigue and dizziness from low blood oxygen concentration, as well as respiratory symptoms like coughing, sneezing, and shortness of breath owing to inflammation and damage (Wright & Kelly, 2017). Furthermore, microplastics disrupt hormone production, release, transport, metabolism, and elimination. This can result in metabolic disorders, developmental disorders, and even reproductive disorders (i.e., infertility, miscarriage, and congenital malformations) as well as endocrine disruption (Vandenberg et al., 2017). Based on the health exposure assessment presented in Table 4.2, it can be determined that residents of Dhaka City, Bangladesh who regularly consume flour are exposed to substantial health hazards of microplastics.

# CHAPTER FIVE

# CONCLUSIONS AND RECOMMENDATIONS

## 5.1 Conclusions and Recommendations

With the growing production and usage of plastics in our society, the risk of microplastic contamination has also been increasing day by day. The current study identified the presence of microplastic contamination in branded and non-branded flour consumed by the people of Dhaka City, Bangladesh, which confirms the hypothesis of our research. All the branded and non-branded flour have been identified to contain microplastics in a significant amount. Among all six flour samples, sample NB1 was found to have the highest abundance of microplastic particle/kg, which was collected from Kawranbazar, Dhaka. Among the branded samples, sample B2 has been found to have the highest abundance of microplastic particle/kg. All five morphotypes of microplastics including fiber, fragment, bead, foam and film have been identified with fiber having the highest prevalence. Several colors and size composition has been determined that indicated dominance of transparent and large microplastics (>600µm). Different polymeric composition of microplastics have also been discovered that include PP, PS, HDPE, LDPE etc. whose sources might be the use of plastic packaging made of such polymers. Furthermore, non-branded flours have been found to have a significant higher abundance of microplastics than branded flour samples, which indicates that the source of microplastic contamination is associated with the production, handling, transportation and storage process of flour in Bangladesh. Furthermore, the estimation of health exposure to microplastics present in flour indicated alarming health risk of the flour consumers of Dhaka City, Bangladesh. The evidence presented underscores the ubiquity of microplastics in flour, highlighting the urgent need for comprehensive strategies to mitigate their impact on our daily diet.

Flour being a major source of carbohydrate in Bangladesh the microplastic contamination in flour has raised an alarming situation. The source of microplastic contamination requires proper investigation and identification to prevent the contamination from its root. Use of plastic materials and packets in flour production, handling and sale might be the most potential source, which requires primary concern. One fundamental step towards addressing microplastic pollution through flour packaging involves a shift towards alternative packaging materials. Traditional plastics pose significant environmental threats, and the development and utilization of more sustainable alternatives can help alleviate this issue. Biodegradable plastics, for instance, break down into natural components over time, reducing the risk of microplastic pollution. Additionally, compostable packaging made from plant-based sources can serve as an environmentally friendly alternative. The adoption of paper-based packaging is another viable option, offering both biodegradability and recyclability. Consideration should also be given to multi-material packaging solutions. Laminates that incorporate a layer of recyclable or biodegradable material alongside a protective layer can strike a balance between functionality and environmental impact. This approach ensures that the protective properties of packaging are maintained while minimizing the ecological footprint. In addition, awareness empowers consumers to make informed choices, encouraging the adoption of packaging materials that have a lower ecological impact regardless of its economic impacts. Understanding the life cycle of products and the persistence of plastic in the environment motivates individuals to seek out and support alternatives.

Recognizing the complexity of the issue, a holistic approach that combines rigorous monitoring, scientific research, and regulatory measures is imperative. Standardized testing protocols should be established to assess the levels of microplastics in different food categories, enabling accurate risk assessments and informed policymaking. Moreover, technology offers practical applications for sustainable packaging alternatives contamination prevention strategies. From biodegradable plastics to advanced recycling processes, technological solutions pave the way for environmentally friendly options. Integrating these technologies into the management of plastic packaging for food items including flour is crucial for achieving meaningful reductions in microplastic contamination. By fostering a symbiotic relationship between research initiatives and technological innovations, a continuous cycle of improvement and adaptation can be established. Additionally, further research is essential to understand the long-term health effects of ingesting microplastics, particularly in relation to bioaccumulation and potential toxicological impacts.

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## APPENDICES

### APPENDIX A: Supplementary Tables

**Table A1:** Quantification of Microplastics in the Flour Samples (branded samples and non-branded samples) including their Statistical Analysis.

| Sample Type | Sample | Sample ID | TMPs (particles/5gm) | TMPs/ Replication (particles/1kg) | TMPs/Sample (particles/1kg) Mean ± SD | TMPs/Type (particles/1kg) Mean ± SD |
|---|---|---|---|---|---|---|
| Branded Flour | B1 | B1a | 134 | 2680 | 2347 ± 289 | 2747 ± 654 |
| | | B1b | 109 | 2180 | | |
| | | B1c | 109 | 2180 | | |
| | B2 | B2a | 189 | 3780 | 3067 ± 620 | |
| | | B2b | 138 | 2760 | | |
| | | B2c | 133 | 2660 | | |
| | B3 | B3a | 119 | 2380 | 2827 ± 916 | |
| | | B3b | 111 | 2220 | | |
| | | B3c | 194 | 3880 | | |
| Non-Branded Flour | NB1 | NB1a | 332 | 6640 | 6993 ± 319 | 6409 ± 625 |
| | | NB1b | 354 | 7080 | | |
| | | NB1c | 363 | 7260 | | |
| | NB2 | NB2a | 291 | 5820 | 5867 ± 214 | |
| | | NB2b | 305 | 6100 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | NB2c | 284 | 5680 | | |
| | NB3 | NB3a | 282 | 5640 | 6367 ± 677 | |
| | | NB3b | 324 | 6480 | | |
| | | NB3c | 349 | 6980 | | |
| **Mean Abundance of MPs in Flour in Dhaka, Bangladesh (particles/kg)** | | | | | | 4578 ± 1984 |
| **One-way ANOVA test** (B1, B2, B3, NB1, NB2, NB3) ; F = 39.5849; p < 0.0001<br>**Student's T test** (Branded, Non-Branded) ; T = -12.149; p < 0.0001 | | | | | | |

**Table A2:** Quantification of Microplastics/Morphotypes in the branded samples and non-branded flour samples.

| Sample Type | Sample | Fiber/kg Mean ± SD | Fragment/kg Mean ± SD | Bead/kg Mean ± SD | Foam/kg Mean ± SD | Film/kg Mean ± SD |
|---|---|---|---|---|---|---|
| **Branded** | **B1** | 2320 ± 295 | 13 ± 13 | 7 ± 12 | 7 ± 12 | 0 |
| | **B2** | 3047 ± 636 | 7 ± 12 | 7 ± 12 | 7 ± 12 | 0 |
| | **B3** | 2800 ± 922 | 13 ± 12 | 0 | 7 ± 12 | 7 ± 12 |
| **Non-Branded** | **NB1** | 6860 ± 370 | 67 ± 31 | 33 ± 23 | 7 ± 12 | 27 ± 12 |
| | **NB2** | 5800 ± 240 | 27 ± 12 | 40 ± 40 | 0 | 0 |
| | **NB3** | 6287 ± 685 | 27 ± 12 | 20 ± 0 | 27 ± 31 | 7 ± 12 |

**Table A3:** Percentage of MPs/Morphotype in the Flour Samples (all samples, branded samples, non-branded samples) collected from Dhaka, Bangladesh including their Statistical Analysis

| Morphotype | Percentage of MPs/Morphotype (all samples) Mean ± SD | Percentage of MPs/ Morphotype (Branded samples) Mean ± SD | Percentage of MPs/ Morphotype (Non-Branded samples) Mean ± SD | Student's T test (B, NB) |
|---|---|---|---|---|
| **Fiber** | 98.48 ± 0.94 | 99.11 ± 1.41 | 98.54 ± 0.41 | t = -0.199<br>p = 0.860 |
| **Fragment** | 0.69 ± 0.51 | 0.40 ± 0.74 | 0.62 ± 0.30 | t = 0.347<br>p = 0.751 |
| **Bead** | 0.42 ± 0.30 | 0.16 ± 0.42 | 0.49 ± 0.19 | t = -0.555<br>p = 0.618 |
| **Film** | 0.30 ± 0.29 | 0.24 ± 0.34 | 0.17 ± 0.22 | t = 1.081<br>p = 0.340 |
| **Foam** | 0.12 ± 0.16 | 0.08 ± 0.14 | 0.17 ± 0.20 | t = -0.575<br>p = 0.596 |

**Table A4:** Percentage of MPs/Color in the Flour Samples (all samples, branded samples, non-branded samples) collected from Dhaka, Bangladesh including their statistical analysis.

| Color | Percentage of MPs/Color (all samples) Mean ± SD | Percentage of MPs/Color (Branded samples) Mean ± SD | Percentage of MPs/Color (Non-Branded samples) Mean ± SD | Student's T test (B, NB) |
|---|---|---|---|---|
| **Red** | 7 ± 4.7 | 11 ± 3.6 | 4 ± 1.6 | t = 5.783<br>p < 0.001 |
| **Blue** | 10 ± 3.7 | 12 ± 4.1 | 8 ± 2.4 | t = 2.242<br>p = 0.039 |
| **White** | 3 ± 3.2 | 7 ± 2.4 | 1 ± 1.2 | t = 5.652<br>p < 0.001 |
| **Brown** | 3 ± 1.8 | 4 ± 1.9 | 3 ± 1.6 | t = 1.355<br>p = 0.194 |
| **Transparent** | 38 ± 11.0 | 28 ± 6.1 | 44 ± 8.1 | t = -4.879<br>p < 0.001 |
| **Purple** | 6 ± 2.7 | 7 ± 2.7 | 5 ± 2.5 | t = 1.362<br>p = 0.192 |
| **Pink** | 11 ± 3.8 | 8 ± 2.5 | 13 ± 3.2 | t = -3.692<br>p < 0.001 |
| **Gray** | 5 ± 2.5 | 6 ± 1.5 | 5 ± 3.3 | t = 0.462<br>p = 0.65 |
| **Black** | 1 ± 2.0 | 2 ± 2.4 | 1 ± 1.0 | t = 1.894<br>p = 0.077 |
| **Green** | 3 ± 1.8 | 2 ± 1.6 | 4 ± 1.7 | t = -1.97<br>p = 0.066 |
| **Yellow** | 4 ± 2.8 | 4 ± 3.7 | 4 ± 1.7 | t = 0.247<br>p = 0.808 |
| **Orange** | 9 ± 2.7 | 9 ± 3.3 | 8 ± 2.1 | t = 0.341<br>p = 0.737 |

**Table A5:** Percentage of MPs/Size in the Flour Samples (all samples, branded samples, non-branded samples) collected from Dhaka, Bangladesh including their Statistical Analysis.

| **Size Range** | **Percentage of MPs/Size (all samples) Mean ± SD** | **Percentage of MPs/Size (Branded samples) Mean ± SD** | **Percentage of MPs/Size (Non-Branded samples) Mean ± SD** | **Student's T test (B, NB)** |
|---|---|---|---|---|
| **< 100 µm** | 2 ± 1.3 | 2 ± 1.6 | 2 ± 1.0 | t = 0.898<br>p = 0.382 |
| **100 - 200 µm** | 9 ± 2.9 | 7 ± 3.0 | 9 ± 2.8 | t = -1.058<br>p = 0.306 |
| **200 - 400 µm** | 18 ± 4.7 | 15 ± 5.7 | 19 ± 2.9 | t = -1.405<br>p = 0.179 |
| **400 - 600 µm** | 14 ± 4.5 | 16 ± 5.8 | 13 ± 2.7 | t = 0.833<br>p = 0.417 |
| **600 - 800 µm** | 11 ± 4.3 | 13 ± 5.5 | 11 ± 1.9 | t = 1.663<br>p = 0.116 |
| **800 - 1000 µm** | 10 ± 3.1 | 8 ± 2.9 | 11 ± 2.2 | t = -2.195<br>p = 0.01 |
| **1000 - 1500 µm** | 15 ± 3.0 | 16 ± 3.6 | 15 ± 2.4 | t = 0.381<br>p = 0.708 |
| **1500 - 2000 µm** | 9 ± 3.0 | 11 ± 3.4 | 8 ± 1.9 | t = 2.154<br>p = 0.047 |
| **> 2000 µm** | 12 ± 3.0 | 12 ± 3.9 | 12 ± 1.8 | t = -0.389<br>p = 0.702 |

**Table A6:** Comparison between the Percentages of Small MPs and Large MPs found in the Flour Samples (all samples, branded samples, non-branded samples) collected from Dhaka City, Bangladesh including their Statistical Analysis.

| **Sample Type** | **Percentage of Small MPs <600 µm SD±mean** | **Percentage of Large MPs >600 µm SD±mean** | **Student's T test (Small MPs, Large MPs)** |
|---|---|---|---|
| **Branded samples** | 41 ± 4.9 | 59 ± 4.9 | t = -7.474<br>p < 0.0001 |
| **Non-Branded samples** | 43 ± 3.7 | 57 ± 3.7 | t = -7.161<br>p < 0.0001 |
| **All samples** | 42 ± 4.3 | 58 ± 4.3 | t = -11.419<br>p < 0.0001 |