CHARACTERIZATION OF A FIBRE-RICH POWDER PREPARED BY LIQUEFACTION OF UNRIPE BANANA FLOUR AND FORMULATION OF FIBRE-RICH BISCUITS

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Department of Applied Food Science and Nutrition

Faculty of Food Science and Technology

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

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The Author

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List of Abbreviations

UBF	Unripe Banana Flour
FRP	Fibre-Rich Powder
DPPH	2,2-diphenyl-hydrazyl-hydrate
E	Extinction Coefficient
GAE	Gallic Acid Equivalent
TPC	Total Phenolic Content
TFC	Total Flavonoid Content
TE	Trolox Equivalent
QE	Quercetin
UV	Ultra Violet
TS	Total Starch
RS	Resistant Starch
WHC	Water Holding Capacity
OHC	Oil Holding Capacity
W/W	Weight per Weight
WHO	World Health Organization
FAO	Food and Agriculture Organization
BBS	Bangladesh Bureau of Statistics
GDP	Gross Domestic Product
USDA	United States Department of Agriculture
LDL	Low-Density Lipoprotein
CVD	Cardiovascular Disease
BPH	Benign Prostate Hperplasia
ROS	Reactive Oxygen Species
RDS	Rapidly Digestible Starch
SDS	Slowly Digestible Starch
SCFA	Short Chain Fatty Acid
SDFs	Slowly Digestible Fibers
GI	Glycemic Index
PPO	Polyphenol Oxidase
BF	Banana Flour
IF	Insoluble Fiber

HI	Hydrolysis Index
SIF	Soluble Ingestible Fiber
rpm	Revolutions per minute
AOAC	Association of Official Agriculture Chemists

Abstract

Unripe banana flour is high in resistant starch and other essential chemicals, has recently been used to make a variety of innovative foodstuffs. The principle aim of this study was to evaluate some chemical and physicochemical properties of a fibre-rich powder prepared by liquefaction of unripe banana flour. Two flour samples prepared were subjected to analysis for determination of proximate composition, minerals composition, TS, RS, total phenolic content, antioxidant activity and total flavonoid content, water and oil holding capacity. Also experimental biscuits were formulated with partially substituting UBF and FRP with wheat flour in different proportions (18%, 29.9% w/w). These biscuits samples were evaluated for proximate composition and sensory attributes. UBF had higher amount of moisture content (6.7%) and ash content (2.6%). FRP had higher amount of carbohydrate content (86.1%). Formulated biscuit samples had highest value of crude fiber (1.7% in sample D), fat content (17.7% in sample B) and crude protein (10.1% in control sample) respectively. When comparing UBF and FRP to wheat flour, sodium, calcium, magnesium, potassium, and chloride were much greater significantly, but phosphorus, copper, zinc, and iron were significantly (p value < 0.05) lower. The two flour samples had antioxidant content that was more similar to each other. FRP had the highest levels of total phenolic content (1.04±0.06 mg GAE / 100 g) and total flavonoid content (7.5±0.04 mg QE / 100 g). The total starch content of UBF 45.8±0.03 (g/100g) was higher than FRP. Resistant starch content 19.13±0.01 (g/100g) was higher in UBF. The highest mean value of available starch 40.4±0.02 (g/100g) was found in FRP. Both WHC (2.6±04) g of water/g of dry sample and OHC (1.1±05) g of water/g of dry sample were higher in unripe banana flour. The consumer profiling of the biscuits made from a blend of UBF and FRP in comparison with that of wheat flour biscuits (control) revealed that cookies made with UBF and FRP attained higher overall acceptability scores than commercial or control biscuits. This finding showed that biscuits formulated from UBF and FRP could be of good consumer and market acceptability. All sensory attributes in the control biscuits were lower, with the exception of color. The sensory attributes of sample C (29.5% FRP) are the best of all the formulated biscuits. As a result, the findings of this study could be used in the bakery industry to fulfill consumer specifications and expectations.

Keywords: Proximate analysis, starch, mineral, FRP, antioxidant activity.

Chapter 1: Introduction

Banana (*Musa spp*.) is a tropical and subtropical plant that covers around 8.8 million hectares **(Mohapatra et al., 2010)** and is one of the world's most important food sources. And it can be eaten fresh or processed. It has also a significant economic influence on developing countries. It is possibly the oldest cultivated plant on the world **(Kumar et al., 2012).** It is also a nutrient-dense fruit crop that grown all over the world and utilized as both a staple food and a nutritional supplement **(Islam et al., 2016).** Musa plant harvesting has now become a significant contribution to human society, since it yields the world's fourth most important food (after rice, wheat, and maize) **(Nelson et al., 2006).** Aside from that, the crop is regarded as essential to the people who are living in the growing areas because it provides a significant portion of their annual income as well as a source of food. After citrus, it is the second most widely grown fruit, accounting for around 16% of global fruit production **(FAO, 2009)**. Banana has a significant socioeconomic impact across Asia and the Pacific.

Bangladesh is primarily an agricultural country. Agriculture directly or indirectly employs more than 80 % of the world's population. Agriculture contributes significantly to the country's Gross Domestic Product (GDP). In the fiscal year 2015-16, agriculture contributed 14.75 % of GDP **(BBS, 2016).** In the 2006-07 season, Bangladesh's total banana cropped area was 145280 acres (58818 ha). The overall production in 2010-2011 was 800840 metric tons, with a planted area of around 130589 acres **(BBS, 2012)**. Bangladesh is ranked 14th among the world's top 20 banana-producing countries. It is an economically important fruit crop grown in both domestic and commercial farms in Bangladesh. Furthermore, bananas rank top among the fruits produced in the country, providing 42 % of the country's overall fruit requirements. Furthermore, when compared to other fruits and field crops, its financial return as a crop is higher **(Haque, 1988)**. Every year, the country produces an average 1.00 million tons of bananas **(Hossain, 2014)**. The overall per capita consumption of banana in Bangladesh is 4.7 kg. This is far less than that consumed in Europe, particularly in Belgium (26.7 kg), Sweden (16.7 kg), and Germany (14.5 kg), whereas the United States consumed 13.1 kg and the United Kingdom 10.5 kg **(Siti Hawa, 1998).** A Bangladeshi's typical dietary consumption is insufficient in calories, vitamins, and minerals. Banana, the cheapest fruit of Bangladesh, has the potential to significantly improve this situation. It is a crop that can be used in a variety of ways. Bananas are high in calories and contain the majority of the vitamins required for human nourishment. They are high in carbohydrate, potassium, and vitamins A, C, and B6, among others. They're low in fat and high in dietary fibre. It is frequently the first solid meal given to an infant. Besides, Bangladeshis' traditional cuisine is ripe banana combined with rice and milk **(Hossain, 2014)**. Bananas can be used for a variety of medical purposes. Along with its potential to balance intestinal functions, green banana is a highly recommended diet for a variety of pathological diseases, including constipation and diarrhea. It is advised in cases of colitis, ulcerative colitis, gastric ulcer, uremia, nephritis, gout, cardiovascular disease, and celiac disease since it has the capacity to increase the proliferation of beneficial acidophilus bacteria in individuals. Banana flour is a starchy food with a high proportion of indigestible components such resistant starch (RS 17.5%) and non-starch polysaccharides, which are included in the dietary fibre content (DF 14.5%) **(Juarez Garcia et al., 2006)**. Unripe banana flour has shown to have nutritional and nutraceutical value **(Pacheco-Delahaye et al., 2004)**.

Regular consumption of green banana flour can be expected to offer beneficial health effects for humans due to the high content of these useful elements, which are connected with indigestible components **(Rodriguez-Ambriz et al., 2008)**. Green banana flour has a distinct advantage in that it contains a wide range of vitamins and minerals in both the pulp and peel. At its early development stage, banana fruits have a high level of minerals and vitamins **(Singh et al., 2017)** and are regarded a prebiotic functional food. Due to the general properties of RS and/or its fermentation, UBF consumption can encourage a reduction in energy intake during subsequent meals. As a result, several non-communicable disorders, such as overweight/ obesity, hyperglycemia, and hyperlipidemia, may be reduced. However, it is needed to confirm whether these effects are consistent over time. UBF is a functional ingredient that is high in resistant starch (around 57 % dry weight) **(Rayo et al., 2015).** Furthermore, its source is banana culture, which is readily available and inexpensive, with a minimum integrated production procedure that protects its natural qualities **(Tribess et al., 2009)**. The present definitions and criteria for nutrient essentiality were recently evaluated, and it was suggested **(Jew et al., 2015)** that these conceptions should be broadened to meet scientific progress. Due to the general variety of metabolites and bioactive compounds found in unripe banana flour, it can improve gastrointestinal function, reduce the glycaemia response of food in the human gut, and lower the risk of several disorders.

Despite the fact that green banana fiber is a valuable nutritional component, its absence from the human diet results in a significant nutritional loss because it contains extractable bioactive compounds that can be used as value-added materials. In addition, a fiber-rich powder made from enzymatic starch liquefaction of UBF seemed to have a dietary fiber content of 31.8 %, and it was suggested that this flour could be a good source of polyphenols, which are natural antioxidants **(Rodriguez- Ambriz et al., 2008)**. Liquefaction may aid in the reduction of RS and the increase of total dietary fiber in fiber-rich powder. Total dietary fibre (TDF) was higher in FRP than in UBF, but total starch (TS) and resistant starch (RS) were lower in processed food products, owing to granular disruption and starch hydrolysis during the liquefaction process, which resulted in lower TS and RS and higher TDF. The loss of granular integrity, which is the primary cause of native banana starch indigestibility, also accounts for the lower RS level. For this reason, the liquefaction process has been used in this study for the preparation of fiber-rich powder. Despite their high yield and numerous health benefits, approximately one-fifth of all banana (Musa spp) harvested is wasted or rejected during storage and transportation. It is a perishable fruit that spoils quickly. Bananas are indeed suitable for industrial processing due to their high soluble solids, mineral content, and low acidity. Banana flour is a perfect way to keep the nutritional value of fresh bananas while reducing postharvest losses **(Aurore et al., 2009)**. However, several studies on the manufacturing of ripe and unripe banana flour in bakery items have been conducted **(Saifullah et al., 2009)**. According to the new economic strategy, food waste should be utilized more effectively and converted into a variety of innovative products. Researchers recently looked at the effect of unripe banana flour added to pasta, noodles, bread **(Juarez-garcia et al., 2006),** edible films, and cookies, and showed that it had a favorable impact on nutrition levels, such as a rise in DF and RS in associated food products. RS intake of between 6g and 12g per meal has been proven to enhance postprandial glucose and insulin levels, with a daily RS intake of around 20g considered sufficient to improve health **(Homayouni et al., 2014)**. All of these food commodities have been demonstrated to have higher nutritional benefits.

The possibility of incorporating UBF in the production of ready-to-eat foods like biscuits offers a significant opportunity to implement bioactive components that are now deficient in these foods **(Aurore et al., 2009)**. Biscuits are one of the most popular bakery items in the human diet because they are convenient, delicious, ready-to-eat, affordable, riches in nutritional value, and have a longer shelf life than other bakery products **(Mahloko et al., 2019)**. These are also a popular staple foodstuff due to their diverse flavor profiles, crispiness, and digestibility. Biscuits in the general markets are often high in carbohydrates, fats, and calories but poor in dietary fibre, vitamins, and minerals, making them an unhealthy food source for daily intake **(Norhayati et al., 2015).** Biscuit fortification was introduced in the industry to improve nutritional and functional quality by using components like unripe banana flour as efficient transporters of nutrient content. Despite the unique position of Bananas among other fruit crops of Bangladesh, just a few research have been conducted. Therefore, in terms of waste management and value added to agro-product, the general focus of this research was to assess the proximate composition (moisture, fat protein, and carbohydrate), minerals, bio-active compounds, and antioxidant capabilities of two banana flours, as well as the development of biscuits with regard to textural and sensory attributes.

Objectives

- To prepare and characterize the fibre-rich powder from unripe banana flour.
- To evaluate the nutritional composition of biscuits enriched with Unripe Banana Flour and Fiber-rich Powder.
- To evaluate the bioactive components of Unripe Banana Flour and Fiber-rich Powder.

Chapter 2: Review of Literature

Bananas are one of the most commonly cultivated tropical fruits in the world. However, due to post-harvest loss, much of this fruit is discarded. Another option for minimizing this loss is to process bananas into a more stable product, such as banana flour **(Aurore et al., 2009)**. Its year-round production, low cost, light coloration, and large volume make it a realistic option for large-scale use in a variety of products.

In recent decades, consumers have been drawn to various innovatively manufactured food varieties because of their health benefits, such as prevention from diet-related diseases **(Soukoulis et al., 2014).** As a result, producer's efforts to make nutritious food products that benefit consumer's health tend to be a challenge. Fibre-rich powder will play a significant function in this instance.

The aim of the present study was to evaluate some chemical and physicochemical properties of unripe banana flour and fibre-rich powder prepared by liquefaction of unripe banana flour and incorporated this two flours in biscuits.

2.1 Banana *(Musa acuminata)*

Bananas, scientifically known as (*Musa acuminata*), are a very popular fruit on the global market. Bananas are harvested in over 130 countries and are the world's second most popular fruit after citrus. The size, color, and firmness of this fruit varies, but it is normally elongated and curved, with soft, starchy flesh covered by a rind that is green, yellow, red, purple, or brown when mature. Banana peel is high in dietary fibers, proteins, vitamins, and minerals including potassium, as it makes up 35 % of the fruit **(Giri et al., 2016)**. Dietary fiber is abundant in banana peel and unripe banana fruit. Dietary fibre (DF) is composed of cellulose, hemicellulose, lignin, pectin, -glucans, and gums from a chemical standpoint **(Figuerola et al., 2005).**This fruit contains indigestible carbohydrates, proteins, essential amino acids, cellulose, hemicelluloses, lignin, starch, resistant starch, polyunsaturated fatty acids and potassium (**Rebello et al., 2014)**. This fruit also contains antioxidant compounds including polyphenols, catecholamines and carotenoids and anthocyanidins **(Bennett et al., 2010).** At different stages of ripening, the presence of essential macro (potassium, phosphorus, calcium, sodium, and magnesium) and trace minerals (iron, zinc, copper, and manganese) in both the pulp and peel of the banana fruit. Furthermore, bananas are the most vitamin-dense fruits (B3, B6, B12, C, and E).

Table 2. 1 Nutritive value of Banana per 100gm

Constituent	Amount µg, mg, g, or percent daily value
Energy	371 kJ (89 kcal)
Water	74.91 g
Carbohydrates	22.84 g
Sugars	12.23 g
Dietary fibre	2.6 g
Vitamins	
• Pantothenic acid (B5)	0.334 mg, (7%)
• Pyridoxine (B6)	0.4 mg, (31%)
• Choline	9.8 mg, (2%)
• Vitamin C	8.7 mg, (10%)
Minerals	
• Magnesium	27 mg, (8%)
• Phosphorus	22 mg, (3%)
• Potassium	358 mg, (8%)
• Sodium	1 mg, (0%)
• Zinc	0.15 mg, (2%)

Source: Wikipedia, Internet, USDA databases

2.2 Major components of Banana and their health benefits

2.2.1 Health benefits of phenolics

Banana pulp is abundant in phenolic compounds such as gallic acid, catechins, epicatechins, tannins, anthocyanins, catecholamines, phenolic acids, carotenoids and flavonoids (**Singh et al., 2016)**. Phenolic chemicals and natural antioxidants in bananas contribute to the astringency of green bananas as well as their storage stability and health benefits **(Aurore et al., 2009)**. Phenolic compounds, which have the potential to donate hydrogen atoms to free radicals, are usually called antioxidants or free radical terminators. In humans, phenolic compounds offer favorable health features such as lowering the risk of coronary heart disease, inhibiting lipoproteins, and acting as an antioxidant **(Borges et al., 2014).**

(Lewis et al. 1999) discovered that a flavonoid called leucocyanidin is a major component of an aqueous extract of unripe banana pulp that exhibits anti-ulcerogenic activity. This flavonoid may have enormous therapeutic potential in the treatment of gastric disease situations. Flavonoids' antioxidant capacity, free radical scavenging ability, and chelating action are all linked to the presence of functional groups in their nuclear structure, according to the structure–activity relationship **(Heim et al., 2002).** Flavonoids have also been demonstrated to have antimutagenic and antitumoral actions as a result of these qualities **(Rice-Evans et al., 1996)**. Many enzymes involved in the manufacture of eicosanoids, such as oxygenases (prostaglandin synthase), are inhibited by flavonoids. As a result, flavonoids block hyaluronidase activity and aid in the maintenance of connective tissue proteoglycans. This would inhibit bacterial metastases from spreading **(Havsteen, 2002)**. Flavonoids are said to prevent the oxidation of the body's natural water-soluble antioxidants, such as ascorbic acid, because they are preferentially oxidized.

Table 2.2 Uses and health benefits of bioactive compounds in banana

Bioactive compound	**Health benefits**
Tannic acid	For the treatment of burns, they're used as medicinal agents.
Catechin	Brachial artery dilatation improved plasma antioxidant activity and fat oxidation through increasing the resistance of LDL to oxidation.
Gallic acid	Antioxidant and hepatoprotective actions.
Cinnamic acid	By enzyme-catalyzed amination to phenylalanine, it is a precursor to the sweetener aspartame.
p-Coumaric acid	Antioxidant characteristics that may help to lower the chances of stomach cancer.
Gallocatechin gallate	Reduction of cholesterol.
Quercetin	Encourages blood flow, which improves overall cardiovascular health.
Ferulic acid	Antioxidant, antibacterial, anti-inflammatory, anti-allergic, anti-carcinogenic, enzyme activity modulation, antiviral, and vasodilator effects.
Trans-α carotene	Vitamin A precursor.
Trans-β carotene	Lower the chances of developing cardiovascular disease and cancer.
Violaxanthin	It is a food coloring agent.
Cryptoxanthin	Lung cancer risk may be reduced by using food colorants.

Serotonin	It contributes to emotions of happiness and well-being.
Dopamine	Reduce oxidative stress in the plasma and improve LDL resistance to oxidative modification.
Catecholamines	Blood pressure, glucose levels, and heart rate are all elevated.
β-Sitosterol	Possibility of lowering blood cholesterol and benign prostatic hyperplasia (BPH).
Campesterol and stigmasterol	Reduces cholesterol absorption in the human intestines.

2.2.2 Health benefits of biogenic amines

Banana peel and pulp are known to be good suppliers of certain biogenic amines (catecholamines) which are made from decarboxylation of amino acids or by the amination of aldehydes and ketones. When people eat bananas, the serotonin in the pulp (which ranges from 8 to 50 g/g) makes them feel good. Dopamine, serotonin, epinephrin, and norepinephrine are all abundant in bananas **(Buckley, 1961)**. They emphasized the importance of dopamine as a neurotransmitter in the human brain and body, as well as its impact on mood and emotional stability. Tryptophan is a dopamine precursor. Many researchers have observed an increase in dopamine concentration in both the peel and the pulp as the fruit matures from unripe to ripe **(Gonzalez-Montelongo et al., 2010).** When researchers compared dopamine to other natural antioxidants including ascorbic acid, reduced glutathione, and phenolic acids (e.g. gallocatechin gallate), they discovered that dopamine was the most effective in vitro (DPPH assay).

2.2.3 Health benefits of carotenoids

Carotenoids are abundant in bananas. Some of them are vitamin A precursors, while others are known to have potent antioxidant properties that can scavenge (reactive oxygen species) ROS. The α-carotene, β-carotene, and β-cryptoxanthin are provitamin A carotenoids which are found in banana fruit, while others such as lycopene and lutein have high antioxidant properties **(Erdman et al., 1993)**. Lycopene has been linked to the reduction in the risk of prostate cancer in men, and lutein has been linked to a reduction in the risk of age-related macular degeneration in humans **(Davey et al.,**

2006). (Davey et al., 2009) investigated over 171 distinct Musa spp. genotypes for provitamin A carotenoids and 47 different genotypes for two minerals (iron and zinc). They discovered a lot of variation in provitamin A levels between cultivars. They proposed that high-provitamin A and trace mineral cultivars be used as development initiatives to boost nutritional health and alleviate micronutrient deficits in Musa-consuming people. Trans-carotene concentration is higher in yellow- and orange-fleshed banana varieties **(Englberger et al., 2006)**. Fruits high in carotenoids have been shown to increase immunity and lower the risk of diseases like cancer, type 2 diabetes, and cardiovascular issues **(Krinsky and Johnson, 2005)**. Certain banana cultivars enriched in provitamin A carotenoids can be produced and consumed by the poorest people, who suffer from severe vitamin A shortage, and eating of such banana fruit can help to ameliorate vitamin A deficiency **(Fungo and Pillay, 2013)**. Individuals with poor carotenoid (provitamin A) intake have been linked to an increased risk of degenerative illnesses **(Adeniji et al., 2006)**.

2.2.4 Health benefits of phytosterols

Food processors are interested in using these naturally occurring plant sterols to create functional foods with greater health advantages. The peel and pulp of bananas have been found to have a significant level of phytosterols **(Akihisa et al., 1986)**. The phytosterol content of unripe bananas has been observed to range from 2.8 to 12.4 g/kg **(Vilaverde et al., 2013)**. They fight with cholesterol for absorption in the intestines because their structures are similar to cholesterol, thus decreasing blood cholesterol levels **(Marangoni and Poli, 2010)**. They observed that taking 3 g of phytosterols on a daily basis lowers LDL cholesterol levels significantly.

2.2.5 Health benefits of antioxidant

Bioactive substances such as phenolics, carotenoids, dopamine, carotenes, norepinephrine, and ascorbic acid have been found in bananas as they are high in antioxidant content. Polyphenols linked to polysaccharides and proteins in cell walls are important components of dietary fibre, according to **(Goi et al., 2009)**. The antioxidant gallocatechin was plentiful in banana, according to **(Someya et al., 2002)**. For this reason, bananas are thought to contain bioactive compounds with high antioxidant potential, which aid in physiological defense against oxidative stress-related diseases and free-radical-mediated processes in biological systems **(Singh et al.,**

2016). Antioxidants also help to combat diseases like arthritis, diabetes, arteriosclerosis, age-related macular degeneration, cancer, inflammation, genotoxicity, and Alzheimer's disease.

2.2.6 Health benefits of resistant starch

Carbohydrates can be classed as digestible or indigestible, glycemic or nonglycemic from a nutritional perspective. There is a nutritional trend to limit glycemic carbohydrate consumption in food items in order to raise the content of indigestible carbohydrates **(WHO/FAO, 2003)** or to enhance the content of slowly digestible starch due to its health benefits. Banana fruits are a globally important food crop source with a high concentration of indigestible carbohydrates, notably resistant starch, also known as slowly digestible starch **(Singh et al., 2017)**. Rapidly digestible starch (RDS) is digested in vitro within just 20 minutes, slowly digestible starch (SDS) is digested in between 20 and 120 minutes, and resistant starch is digested in between 20 and 120 minutes (RS). In the duodenum and proximal parts of the small intestine, RDS is rapidly digested and absorbed, resulting in a rapid response to high blood glucose and, in most cases, a subsequent episode of hypoglycemia. SDS could offer a longer, more constant source of systemic glucose with a low glycemic reaction, which is useful in products used by athletes, as well as a longer, more consistent source of systemic glucose with a low glycemic response **(Englyst et al., 1992).**

While RS is a slowly digesting starch, it is fermented by microorganisms in the colon **(Fu L et al., 2008)** to form short chain fatty acids (SCFA), which raise the pH level of the large intestine, preventing harmful bacteria from growing while promoting the growth of helpful bacteria. SCFAs provide the body with additional energy as well as a high proportion of butyrate, which is favorable to colon health and has anti-cancer properties. With a low initial glycaemia and a delayed and extended release of glucose, RS is digested slowly across the entire small intestine, enabling sustained glucose release. This extended glucose secretion aids in diabetic management. A RS intake of between 6g and 12g per meal has been proven to enhance postprandial glucose and insulin levels, with a daily RS intake of around 20g thought sufficient to improve health. Resistant starch has been shown to improved blood glucose **(Brand-Miller et al., 2003)**, insulin sensitivity, insulin secretion, blood lipids, cognition, hunger, athletic performance, constipation, diverticulitis, and weight management **(Thakorlal et**

al.,2010). When resistant starch was introduced to the hydration pretreatment, **(Ramakrishna et al., 2000)** observed a significant reduction in the frequency of diarrhea. Intestinal inflammation was reduced by resistant starch from bananas, with fewer macroscopic and microscopic lesions, suggesting that incorporating green banana flour into the diet could be a useful supplement in the treatment of patients with intestinal inflammation **(Scarminio et al., 2012)**.

2.2.7 Health benefits of dietary fiber

The fiber content of bananas is substantial, and it has been shown that different forms of dietary fiber (DF) can alter the profile of gastrointestinal hormone release related to satiety and energy intake **(Sánchez et al., 2012)**. For this reason, increasing the supply and consumption of DF-containing foods and products is crucial for human diet. Several physiological and metabolic effects have been linked to a high DF intake **(Drzikova et al., 2005)**. Humans are unable to digest fibers, as well as resistant starches, due to a lack of enzymes capable of breaking down their structure. Undigested fiber is fermented by the colonic flora, resulting in a variety of health-promoting by-products. DF has a buffering action in the digestive tract, which helps to reduce excess acid in the stomach, enhance fecal volume, and encourage intestinal evacuation, as well as providing a favorable environment for the growth of good intestinal bacteria. Fiber has the ability to bind to a variety of molecules, including cholesterol, decreasing cholesterol levels **(Jimenez-Escring & Sanchez-Muniz, 2000)**. Apart from that, DF is useful in the prevention and treatment of obesity, atherosclerosis, coronary heart disease, colorectal cancer, and diabetes **(Peters, 2003).** Certain fibers, notably highly viscous slowly digested fibers (SDFs), are associated with mild postprandial glycemic reactions, which is important in the dietetic treatment of diabetes **(Schneeman, 1987).**

2.2.8 Health benefits of butyrate

Butyrate, one of the short fatty acids found in bananas, suppresses the growth of malignant cells that can turn cancerous. It enhances colon health by increasing faucal bulking and also functions as a rehydrating agent for patients suffering from diarrhea **(Fuentes et al., 2010)**. It facilitates in the slow digestion of glucose and insulin levels in the blood. Low insulin levels may indicate the body to burn fat, which aids in the prevention of type 2 diabetes and the management of obesity **(Penn-Marshall et al., 2010).** It binds bile acids and cholesterol in the digestive system, preventing them from being absorbed into the bloodstream. Apart from that, it assists in the prevention of osteoporosis by enhancing calcium and other mineral absorption, such as magnesium and iron. It also transfers probiotics to the large intestine, where they can maximize their effects, such as immune system strengthening.

2.2.9 Health benefits of minerals

Bananas have a lot of precious minerals in them. Minerals help the body's catalytic, structural, and regulatory activities, according to **(Freeland-Graves and Trotter, 2003)**. Minerals act as electrolytes, bringing fluid equilibrium, stomach acidity and acid-base balance to the body. Minerals have also been connected to cellular and basal metabolism, where they perform physiological tasks such as active transport, blood pressure regulation, cell membrane potential, muscular contraction, and nerve transmission **(Sulaiman et al., 2011)**. The high potassium content of bananas has been discovered to help reduce high blood pressure and muscular cramps. Unripe banana, on the other hand, has been shown to protect the gastric mucosa against aspirin-induced damage. According to a recent study **(Heo et al., 2008)**, eating fresh fruits such as bananas may protect neuron cells from oxidative stress-induced neurotoxicity and may help to reduce the risk of neurodegenerative conditions such as Alzheimer’s disease.

2.3 Application of banana flour in different kind of food products

The integration of good quality proteins, essential amino acids, vitamins, minerals, and dietary fibers to baked items made from composite flours has garnered significant attention **(Chandra et al., 2015)**. In this connection, unripe banana flour has been effectively transformed into a variety of baked goods, including slowly digestible cookies, high-fiber bread, snacks, noodles, spaghetti, and cakes **(Segundo et al., 2017).** In recent years, there has also been a lot of interest in the intention of promoting diabetic patient control by manipulating the glycemic impact of the carbohydrates they consumed. The glycemic index (GI) idea is a technique for ranking foods based on their tendency to raise blood glucose levels, which leads to the preference for "low carbohydrate" foods and diets **(Bjorck et al., 1994)**. The RS content of a food or complete meal is one nutritional characteristic that may be associated to low GI features, even though it still cannot be used as a general rule. Several publications have asserted the nutritional/nutraceutical potential of unripe banana starch and fiber in this regard **(Pacheco-Delahaye et al., 2004).**

2.3.1 Biscuits and cookies

Biscuit or cookie preparations are quite popular among baked goods because of their ready-to-eat form, palatability, aroma, texture quality, and relatively longer shelf life. There has been an increase in cookie consumption in recent years. The use of unripened banana flour in biscuits by Agama-Acevedo et al**. (Agama-Acevedo et al., 2012)** significantly increased the level of fiber and resistant starch, lowering starch digestibility and glycemic index. To avoid fat and digestible carbohydrates, cookies using unripe banana flour (UBF) were made with only a few components. When the UBF level in the cookie was increased, moisture and dietary fiber content increased, while protein and fat content diminished. Cookies made with 15 g/100 g of UBF and control cookies had identical fat content, and as the amount of UBF in the formulation grew, fat content reduced. This phenomenon can be associated with the viscosity induced by the UBF dietary fiber, which prevented fat extraction during the experiment **(Rodrguez-Ambrz et al., 2008)**. In cookies made with UBF, there was a small rise in ash content. This behavior is linked to UBF's greater ash content, which is attributable to the fruit's potassium concentration **(Bello-Perz et al., 2006)**. In cookies partially substituted with UBF, total dietary fiber content increased, and this quantity improved as the concentration of UBF in the formulation increased. When UBF was increased in the formulation, total starch increased. The greater starch content (73.4 g/100 g) in UBF is related to this trend **(Juárez-Garca et al., 2006)**. The amount of resistant starch (RS) in cookies made with UBF increased. When UBF was increased 30% in the formulation compared to the control sample (2.3 g/100 g), an increase in RS content of up to 100% (5.44 g/100 g) was calculated. The natural product with the greatest RS concentration is an unripe banana. Cookies manufactured with conventional wheat flour contained 1.48 g/100 g resistant starch, whereas those containing resistant starch-rich banana powder included 8.42 g/100 g **(Aparicio-Saguilán et al., 2007)**. The GI value in control cookie was the greatest, but GI value dropped when the UBF in the cookie increased. This pattern matches the RS content determined by UBF in the cookies. Cookies containing UBF exhibited lower rapidly digestible starch (RDS) than control cookies, and RDS declined as the amount of UBF in the cookie increased. The inclusion of indigestible carbohydrates from UBF, has a diluting effect, lowering the RDS. The amount of slowly digested starch (SDS) in the control cookie and the cookie made with

15 g/100 g of UBF was similar. When the amount of UBF in the cookie was increased, there was an increase in SDS.

2.3.2 Cake batter

Previous research has found that the specific gravity of cake batter has a direct impact on the taste, volume, and texture of bakery end goods. The use of UBF in cake batter dramatically reduced the specific gravity value while significantly raising the concentration level. The potential of cake batter formulation with UBF to increase air bubbles incorporation into the oil in water cake batter emulsion was demonstrated by the lower specific gravity of all UBF samples compared to the control sample **(Celik et al., 2007)**. In addition, there is a link between specific gravity and the amount and types of proteins in cake batter. It appears that the amount and network of wheat flour proteins in the testifier sample has a significant impact on the air trap in cake dough **(Itthivadhannapong & Sangnark, 2016)**. As the concentration of UBF in the cake batter increases, the color of the batter darkens. According to **(Walker and Ferrar, 1998)**, the oxidation of phenolic components by polyphenol oxidase (PPO) is the principal cause of enzymatic browning in a variety of fruits**.**

2.3.3 Ice-cream

In recent years, consumers have been drawn to various innovatively created food varieties because of their health benefits, such as prevention from diet-related disorders **(Soukoulis et al., 2014**). Ice cream can fulfill this role by supplying probiotics and prebiotics to the body. As a prebiotic agent, dietary fiber can have a variety of functions. Depending on their chemical and functional qualities, green banana pulp and peel flour may be used in a variety of ways **(Alkarkhi et al., 2011)**. Using fruit fiber in ice cream production has several advantages, including improved ice cream structure due to fibrous framework and melting properties, reduced recrystallization, resulting in longer shelf life, and increased ice cream viscosities, allowing freezing at higher overrun without affecting ice crystal sizes and resulting in more homogeneous air bubble formation in the ice cream **(Dervisoglu & Yazici, 2006).** Ice cream manufacturers are always attempting to keep up with innovative flavors, and since fruits may enhance the taste and aroma of the ice cream, they are often used to do so **(Karaman et al., 2014)**. In this regard, green bananas are appropriate as a source of dietary fiber and a foodstuff ingredient. With the addition of green banana flour, the ice cream's dry matter level increased. The volume of air used in the production process is relevant to overrun and melting. Because the air in the ice cream provides a light texture and affects various physical attributes like melting and hardness, this property can form the final product's structure. Although adding green banana flour to the ice cream samples reduced overrun rates, the control samples had higher overrun rates than the green banana flour samples. Since this viscosity of ice cream increased with the addition of banana flour, it's suspected that during batch freezing, less air was included in the ice cream mixture with unripe banana flour, resulting in less overflow than in the control (without unripe banana flour). The results of using banana flour to reduce overrun rates in ice cream samples were found to be consistent with those reported in the literature **(Temiz & Yesilu, 2010)**. The amount of K, Mg, and P levels in ice cream samples increased considerably. The high mass fractions of K, Mg, and P in banana may account for the rise in mineral content. Green banana flour contains trace amounts of Fe, Zn, Ni, and Mn, all of which contribute to the fruit's antioxidant activity **(Puentle et al., 2011)**.

2.3.4 Bread

The moisture content of BF bread was higher than that of control bread. Because the former components are hydrophilic and have the capacity to join more water molecules, the high moisture content in BF bread could be linked to the high protein and starch composition and low lipid level. The texture of bread with a high moisture content is softer than bread with a low moisture content.

The high ash concentration of the BF flour had an impact on the ash content of bread made with it, as ash content was higher in BF bread than in control bread. Protein and carbohydrate content were higher in BF bread than in control, which is significant since both nutrients play important metabolic roles in our bodies. However, the lipid content of BF bread was lower than that of the control, which an important observation is given the possibility of the creation of an amylose-lipid complex. Reduced lipid content and an increase in the indigestible fraction of starch have been connected to this type of complex. The total starch content of BF bread was greater than that of control bread, but the accessible starch content was lower in the former. This data supports the creation of an amylose-lipid complex during the processing of starchy foods. The resistant starch content of the BF-added bread was higher (6.74%) than that of the control bread (1.0 %). Regular wheat bread includes a lot of digestible starch fractions **(Granfeldt, 1994)**;

also, bread made with regular baking has an RS content of 3.0%, and when long-time baking and other components were examined, the RS level ranged from 5.1 to 7.7% **(Liljeberg et al., 1996)**. The dietary fiber level of the bread manufactured with BF was higher than the control bread sample. As there is an RS percentage correlated with the insoluble residue of dietary fiber, this pattern coincides with the greater RS level seen in BF bread. The total indigestible fiber (TIF) content of the BF bread sample (26.11%) was higher than the control bread sample (18.36%) **(Saura-Calixto et al., 2000)**. Bakery items manufactured with BF have a high IF content and may be a good option for persons who have particular calorie needs. At 180 minutes, control bread baked in the lab had a 39 % hydrolysis rate, which was lower than BF bread (32%). The control bread was absorbed faster than the BF products. The HI of BF bread was lower than that of control bread, which is consistent with the chemical properties of both products (e.g. RS, DF, IF, etc.). Because BF bread has a low glycemic response, it has a substantial effect on the rates of digestion and absorption (simulated by the dialysis phase) of the starch component of the meals.

2.3.5 Pasta or spaghetti

Pasta is a classic dish whose popularity has grown as a result of its convenience in transportation, handling, cooking, and preparation. Pasta is low in sodium and lipids, cholesterol-free, and high in complex carbohydrates, resulting in a low postprandial reaction to blood glucose and insulin **(Cleary & Brennan, 2006)**. With the addition of banana flour to the spaghetti, the amount of digestible starch (DS) in the spaghetti reduced. This pattern was comparable to that described in spaghetti with chickpea flour by **(Goni and Valentn-Gamazo, 2003)**. Although banana flour had the lowest DS content (25.57%) and durum wheat flour had the highest (75.10%), a diluting impact in this parameter was found in the banana flour-added spaghetti. The addition of banana flour to the spaghetti resulted in a substantial rise in resistant starch (RS) levels. The control sample had the lowest RS value (1.11 %), whereas the spaghetti with 45% banana flour had the highest value (12.42%). This is because banana flour has a high RS concentration. Unripe banana flour was found to have RS levels of 57.2 and 47.3 % using two independent methods, indicating that it is the natural product with the greatest RS content **(Faisant et al., 1995)**. When the amount of banana flour in the spaghetti increased, the (insoluble indigestible fiber) IIF increased and the (soluble indigestible fiber) SIF decreased. The IIF content of spaghetti with additional banana flour varied, with the control sample having the lowest content **(Goni & Valentn-Gamazo, 2003)**. The increase in the IIF is due to the high concentration of this fraction in banana flour (57.75%), whereas the decrease in the SIF is due to the cooking loss value, which increased as the amount of banana flour in the spaghetti increased. Because only extractable polyphenols are documented, there are very few investigations on non-extractable polyphenols. The values calculated in the banana flour-added spaghetti were lower than those calculated in the control sample. The sample with 45% additional banana flour had the highest polyphenolic compounds value, especially in terms of condensed tannin concentration. Banana fruits are known to be high in polyphenols, as well as anthocyanins and proanthocyanidins. When compared to the control sample, the addition of banana flour to the spaghettis improved the antioxidant capacity. Antioxidant activity is highest in the sample with 45 % additional banana flour. The banana flour used to prepare the spaghetti was made from green or immature bananas, which have an astringent flavor due to the presence of condensed tannins or proanthocyanidins at this stage. Due to the condensed tannins in banana flour, pasta with added banana flour has a higher antioxidant capacity. The antioxidant activity of the fruit is impressive, according to **(Kondo et al., 2005)**, since they are rich in polyphenolic chemicals and vitamins, which play a vital role in the absorption of free radicals. According to chemical point of view, unripe banana flour and fiber-rich powder which is produced by liquefaction of unripe banana flour have high total dietary fiber, indigestible fraction contents, total starch levels, and resistant starch content. Preparation of biscuits fortified with high-fiber unripe banana flour and fiber-rich powder. The fiber-rich powder appeared to be a potential ingredient for functional foods due to its high total dietary fiber and indigestible fraction percentages.

Conclusion:

According to the chemical composition, unripe banana flour and fibre-rich powder prepared by liquefaction of unripe banana flour have high total dietary fibre, indigestible fraction contents, total starch levels, and resistant starch content. The fibre-rich powder appears to be an effective ingredient for functional foods due to its high total dietary fibre and indigestible fraction concentrations.

Chapter 3: Materials and Methods

3.1 Study period and study area

The research work was conducted for a period of five months from June 2020 to October 2020. The research work was conducted in the laboratory of the Department of Applied Food Science and Nutrition, Department of Food Processing and Engineering, Department of Physiology, Biochemistry and Pharmacology, Department of Animal Science and Nutrition at Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh.

3.2 Experimental design

At first, local market of Jawtola, located in the area of Pahartali in Chattogram city was selected from where unripe bananas were collected. I was selected this market because of higher availability and lower cost of Bananas. After collection of samples, they were used for preparation of unripe banana flour. Then this unripe banana flour was used for formulation of fiber-rich powder by liquefaction process. After processing of these flours (unripe banana flour & fiber-rich powder), were used to determine proximate composition (moisture, ash, crude fat, protein, crude fiber and carbohydrate) and mineral (Sodium, Potassium, Calcium, Magnesium, phosphorus Iron, Chloride, Copper and Zinc) contents. Also bioactive compounds (flavonoid, polyphenol) and antioxidant capacity were measured. Furthermore, unripe banana flour and fiber-rich powder were incorporated into biscuits and compare with control. All formulated biscuits were used to determine proximate composition and consumer's acceptability test.

3.3 Samples collection

Mature and fresh unripe bananas (*Musa acuminata*) were purchased from Wireless Jawtola market, Chattogram metropolitan city. Other ingredients for the formulation of experimental biscuits like Fresh wheat flour, Aarong butter, eggs, ACI sugar, Family Food baking powder, Confidence salt were individually purchased from the Basket supermarket in Chattogram metropolitan city, Bangladesh.

3.4 Preparation of unripe banana flour

The method for UBF production was followed from **Saifullah et al., (2009)** with some modification. The bananas were washed with the potable water to remove any dirt on the fruits to avoid physical contamination. The bananas then were peeled manually and sliced longitudinally to about 1cm thick and dipped into (0.5% w/v)/ (0.5 g/100 mL) citric acid solution for 10 minutes. After that, the sliced pulps were dried at 60°C for 48 hours in a cabinet dryer. After drying, the dried samples were taken and ground using a grinder until powder obtained to ensure homogeneity. After that, the powdered sample was sieved for removal large particles. Then unripe banana flour samples were packed into zip-lock plastic bag and kept at 4℃ in the refrigerator for further examinations.

Unripe Banana
▼
Peeling
▼
Cutting in to small sizes
▼
Dipping into water containing citric acid (0.5w/v)
▼
Draining
▼
Drying in cabinet oven at 60^0C 48 hrs
▼
Grinding
▼
Sieving
▼
Unripe Banana Flour

Flow chart: Processing of unripe banana flour.

3.5 Preparation of fiber-rich powder

At the laboratory level, liquefaction was performed using the method of **Flores-Gorosquieta et al., (2004)** with some modifications: a suspension of banana flour in distilled water (20 % w/v) was prepared in a beaker; the dispersion was then gelatinized at 80°C for 10 minutes after the pH was adjusted to 7.3. The gelatinized slurry was cooled down to 70°C, then combined with *Bacillus subtilis* α-amylase (0.2 % v/v) and incubated for 3 hours at 70°C. After that, the enzyme was inactivated by lowering the pH to 2.0 using HCl. After that, the mixture was centrifuged for 15 minutes at 1700 rpm to separate the insoluble fiber-enriched fraction. To obtain the fiber-rich powder, the material was dried at 40°C for 48 hours, then milled, and sieved.

3.6 Formulation and processing of biscuits

Biscuits were prepared by the method of **Laguna et al., (2011)**. The flour, baking powder, sugar and salt were mixed thoroughly for 60 s inside a bowl before they were mixed with butter and egg manually for 2 mints. The batter was shaped by a circle and other shaped cookie cutter before being placed inside the oven at a temperature of 180°C for 20 min. After cooling down, the biscuits were packed in Ziploc bags. Cookie cutter was used to cut the biscuit dough as well as giving the biscuits uniform shape. For flour, two treatments were prepared: control (wheat flour, no addition of UBF and fiber-rich powder), 18% UBF and fiber-rich powder, 29.5% UBF and fiber-rich powder. Five formulations were prepared with different percentages of UBF and fiber-rich powder with wheat flour from which one as control formulation.

Table 3.1 The Percentage of ingredient use in composited UBF and FRP biscuits formulation (%)

Ingredient (%)	Sample A(Control)	Sample B	Sample C	Sample D	Sample E
Wheat flour	59	41	29.5	41	29.5
UBF	0	0	0	18	29.5
FRP	0	18	29.5	0	0
Butter	13.2	13.2	13.2	13.2	13.2

Sugar	13.2	13.2	13.2	13.2	13.2
Egg (ml)	13.7	13.7	13.7	13.7	13.7
Baking powder	0.6	0.6	0.6	0.6	0.6
Salt	0.3	0.3	0.3	0.3	0.3
Total	100	100	100	100	100

3.7 Proximate analysis of biscuits, unripe banana flour and fiber-rich powder

Moisture, protein, fat and ash contents of (Unripe banana flour, fibre-rich powder and 5 types of formulated biscuits) samples were measured in triplicate according to AOAC methods. The moisture was measured by oven drying at 105℃ to constant weight **(AOAC, 2016).** The crude protein content was measured by the Kjeldahl procedure (6.25×N). Total lipid was extracted by the **AOAC (2016)** method using the Soxhlet apparatus. Ash was measured gravimetrically in a muffle furnace by heating at 550℃ to constant weight **(AOAC, 2016).**

3.8 Bioactive compounds of unripe banana flour and fiber-rich powder

3.8.1 Preparation of extracts

Preparation and identification of phenolic acids were determined according to a modified method described by **Ferreres et al., (2008)**. Dried samples were transferred into respective beakers added with absolute ethanol, and left to shake on a shaker for 72 hrs at room temperature. The solvent was then separated from residue by straining. The filtrate was collected and stored at room temperature while the residue was re-extracted twice, each time with fresh solvent. Finally, all the filtrates were combined and evaporated under reduced pressure at 60℃ using a rotary evaporator to obtain the crude extracts. The crude extracts were weighed and stored at 4℃ until further analysis.

3.8.2 Total flavonoid content (TFC)

Total flavonoid content of the (sample) extracts was determined using the aluminum chloride colorimetric method described by **Chang et al., (2002)**. Stock solution (1 mg/mL) of extracts was prepared. Quercetin was dissolved in 80% ethanol to make standard solutions (1.0, 3.0, 5.0, and 7.0 mg/mL) to plot a standard curve. Aliquots of

0.5 mL of diluted extract or standard solution was mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 mol/L potassium acetate, and 2.8 mL of distilled water in the cuvette. The mixture was left at room temperature for 30 mints. The absorbance was read at wavelength 415 nm in UV-visible spectrophotometer. For the blank, 10% aluminum chloride was substituted with distilled water of the same amount. TFC was calculated and expressed as milligrams quercetin equivalents (QE) per gram of extract (mg QE/g).

3.8.3 Total phenolic content (TPC)

Total phenolic content of the (sample) extracts was determined according to the method described with slight modifications **(Azizi et al., 2010)**. Stock solutions (1mg/mL) of extracts and standard solutions of gallic acid (1.0, 2.0, 4.0, 6.0, 8.0 mg/mL) was prepared. Extracts or gallic acid standard solution (0.3 mL) was pipetted into a cuvette. Diluted Folin-Ciocalteu reagent (1.5 mL) was then added and mixed. The mixture was left for 3 mints before adding 1.5 mL of sodium carbonate (75 g/L) solution and was left for 60 mints. The absorbance was read at wavelength 765 nm using a UV spectrophotometer and ethanol was used as the blank. TPC was calculated and expressed as milligrams of gallic acid equivalents (GAE) per gram of extracts (mg GAE/g).

3.8.4 Antioxidant activity

Antioxidant capacity of the extracts was determined using DPPH assay as described by **Azlim Almey et al., (2010)** with slight modifications. Stock solution (1 mg/mL) of extract was diluted to concentrations of (0.50, 1.00, 1.50, 2.00, 2.50) mg/mL in methanol. Methanolic DPPH solution was prepared by dissolving 6 mg of DPPH in 100mL methanol. The methanolic DPPH solution (2 mL) was added to 1 mL of each extract solution of different concentrations and the mixture was left for 30 min and the absorbance was read at wavelength 517 nm. Control was prepared by mixing 1 mL of methanol with 2 mL of DPPH solution. Methanol was used as a blank while Trolox was used as a standard. Antioxidant capacity based on the DPPH free radical scavenging ability of extracts was calculated and expressed as milligrams of Trolox equivalents (TE) per gram of extracts (mg TE/g).

3.9 Mineral analysis

This method involves the extraction of minerals from the organic food matrix by digestion through wet digestion. The mineral contents in the digested compounds was determined by spectrophotometer (Humalyzer 3000®). Commercially available biochemical kit (Randox) was used for biochemical assay. The whole procedure was done in the Postgraduate Research lab under the Department of Physiology, Biochemistry and Pharmacology at Chittagong Veterinary and Animal Sciences University. All the analyses were done in triplicates. Beaker, measuring pipets, volumetric flask, analytical balance, heating mantle or hot plate, Filter paper, (Whatman® No. 541) apparatus were used for mineral analysis. Also Nitric acid and Perchloric acid reagent were used for mineral analysis.

Procedure of sample extraction

One (01) g of dry sample was weighted in a conical flask. For dried samples, 7.5 ml conc. HNO_3, and 2.5ml conc. $HClO_4$ in the ratio of 2:1 was prepared. For wet sample, 5 mL HNO_3 and 1 ml $HClO_4$ was added (HNO_3: $HClO_4$ = 5:1). Then the flask was placed in a hot plate at 200W for 1-2 hours until full digestion. After digestion, it was cooled at room temperature. Then transferred the digested samples into 100 ml volumetric flask and diluted up to 100 mark with Deionized water and mixed well. Later, the solution was filtered through Whatman® filter paper No. 1 and transfer to Eppendorf Tube for mineral quantification **(Schoenfeld et al., 1964; Chauhan et al., 1969; AACC, 2000).**

3.9.1 Determination of Sodium (Na)

Sodium is precipitated as a triple salt with magnesium and Uranyl acetate. The excess of uranyl ions are reacted with ferrocyanide in an acidic medium to develop a brownish color. The intensity of the color produced is inversely proportional to the concentration of sodium in the sample. At first precipitation was completed for Sodium determination. For blank, 1 ml precipitating reagent, 20 µl sodium standard and 0 µl ml sample were added into cuvette by using pipette. For standard, 1 ml precipitating reagent, 0 µl sodium standard and 20 µl sample were added into cuvette. Mixed well and let stand at retention time for 5 mints. With shaking well intermittently. Centrifuged at 2500 to 3000 rpm to obtain a clear supernatant. Color development was the second step of

Sodium determination. For the preparation of blank solution, 1ml acid reagent (L2), 20 µl precipitating reagent (L1) and 100 µl color reagent were added into cuvette with the help of pipette. For standard solution, 1ml acid reagent (L2), 20 µl supernatant from step 1 and 100 µl color reagent were added. For sample preparation, 1ml acid reagent (L2), 20 µl supernatant from step 1 and 100 µl color reagent were added. The quantity of Sodium was estimated (mmol / L) by subtracting the absorbance of sample from the absorbance of blank sample, dividing by the result obtained by subtracting the absorbance of standard from the absorbance of blank, and lastly multiplying the obtained value by the concentration of the standard.

3.9.2 Determination of Chloride Ion (Cl^-)

Chloride ions combine with free mercuric ions and release thiocyanate from mercuric thiocyanate. The thiocyanate released combines with the ferric ions to form a red brown ferric thiocyanate complex. Intensity of the color formed is directly proportional to the amount of chloride present in the sample. For the preparation of blank solution, 0.01 ml deionized water, 1.0 ml reagent were added into cuvette. 0.01 ml standard and 1ml reagent were added for standard solution preparation. 0.01 ml sample extract and 1ml Reagent were added for sample solution preparation. The quantity of Chloride Ion was estimated (mmol / L), dividing the absorbance of sample by the absorbance of standard, and lastly multiplying the obtained value by the concentration of the standard.

3.9.3 Determination of Calcium (Ca^{++})

Calcium ions form a violet complex with O-Cresolphthalein complexone in an alkaline medium. For the preparation of reagent blank solution, 25 µl distilled water and 1ml working reagent were added into cuvette. For standard, 25 µl (Ca^{++}) standard and 1ml working reagent were added. 25 µl ml sample extract and 1ml working reagent were added for the preparation of sample solution. The quantity of Calcium was estimated (mg/dl), dividing the absorbance of sample by the absorbance of standard, and lastly multiplying the obtained value by the concentration of the standard.

3.9.4 Determination of magnesium (Mg)

The method is based on the specific binding of calmagite, a metallochromic indicator and magnesium at alkaline pH with the resulting shift in the absorption wavelength of the complex. The intensity of the chromophore formed is proportional to the concentration of magnesium in the sample. For the preparation of reagent blank solution, 1ml reagent was added into cuvette. For magnesium standard preparation, 10 µl magnesium standard and 1ml reagent were added. 10 µl ml sample extract and 1ml reagent were added for the preparation of sample solution. The quantity of Magnesium was estimated (mg/dl), dividing the absorbance of the sample by the absorbance of the standard, and lastly multiplying the obtained value by the concentration of the standard.

3.9.5 Determination of Phosphorus (P)

Inorganic phosphate reacts with ammonium molybdate in the presence of sulfuric acid to form a phosphomolybdic complex which is measured at 340nm. For the preparation of reagent blank solution, 1ml phosphorus reagent was added into cuvette. For phosphorus standard preparation, 10 µl phosphorus standard and 1ml phosphorus reagent were added. 10 µl ml sample extract and 1ml phosphorus reagent were added for the preparation of sample solution. The quantity of Phosphorus was estimated (mg/dl), dividing the absorbance of sample by the absorbance of standard, and lastly multiplying the obtained value by the concentration of the standard.

3.9.6 Determination of Potassium (K^+)

Sodium tetraphenyl boron reacts with potassium to produce a fine turbidity of potassium tetraphenyl boron. The intensity of turbidity is directly proportional to the concentration of potassium in the sample. For the preparation of blank solution, 0.02 ml deionized water and 1.0 ml K^+ Reagent were added into cuvette by using pipette. 0.02 ml standard and 1ml K^+ Reagent were added for standard solution preparation. 0.02 ml sample extract and 1ml K^+ Reagent were added for sample solution preparation. The quantity of Potassium was estimated (mmol / L), dividing the absorbance of sample by the absorbance of standard, and lastly multiplying the obtained value by the concentration of the standard.

3.9.7 Determination of Iron (Fe)

The iron is dissociated from transferring-iron complex in weakly acid medium. Liberated iron is reduced into the bivalent form by means of ascorbic acid. Ferrous ions give with FerroZine (A ferroin compound that forms a stable magenta-colored solution with the ferrous ion) a colored complex. The intensity of the color formed is proportional to the iron concentration in the sample. For the preparation of blank solution, 1ml Fe reagent was added into cuvette with the help of pipette. For standard preparation, 200 µl Fe standard and 1ml Fe reagent were added. 200 µl sample extract and 1ml Fe reagent were added for the preparation of sample solution. The quantity of Iron was estimated (µg / dl) by subtracting the absorbance of the blank sample from the absorbance of the sample, dividing by the absorbance of standard, and lastly multiplying the obtained value by the concentration of the standard.

3.9.8 Determination of Zinc (Zn)

Zinc in an alkaline medium reacts with Nitro-PAPS to form a purple colored complex. Intensity of the complex formed is directly proportional to the amount of Zinc present in the sample. For the preparation of blank solution, 50 µl distilled water and 1ml working reagent were added into cuvette. For standard solution preparation, 50 µl Zinc standard and 1ml working reagent were added. 50 µl ml sample extract and 1ml working reagent were added for the preparation of sample solution. The quantity of Zinc was estimated (µg/dl), dividing the absorbance of sample by the absorbance of standard, and lastly multiplying the obtained value by the concentration of the standard.

3.9.9 Determination of Copper (Cu)

Copper, reacts with Di-Br-PAESA to from a colored complex. Intensity of the complex formed is directly proportional to the amount of Copper present in the sample. For the preparation of blank solution, 500 µl buffer reagent (L1), 500 µl color reagent (L2) and 50 µl distilled water were added into cuvette with the help of pipette. For standard solution, 500 µl buffer reagent (L1) color reagent (L2) and 50 µl copper standard were added. For sample solution preparation, 500 µl buffer reagent (L1), 500 µl color reagent (L2) and 50 µl sample extract were added. The quantity of Copper was estimated (µg/dl), dividing the absorbance of sample by the absorbance of standard, and lastly multiplying the obtained value by the concentration of the standard.

3.10 Determination of Starch (Total Starch, Resistant Starch, Available Starch)

Total starch (TS) content of the UBF were analysed according to method of **Goni et al., (1997).** Briefly, 50 mg ground sample of flour was dispersed in 6 ml of 2M KOH and the mixture was incubated for 30 minutes at room temperature. The solubilized starch was then be hydrolyzed by adding 60 μL of amyloglucosidase and incubating at 60°C for 45 minutes by shaking in water bath. After centrifugation (15 minutes, 4500 g), the supernatant was measured using glucose oxidase-peroxidase kit. The total starch content in the sample was calculated as glucose x 0.9. The Resistant Starch (RS) analysis was conducted according to AOAC method 2002.02 **(McCleary & Monaghan, 2002).** A sample of boiled beans was used as in-house reference material. Glucose was quantified in the supernatants with a GOD/POD/ABTS mixture.

Potentially available starch content was determined by the subtracting the difference of total starch by resistant starch.

Available starch = Total starch – Resistant starch

3.11 Water and oil holding capacity of unripe banana flour and fiber-rich powder

With slight modifications, the method of **Rodriguez-Ambriz et al., (2007)** was used to measure the water and oil absorption capabilities of two flour (UBF and fiber-rich powder) samples. For experiment 1 g of dry material was combined with 25 mL of distilled water or commercial olive oil. The solution was mixed for 1 minute before being incubated for 1 hour at room temperature. The supernatant was poured out after the tubes were centrifuged at 3000×g for 20 minutes. After that, the tubes were drained for 10 minutes at a 45° angle. Water holding capacity (WHC, in g of water per 100 g of sample) and oil holding capacity (OHC, in g of oil per 100 g of sample) are calculated after the residue was weighed.

3.12 Sensory evaluation of the biscuits

Sensory evaluation of the biscuits was carried within 24 h of baking by 15 panelists. For the selection of panelists, regular biscuit consumers were given priority, they were well informed and required consent was obtained for their participation. All four biscuit samples were simultaneously placed to all the consumers in a randomized manner. During the procedure, they were instructed to cleanse their mouths in between consuming each sample to minimize any residual effect also briefed on other procedure before evaluation. The panelists were the students of the faculty of Food Science and Technology, Chattogram Veterinary and Animal Sciences University, Bangladesh who had previous experience products evaluation. Evaluation was done at room temperature in the laboratory of the department of Applied Food Science and Nutrition, Chattogram Veterinary and Animal Sciences University. Score card was prepared keeping in view the quality characteristics of the products. The responses of the individuals panelist were obtained concerning physical appearance, color, taste, aroma, shape, crispiness and overall acceptability of the samples using a nine-point hedonic scale (Like extremely = 9, Like very much = 8, Like moderately = 7, Like slightly = 6, Neither like nor dislike = 5, Dislike slight = 4, Dislike moderately = 3, Dislike very much = 2, Dislike Extremely = 1.).While scoring, highest score (9) was assigned to most preferred characteristic and least score (1) to the least desired characteristic.

3.13 Statistical Analysis

Statistical analysis was performed by using MS Excel 2013 and Statistical Package for Social Science (SPSS 25th version). Values are expressed as means ± standard deviation (SD). One-way ANOVA and post-hoc "Tukey" tests were used to identify the variation within the sample groups. Statistical significance was set at $P<0.05$.

Chapter 4: Results

4.1 Proximate analysis of unripe banana flour, fiber rich powder and five formulated biscuits

The result of proximate analysis of banana flour, fiber rich powder and five formulated biscuits (where one is control biscuit) are presented in Table 4.1. In case of two flour UBF had the highest moisture content (6.7±.05) %. FRP had the highest carbohydrate content (86.1±.53) % whereas UBF had the lowest value (83.7±.03) %. Crude fiber content was higher (1.6±.04) % in UBF Sample whereas lower value (1.5±.06) % was found in FRP Sample. The highest value of ash content (2.6±.02) % was found in UBF sample and the lowest value (1.6±.03) % was for FRP Sample. Fat content were similar to UBF and FRP flour. Crude protein was higher in UBF and lower in FRP sample.

In case of formulated biscuits, the highest value of ash content (1.8±.05) % was found in sample E and the lowest value (1.2±.04) % was for control Sample. Sample D had the highest moisture content (3.4±.05) % whereas Sample E had the lowest value (1.4±.05) %. Fruit and vegetable contain less protein and crude fat. Crude fiber content was higher (1.7±.03) % in Sample D whereas lower value (0.5±.04) % was found in control Sample. Carbohydrate content (81.4±.04) % was higher in control sample comparatively than other formulated biscuits. Fat content was highest in sample B and lowest in sample D. Crude protein was higher in control sample and lower in sample E. Descriptive statistics and Post hoc Tukey test in One-way ANOVA procedures were conducted to analyze data statistically at 5% level of significance.

Table 4.1 Proximate analysis of UBF, FRP and five formulated biscuits

Sample	CHO (%)	MC (%)	CF (%)	Ash (%)	Fat (%)	CP (%)
UBF	$83.7\pm.03^{e}$	$6.7\pm.05^{a}$	$1.6\pm.04c^{d}$	$2.6\pm.02^{a}$	$1.0\pm.04^{e}$	$4.5\pm.05^{f}$
FRP	$86.1\pm.53^{d}$	$5.6\pm.03^{b}$	$1.5\pm.06^{d}$	$1.6\pm.03^{c}$	$1.0\pm.04^{e}$	$4.3\pm.05^{g}$
Sample A(Control)	$81.4\pm.04^{c}$	$3.3\pm.03^{c}$	$0.5\pm.04^{f}$	$1.2\pm.04^{e}$	$13.8\pm.05^{c}$	$10.1\pm.05^{a}$
Sample B	$68.9\pm.05^{b}$	$2.4\pm.03^{e}$	$1.1\pm.04^{e}$	$1.4\pm.05^{d}$	$17.7\pm.05^{c}$	$8.5\pm.05^{b}$
Sample C	$79.9\pm.04^{c}$	$3.0\pm.05^{d}$	$1.6\pm.05^{ab}$	$1.6\pm.03^{c}$	$13.9\pm.04^{b}$	$7.8\pm.07^{d}$
Sample D	$71.4\pm.05^{c}$	$3.4\pm.05^{c}$	$1.7\pm.03^{a}$	$1.5\pm.05^{c}$	$13.6\pm.05^{d}$	$8.2\pm.07^{c}$
Sample E	$73.0\pm.04^{a}$	$1.4\pm.05^{f}$	$1.6\pm.04^{bc}$	$1.8\pm.05^{b}$	$14.6\pm.05^{a}$	$7.6\pm.03^{e}$

.All values showed (ME±SD= Mean ± Standard Deviation) of proximate composition of five formulated biscuits where one was control biscuit and two flours (unripe banana flour and fiber-rich powder).Significant at $P < 0.05$; Mean values followed by different superscript letters in the same coloum denote a significant difference comparison done across formulation

Legends: Sample A (Control) = 59% of wheat flour, Sample B = 18% fiber-rich powder, Sample C = 29.5% fiber-rich powder, Sample D= 18% unripe banana flour, Sample E = 29.5% unripe banana flour. UBF=Unripe banana flour, FRP=Fiber-rich powder.

CHO= Carbohydrate, MC= Moisture Content, CF= Crude Fiber, CP= Crude Protein.

4.2 Mineral analysis of unripe banana flour, fiber rich powder and wheat flour

The result of mineral contents of banana flour, fiber rich powder and wheat flour are presented in Table 4.2. Magnesium, Phosphorus, Potassium and Copper were higher in unripe banana flour Sample. Sodium, Chloride, Zinc, Calcium and Iron were higher in fiber-rich powder Sample. Wheat flour had lower levels of all minerals.

The highest Calcium, Magnesium, Phosphorous, Chloride , Potassium, Sodium, Copper, Zinc and Iron and were (1.1±0.04) mg/dl, (1.3±0.05) mg/dl, (1.6±0.04) mg/dl, (54.0±0.05) mg/dl, (48.6±0.03) mg/dl, (275.4±0.06) mg/dl, (0.06±0.03) mg/dl, (0.07±0.03) mg/dl ,(0.124±0.05) mg/dl respectively and the lowest values were (0.3±0.5) mg/dl, (0.3±0.05) mg/dl, (0.11±0.04) mg/dl, (27.0±0.05) mg/dl, (28.8±0.04) mg/dl, (129.6±0.06) mg/dl, (0.03±0.03) mg/dl, (0.03±0.02) mg/dl , (0.03±0.02) mg/dl respectively.

Table 4.2 Mineral contents of UBF, FRP and wheat flour

Mineral	Unripe Banana flour	Fiber-rich powder	Wheat flour
Ca(mg/dl)	$0.64±0.05^{b}$	$1.09±0.04^{a}$	$0.3±0.5^{c}$
Mg(mg/dl)	$1.3±0.05^{a}$	$0.7±0.05^{b}$	$0.3±0.05^{c}$
P(mg/dl)	$1.6±0.04^{a}$	$0.8±0.03^{b}$	$0.11±0.04^{c}$
Cl(mg/dl)	$41.4±0.04^{b}$	$54.0±0.05^{a}$	$27.0±0.05^{c}$
K(mg/dl)	$48.6±0.03^{a}$	$36.0±0.05^{b}$	$28.8±0.04^{c}$
Na(mg/dl)	$167.4±0.03^{b}$	$275.4±0.06^{a}$	$129.6±0.06^{c}$
Cu(mg/dl)	$0.06±0.03^{a}$	$0.03±0.05^{a}$	$0.03±0.03^{a}$
Zn(mg/dl)	$0.06±0.03^{a}$	$0.07±0.03^{a}$	$0.03±0.02^{a}$
Fe(mg/dl)	$0.09±0.05^{a}$	$0.124±0.05^{a}$	$0.03±0.02^{a}$

All values showed (ME±SD= Mean ± Standard Deviation) of data. Significant at $P < 0.05$; Mean values followed by different superscript letters in the same raw denote a significant difference comparison done across formulation.

Legends: UBF=Unripe banana flour, FRP=Fiber-rich powder.

Each samples were repeated three times and average values of UBF and FRP were found explicitly more than wheat flour. Among three flours most of the mineral content are higher in fiber-rich powder than another two flours and banana flour was very close to that value.

4.3 Antioxidant capacity of unripe banana flour and fiber rich powder

The result of antioxidant capacity of unripe banana flour and fiber-rich powder are presented in Table 4.3 and which were expressed as Trolox Equivalent. UBF had a greater antioxidant capacity value of 3.39±.04 (mg TE/100 g) than FRP, which had a lower concentration of 3.36±.05 (mg TE/100 g). In addition, the antioxidant content of the two flour samples was more similar to each other.

Table 4.3 Antioxidant capacity of UBF and FRP

Sample	Sample after 10 minutes(mg TE / 100 g)	Sample after 20 minutes(mg TE / 100 g)	Sample after 30 minutes(mg TE / 100 g)
UBF	3.39±.04	3.36±.04	3.34±.05
FRP	3.36±.05	3.35±.05	3.34±.05

There was a significant difference ($p<0.05$) of antioxidant activity between unripe banana flour and fibre-rich powder. All values showed ME±SD of data where ME = Mean and SD = Standard Deviation.

Legends: UBF=Unripe banana flour; FRP=Fiber-rich powder.

In table 4.3, the highest (ME±SD) antioxidant scores (3.39±.04) (mg TE/100 g) were obtained in BF after 10 minutes, and similar values (3.34±.05) (mg TE/100 g) were observed in both UBF and FRP after 30 minutes. Each sample was tested three times, and UBF and FRP had antioxidant capacities that were more similar to one another.

4.4 Total phenolic content and total flavonoid content (TPC, TFC) of unripe banana flour and fiber rich powder

Table 4.4 showed (ME±SD) of the total phenolic content and total flavonoid contents of unripe banana flour and fiber-rich powder which were expressed as Gallic Acid Equivalent (GAE). Both TPC 1.04±0.06 (mg GAE / 100 g) and TFC 7.5±0.04 (mg QE / 100 g) were higher in FRP.

Table 4.4 Total phenolic content and total flavonoid content of UBF and FRP

Sample	TPC (mg GAE / 100 g)	TFC (mg QE / 100 g)
UBF	0.72±0.02	5.6±0.05
FRP	1.04±0.06	7.5±0.04

There was a significant difference ($p<0.05$) of antioxidant activity between unripe banana flour and fibre-rich powder. All values showed ME±SD of data where ME = Mean and SD = Standard Deviation.

Legends: UBF=Unripe banana flour, FRP=Fiber-rich powder, TPC= Total polyphenol content, TFC=Total flavonoid content.

4.5 Total starch, resistant starch and available starch of unripe banana flour and fiber-rich powder

Table 4.5 showed (ME±SD) of total starch of banana flour and fiber-rich powder. The total starch content of UBF 45.8±0.03 (g/100g) was higher than FRP. Resistant starch content 19.13±0.01 (g/100g) was higher in UBF. The highest mean value of available starch 40.4±0.02 (g/100g) was found in FRP.

Table 4.5 Total starch, resistant starch and available starch of UBF and FRP

Sample	TS (g/100g)	RS (g/100g)	AS (g/100g)
UBF	45.8±0.03	19.13±0.01	26.7±0.04
FRP	43.5±0.05	2.3±0.06	40.4±0.02

There was a significant difference ($p<0.05$) of antioxidant activity between unripe banana flour and fibre-rich powder. All values showed ME±SD of data where ME = Mean and SD = Standard Deviation.

Legend: UBF=Unripe banana flour, FRP=Fiber-rich powder, TS= Total starch, RS= Resistant starch, AS= Available starch.

4.6 Sensory evaluation of five formulated biscuits

Sensory attributes of five formulated biscuits where one is control biscuit are presented in Table 4.6. In case of control highest score of color was recorded (8.14±.74). In the instance of sample C, aroma, appearance, taste, shape and overall approval were reported as higher (8.54±.51), (8.3±1.28), (8.5±.51), (8.7±.49) and (8.54±.51) respectively. Highest score of crispiness was recorded in case of (6.2±.86) sample D.

Except for color, all sensory qualities in the control biscuit were lower. In terms of sample C, a lower crispiness score (5.6±1.05) was reported.

Table 4.6 Sensory evaluation of five formulated biscuits

Sample	Sample A (control)	Sample B	Sample C	Sample D	Sample E
Color	**8.14±.74^{a}**	7.14±1.19^{b}	7.7±.98^{ab}	6.94±.59^{b}	6.8±.68^{b}
Aroma	6.4±.82^{c}	7.74±1.03^{ab}	**8.54±.51^{a}**	7.5±.91^{b}	7.6±.50^{b}
Appearance	6.34±.82^{b}	7.54±.92^{a}	**8.3±1.28^{a}**	7.6±.74^{a}	7.6±.63^{a}
Crispiness	5.9±.99^{a}	6.2±.68^{a}	5.6±1.05^{a}	**6.2±.86^{a}**	5.8±.68^{a}
Taste	6.34±7.2^{b}	8.0±.92^{a}	**8.5±.51^{a}**	8.07±.59^{a}	8.0±.38^{a}
Shape	7.94±.60^{b}	8.2±.68^{ab}	**8.7±.49^{a}**	8.5±.52^{ab}	8.3±.46^{ab}
Acceptability	6.54±.91^{b}	8.2±.78^{a}	**8.54±.51^{a}**	8.14±.51^{a}	8.3±.46^{a}

* Significant at P <0.05; Mean values followed by different superscript letters in the same raw denote a significant difference comparison done across formulation. All values showed (ME±SD= Mean ± Standard Deviation) of proximate composition of five formulated biscuits where one was control biscuit.

Legends: Sample A (Control) = (59% of wheat flour), Sample B = (18% fiber-rich powder), Sample C = (29.5% fiber-rich powder), Sample D= (18% unripe banana flour), Sample E = (29.5% unripe banana flour).

4.7 Water holding capacity (WHC) and oil holding capacity (OHC) of unripe banana flour and fiber-rich powder

The result of Water and oil holding capacity of banana flour and fiber-rich powder are presented in Table 4.7 The results of WHC and OHC of Unripe Banana flour and fiber-rich powder were ranged from (1.6±.05 to 2.6±.04) g of water/g dry sample and (0.9±.03 to 1.1±.05) g of oil/g dry sample respectively. In the case of unripe banana flour, both WHC (2.6±.04) g of water/g of dry sample and OHC (1.1±.05) g of water/g of dry sample were greater.

Table 4.7 Water holding capacity and oil holding capacity of UBF & FRP

Sample	WHC (g of water/g dry sample)	OHC (g of oil/g dry sample)
UBF	2.6±.04	1.1±.05
FRP	1.6±.05	0.9±.03

There was a significant difference ($p<0.05$) of antioxidant activity between unripe banana flour and fibre-rich powder. All values showed (ME±SD= Mean ± Standard Deviation) scores of WHC and OHC of unripe banana flour.

Legends: UBF=Unripe banana flour, FRP=Fiber-rich powder, WHC=Water holding capacity, OHC= Oil holding capacity.

Chapter 5: Discussions

5.1 Proximate analysis of unripe banana flour, fiber rich powder and five formulated biscuits

The CHO content is higher in FRP (86.1±.53) % than in UBF (83.7±.03) %. In the case of CHO content, a one-way ANOVA showed that the samples were significantly different ($p<0.05$). The content of carbohydrate was high in both types of flours and this is expected since unripe banana is known to contain high level of starch and dietary fibers. The carbohydrate content of unripe banana flour was higher than that of unripe plantain *(musa paradisiaca*) flours, according to **Pacheco-Delahaye et al., (2008). (Bezerra et al., 2013)** observed similar values for unpeeled banana flours and lower values observed for peeled banana. Similar results also observed by **Martinez et al., (2009)** for unripe banana flour in the ingredient to the increase of carbohydrate indigestible paste.

Rodrguez-Ambriz et al., (2008) found that the carbohydrate content of fiber-rich powder was greater. The carbohydrate contents of unripe banana flour biscuits and fiber-rich powder biscuits were similar to those reported by **Asif-Ul-Alam et al., (2014)** flour biscuits and **Adeola et al., (2018)** reported a higher carbohydrate content for biscuits made using unripe banana flour blends.

The results of total moisture content of banana flour and fiber-rich powder were ranged from (5.6-6.7) %. **Juarez-Garcia et al. (2006**) found that the overall moisture content of unripe banana flour was lower; **Liao et al., (2015)** also reported lower moisture content for chemical composition of green banana flour powder. **Rodrguez-Ambriz et al., (2008)** reported a reduced total moisture content of fiber rich powder. The total moisture content of unripe banana flour biscuits and fiber-rich powder biscuits was (1.4-3.4) % lower than the chemical composition of cookies combined with unripe banana flour (g/100 g) reported **by Agama-Acevedo et al., (2012);** similar reported by **Chakraborty et al., (2021)** for banana pseudostem substitution in wheat four biscuits enriches the nutritional and antioxidative properties with considerable acceptability. These readings were within the allowed range (less than 20%) for a long shelf life. According to **Mahloko et al., (2019)**, if the moisture level of the flour is less than 14%, it can prevent microbial growth and enhance the storage. Biscuits have a low water content, which extends the shelf life of the product. The moisture content and water activity of powder items are crucial factors that can influence other physical and chemical parameters of foods. They're also important for food security and shelf life. When compared to ripe banana flour, unripe banana flour absorbs less moisture. It's because UBF contains a lot of starch, which has a crystalline structure with amylase (1, 4 glycosidic linkage) and amylopectin (branched structure 1-4 glycosidic linkage) and doesn't effectively participate in hydrogen connect with water molecules.

The results of crude fiber ranged from (1.5 to 1.6) % for fiber-rich powder and unripe banana flour. A one-way ANOVA showed that the samples were significantly different ($p<0.05$). **Norhidayah et al., (2014)** showed crude fiber (1.65 ± 0.06) % for chemical composition of banana flour (BF) prepared from unripe banana. The result of crude fiber for fiber rich powder was found (1.6±06) % lower than that of the results of **Rodríguez-Ambriz et al., (2008).** The recommended daily fiber intake is 25 to 30 g, with insoluble fibers making up the majority of the consumption (70 to 75 %). The soluble fiber/insoluble fiber content, which is mentioned in the literature, is crucial for the assessment of functional characteristics **(Figuerola et al., 2005)**.UBF exhibited a 2.6% ash content, a value that decreased in the fiber-rich powder 1.6%. It may be concluded that during the liquefaction process, the washing stage removed some minerals present in UBF. The ash content of the UBF was found to be higher than that of other study findings **(Ovando-Martinez et al., 2009).**Moreover, our result is supported by the study of **Liao et al., (2015).** On the other hand our result is lower than reported by **Juarez-Garcia et al., (2006), Aguirre-Cruzet al., (2006).** The higher the mineral concentration, the higher the ash content, which is depends on flour quality. Two experimental flours and all formulated biscuits had a greater ash level, indicating that they are better mineral sources. The results of ash content ranged from (1.4 to 1.8) % for UBF and FRP biscuits lower than reported by **Shareenie et al., (2021)**for the effects of unripe Saba banana composite flour on acceptance and physicochemical characteristics of biscuits .

Fat contents were slightly low in both flour types. As the same quantity of fat added, the fat content of both flours biscuits has not differed much. Moreover, The results of fat content of unripe banana flour and fiber-rich powder(1.0±.04) % were similar and this result was higher than the values reported by others, being estimated to be between (0.33 and 0.82) % **(Da Mota et al.,2000)**. Fat content ranged from (13.6 to 17.7) % for UBF and FRP biscuits. In case of fat content, a one-way ANOVA showed that the samples were significantly different ($p<0.05$). **Liao et al., (2015); Ovando-Martinez et al., (2009)** represent fat content (0.25 ± 0.07) %, (0.54 ± 0.03) % and (0.94±0.04) % respectively for banana flour and fiber-rich banana flour. Fat content of UBF biscuits and FRP biscuits higher than that of **(Chakraborty et al., 2021)** for banana pseudostem substitution in wheat four biscuits. According to **Emaga et al., (2007),** banana and plantain peels are rich in polyunsaturated fatty acids, particularly linoleic acid and a-linolenic acid.

The results of crude protein content were higher in all biscuits which ranged from (7.6 to 10.1) % than in UBF and FRP flours. In addition, protein content is highest in FRP biscuits than UBF biscuits. In the case of crude protein content, a one-way ANOVA showed that the samples were significantly different ($p<0.05$). Protein is essential for the growth, recovery, and maintenance of the human body, as well as the maintenance of fluids, and protein functions as enzymes, hormones, and other substances. Because eggs were used in the biscuit production as egg is a good protein source, all biscuits had a much greater protein content than the corresponding flour **(Adeola and Ohizua, 2018)**.The crude protein of UBF were lower than the findings reported by **Haslinda et al., (2021)** for Chemical composition and physicochemical properties of green banana *(Musa acuminate balbisiana Colla v. Awak*) and higher than the value observed by **Rodríguez et al., (2008)**. The amylolytic phase of flour liquefaction, which leads to fiber-rich powder, is responsible for this result because it removes water-soluble proteins from the supernatant. In the case of biscuits, the crude protein result is lower than the findings of **Chakraborty et al., (2021).**

5.2 Mineral content of unripe banana flour, fiber rich powder and wheat flour

Calcium, Copper, and Zinc mineral concentration was determined to be similar to **Anajekwu et al., (2020)**, but lower than the value recorded by **Haslinda et al. (2009)** for chemical composition and physicochemical properties of green banana (*Musa acuminate balbisiana Colla cv. Awak*) flour. The value of potassium (K) of this study was higher than the value found by **Anajekwu et al., (2020)** for physicochemical properties and total carotenoid content of high-quality unripe plantain flour from varieties of hybrid plantain cultivars and lower than the value found the by **Haslinda et al., (2009**). Sodium (Na) content is higher than **Anajekwu and their team (2020).** In comparison to **Abbas et al., (2009)** findings UBF and FRP had lower levels of magnesium (Mg), phosphorus (P), chloride (Cl), and iron (Fe) for physicochemical properties and total carotenoid content of high-quality unripe plantain flour from varieties of hybrid plantain cultivars.

5.3 Antioxidant capacity of unripe banana flour and fiber-rich powder

Banana being abundant in antioxidants have contained bioactive compounds include phenolics, carotenoids, dopamine, dopa, carotenes, norepinephrine, and ascorbic acid (vitamin C), all of which can protect the body from oxidative stress. Antioxidant compounds found in banana flour are frequently linked to an increase in free radical scavenging abilities. According to **Goni et al., (1997)** polyphenols associated with polysaccharides and proteins in cell walls, are important components of dietary fiber. The highest value of antioxidant in UBF initially was (3.39±.04) mg TE/100 g and it has gradually decreased in proportion to time, after a few minutes (30 minutes), the antioxidant result in both UBF and FRP became the lowest (3.34±.05) mg TE/100 g lower than the results recorded by **Ovando-Martinez et al., (2009)** for antioxidant capacity (ABTS method) (l mol Trolox eq/g).

5.4 Total phenolic content (TPC) and total flavonoid content (TFC) of unripe banana flour and fiber rich powder

Phenolic compounds, which have the capability to donate hydrogen atoms to free radicals, are called main antioxidants or free radical terminators. Gallic acid, catechin, epicatechin, tannins, anthocyanins, catecholamines, phenolic acids, and flavonoids are some of the phenolic compounds found in bananas **(Singh et al., 2016)**. The most essential antioxidants are polyphenols, which are found in fruits and vegetables. The results of total phenolic content (TPC) were ranged from (0.72-1.04) mg GAE/100g for unripe banana flour and fiber-rich powder. The total phenolic content of UBF and FRP was higher than the value reported by **Savlak et al. (2016)** for unripe banana flour (UBF) for different particle size distributions. The polyphenol content of fiber rich powder was lower than the value found by **Rodríguez-Ambriz et al., (2008)** for characterization of a fiber-rich powder prepared by liquefaction of unripe banana flour. Total phenolic content of unripe banana flour was lower than the findings reported by **Khoza et al., (2021)** for green banana flour.

Unripe banana flour is high in TFC compared to wheat flour, and the main classes of flavonoids detected in bananas are quercetin, myricetin, and kaempferol. Flavonoids are active compounds against many infectious diseases (bacterial and viral diseases), cardiovascular diseases, cancers, and other age-related diseases. In this work, unripe banana flour had obtained lower TFC than fiber-rich powder. The results of total flavonoid content (TFC) were ranged from (5.6-7.5) mg QE/100g for UBF and FRP. According to **Khoza et al., (2021)** unripe banana flour has a lower total flavonoid content than fibre-rich powder.

5.5 Total starch, RS and AS of unripe banana flour and fiber-rich powder

The amount of glucose liberated during product digestion is directly proportional to the total starch content. The result of total starch was higher in unripe banana flour than that in the fiber-rich powder. Such high starch levels may lead to the formation of resistant starch (RS) during the processing of BF-based products, as has been seen in banana starch extrudates. The total starch (TS) content in the fiber-rich powder was lower (43.5) g/100g than that in the UBF. Because, un-gelatinized banana starch is not fully hydrolyzed by a-amylase. In this study TS in UBF (45.8±0.03) (g/100g) and in FRP (43.5±0.05)(g/100g) is higher than **Haslinda et al., (2009)** for chemical composition and physicochemical properties of green banana (*Musa acuminata ,balbisiana Colla cv. Awak*) flour lower than (range 68.42 to 76.77)(g/100g) reported by **Juarez-Garcia et al., (2006)**

Surprisingly, banana flour is an excellent source of RS. When RS exhibits physiological effects that are similar to those generated by dietary fibre, it can be employed as a source of DF. RS has received a lot of interest in the last two decades because of its possible health benefits (similar to soluble DF), such as how it affects the digestive tract, microbiome, blood cholesterol levels, glycemic index, and diabetes control. The result of resistant starch of UBF and FRP are presented in Table 4.5. Where resistant starch higher in BF than FRP. When resistant starch (RS) was assessed in banana flour, a high content (19.13) g/100g was observed, but the value dropped dramatically in fiber-rich powder (2.3) g/100g, confirming the effect of boiling during liquefaction of the sample, which destroyed the starch granular crystalline structure, resulting in a decrease in RS content (Table 4.5). UBF has a resistant starch of 19.13± 0.04 (g/100g), which is higher than the value found by **Juarez-Garcia et al., (2006)** but lower than the recorded value of **Liao et al., (2015)**, who have a resistant starch of 30.30±2.66 (g/100g).;also lower than the value 25.33±0.14 (g/100g) for resistant starch (RS) by **Norhidayah et al.,**

(2014) in composite flours consist of banana flour (BF). In my study the resistant starch found in FRP was 2.3±0.06 (g/100g), although **Rodrguez-Ambriz et al. (2008)** reported a resistant starch of 3.6 ± 0.4 (g/100g), which was lower than the results of this investigation.

Available starch refers to the portion of total starch that can be hydrolyzed by digestive enzymes. Available starch was highest in FRP 40.4±0.02 (g/100g) and lowest in UBF 26.7±0.04 (g/100g) and other study represented (27.78 to 56.3) g/100g by **Juarez-Garcia et al., (2006).**

5.6 Sensory evaluation of five formulated biscuits

Organoleptic tests of the biscuits depend on its color, aroma, appearance, crispness, taste, shape and overall acceptability of the sample. Table 4.6 shows the summary of sensory attributes of UBF and FRP biscuits and control biscuits. There were significant differences ($p<0.05$) between color, aroma, appearance, crispness, taste, shape, and overall acceptability between the five biscuit samples as ranked by the panelists The control biscuit sample scored highest (8.14±.74) for color and lowest in sample E. It was observed that with the increased in UBF substitution, there is decrease in color of the biscuits. The low acceptability in color for samples with substitution of the UBF may be due to banana flour that initially darker in color compared to the wheat flour that lighter in color. The color of the biscuit become darker as the baking process takes place.

Sample C scored highest (8.54±0.51) for aroma and lowest in control biscuit. In terms of aroma the butter used in the production of the biscuits produced its own distinctive aroma which might have been more dominant. Sample C scored highest (8.3±1.28) for appearance and lowest in control. Sample D scored highest (6.2±.86) for crispiness and lowest in sample C. Sample C scored highest (8.5±0.51) for taste and lowest in control. Sample C scored highest (8.7±.49) for shape and lowest in control. Biscuits structures are primarily affected by three components which were sugar, flour, and eggs. According to **Weaver, (2006)** flour has three effects on the construction of biscuits. Starches is gelatinizing and will form the structure of the cookies when they are bake, gluten strands in the flour generate a chewy texture, and high protein level contributes in the production of a considerably chewier texture since more gluten strands are formed with a greater protein concentration **(Weaver, 2006)**. Overall acceptability was highest for sample C (8.54±.51) and lowest for control biscuit. Statistical analysis for taste, appearance, and overall acceptance showed a significant difference ($p<0.05$) in the level of acceptance of the biscuits. This could have occurred due to the higher percentage of fiber-rich powder content in sample C which triggered a strong better taste intensity; this heightened betterness resulted in the highest acceptance.

5.7 Water holding capacity (WHC) and oil holding capacity (OHC) of unripe banana flour and fiber-rich powder

Water holding capacity of the UBF increased with time due to hygroscopic nature of flours. Hygroscopicity is due to the attainment of equilibrium between product and surrounding environment at particular relative humidity and temperature conditions. Due to increase in water activity bulk density of the flours also increased. Bulk density relates with the flowability of the flour therefore it can affect the conveying and storage properties. The unripe banana flour (2.6±.04) g of water/g dry sample had higher than had the fiber-rich powder (1.6±.05) g of water/g dry sample. This may be explained by the high total starch content in the native banana flour. The Water holding capacity of unripe banana flour (2.6±.04) g of water/g dry sample lower than represented by **Pragati et al., (2014)** for comparative study of ripe and unripe banana flour during storage.

Another important functional property of fiber ingredients is the OHC. Good oil absorption capacities of the flours suggest that they may be useful in food preparations that involve oil mixing, such as in bakery products where oil is an important ingredient. In unripe banana flour, which was approximately (1.1±.05) g of oil/g dry sample .The fiber-rich powder held (0.9±.03) g of oil/g dry sample which is lower than UBF sample. Other fiber concentrates exhibit notably lower OHC, e.g oil-holding capacity (OHC) in unripe banana flour (0.80±.05) g of oil/g (**Alkarkhi et al., 2011)** and higher in fiber-rich powder (1.8 g of oil/g) (**Rodríguez-Ambriz et al., 2008)**. Accordingly, the use of fiber-rich powder may be appropriate in products where emulsifying properties are not required.

Chapter 6: Conclusion

Bananas are one of the fruits that can be regarded as improving consumer health. The goal of this study was to determine the proximate composition, bioactive compounds and physicochemical properties of unripe banana flour and fiber-rich powder obtained from unripe banana flour (*Musa acuminata*), as well as the proximate composition of biscuits made with both banana flours. The development of such functional foods (as biscuits) not only improves the nutritional status of the general population but also helps those suffering from degenerative diseases associated with today's changing life styles and environment. At the same time, the study attempts to address the problem of bio-resource wastage as huge amounts of unripe banana are wasted every year after harvesting of the fruits. Banana flour is favorable for industrial processing due to its rich content of soluble solids, minerals, dietary fiber, resistant starch and free radical scavenging properties. It was pointed out that these flours might be an important source of polyphenols, compounds that are regarded as natural antioxidants. Fiber and resistant starch helps in preventing colon cancer because they are substrate for the colonic microorganisms. The rapidly digestible starch decreased and the slowly digestible starch increased in UBF which could be a nutritional alternative for people with health problems such as diabetes and obesity. The data obtained in this study will be successfully used to predict the shelf life of unripe banana flour, fiber-rich powder and formulated biscuits and understand their neutraceutical properties. Therefore, the data in our study may provide the basis for future research.

Chapter 7: Recommendations & future perspectives

According to the new economic strategy to increase waste utilization of food products and convert them into various innovative products. Banana includes the production of banana flour when the fruit is unripe, and to incorporate the flour into slowly digestible biscuits. Intake of flour from unripe banana can improve the gastrointestinal function, decrease the glycaemia response of food in the human gut as well as reduce the risk of several disorders due to its variety of metabolites and bioactive compounds. Therefore, in view of waste management and value added to agro byproducts, the present study elucidates the effects of two banana flour on the chemical composition and their functional properties. Besides adding nutritional value to biscuits, banana flour also stands out for increasing farmer's income, representing the complete use of the fruit and benefits in humans in the future.

The following are the recommendations based on the findings of this study:

- Cultivar variation, method of extraction, climatic differences and agricultural practices could have a major impact in the chemical composition of flours. Also processing methods of cabinet drying and heat treatment of liquefaction used in this study showing little effect. So, further evaluation should be needed in case of nutritional composition.
- More investigations on applying the unripe banana to other food products are needed. Because color and taste of banana flour might affect some quality aspect of food products.
- Further studies on the simulated gastrointestinal digestion of the biscuits in future should be carried out to present an accurate picture of the nutritional and health benefits.
- Drying and liquefaction procedures should also be improved in order to produce higher-quality flour.
- Bioactive compounds, minerals and microbiological analysis in biscuits were not examined due to a lack of time and availability of facilities. To evaluate these compositions, more research will be required. Microbial analysis may also improve the amount of product acceptance.

References

AACC. 2000. Approved Method of the AACC. 10 th edition. American Association of Cereal.

Abbas FM, Saifullah R, Azhar ME. 2009. Differentiation of ripe banana flour using mineral composition and logistic regression model. International Food Research Journal, 16(1): pp. 83-87.

Adeola AA and Ohizua ER. 2018. Physical, chemical, and sensory properties of biscuits prepared from flour blends of unripe cooking banana, pigeon pea, and sweet potato. Food Science and Nutrition, 6 (3): pp.532-540.

Agama-Acevedo E, Islas-Hernández JJ, Pacheco-Vargas G, Osorio-Díaz P, Bello-Pérez LA. 2012. Starch digestibility and glycemic index of cookies partially substituted with unripe banana flour. LWT-Food Science and Technology, 46(1): pp.177-82.

Aguirre-Cruz A, Álvarez-Castillo A, Yee-Madeira H, Bello-Pérez LA. 2008. Production of fiber-rich powder by the acid treatment of unripe banana flour. Journal of applied polymer science, 109 (1): pp.382-387.

Akihisa T, Shimizu N, Tamura T and Matsumoto T. 1986. (24S)-14α, 24-dimethyl-9β, 19-cyclo-5α-cholest-25-en-3β-ol: A new sterol and other sterols in Musa sapientum, 21(8): pp.494-497.

Alkarkhi AF, bin Ramli S, Yong YS, Easa AM. 2011. Comparing physicochemical properties of banana pulp and peel flours prepared from green and ripe fruits. Food Chemistry, 129(2): pp.312-318.

Anajekwu EO, Maziya-Dixon B, Akinoso R, Awoyale W, Alamu EO. 2020. Physicochemical properties and total carotenoid content of high-quality unripe plantain flour from varieties of hybrid plantain cultivars. Journal of Chemistry, pp.68-71.

AOAC. 2016. Official methods of analysis. 20th ed. Washington DC: Association of Official Analytical Chemists.

Aparicio-Saguilán A, Sáyago-Ayerdi SG, Vargas-Torres A, Tovar J, Ascencio Otero TE & Bello-Pérez LA. 2007. Slowly digestible cookies prepared from resistant starch-rich lintnerized banana starch. Journal of Food Composition and Analysis, 20 (3-4): 175-181.

Asif-Ul-Alam SM, Islam MZ, Hoque MM, Monalisa K. 2014. Effects of drying on the physicochemical and functional properties of green banana (Musa sapientum) flour and development of baked product. American Journal of Food Science and Technology, 2(4): pp.128-33.

Aurore G, Parfait B, Fahrasmane B. 2009. Bananas, raw materials for making processed food products. Trends Food Science Technology, 20(2): pp.78–91.

Azizi J, Ismail S, Mordi MN, Ramanathan S, Said MIM, Mansor SM. 2010. In vitro and in vivo effects of three different Mitragyna speciosa Korth leaf extract on phase II drug metabolizing enzymes—glutathione transferases (GSTs). Molecules, 15 (1): pp. 432-441.

Azlim Almey AA, Ahmed Jalal Khan C, Syed Zahir I, Mustapha Suleiman K, Aisyah MR and Kamarul Rahim K. 2010. Total phenolic content and primary antioxidant activity of methanolic and ethanolic extracts of aromatic plants' leaves. International Food Research Journal, 17(4).

BBS (Bangladesh Bureau of Statistics). 2016. Statistical Year Book of Bangladesh, Ministry of Planning, Government of the People's Republic of Bangladesh, Dhaka, Bangladesh.

BBS 2012. Statistics year book of Bangladesh. Bangladesh bureau of statistics. Ministry of Planning, Government of the people's Republic of Bangladesh, Dhaka.

Bello-Pérez LA, Rendon-Villalobos R, Agama-Acevedo E & Islas-Hernández JJ. 2006. In vitro starch digestibility of tortillas elaborated by different masa preparation procedures. Cereal Chemistry, 82: pp.188-193.

Bennett RN, Shiga TM, Hassimotto NM, Rosa EA, Lajolo FM. and Cordenunsi BR. 2010. Phenolics and antioxidant properties of fruit pulp and cell wall fractions of postharvest banana (Musa acuminata Juss.) cultivars. Journal of Agricultural and Food Chemistry, 58(13): pp.7991-8003.

Bezerra CV, Rodrigues AM, Amante ER, Silva LH. 2013. Nutritional potential of green banana flour obtained by drying in spouted bed. Revista Brasileira de Fruticultura, 35: pp.1140- 1146.

Bjorck IM, Granfeldt Y, Liljeberg H, Tovar J, Asp N .1994. Food ¨ properties affecting the digestion and absorption of carbohydrates, The American Journal of Clinial Nutrition, 59(3): pp. 699–705.

Borges CV, Amorim VBO, Ramlov F, Ledo CAS, Donato M, Maraschin M, & Amorim EP. 2014. Characterisation of metabolic profile of banana genotypes, aiming at biofortified *Musa spp*. cultivars. Food Chemistry, 145: pp. 496–504.

Brand-Miller J, Hayne S, Petocz P & Colagiuri S. 2003. Low-glycemic index diets in the management of diabetes: a meta-analysis of randomized clinical trials. Diabetes Care, 26 (8): pp.2261-2267.

Buckley EH. 1961. Further studies on the biosynthesis of 3-hydroxytyramine in the peel of the banana. Plant Physiology, 36: pp.315–320.

Celik I, Yilmaz Y, Isik F, Ustun O. 2007. Effect of soapwort on physical and sensory properties of sponge cakes and rheological properties of sponge cake batters. Food Chemistry. 101: pp.907-911.

Chakraborty R, Sabruna S, Roy R, Majumdar S, Roy S. 2021 Jan. Banana pseudostem substitution in wheat flour biscuits enriches the nutritional and antioxidative properties with considerable acceptability. SN Applied Sciences, 3(1): pp.1-2.

Chandra S, Singh S, Kumari D. 2015. Evaluation of functional properties of composite fours and sensorial attributes of composite four biscuits. The Journal of Food Science Technology, 52(6): pp.3681–3688.

Chang CC, Yang MH, Wen HM, Chern JC. 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of food and drug analysis, 10 (3): pp.40-44.

Chauhan UPS and Sarkar BR. 1969. Use of calmagite for the determination of traces of magnesium in biological materials. Analytical biochemistry, 32(1): pp.70-80.

Chemist, INC .st., Paul, Minnesota, USA.

Choudhary SP, Tran LS. 2011. Phytosterols: perspectives in human nutrition and clinical therapy. Current Medicinal Chemistry, 18: 4557–4567.

Cleary L & Brennan C. 2006. The influence of a (1- 3) (1-4)-b-D-glucan rich fraction from barley on the physico-chemical properties and in vitro reducing sugars release of durum wheat pasta. International Journal of Food Science and Technology, 41: pp.910–918.

Da Mota RV, Lajolo FM, Ciacco C, Cordenunsi BR. 2000. Composition and functional properties of banana flour from different varieties. 52 (2-3): pp.63–68.

Davey MW, Bergh IV, Markham R, Swennen R, Keulemans J. 2009. Genetic variability in Musa fruit provitamin A carotenoids, lutein and mineral micronutrient contents. Food Chemistry, 115(3): pp.806–813.

Davey MW, Keulemans J, Swennen R. 2006. Methods for the efficient quantification of fruit provitamin A contents. Journal of Chromatography, 1136(2): pp.176–184.

Dervisoglu M, Yazici F. 2006. Note. The effect of citrus fibre on the physical, chemical and sensory properties of ice cream. Food Science and Technology International, 12(2): pp.159–164.

Drzikova B, Dongowsky G, Gebhardt E, Habel A. 2005. The composition of dietary fibre-rich extrudates from oat affects bile acid binding and fermentation in vitro. Food Chemistry, 90(1-2): pp.181–192.

Emaga TH, Andrianaivo RH, Wathelet B, Tchango JT, Paquot M. 2007. Effects of the stage of maturation and varieties on the chemical composition of banana and plantain peels. Food Chemistry, 103(2): pp. 590-600.

Englberger L, Wills R B, Blades B, Dufficy L, Daniells JW, Coyne T. 2006. Carotenoid content and flesh color of selected banana cultivars growing in Australia. Food and Nutrition Bulletin, 27(4): pp.281–291.

Englyst HN, Kingman SM & Cummings JH. 1992. Classification and measurement of nutrionally important starch fractions. European Journal of Clinical Nutrition, 46: pp.33-50.

Erdman JW, Jr Bierer TL, Gugger ET. 1993. Absorption and transport of carotenoids. Annals of the New York Academy of Sciences, 691: pp.76–85.

Faisant IN, Gallant DJ, Bouchet B & Champ M. 1995. Banana starch breakdown in the human small intestine studied by electron microscopy. European Journal of Clinical Nutrition, 49(2): pp.98–104.

FAO, 2009. Production Year Book. Food and Agriculture Organization of the United Nations, Rome, Italy.

Ferreres F, Valentão P, Pereira J, Bento A, Noites A, Seabra RM, Andrade PB. 2008. HPLC-DAD-MS/MS-ESI screening of phenolic compounds in Pieris brassicae

L. reared on Brassica rapa var. rapa L. Journal of agricultural and food chemistry, 56(3): pp. 844-853.

Figuerola F, Hurtado ML, Estévez AM, Chiffelle I, Asenjo F. 2005. Fibre Concentrates From Apple pomace and citrus peel as potential fibre sources for food enrichment. Food Chemistry, 91(3): pp. 395-401.

Flores-Gorosquieta, E, Garcıa-Suarez, FJ, Flores-Huicochea E, Nunez-Santiago MC, Gonzalez-Soto RA & Bello-Perez LA. 2004. Multi-objective optimization of process conditions in the manufacturing of banana (*Musa paradisiaca L.*) starch/natural rubber films. Carbohydrate Polymers, 55: pp.86–90.

Freeland-Graves JH & Trotter PJ. 2003. Minerals – dietary importance. In B Caballero L, Trugo & P Finglas (Eds.). Encyclopedia of food science and nutrition, California, San Diego: Academic Press, pp. 4005–4012.

Fu L, Tian JC, Sun CL, Li C. 2008. RVA and farinograph properties study on blends of resistant starch and wheat starch. Agricultural Sciences in China, 7: pp.812-822.

Fuentes ZE, Riquelme NMJ, Sanchez ZE, Perez AJA. 2010. Resistant starch as a functional ingredient: A review. Food Research International, 43: pp.931-942.

Fungo R, Pillay M. 2013. β-Carotene content of selected banana genotypes from Uganda. African Journal of Biotechnology, 10: pp.5423–5430.

Giri SS, Jun JW, Sukumaran V and Park SC. 2016. "Dietary administration of banana (Musa acuminata) peel flour affects the growth, antioxidant status, cytokine responses, and disease susceptibility of Rohu, Labeo rohita," Journal of Immunology Research.

Goñi I & Valentín-Gamazo C. 2003. Chickpea flour ingredient slows glycemic response to pasta in healthy volunteers. Food Chemistry, 81(4): pp.511–515.

Goñi I, Díaz-Rubio ME, Pérez-Jiménez J, Saura-Calixto F. 2009. Towards an updated methodology for measurement of dietary fibre, including associated polyphenols, in food and beverages. Food Research International, 42: pp.840–844.

Goñi, I, Garcia-Alonso A, Saura-Calixto F. 1997. A starch hydrolysis procedure to estimate glycemic index. Nutrition Research, 17(3): pp.427–437.

González-Montelongo R, Lobo MG, González M. 2010. Antioxidant activity in banana peel extracts: testing extraction conditions and related bioactive compounds. Food Chemistry, 119(3): 1030–1039.

Granfeldt Y .1994. Food factors affecting metabolic responses to cereal products. Doctoral Dissertation, University of Lund, Sweden, pp. 27–29.

Haque MA. 1988. Kolar Bagan (In Bengali). 3rd Edition. Banana Research Project, Bangladesh Agricultural University, Mymensingh, pp. 24.

Haslinda WH, Cheng LH, Chong LC, Aziah AN. 2009. Chemical composition and physicochemical properties of green banana (Musa acuminata× balbisiana Colla cv. Awak) flour. International journal of food sciences and nutrition, 60(4): pp. 232-239.

Havsteen BH. 2002. The biochemistry and medical significance of the flavonoids. Pharmacology & Therapeutics, 96(2-3): pp.67–202.

Heim KE, Tagliaferro AR, Bobilya DJ. 2002. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. The Journal of Nutritional Biochemistry, 13(10): pp.572–584.

Heo HJ, Choi SJ, Choi SG, Shin DH, Lee JM, Lee CY. 2008. Effects of banana, orange, and apple on oxidative stress-induced neurotoxicity in PC12 cells. Journal of Food Science, 73(2): pp.28-32.

Homayouni A, Amini AK, Keshtiban AM, Mortazavian K, Esazadeh, and S Pourmoradian. 2014. Resistant starch in food industry: A changing outlook for consumer and producer, 66(1–2): pp.102–114.

Hossain MF. 2014. A Study of Banana Production in Bangladesh: Area, Yield and Major Constraints, ARPN Journal of Agricultural and Biological Science, 9(6): pp.206.

Islam MR, Azad, AK, Abuhena M, Haldar R. and Rahman S. 2016. Analysis of Genetic Variation between five Banana Fruit Varieties by RAPD Markers, Journal of Biological Engineering Research and Review,3(1).

Jew S, Antoine JM, Bourlioux P, Milner J, Tapsell L C, Yang Y & Jones PJH. 2015. Nutrient essentiality revisited. Journal of Functional Foods, 14: pp. 203–209.

Jimenez-Escrig A & Sanchez-Muniz FJ. 2000. Dietary fibre from edible seaweeds: Chemical structure, physicochemical properties and effects on cholesterol metabolism, Nutrition Research, 20(4): pp.585–598.

Juarez-Garcia E, Acavedo-Agama E, Ayerdi-Sayago SG, Ambrez Rodriguez SL. 2006. Composition, digestibility and application in bread making of banana flour. Plant Foods Human Nutrition, 61(3): pp.131-137.

Karaman S, Toker OS, Yuksel F, Cam M, Kayacier A, Dogan, M. 2014 .Physicochemical, bioactive, and sensory properties of per simmon-based ice cream: technique for order preference by similarity to ideal solution to determine optimum concentration. Journal of Dairy Science, 97(1): pp.97–110.

Khoza M, Kayitesi E, Dlamini BC. 2021. Physicochemical Characteristics, Microstructure and Health Promoting Properties of Green Banana Flour, 10(12): pp.2894.

Kondo S, Kittikorn M & Kanlayanarat S. 2005. Preharvest antioxidant activities of tropical fruit and the effect of low temperature storage on antioxidants and jasmonates. Postharvest Biology and Technology, 36(3): pp.309–318.

Krinsk NI, Johnson EJ. 2005. Carotenoid actions and their relation to health and disease. Molecular Aspects of Medicine, 26(6): pp.459–516.

Kuklin AI, Conger BV. 1995. Catecholamines in plants. Journal of the Plant Growth Regulation, 14(2): pp. 91–97.

Kumar KPS, D Bhowmik, S Duraivel and M Umadevi. 2012. Traditional and medicinal uses of banana. The Journal of Pharmacognosy Phytochemistry, 1(3): pp.51-63.

Laguna L, Salvador A, Sanz T and Fiszman SM. 2011. Performance of a resistant starch rich ingredient in the baking and eating quality of short-dough biscuits. LWT - Food Science and Technology, 44(3): pp.737-746.

Lewis DA, Fields WN, Shaw GP. 1999. A natural flavonoid present in unripe plantain banana pulp (*Musa sapientum L.* Var. *paradisiaca*) protects the gastric mucosa from aspirin-induced erosions. Journal of Ethnopharmacology, 65(3): pp.283–288.

Li C, Ford ES, Zhao G, Balluz LS, Giles WH, Liu S. 2011. Serum α-carotene concentrations and risk of death among US adults: the third national health and nutrition examination survey follow-up study. Archives of Internal Medicine, 171: pp.507–515.

Liao HJ, Hung CC. 2015. Chemical composition and in vitro starch digestibility of green banana (cv. Giant Cavendish) flour and its derived autoclaved/debranched powder. LWT-Food Science and Technology, 64(2): pp.639-644.

Liljeberg H, Akerberg A, Bjorck I .1996. Resistant starch formation ¨ in bread as influenced by choice of ingredients or baking conditions, Food Chemistry, 56(4): pp.389–394.

Mahloko LM, Silungwe H, Mashau ME and Kgatla, TE. 2019. Bioactive compounds, antioxidant activity and physical characteristics of wheat-prickly pear and banana biscuits, Heliyon, 5(10): pp.2479.

Marangoni F, Poli A. 2010. Phytosterols and cardiovascular health. Pharmacological Research, 61(3): pp.193–199.

Martinez MO, Ayerdi SS, Agama EC, Goni I, Bello PL. 2009. Unripe banana flour in the ingredient to the increase of carbohydrate indigestible paste. Food Chemistry, 113: pp.121-126.

McCleary BV, Monaghan DA. 2002. Measurement of resistant starch. Journal AOAC International, 85(3): pp.665–675.

Mohapatra DS, Mishra and Sutar N. 2010. Banana and its by-product utilization: An overview. Journal of Science Industry Research, 69: pp.323-329.

Nelson SC, Ploetz RC and Kepler AK. 2006. Musa species (banana and plantain), Musaceae (banana family), 15(2): pp.251-259.

Norhayati MK, Fairulnizal MM, Zaiton A, Wan Syuriahti W, Rusidah S, Aswir A, Ang J, Naeem M, Suraiami M, Azerulazree J. and Vimala B, 2015. Nutritional composition of selected commercial biscuits in Malaysia. *Sains Malaysiana*, 44(4): pp.581-591.

Norhidayah M, Noorlaila A, Izzati NF. 2014. Textural and sensorial properties of cookies prepared by partial substitution of wheat flour with unripe banana (Musa x paradisiaca var. Tanduk and Musa acuminata var. Emas) flour. International Food Research Journal, 21(6): pp.21-33.

Ovando-Martinez M, Sáyago-Ayerdi S, Agama-Acevedo E, Goñi I, Bello-Pérez LA. 2009. Unripe banana flour as an ingredient to increase the undigestible carbohydrates of pasta. Food Chemistry, 113(1): pp.121-126.

Pacheco-Delahaye E, Maldonado R, Pérez E, Schroeder M. 2008. Production and characterization of unripe plantain (Musa paradisiaca L.) flours, 33(4): pp. 290-296.

Pacheco-Delahaye E, Perez R & Schnell M. 2004. Nutritional and sensory evaluation of powder drinks based on papaya, green plantain and rice bran glycemic index. Interciencia, 29: pp.46-51.

Penn-Marshall M, Holtzman GI, Barbeau WE .2010. African americans may have to consume more than 12 grams a day of resistant starch to lower their risk for type 2 diabetes. Journal of Medicinal Food 13(4): pp.999-1004.

Pérez-Jiménez J, Arranz S, Tabernero M, Díaz-Rubio ME, Serrano J, Goñi I, Saura-Calixto F. 2008. Updated methodology to determine antioxidant capacity in plant foods, oils and beverages: extraction, measurement and expression of results. Food Research International, 41(3): pp. 274–85.

Peters U. 2003. Dietary fibre and colorectal adenoma in a colorectal cancer early detection programme. The Lancet, 361(9368): pp.1491–1495.

Pragati S, Genitha I, Ravish K. 2014 Jan. Comparative study of ripe and unripe banana flour during storage. Journal of Food Processing & Technology, 5(11): pp.1.

Puente LA, Pinto-Muñoz CA, Castro ES, Cortés M. 2009. Physalis peruviana Linnaeus, the multiple properties of a highly functional fruit: a review. Food Research International, 44: pp.1733–1740.

Ramakrishna BS, Venkataraman S, Srinivasan P, Dash P, Young GP, Binder HJ. 2000. Amilase-Resistant Starch Plus Oral Rehydration Solution for Choleration. New England Journal of Medicine, Waltham, 342: pp.308-313.

Rayo LM, Chaguri e Carvalho L, Sardá, FAH, Dacanal GC, Menezes EW & Tadini CC. 2015. Production of instant green banana flour (Musa cavendischii, var. Nanicão) by a pulsed-fluidized bed agglomeration. LWT – Food Science and Technology, 63(1): pp.461–469.

Rebello C, Greenway FL & Dhurandhar NV. 2014. Functional foods to promote weight loss and satiety. Current Opinion in Clinical Nutrition and Metabolic Care, 17(6): pp.596–604.

Rice-Evans CA, Miller NJ, Paganga G. 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Biology & Medicine, 20(7): 933–956.

Rodríguez-Ambriz SL, Islas-Hernández JJ, Agama-Acevedo E, Tovar J, Bello-Pérez LA. 2007. Characterization of a fibre-rich powder prepared by liquefaction of unripe banana flour. Food Chemistry, 107(4): pp. 1515–1521.

Saifullah R, Abbas FMA, Yeoh SY, Azhar ME. 2009. Utilization of green Banana flour as a functional ingredient in yellow noodle. Internatioal Food Research Journal, 16(3): pp.373-379.

Sánchez D, Miguel M & Aleixandre A. 2012. Dietary fiber, gut peptides, and adipocytokines. Journal of Medicinal Food, 15(3): pp.223–230.

Saura-Calixto F, García-Alonso A, Goñi I & Bravo L. 2000. In vitro determination of the indigestible fraction in foods: an alternative to dietary fibre analysis. Journal of Agricultural and Food Chemistry, 48(8): pp. 3342–3347.

Savlak N, Türker B, Yeşilkanat N. 2016. Effects of particle size distribution on some physical, chemical and functional properties of unripe banana flour. Food Chemistry, 213: pp.180-186.

Scarminio V, Fruet AC, Witaicenis A, Rall VL, Di Stasi LC. 2012. Dietary Intervention With Green Dwarf Banana Flour (Musa Sp Aaa) Prevents Intestinal Inflammation In A Trinitrobenzesulfonic Acid Model Of Rat Colitis. Nutrition Research, Tarrytown, 32 (3): pp.202-209.

Schneeman BO. 1987. Soluble vs insoluble fiber-different physiological responses. Food Technology, 47: pp.81–82.

Schoenfeld RG, Lewellen CJ. 1964. A colorimetric method for determination of serum chloride. Clinical Chemistry, 10(6): pp.553-539.

Segundo C, Román L, Gómez M, Martínez MM. 2017. Mechanically fractionated four isolated from green bananas (M. cavendishii var. nanica) as a tool to increase the dietary fber and phytochemical bioactivity of layer and sponge cakes. Food Chemistry, 219: pp.240–248.

Shareenie MA, Matkhir AA, Akanda JH, Mamat H, Hamid MA. 2021 Jan. The Effects of Unripe Saba Banana Composite Flour on Acceptance and Physicochemical Characteristics of Biscuits. Scientific Research Journal, 18(1): pp.55-70.

Singh B, Singh JP, Kaur A & Singh N. 2016. Bioactive compounds in banana and their associated health benefits – a review. Food Chemistry, 206: pp.1–11.

Singh R, Ranvir S & Madan S. 2017. Comparative study of the properties of ripe banana flour, unripe banana flour and cooked banana flour aiming towards effective utilization of these flours. International Journal of Current Microbiology and Applied Sciences, 6(8): pp.2003–2015

Siti Hawa J. 1998. Commercial exploitation of the banana diversity in Malaysia. Proceedings of the 1st National Banana Seminar in Malaysia, pp.45-51.

Someya S, Yoshiki Y and Okubo K. 2002. "Antioxidant compounds from bananas (*Musa Cavendish*)," Food Chemistry, 79(3): pp.351–354.

Soukoulis C, Fisk ID, Bohn T. 2014. Ice cream as a vehicle for incorporating health promoting ingredients: conceptualization and overview of quality and storage stability. Comprehensive Review of Food Science and Food Safety, 13 (4): pp.627–55.

Sulaiman SF, Yusoff NAM, Eldeen IM, Seow EM, Sajak AAB, Supriatno & Ooi KL. 2011. Correlation between total phenolic and mineral contents with antioxidant activity of eight Malaysian bananas (Musa sp). Journal of Food Composition and Analysis, 24(1): pp.1–10.

Temiz H, Yesilsu AF. 2010. Effect of pekmez addition on the physical, chemical, and sensory properties of ice cream. Czech Journal of Food Sciences, 28(6): pp.538–546.

Thakorlal J, Perera CO, Smith B, Englberger L, Lorens A .2010. Resistant starch in Micronesian banana cultivars offers health benefits. Pacific Health Dialog, 16(1): pp.49-59.

Tribess TB, Hernández-Uribe JP, Méndez-Montealvo MGC, Menezes EW, Bello-Perez LA and Tadini CC. 2009. Thermal properties and resistant starch content of green banana flour (*Musa cavendishii*) produced at different drying conditions. LWT-Food Science and Technology, 42(5): pp.1022-1025.

Villaverde JJ, Oliveira L, Vilela C, Domingues RM, Freitas N, Cordeiro N, Freire CS, Silvestre AJ. 2013. High valuable compounds from the unripe peel of several Musa species cultivated in Madeira Island (Portugal). Industrial crops and products, 42: pp.507-512.

Weaver. 2006. Ingredients, techniques and recipes for successful baking in your kitchen. The Prepared Pantry, pp.1-32.

WHO/FAO Consultation. .2003. Diet, nutrition and the prevention of chronic diseases. Report of a Joint WHO/FAO Expert Consultation, WHO Technical Report Series 916. Geneva: World Health Organization.

Wilt T, Ishani A, MacDonald R, Stark G, Mulrow C, Lau J. 1999. Beta-sitosterols for benign prostatic hyperplasia. Cochrane Database Systematic Reviews, (3).

Yin X, Quan J, Kanazawa T. 2008. Banana prevents plasma oxidative stress in healthy individuals. Plant Foods for Human Nutrition, 63: pp.71–76.

Young SN. 2007. How to increase serotonin in the human brain without drugs. Journal of Psychiatry & Neuroscience, 32(6): pp. 394–399.

Appendices

Appendix A: Sensory evaluation form

Acceptance Test

Date: Sample code:

Gender: Male/ female Age:

Instruction:

You are given five samples. Please start your evaluation from left to right. Evaluate each attributes by circling the appropriate scale which indicates your degree of liking. Rinse your mouth with plain water before tasting the each sample.

Color

Dislike extremely Like extremely

Aroma

Dislike extremely Like extremely

Appearance

Dislike extremely Like extremely

Crispiness

Dislike extremely Like extremely

Taste

Dislike extremely Like extremely

Shape

Dislike extremely Like extremely

Overall acceptability

Dislike extremely Like extremely

Comment (if any)

Appendix B: Sensory evaluation panelist work

Sensory panelists don’t do it

1. Eat, Drinks or Smoke within 90 minutes.
2. Use gum, mints etc. flavored item within 30 minutes.
3. Wear perfumes, cologne and fragrance item during test.
4. Talk and comment during evaluation.
5. Taste if you have a lot of prior knowledge about the manufacturing which you may dislike.
6. Taste if you have a cold.
7. Share the product with other.

Sensory panelists should

1. Be attractively dressed and well groomed.
2. Be tactful and concerned about the exhibitors and their feelings.
3. Have a pleasant manner; smile and be prompt.
4. Avoid consulting with spectators.
5. Hide personal likes and dislikes.
6. Be familiar with the products being judged.
7. Take the time to get a general picture of the entries.
8. Recognize quality standards.
9. Don’t give top placing if entries are not worthy.
10. Don’t rule out unfamiliar ways of doing things if the results obtained are satisfactory. Judge the results that you see, rather than what ‘‘might’’ have been done.
11. Make quick and firm decisions.
12. Offer reasons for decisions, encourage the exhibitor to continue, to learn and to improve.
13. Offer compliments and constructive criticism.
14. Be fragrance neutral.
15. Participate regularly.
16. Take sensory evaluation seriously.
17. Take your time and focus during test.
18. Follow the method and instruction precisely.
19. Be confident in initial judgment.
20. Rest and cleanse your palate for next sample.

Remember

No food entry is so poorly done that it is not worthy of an encouraging comment.

No food entry is so well done that some improvement may not be made

Appendix C: Photo gallery

Unripe banana flour preparation

1. Washing Bananas

2. Peeling

3. Cutting

4. Drying

5. Weighing

6. Grinding

7. Sieving

8. Unripe banana flour

Fiber Rich-powder preparation

 1. Weighing UBF	 2. Making suspension	 3. Adjusting pH
 4. Gelatinization	 5. Adding α-amylase	 6. Incubation
 7. Acidification	 8. Centrifugation	 9.Drying
 10. Grinding	 11. Fibre-rich powder	

Biscuit Preparation

 1. Mixing	 2. Making dough	 3. Cutting
 . 4.Baking biscuits	 5.Biscuits	 6.Sensory evaluation

Laboratory Activities

 Moisture	 Ash	 Protein
 Fat	 Fiber	 Sample extraction
 Sampling for bioactive compounds	 UV-Visible Spectrophotometer	 Sample extraction for Mineral analysis

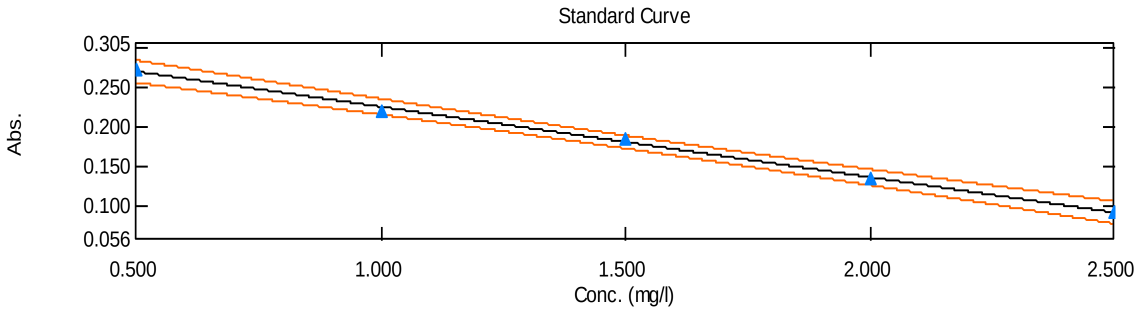
 Sampling of minerals	 Analyzing Minerals	 WHC & OHC

Appendix D: Table & graph for bioactive compounds

Antioxidant capacity standard table

Serial no.	Sample ID	Ex	Conc. (ppm)		WL (517.0)
1.	Std1	Standard	0.500		0.272
2.	Std2	Standard	1.000		0.221
3.	Std3	Standard	1.500		0.185
4.	Std4	Standard	2.000		0.133
5.	Std5	Standard	2.500		0.092

Standard Curve



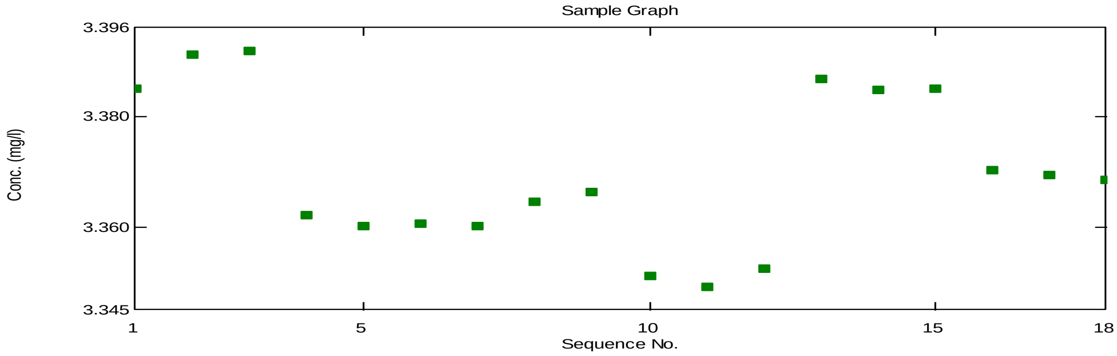
y = - 0.0894539 x + 0.314536
r2 = 0.99735

Sample table

Serial no.	Sample ID	Ex	Conc. (ppm)	WL 517.0
1	UBF 10min	Unknown	3.385	0.012
2	UBF 10min	Unknown	3.391	0.011
3	UBF 10min	Unknown	3.392	0.011
4	FRP 10min	Unknown	3.362	0.014
5	FRP 10min	Unknown	3.360	0.014
6	FRP 10min	Unknown	3.361	0.014
7	UBF 20min	Unknown	3.360	0.014
8	UBF 20min	Unknown	3.365	0.014

9	UBF 20min	Unknown	3.366	0.013
10	FRP 20min	Unknown	3.351	0.015
11	FRP 20min	Unknown	3.349	0.015
12	FRP 20min	Unknown	3.353	0.015
13	UBF 30min	Unknown	3.387	0.012
14	UBF 30min	Unknown	3.385	0.012
15	UBF 30min	Unknown	3.385	0.012
16	FRP 30min	Unknown	3.370	0.013
17	FRP 30min	Unknown	3.370	0.013
18	FRP 30min	Unknown	3.369	0.013

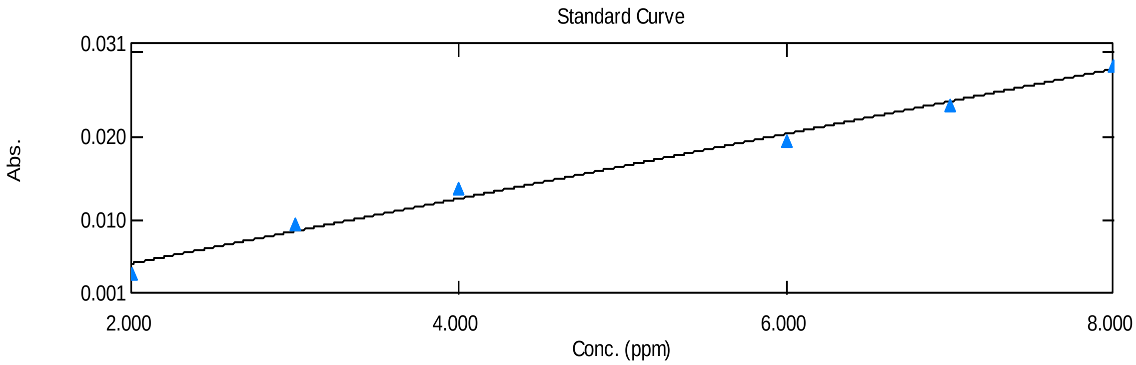
Sample graph



TFC standard table

Serial no.	Sample ID	Ex	Conc. (ppm)	WL 415.0	Wgt. Factor
1	Std1	Standard	2.000	0.004	1.000
2	Std2	Standard	3.000	0.010	1.000
3	Std3	Standard	4.000	0.014	1.000
4	Std4	Standard	6.000	0.020	1.000
5	Std5	Standard	7.000	0.024	1.000
6	Std6	Standard	8.000	0.029	1.000

Standard Curve

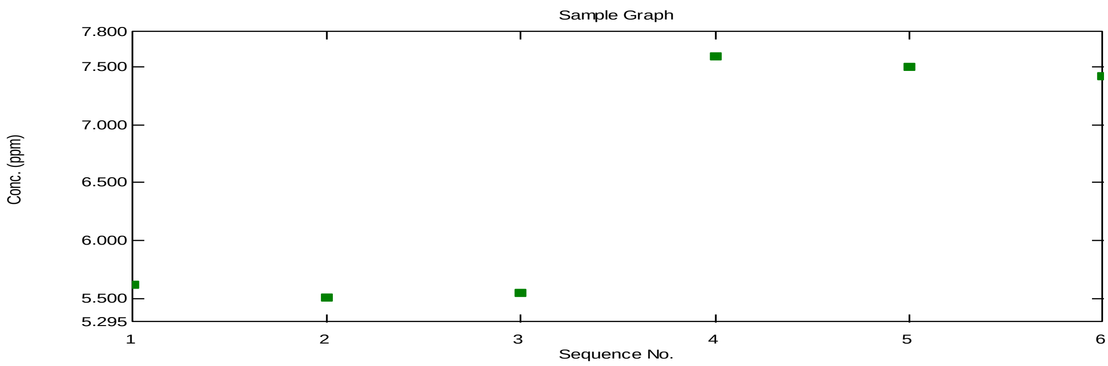


y = 0.00385110 x - 0.00271158
r2 = 0.98868

Sample Table

Serial no.	Sample ID	Ex	Conc. (ppm)	WL 415.0
1	UBF	Unknown	5.621	0.019
2	UBF	Unknown	5.504	0.018
3	UBF	Unknown	5.542	0.019
4	FRP	Unknown	7.591	0.027
5	FRP	Unknown	7.503	0.026
6	FRP	Unknown	7.414	0.026

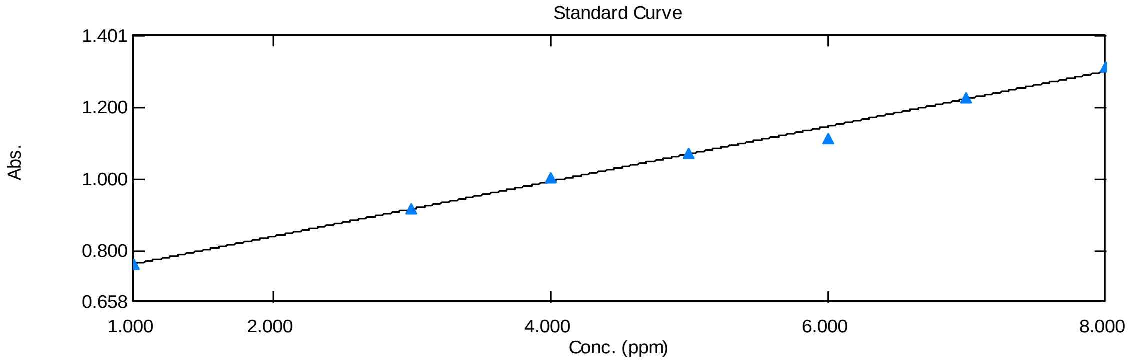
Sample graph



TPC Standard Table

Serial no.	Sample ID	Ex	Conc. (ppm)	WL 760.0
1	Std1	Standard	1.000	0.763
2	Std2	Standard	2.000	0.780
3	Std3	Standard	3.000	0.920
4	Std4	Standard	4.000	1.007
5	Std5	Standard	5.000	1.074
6	Std6	Standard	6.000	1.115
7	Std7	Standard	7.000	1.230
8	Std8	Standard	8.000	1.314

Standard curve

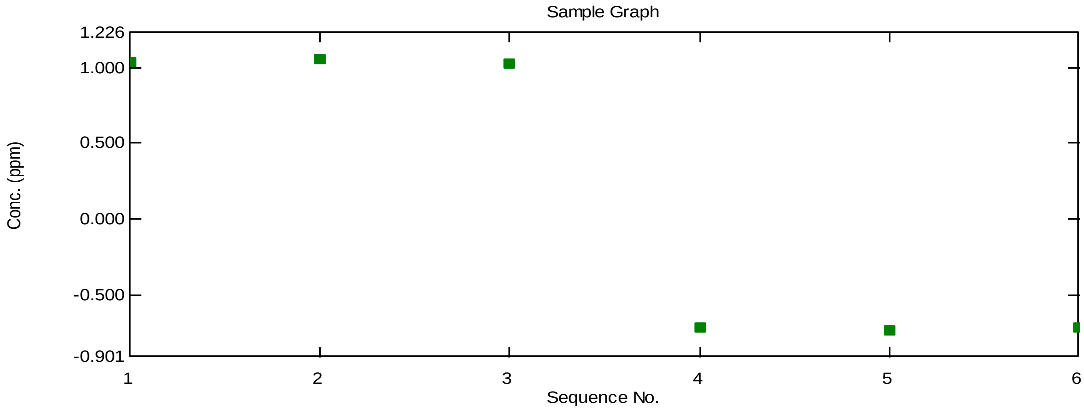


y = 0.0768527 x + 0.687090
r2 = 0.99301

Sample table

Serial no.	Sample ID	Ex	Conc. (ppm)	WL 760.0
1	FRP	Unknown	1.033	0.766
2	FRP	Unknown	1.049	0.768
3	FRP	Unknown	1.029	0.766
4	UBF	Unknown	0.712	0.632
5	UBF	Unknown	0.723	0.631
6	UBF	Unknown	0.713	0.632

Sample graph



Brief Biography

Fatema Akther passed the Secondary School Certificate Examination in 2010 and then Higher Secondary Certificate Examination in 2012. She obtained his B.Sc. (Hon's) in Food Science and Technology from the Faculty of Food Science and Technology of Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh. Now, she is a candidate for the degree of Master of Science in Applied Human Nutrition and Dietetics under the Department of Applied Food Science and Nutrition, Faculty of Food Science and Technology, Chattogram Veterinary and Animal Sciences University (CVASU). Her research interests are functional food product development and nutritional value analysis, processing, preservation and development of modified food products, functional food product development , quality control and quality assurance regarding food, chemical and microbial analysis of food, new techniques to measure food quality, taste and flavor, control of unit operation in food processing and instrumental food analysis with UV-Visible spectroscopy, Atomic Absorption Spectroscopy (AAS), High Performance Liquid Chromatography (HPLC), Gas Chromatography (GS) etc.