FORMULATION OF BISCUIT WITH BANANA (*Musa sapientum*) PEEL TO ENHANCE FIBRE, ANTIOXIDANT CAPACITY AND BIOACTIVE PROPERTIES

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The thesis submitted in the partial fulfilment of the requirements for the Degree of Master of Science in Food chemistry and Quality Assurance

Department of Applied Chemistry and Chemical Technology

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JUNE 2022

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TO MY RESPECTED AND BELOVED PARENTS

DEDICATED AND TEACHERS

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List of abbreviation

Abbreviation	Elaboration
DPPH	2,2-Diphenenyl-hydrazyl-hydrate
FD&C	Food, Drug, and Cosmetic Act
FDA	Food and Drug Administration
FAO	Food and Agriculture Organization
GABA	Gallic Acid Equivalent
IARI	Indian Agriculture Research Institute
TAC	Total Anthocyanin Content
SDA	Sabouraud Dextrose Agar
BTH	Butylated Hydroxy Quinone
CDA	Cardiovascular Diseases
DW	Distilled Water
FAOSTAD	Food and Agriculture Organization Statistics Database
H_2O_2	Hydrogen Peroxide
HNO_3	Nitric Acid
TRAP	Total Radical Antioxidative Potential
CVASU	Chottagram Veterinary and Animal Sciences University
CAC	Codex Alimentarius Commission
TEAC	Trolox Equivalent Antioxidant Capacity
TPC	Total Phenolic Content
ROS	Reactive Oxygen Species
BBS	Bangladesh Bureau of Statistics
Abs.S	The absorbance of the standard
Abs.T	The absorbance of the test Sample

Abstracts

The focus of the research was to see how banana peel powder combined with wheat flour could be used to make biscuits with a high phytochemical composition (bioactive compounds, antioxidant capacity, and crude fibre) and to see how it affected the nutritional profile and sensory attributes of the biscuits. Because of the results of many observational studies from all over the world, phytochemical components are the most important qualitative qualities of fruits for lowering progressive chronic disorders. The banana peel powder was made by drying the banana peels. All of the findings were statistically examined to see just how significant the fluctuation in observations effects involve in the biscuit's recipe was. Using a one-way analysis of variance (ANOVA), the threshold of significance at $P<0.05$ was calculated. The aesthetic rank of wheat flour with banana peel powder of all kinds sample D (10 percent banana peel) was the highest among four distinct formulations. When compared to wheat flour, fortified biscuits had lesser moisture, protein, fat, fibre, ash, and some bioactive compounds (polyphenol, flavonoids, and anthocyanin) and more fat, fibre, ash, and some bioactive compounds. When 10% banana peel powder was replaced for wheat flour, superior quality, nutritious, and fibre-rich biscuits were produced (moisture 3.87 %, carbohydrate 64.83%, protein 10.13%, fat 18.18 %, ash 1.65% and fibre 0.75%). The produced biscuit had larger levels of phytochemical substances (Polyphenol 4.22%, flavonoid 23,87%, anthocyanin 13.41%, and antioxidant capacity 2. 85%) than sample A. (reference biscuit). The 10% banana peel with wheat flour combination had the highest value for fragrance (8.0), taste (8.10), colour (6.80), texture (5.40) and overall acceptance (7.60). The substitution of 10% banana peel powder for wheat flour can serve as a compatible resource for value-added product manufacture and waste utilization, potentially improving food security in Bangladesh, where bananas are plentiful. In regarding the nutritional and organoleptic, the finished product was determined to be somewhat more acceptable behaviour.

Keywords: Banana peel, Phytochemical, Antioxidant capacity and Fortified biscuit

Chapter 1: Introduction

Bangladesh is a country where major portion of the population earns their livelihood through farming. The majority of people rely on agriculture, either explicitly or implicitly. The country's gross domestic product is significantly increased by agriculture (GDP). In 2015–16, agriculture contributed around 14.75 % to the GDP (BBS, 2016). Numerous Musa species belonging to the Musaceae family are included in the banana taxonomy. Tropical areas are where bananas are mostly farmed. Although they are currently grown all over the world, bananas are said to have originated in the tropical regions of southern Asia (Anhwange et al., 2009). It is the fourth most significant crops produced on a global scale as banana is the most popular fruit among people (Aurore et al., 2009). Being among the list of most popular fruits, the annual production of banana is around 102 million tonnes (Faostat, 2013).

Based on the most recent FAO statistical report, Asia is the most dominant country in banana production accounting for 54.4% of global banana output. Banana is one of the world's primary food crops, following rice, wheat, and maize, with an average consumption of 12 kilograms per capita (Faostat, 2017). Every year, the country produces over 1,000,000 tons of bananas, for this rate of banana production, Bangladesh is ranked 14th among the world's top 20 banana producing countries (Hossain, 2014). The entire cultivated area of horticultural crops in Bangladesh is around .69 million hectares, accounting for approximately 5% of total planted area. The production of banana from 119325 acres is 774286 metric tons where unripe/green banana (as vegetable) output totals 144135 metric tons, with a total area of 25479 acres (BBS, 2013).

Peel and the pulp are two parts of banana fruit. Peel is around 35% to 40% of the whole weight of banana and it is a by-product of this fruit. As there is no such use of banana peel, it is dumped as by-product of banana and this adds up to the large amount of organic waste material to be managed. This by-product is a threat to our environment as the peel consists of large amount of nitrogen as well as high level of phosphorus. Banana peel is also a favourable spot for microbial growth due to its high-water activity and may modify it. With the aim of utilization of banana peel, many researchers have studied the composition of banana peel and for its possible use (Agama-Acevedo et al.,

2016). The edible part of banana is its pulp and it is very high in nutritional aspects. The extraction and separation of multiple health-beneficial substances such as different types of starch, cellulose, and biologically active compounds as well as the use of banana pulp as a food additive are just a few of the issues that have been the subject of banana pulp study (Singh et al., 2016). Bioactive substances are compounds with additional nutritive value that exist naturally in minute concentrations in plants and foods. They promote the growth of probiotics, have a beneficial biological impact and help to avoid cancer and cardiovascular disease (Kris-Etherton et al., 2002). Phenolics, biogenic amines, carotenoids, flavonoids, phytosterols, flavonoids and other phytochemicals are some of the most prevalent bioactive substances discovered by specialists in bananas and banana peels (Pereira and Maraschin, 2015). Given that these components are present, bananas and their peels possess more antioxidant properties than a variety of other fruits and herbs (Moongngarm et al., 2014). As the demand of functional foods are rising rapidly and consumers wellbeing is being prioritize in the recent years, there is an increasing interest in vitamins, minerals, bioactive substances, unsaturated fatty acids and fibre in dietary goods. Utilizing banana leftovers in research to look at how they affect food characteristics has become a popular practice (Kaur et al., 2015). Nearly one - third of a banana is wasted because people only eat fruits that are fully ripe. That's why utilization of banana in its all-ripening stages have also attracted increasing attention in recent years (Sheikh et al., 2017).

Banana peel's chemical makeup determines its useful possible applications. Proteins, soluble fibre, PUFA, vital amino acids, and potassium are all found in banana peels (Emaga et al., 2007). Banana pulp contains significant amounts of phytonutrients, especially vitamins (B3, B6, B12. C and E). Additionally, it includes dietary fibre, carotenoids, flavonoids, and amine chemicals. Fibre content refers to the non - digestible carbohydrate polymers found in food. Depending on how easily they dissolve in water, they are divided into two categories: soluble fibres (pectin and certain hemicelluloses) and insoluble fibres (cellulose and starch) (Alba et al., 2018). Reports showed that banana peel contains more dietary fibres than banana pulp (Garcia-Amezquita et al., 2018). It might be utilized to produce cellulosic ethanol (Emaga et al., 2008). Pectin extract might be utilized as a substitute. Antioxidant chemicals have been shown to have synergistic effects and protective characteristics against a variety of degenerative illnesses, such as cancer, stroke, heart disease and Parkinson's disease (Abdel-Hameed, 2009).

Intake of fruits is very essential for well-being of humans. Risk of various chronic diseases are reduced due to consumption of fruits. The food industry has recently struggled with a rise in food waste brought on by the processing of fruit into diverse products including juices, wines, jellies, fruit smoothies, and so on (Bertagnolli et al., 2014). Worldwide fruit production and consumption have increased dramatically owing to flavour and health advantages due to the inclusion of nutrients such as minerals, vitamins, fibre, and other bioactive substances required by the human body for a healthy life (Guo et al., 2003). Food waste is a huge issue all over the globe consequently, studying the peel and seed of fruits might disclose vital natural sources of nutrients as well as country economic indexes (Berto et al., 2015).

Biscuits are a form of confectionery with a low moisture content that may be used as a transportation tool for key nutrients if made widely available to the public (Chinma and Gernah, 2007). Wheat flour is a rich source of energy and other nutrients but in the refining process its antioxidant capacity is reduced. To boost the wheat flour's antioxidant capability, combine it with banana peel flour (Fatemeh, 2012). Due to particular eating habits, changing consumption patterns, economic considerations, and commercial demands, several studies have been done in which wheat flour was substituted with flour made from fruit by-products to produce bakery goods such as biscuits (Perez, 2017).

Reusing banana manufacturing waste, such as skin may boost input materials productivity and alleviate the massive waste disposal challenges faced by the food sector (Kobori and Jorge, 2005). Because both fruits have significant antioxidant potential and are rich in phenolic compounds as well as vitamin C, producing flour from banana peels to develop a product like biscuits or partially blending these flours with wheat flour to increase the nutrient benefit of biscuits will therefore be the most technologically and economically feasible option (Caleja et al., 2017). Around the world, people are becoming more aware of the need of consuming wholesome foods and health-conscious customers favour foods that offer benefits beyond just basic nutrition (Baba et al., 2015). As a result, functional biscuits produced with wheat flour and components that promote health created with non-wheat flours are becoming increasingly popular (Dewettinck et al., 2008). The health-protective grain components found in biscuits, such as dietary fibre and phytochemicals, are absent due to the use of processed wheat flour and other additions (Fardet, 2010). The right flour selection and processing techniques, including as combining, aeration, fermentation, baking and

packaging, are necessary for the production of biscuits of acceptable quality (Agu et al., 2014).

1.1 Objectives

1. To assess the levels of bioactive components in banana peel (total polyphenols, total flavonoids, and total anthocyanins).
2.To develop a new food product which is enriched with crude fibre and bioactive compounds and other nutrients.
3. To determine nutritional profile of the developed product as well as assessing its nutritional properties.

1.2 Anticipated outcomes

1. Estimated the concentration of bioactive compounds, crude fibre and antioxidant capacity of banana peel.
2. A clear relationship among antioxidant, bioactive compounds, crude fibre and human body discussed.
3. Reduction of discarded waste from processing industries as well as consumption of banana by people that may facilitate to our environment safety.
4. The agro-processors of the country will get a clear idea about developing the tools to isolate, identify and utilization thousands of bioactive compounds available in plants.

Chapter 2: Review of literature

2.1 Originality and distribution of banana

In New Guinea, cultivation dates back to the 4th century BCE, making it one of the most important and ancient food crops in the world (Denham et al., 2003). Dessert and cooking-related bananas are separated into two groups. Dessert bananas are consumed uncooked when ripe, but bananas are fried, boiled, mashed, or roasted before consumption. Approximately one-third of all banana production is made up of the most popular cooking bananas, plantains. Several plantain and banana cultivars are utilized for dessert and cookery in various nations. Numerous islands in southeast Asia as well as the west tropical Pacific Ocean, such as Bangladesh, Sri Lanka, and India's peninsular, and northeaster regions, are home to several banana varieties. Among the nations engaged are Cambodia, Myanmar, Malaysia, Thailand, Vietnam, China, Laos, Indonesia and New Guinea. The distribution of *Musa balbisiana* could be a little haphazard. The highest species diversity is found in Both Malaysia and Indonesia (Argent, 1976). Wild bananas are exclusively warm-weather plants. Although some species and groups can survive in extremely cold temperatures, the genus has a narrow range of temperature tolerance. No species can withstand drought. The Eumusa species are more resistant to drought than the Australimusa and Callimusa species, with *Musa balbisiana* and the two *Musa acuminata* subspecies siamea and burmannica exhibiting the highest resistance.

Bangladesh is home to a wide range of bananas that occupy various ecological niches. Fruit size, shape, colour and scent preferences. As well as nutritional, medicinal, and cultural aspects, all contribute to the variety. There are cultivars for both dessert and cooking. After the final hands have set, commercial growers frequently remove the male bud (Islam et al., 2016).

2.2 Banana production

Bangladesh is mostly an agricultural country. The majority of people are directly or indirectly dependent on agriculture. Agriculture accounts for around 14.75 % of GDP in 2015-16 (BBS, 2016). It holds a key place among the fruits of the country. It is produced the most fruits, but also because of its growing popularity among many farmers as an economic crop and among many people as a healthy fruit. After rice,

wheat, and maize, Musa spp., banana, and plantain are the world's fourth most significant staple food commodities (Islam et al., 2016).

Every year, the country produces over 1,000,000 tons of bananas (Hossain, 2014). In our nation, the major banana-growing districts are, Faridpur, Gazipur, Rangpur, Bogra, Narsingdi, Nator, Pabna, Noakhali, Khulna, Rangamati, Sylhet, Moulvibazar, Netrokona, Khagrachhari, and Bandarban are wild grown banana districts. Banana plants may be found in most rural homesteads around the country. Bangladesh has a variety of banana. Commercial cultivars include BARI Kola-1, Amritsagar, Sabri, Champa, and Kabri. Genasundari, Maher sagar, Kanai banshi, Duds agar, Agni war, Genasundari, Basrai, Binisuta, and more cultivars are also available (Mukul et al., 2013). The overall cropped area in Bangladesh is 36669 acres, with a cropping intensity of 90%. The country's agroecology is organized into 30 AEZs. The entire cultivated area of horticulture crops is around 0.69 million hectares, accounting for approximately 5% of total cropped area. The entire area is 119325 acres and the total banana production is 774286 metric tons. Green banana (as a vegetable) output totals 144135 metric tons, with a total area of 25479 acres (BBS, 2013).

2.2 Composition of banana peel

Peels account for around 30-40 g/100 g of weight as industrial by-product. This led to the creation of 200 tons of rubbish made from banana peels per day, and this amount often increases with time (Pangnakorn, 2006). Municipal landfills frequently get the waste from banana peel disposal, which adds to the existing environmental problems. Utilizing its high-added-value components, such as the nutritive fraction, which has great potential for the production of functional meals, can address the problems. Dietary fibre has been shown to protect against a number of illnesses, such as colon cancer, diabetes, constipation, cardiovascular disease, and irritable bowel syndrome (Rodriguez et al., 2006).

Because banana peels have nutritional value, they are utilized as feedstock. On small farms in nations where bananas are grown, banana peels are frequently used for this purpose. Concerns exist over the impact of the tannins in peels on mammals that consume them (Onwuka et al., 2005). Peels nutritional value varies with respect to cultivar and maturity stage, for instance, plantain peels have less fibre than dessert banana skins, and lignin content rises as fruit ripens (from 7 to 15% dry matter). On

average, banana peels have 30% fibre and 6-9% protein dry matter (measured as NDF). Starch, which is transformed into sugars during ripening, makes up 40% of green banana peels. While mature banana peels can contain up to 30% natural sugars, green peels only have about 15% starch (Happi et al., 2007).

2.3 Important bioactive compound available in banana peel

Bioactive compounds can be found in vegetables and whole grains. They consist of a wide range of heterogeneous molecules with various chemical structures, such as phytosterols, carotenoids, tocopherols, and organosulfur compounds.

2.4 Types of bioactive compounds

2.4.1 Flavonoids

Flavonoids are water-soluble polyphenol compounds that contain 15 carbon atoms. Flavonoid, flavanol, and flavanone are the three major compounds. Numerous biological activities, such as antioxidative, antimicrobial, anticarcinogenic, and neuroprotective effects properties, are provided by flavonoids. Quercetin is a flavonoid of the flavanol type that is consumed almost daily and is present in a wide variety of foods, such as garlic, tea, and apple. The daily consumption of quercetin in the western diet is predicted to be between 0 to 30 mg (D'Andrea, 2015). Epicatechin, epigallocatechin, gallate, gall catechin, ellagic acid and gallo catechin are some of the flavanol catechins. The main constituents of tannins are catechins, and when grapes ripen, their amounts decrease (Gadkari and Balaraman, 2015). Citrus fruits contain the flavanone hesperidin, which has a low bioavailability, a low water solubility and a brief biological life (Parthasarathy et al., 2009). Other natural flavanone presents in grapefruit and oranges called nigenin enhances brain insulin signaling and cognitive performance (Ghofrani et al., 2015).

2.4.2 Phenolic compound

Polyphenols feature many hydroxyl groups connected along with benzene rings. Due to their abundance in food, capacity to act as antioxidant, and potential role in the protection of several diseases linked to oxidative stress, phenolic compounds have drawn attention. The major dietary supplies of phenols are fruits and beverages (fruit juice, tea, and coffee), with minor amounts present in cereals, vegetables, and legumes.

Fruits including apples, grapes, pears, cherries, and other berries provide up to 200–300 mg of phenolic compounds per 100 g of fresh weight; a cup of red wine, tea, or coffee has about 100 mg. Around 1 g of phenolics are consumed on a daily basis on average (Scalbert et al., 2005).

2.4.3 Anthocyanin

Fruits' range of colours, including red, green and purple, are produced by water-soluble pigments called anthocyanins. They are mostly found as acyl glycosides of the corresponding aglycone anthocyanins and aglycone in fresh plant materials. The most common anthocyanidins found in plants include delphinidin, cyanidin, delphinidin, peonidin, peonidin, and malvidin. They are available in the flavours of strawberry, grape, raspberry, berry, red apple and cranberry. Due to genetic and agronomic factors, temperature, light type and intensity, post-harvest treatments, processing, and storage, fruit and vegetable anthocyanin concentration differs from fruit to fruit of the same variety (De Pascual-Teresa, Sanchez-Ballesta, 2008).

2.5 Health benefits of bioactive compounds

Bioactive compounds have antioxidant, free radical scavenging, and chelating properties because functional groups are present in their structure. They also attributed the majority of the health advantages of flavonoids ingestion. Flavonoids have antimutagenic, and antitumoral properties. Many enzymes, including oxygenises (prostaglandin synthase), which are essential in the formation of eicosanoids, are inhibited by flavonoids. Therefore, flavonoids can inhibit hyaluronidase activity and support the upkeep of connective tissue proteoglycans. This procedure would inhibit the spread of bacterial or tumour metastases (Havsteen, 2002).

2.6 Antioxidants

Antioxidants are natural compounds that have a low concentration in contrast to the major oxidizable substrate and protect it from oxidizing (Halliwell, 2007). In the present literature, more than 170 antioxidants have been introduced (Zhou, 2012). As according Boxin et al. (2002), ROS have a beneficial impact on the body's defence mechanisms and can function as agents with anticancer, immunity-boosting, antibacterial, antimicrobial, antifungal, cholesterol-lowering, antiparasitic and anti-

inflammatory properties (Bub et al., 2003). Antioxidants can be present in fruits and vegetables in the form of fibre, polyphones, conjugated dimers of linolenic, limonene, epigallocatechin, soy protein, isoflavones and vitamins A, B, C, and E. There are several components, including calcium, chlorophyllin, alipharin, sulfur compounds, tetrahydro curcumin, glutathione, lipoic acid, indoles, thiocyanates, protease inhibitors, and seas aminol (Karakaya et al., 2001).

2.6.1 Types of antioxidants

2.6.1.1 Natural antioxidant

There is a great deal of public and scholarly interest in fruits and vegetables because they often contain high natural antioxidants (Diwani et al., 2009). Reactive oxygen species (ROS), which may be present in all parts of a plant, as well as prooxidants created both endogenously and exogenously (by heat and light), constantly induce oxidative stress (H_2O_2 and transition metals). To control free radicals, catalysts for lipid oxidation, intermediates of oxidative stress, and products of secondary breakdown, many of these tissues include antioxidant systems (Brown and Kelly, 2007). These naturally occurring substances, including as quercetin, phenolic acids, carotene, and tocopherols, help reduce Fe-induced oxidation and scavenge free radicals. They are present in foods such fruits, vegetables, meats and dairy products. The most prevalent natural antioxidants in everyday foods include ascorbic acid (vitamin C), tocopherols (vitamin E), carotenoids (vitamin A), and other polyphenols such flavonoids, polyphenols, and lycopene (Ozsy et al., 2009).

2.6.1.2 Endogenous antioxidants

Antioxidants that are created by the body's metabolism, that might be enzymatic or non-enzymatic, are known as endogenous antioxidants. Superoxide dismutase is an enzyme antioxidant with a strong resistance. Because of the evolutionary environment in living creatures, several roles and defensive mechanisms against the detrimental impact of free radicals have been produced and chosen. These systems work in conjunction with dietary exogenous antioxidants, and are built into cells (both at the intra- and extracellular levels).

2.6.1.3 Synthetic antioxidant

Synthetic antioxidants are created chemically and are not present naturally (Shahidi et al., 1992). Based on how they work, these antioxidants are divided into two categories. Antioxidants come in primary and secondary forms. The main antioxidants assist in reducing the generation of free radicals during oxidation. Traditional antioxidants that have been used for many years include butylated hydroxy anisole (BHA), tert-butylhydroquinone (TBHQ), butylated hydroxytoluene (BHT), and propyl gallate (PG).

2.6.1.4 Dietary antioxidants

Carotenoids, ascorbates, tocopherols and ascorbates are very well antioxidants and studies demonstrate a link between their health advantages (Boskou et al., 2005). Vitamins C, vitamin E and beta carotene, as well as other carotenoids and ox carotenoids such as lycopene and lutein, are well recognized dietary antioxidants.

2.6.2 Source of antioxidants

Vitamin C, vitamin E, polyphone, lycopene, copper, a-carotene, cysteine and sialic acid are the main sources of antioxidants. High quantities of antioxidants such polyphenols, vitamin C, vitamin E, carotene, and lycopene may be found in fruit juices, drinks, and hot liquids (Ramadan-Hassanien, 2008).

2.6.3 Functions of antioxidants

The protective effects of plant-based diets are significantly influenced by antioxidants. Daily consumption of fruits and vegetables reduces the chance of developing chronic diseases (Dembinska et al., 2008). An antioxidant-rich diet has been shown to provide long-term health benefits (Sin et al., 2013). Antioxidants now relate cell damage, cancer prevention and longevity to free radicals (Kalcher et al., 2009). The antioxidant system, which is in charge of guarding against the negative effects of free radicals and toxic by-products of their metabolism, is how all antioxidants work. These include the addition of hydrogen, electron, and lipid to antioxidants, followed by the formation of a complex between lipid and antioxidants. As dietary components combat chronic disorders, emphasis is being paid to photo-chemicals, plant-derived molecules with constant antioxidant ability (Peter, 2007).

2.7 Mechanism of antioxidant activity in human body

Reactive oxygen species (ROS) levels rise as a result of oxidative stress, which then disrupts cellular function, when reactive oxygen species (ROS) formation and detoxification are in balance. Biological macromolecules including lipid peroxidation acid, and protein are all harmed by ROS. But, as soon as the level of ROS crosses this cut off, ROS production rises. This might result in the cell receiving an excessive number of impulses, directly harming essential elements in signalling pathways. Critical macromolecules are permanently damaged by ROS. Protein-bound thiol and non-protein thiol are the two primary cytosolic low molecular weight sulfhydryl molecules that function as cellular reductants. Thiol is widely used as the first line of defence against oxidative stress as a result. There is proof that oxidative stress causes cellular macromolecules to suffer long-term harm, which triggers the development of diseases such atherosclerosis, coronary heart disease, liver cancer, diabetes and carcinogenesis. Antioxidants have the power to reduce the release of free radicals and the production of reactive oxygen species (Shahidi and Ambigaipalan, 2015). The review concludes that consuming a lot of organic foods rich in antioxidants can provide you with stronger defence against harmful substances and illnesses associated with scavenging (Adwas et al., 2019).

2.8. Fibre

Fruit fibre is preferable to other fibre sources because of its high total and soluble fibre content, capacity to store water and oil, ability to be fermented in the colon, low phytic acid level and low-calorie value. Banana peel is a significant source of dietary fibre because of its high total fibre content (approximately 50 g/100 g). It has been discovered that the maturity of banana fruits affects the dietary fibre composition-contents of banana peels (Happi et al., 2007). However, banana peels are discarded as waste and farmers have been known to utilize them as animal feed. As a result, the potassium content of some Nigerian bananas must be determined, as well as the oils extracted from their peels. Banana is consumed by people from all walks of life all around the world. It is known to contain potassium and has been suggested that it may be used as a potassium source (Anhwange, 2008).

Chapter 3: Materials and methods

3.1 Study areas and sample collection

The experiment lasted six months, from January 1st, 2022, to June 27th, 2022. Relatively fresh banana (*Musa sapientum*) samples were collected from local markets in Rangamati, Narsingdi, Kishoregonj and Chattogram district. Bananas that were fully developed and ripe were chosen for investigation and packed in plastic bags They were then sent to Chattogram Veterinary and Animal Sciences University (CVASU), Khushi, Chattogram, Bangladesh, to the Faculty of Food Science and Technology.

3.2 Chemicals and reagents

•Absolute methanol
•Gallic acid
•Potassium acetate
•Folin Ciocatea (FC) Reagent.
•Absolute ethanol
•2,2-Diphenyl-1-picryl hydrazyl (DPPH)
•Aluminum Chloride
•Sodium carbonate
• Quercetin

3.3 Equipment

•Conical flasks
•UV visible spectrophotometer
•Beakers
•Falcon tubes
•Digital analytical balance
•Volumetric flask
•Electric blender.
•Test tubes

3.4 Processing of banana peel into banana peel powder

Initially, bananas were washed numerous times under running water. The banana peels were then blenched for (below 100°C for 1-2) minutes, dried (60°C for 24 hours at cabinet dryer) and processed into powder in a grinder. This powder was then sieved and stored in an airtight container at 4°C described by Yatnatti et al. (2014).

3.4.1 Banana peel powder and biscuits processing

Biscuits were made according to Al khalifa. (1998) modified recipe. Table 3.1 displays formulations for a wheat and banana peel powder blend for biscuits. Doughs having 2%, 5%, and 10% peel powder as equivalent amounts for wheat flour were utilized to make biscuit samples using the method described by Ashoush and Gadallah. (2011). According to Leelavathi and Haridas Rao. (1993). Biscuit dough was created by combining wheat flour, peel powder, and other ingredients. The formulated mixtures were hand blended for 10 minutes. The dough pieces were sheeted and flattened with a roller into an 8mm thick sheet before being cut into various shapes. Samples were roasted in an electric oven for 20 minutes at 220°C. Following baking, the biscuits were let to cool at room temperature before being stored in an airtight container.

Table 3.1: Formulation of dough containing banana peel powder and other ingredients for biscuits development.

Ingredients	Sample A (Control)	Sample B (2% peel powder)	Sample C (5% peel powder)	Sample D (10% peel powder)
Wheat flour (g)	500	490	475	450
Peel powder (g)	0	10	25	50
Butter (g)	300	300	300	300
Grind sugar (g)	230	230	230	230
Milk powder (g)	3	3	3	3
Baking powder ($NaHCO_3$) (g)	5	5	5	5
water (ml)	100	100	100	100
Salt (g)	50	50	50	50
Flavour (ml)	2	2	2	2

Biscuit preparation:

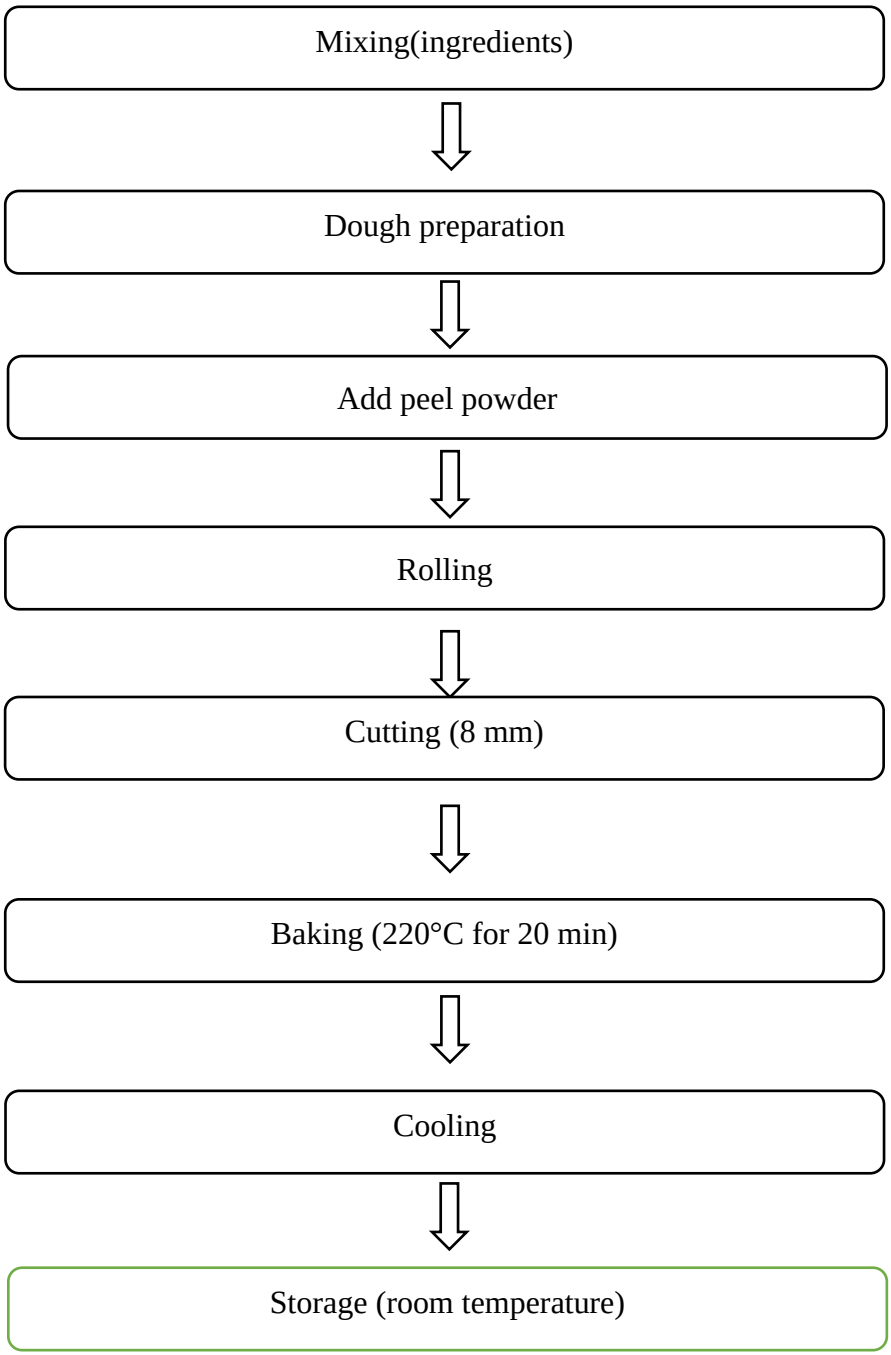

Figure 3.1: Biscuit preparation

3.5 Determination of Antioxidant capacity by DPPH scavenging method
Extract preparation

One gm sample was collected in a Felcon tube. Thereafter, 10 ml of 100% methanol was added and then mixture was allowed for 72 hours. After every 4 hours, continuous straining was performed. After 72 hours, the filtrate was collected, and methanoic extract was discovered.

Procedure

Antioxidant mobility of the extracts was measured using DPPH test, which was slightly modified from the process described by Azlim et al. (2010). In 100 mL 100% methanol, 6 mg of DPPH was dissolved to make a methanoic DPPH solution.

Then, 2 mL of DPPH solution was added to the methanoic extract to dilute it. After giving the mixture a gentle shake, it was allowed to sit at room temperature in the dark for 30 minutes. A UV-VIS spectrophotometer set at 517 nm was used to measure the absorption (UV-2600, Shimadzu Corporation, USA). One ml of methanol was used as a blank to create the control by mixing it with two ml of DPPH solution. By contrasting the samples' absorbance with that of DPPH reference solution, the scavenging mobility was ascertained. The antioxidant capability of extracts is determined using the following equation based on their DPPH free radical scavenging mobility:

$$\% \text{ of inhibition} = \frac{\text{Blank absorbance} - \text{Sample absorbanc}}{\text{Blank absorbance}} \times 100$$

Trolox was utilized as the standard, and the validation standard curve was TEAC composite (Trolox equivalent antioxidant mobility). The results were reported in milligrams (mg) per 100 grams of powder on a dry weight (DW) basis.

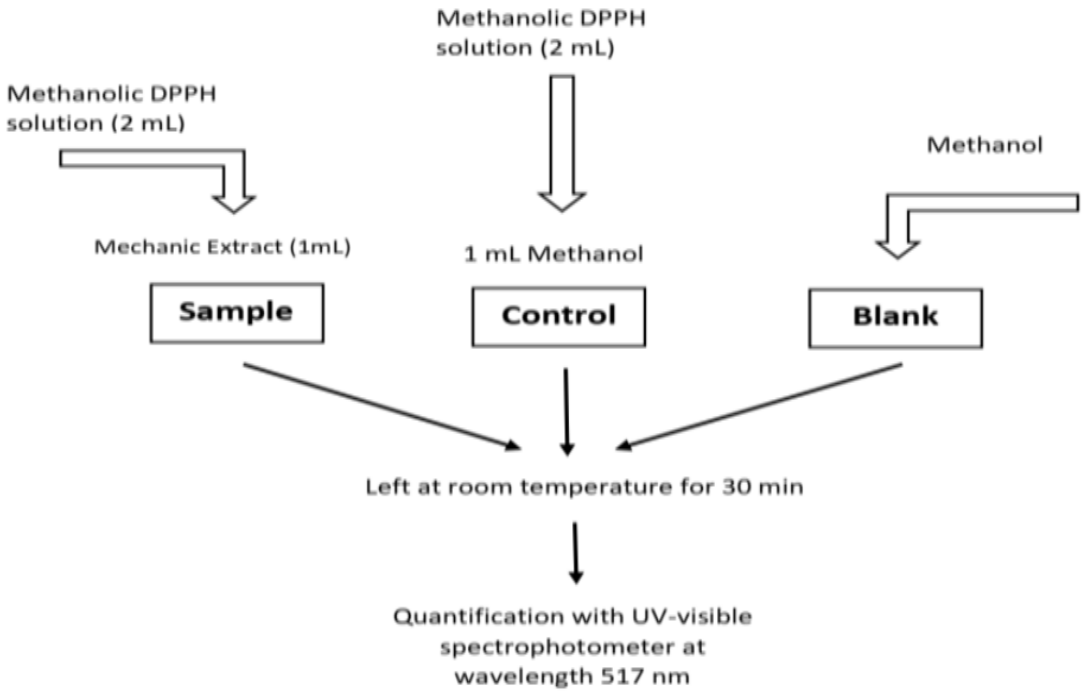

Figure 3.2: Determination of antioxidant capacity

3.6 Determination of bioactive compounds

Extract preparation

In Felcon tube, 5 gm of TAC sample and 1 gm of other TPC and TFC sample were obtained. After that, 10 mL of 100% ethanol was added and mixture was left for 72 hours. After every 4 hours, continuous straining was performed. After 72 hours, the filtrate was collected and an ethanoic extract was discovered.

3.6.1 Total phenolic content (TPC)

The Folin-Ciocalteu reagent technique, with minor changes, was used to quantify TPC of the extracts (Al-Owaisi et al., 2014). The Folin-Ciocalteu technique, as reported by Vergani et al. (2016), was applied to determine the total polyphenol content (TPC) of sample. 1 ml of ethanoic extract and 1.5 ml of FC reagent were combined in a falconer tube and left at room temperature for 3 minutes. After that, the mixture was exposed to 1.5 ml of 7.5% Na_2CO_3 for 60 minutes. The absorbance at 765 nm was measured using a UV-VIS Spectrophotometer (UV2600, Shimadzu Corporation, USA) with C_2H_5OH acting as the blank. In terms of gallic acid equivalents (GAE), TPC was calculated to be mg GAE/g of extract.

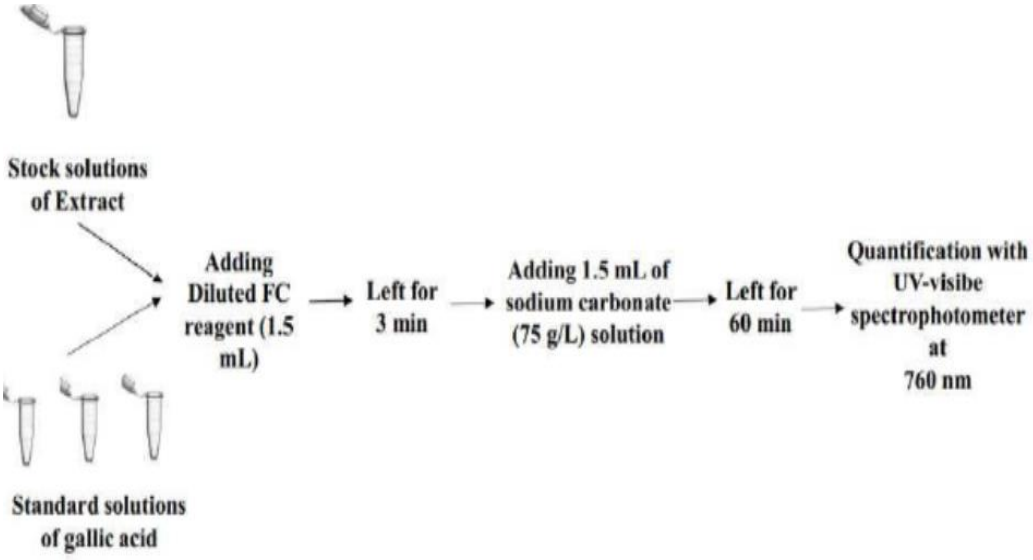

Figure 3.3: Determination of Total phenolic content (TPC)

3.6.2 Total flavonoid contents (TFC)

Using the aluminium chloride colorimetric technique described by Chang et al. (2002) with a few minor modifications, the total flavonoid content (TFC) of the fruit samples was determined. 5ml of diluted extract and 1.5 mL of 95% ethanol were blended in aliquots and placed in a cuvette along with extract stock solutions (1 mg/ml). Following that, the cuvette was stocked with 0.1 mL of 10%$AlCl_3$, 0.1 mL of 1 mol/L potassium acetate, and 2.8 ml of pure water. 30 minutes were spent letting the mixture warm up to room temperature. The blank was 10 percent aluminium chloride replaced with almost the same volume of distilled water, and the absorbance was measured at 415 nm using a UV-visible spectrophotometer (UV-2600, Shimadzu Corporation, USA). By dividing the sample extract absorbance by a quercetin standard curve, the total flavonoid content was calculated. TFC is evaluated and quantified in milligrams of extract per gram of quercetin equivalents (mg QE/g).

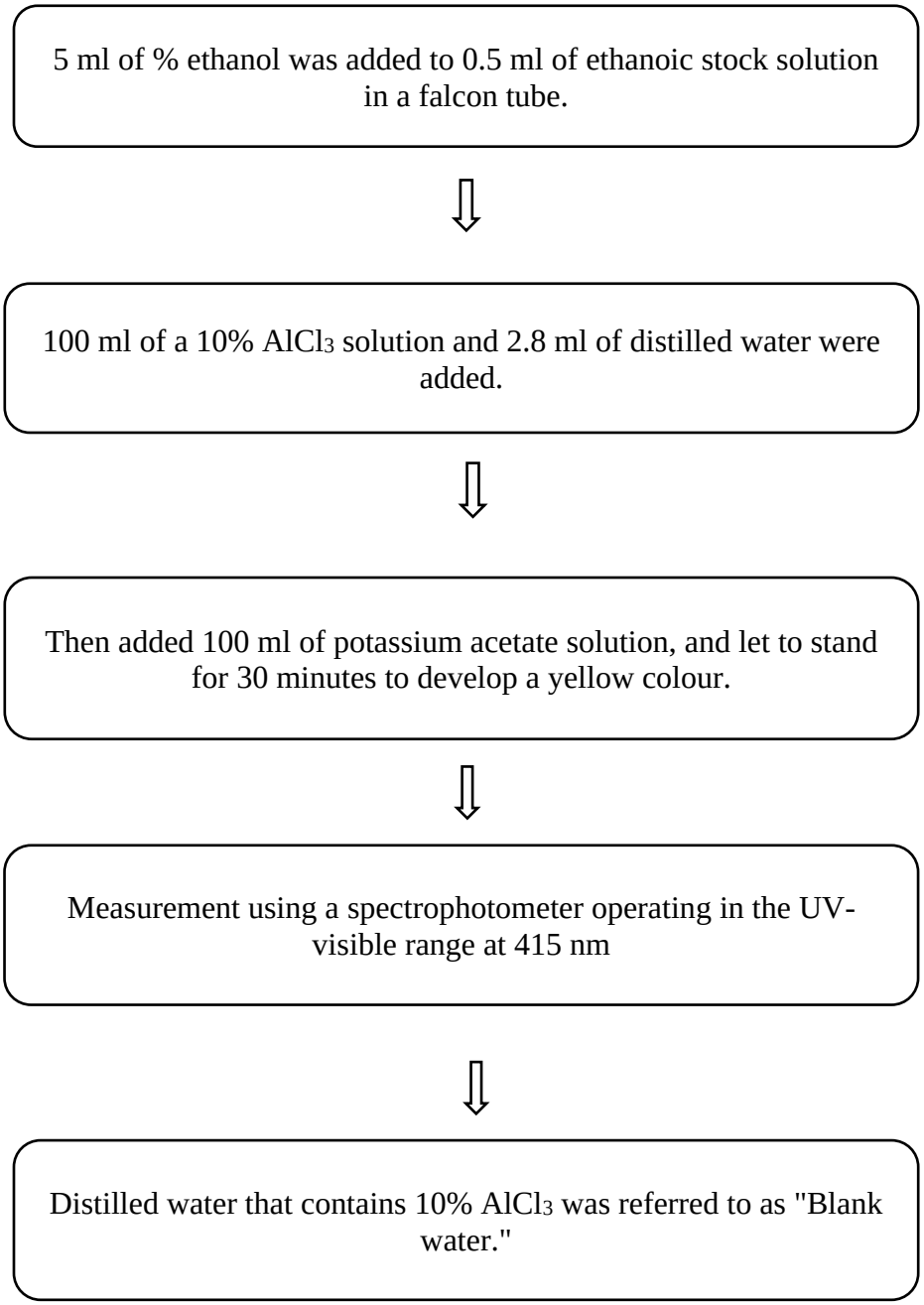

Figure 3.4: Total flavonoid contents (TFC) determination procedure flow diagram

3.6.3 Total anthocyanin contents (TAC)

Samples TAC would be calculated calorimetrically using the aforementioned technique, with a few minor modifications (Selim et al., 2008). A UV-VIS spectrophotometer was used to detect the colour intensity at 520 nm after adding 3 ml of ethanoic extract to a cuvette (UV-2600, Shimadzu Corporation, USA). As a control, ethanol was used. TAC was calculated and expressed as mg per 100 g (mg/100 g) using the following equation.

$$TAC = \text{Absorbance of sample} \times DF \times 100/M \times E$$

Where,

DF stands for dilution factor

M means weight of sample used to make stock solution

E refers to extinction coefficient (55.9)

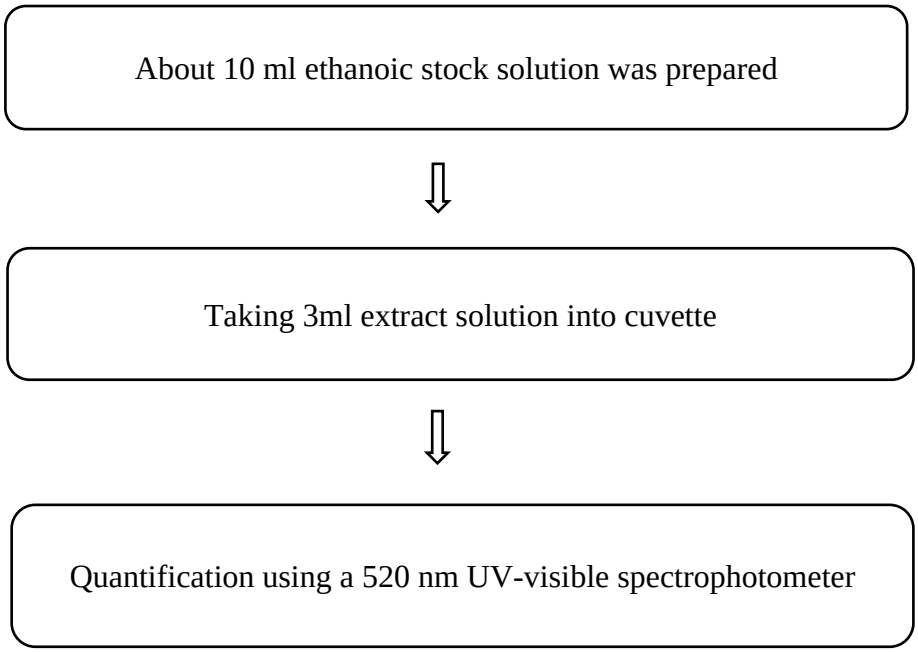

Figure 3.5: Determination of Total Anthocyanin Contents (TAC)

3.7 Proximate analysis of fortified biscuits

3.7.1 Moisture content

AOAC method number 925.09 (2000), was used to measure the moisture content of enriched biscuit and peel powder. The crucibles were cleaned, dried for three hours in an oven set at 105 ° C, and then chilled in desiccators. The crucibles were then weighed after that. About 2 g of the substance were placed in the crucible. The sample was put in the crucible and dried for around 48 hours at 105°C in the oven. The crucibles were dried, then put in desiccators to cool. After cooling, the crucibles were weighed and recorded. The formula stated in equation was then used to obtain the percentage moisture content.

Calculation:

$$\%\ of\ Moisture\ Content = \frac{W1-W2}{W2}$$

Where,

W_1= weight of sample (g) before drying and

W_2 = weight of sample (g) after drying

3.7.2 Crude protein

Crude protein content of enriched biscuits and peel powder was calculated using the macro Kjeldahl technique number 920.87. On oiled filter paper, a sample piece weighing around 1g was measured. The supplies were put in a 100 cc Kjeldahl digestive tube after being safely wrapped. An empty 100 ml digestive tube was made into a blank by inserting a piece of filter paper in to it and e ach tube received 5.0 ml of concentrated sulfuric acid and 2 g of Kjeldahl catalyst. The samples were further digested to allow the nitrogen contained in the heterocyclic ring to be liberated after generating a clear blue solution. Before adding 20 cc of distilled water to dissolve the ingredients, the digest was refrigerated. the diluted. 50 cc of sodium hydroxide at a 40% concentration was added to the digest to promote ammonia release. Ammonia was extracted by steam distillation and collected in a 50 mL flask containing 4 percent boric acid. The distillate was titrated with 0.1520 N HCl standard solution while using a bromocresol green/methyl red combination as an indicator. The following formula was used to calculate the nitrogen content.

$$\% \text{ of Nitrogen} = \frac{\text{Titre (blank)in ml} \times \text{Conc.of acid N/mol}}{\text{Weight of sample(g)}} \times 100$$

Percentage protein was calculated from the percentage nitrogen using the factor 6.25 for plant materials as shown in equation:

$$\%of\ CP = \%N \times Factor \ (6.25)$$

3.7.3 Fibre

Method 920.86 was used to calculate the dietary fibre content of fortified biscuits and peel powder. For crude fibre determination, one gram of each sample was obtained. The materials were first digested for 30 minutes in 0.125M diluted sulfuric acid, followed by three hot water rinses. After another 30 minutes of mild alkali digestion (0.125M KOH), the residue was rinsing three times in hot water. After being cooked for five hours, the digested remnant was chilled and weighed. After burning for two hours at a temperature of 525°C in a muffle furnace, the residue was cooled and weighed once again. The following calculation was used to calculate the total amount of fibre:

$$\%\ of \text{ fibre} = \frac{W1 - W2}{W}$$

Where,

W_1= weight of sample (g) before drying

W_2 = weight of sample (g) after drying

W = weight of dry sample taken for determination (g)

3.7.4 Crude fat

To ascertain the samples' crude fat content, the AOAC (2000) technique uses the Soxhlet equipment.

Procedure

Dried samples were put in a thimble, which had its top filled with a piece of cotton fibre devoid of fat. The fat extraction tube, which was attached to a Soxhlet device, was where the thimble was put. Through the sample in the tube, around 75ml of anhydrous

petroleum ether was added to the flask. The top of the fat extraction tube was joined to the condenser. In a water bath heated to (70-80) °C, the sample was extracted for at least 16 hours. The thimble from the equipment was taken out after the feature extraction phase, and petroleum ether was then distilled or gathered in a Soxhlet tube. When the petroleum had become tiny, it was purified by passing it through a small funnel containing plug cotton into a small, dry (previously weighted) beaker. The flask was carefully washed and filtered with petroleum ether. The petroleum ether was evaporated on a steam bath at room temperature, then dried for 1 hour at 100°C, chilled, and weighted. The ether soluble elements in the sample were determined by the weight difference. The crude fat content was expressed as follows:

$$\% \text{ Of Crude fat} = \frac{\text{Weight of ether solube material}}{\text{Weight of sample taken}} \times 100$$

3.7.5 Carbohydrate

Total carbohydrate content of the sample was determined as total carbohydrate by difference, that is by subtracting the measured protein, fat, ash and moisture from 100 which is followed by Person method.

3.8 Statistical analysis

To assess statistical analysis, data were collected and kept on a Microsoft Excel 2019 spread sheet. Three duplicates of each sample were used. For the sensory evaluation of enriched biscuits and banana peel, descriptive statistics (mean and standard deviation) were performed. Minitab version 19 was used to sort, code, and record the data. Following that, statistical analysis was done. One-way ANOVA techniques were used to examine the facts on proximate composition, phytochemicals, antioxidant capacity, and sensory assessment in order to determine the degree of significant variance at a 95% confidence interval. To discover the variance among the sample groups, a post hoc "Fisher" test was used. A 5% threshold of significance ($p \leq 0.05$) was used for the statistical analysis.

Chapter 4: Result

4.1 Proximate composition of different banana peel

Table-4.1 shows the proximate analysis of two varieties of banana peel. Bangla kola had crude protein, moisture content, crude fat, total ash, total carbohydrate, and crude fibre of 7 %, 26.29 %, 15%, 10.22%, 33.6 %, and 15 %, respectively. Sagor kola had crude protein, moisture content, crude fat, total ash, carbohydrate, and fibre of 8.58%, 19.7%, 8.58%,8.67%,47.7% and 9.34% Protein content varies between 7 and 8.58 %, whereas crude fibre content varies between 18.11 and 15 %.

Table 4.1: Proximate composition of different banana peel

Banana	Carbohydrate (%)	Protein (%)	Crude fibre (%)	Moisture (%)	Crude Fat (%)
Bangla kola	31.6	7	18.11	26.29	15
Sagar kola	47.7	8.58	15	19.7	9.34

4.2 Proximate composition of fortified biscuits

Table-4.2 shows the proximate analysis of biscuits manufactured using wheat flour and banana peel powder at numerous mix ratios. Control (wheat flour) had crude protein, moisture content, crude fat, total ash, total carbohydrate, and crude fibre of 11.33 %, 2.13 %, 15.52 %, 1.52 %, 68.78 %, and 0.60 %, respectively. Protein content varies between 11.33 and 10.13 %, whereas fat content varies between 14.64 and 18.18 %. Among the biscuits made with a combination of flours.

Table 4.2: Proximate compositions of fortified biscuits (Bangla kola)

Variables	Sample A	Sample B	Sample C	Sample D
Moisture (%)	2.133±0.252^{c}	3.873±0.0666^{b}	3.946±0.08^{b}	6.747±0.257^{a}
Carbohydrate (%)	68.782±0.85^{a}	65.445±0.175^{c}	65.471±0.25^{c}	64.830±0.215^{c}
Protein (%)	11.337±0.437^{a}	11.067±0.1493^{a}	10.133±0.41^{b}	10.211±0.265^{b}
Fat (%)	15.527±0.426^{c}	14.647±0.420^{d}	18.183±0.0^{a}	16.706±0.17^{b}
Crude fibre (%)	0.606±0.0152^{c}	0.6567±0.015^{b}	0.673±0.02^{b}	0.751±0.0265^{a}
Ash (%)	1.523±0.058^{b}	1.466±0.0436^{b}	1.627±0.02^{a}	1.653±0.030^{a}

*Data in the same row with different letters are significantly different ($p<0.05$). Control: Biscuits made with wheat flour (100%), B: Biscuits made with wheat flour + mixed banana peel powder (2%), C: Biscuits made with wheat flour+ mixed banana peel powder (5%), D: Biscuits made with wheat flour+ mixed banana peel powder (10%).

Table 4.3: Total phenolic, flavonoid, anthocyanin contents and antioxidant capacity of fortified biscuits

Variables	Sample A	Sample B	Sample C	Sample D
TPC (mg GAE/g)	4.147±0.005^{c}	4.1576±0.01^{c}	4.1823±0.00^{b}	4.228±0.04^{a}
TFC (mg QE/g)	15.531±0.0^{d}	16.827±0.303^{c}	19.013±0.331^{b}	23.879±0.04^{a}
Antioxidant (mg/100 g)	1.522±0.00^{d}	2.0636±0.00^{c}	2.450±0.003^{b}	2.859±0.005^{a}
TAC (TA/100 g)	8.571±0.323^{d}	9.787±0.365^{c}	10.897±0.271^{b}	13.416±0.559^{a}

*Data in the same row with different letters were significantly different ($p<0.05$). Control: Biscuits made with wheat flour (100%), B: Biscuits made with wheat flour mixed peel powder (2%), C: Biscuits made with wheat flour mixed peel powder (5%), D: Biscuits made with wheat flour mixed peel powder (10%).

4.3 Antioxidant capacity of fortified biscuit

The total antioxidant capacity of fortified biscuit was measured using the DPPH test. The antioxidant content of the fortified biscuit is shown in Table 4.3. The percentage inhibition was plotted against the Trolox assay concentration, which was expressed as mg/100 g, to create a calibration curve.

4.4 Bioactive compounds of fortified biscuit

The most prevalent bioactive substances in fruits, vegetables, tea, meat, and other animal proteins are polyphenols, flavonoids, and anthocyanins, and they have a range of health-promoting effects on people. Sample physicochemical extracts were used to extract bioactive compounds, which were then compared to relevant standards. The results of bioactive compounds are as follows.

4.4.1 Total polyphenol contents (TPC) in fortified biscuits

Using the Folin-Ciocalteu method, the total polyphenol content (TPC) of biscuits was ascertained. Table 4.3 displays the total polyphenol content of the enriched biscuits. In this instance, the result was presented as mg GAE/g. The extract's overall polyphenol content varied from 4.14 to 4.22 mg GAE/g (gallic acid equivalent). The total polyphenol content (TPC) of sample D (4.228670.0416 mg GAE/g) was substantially greater than that of the other samples in this investigation, whereas the TPC of sample C (mg GAE/g), samples B (4.157670.01629(mg GAE/g), and sample A (4.1470.005 mg GAE/g) were significantly lower.

4.4.2 Total flavonoid contents (TFC) in fortified biscuits

The total flavonoid content (TFC) of the fruit extract was measured using a slightly modified aluminium chloride colorimetric technique. Table 4.3 displays the total flavonoid result. In this case, the result was represented as mg QE/g extract. The TFC content ranges from sample A (15.5313±0.0785 mg QE/g) to sample D (23.8790±0.0416 mg QE/g). Other biscuits include, in progressive sequence, sample B (4.15767±0.01629 mg QE/g), and sample C (19.013±0.331 mg QE/g).

4.4.3 Total anthocyanin contents (TAC) in fortified biscuits

The total anthocyanin level of the fortified biscuits is shown in Table 4.3. The result was expressed as mg TA/100 g extract in this case. Sample D (13.4160.559 mg TA/100 g) contains the most anthocyanin, while sample A (mg TA/100 g) contains the least. The average anthocyanin concentration in the biscuit is 30 mg TA/100 g. The following is the order in which total anthocyanin level was discovered: sample D is greater than sample C, which is greater than sample B, which is greater than sample A.

Table 4.4: Sensory evaluation of biscuits

Sample ID	Smell	Taste	Texture	Colour	Overall acceptability
Sample A	7.10±0.876^{ab}	7.200±0.78^{ab}	7.500±1.179^{a}	7.200±0.919^{ab}	6.600±1.075^{b}
Sample B	7.000±0.667^{bc}	6.900±0.73^{b}	6.800±1.317^{b}	8.000±1.054^{a}	6.200±0.919^{b}
Sample C	6.100±1.197^{c}	5.900±1.66^{c}	6.100±1.524^{bc}	5.600±1.430^{c}	7.000±1.054^{ab}
Sample D	8.000±1.155^{a}	8.100±0.87^{a}	5.400±0.843^{c}	6.800±0.789^{b}	7.60A±1.075^{a}

*Data in the same row with different letters are significantly different ($p<0.05$). Control: Biscuits made with wheat flour (100%), B: Biscuits made with wheat flour mixed plant powder (2%), C: Biscuits made with wheat flour mixed plant powder (5%), D: Biscuits made with wheat flour mixed plant powder (10%).

4.5 Sensory evaluation of biscuits

Table 4.4: displays the results of sensory qualities such as colour, flavour, taste, texture, and overall acceptability. The addition of banana peel powder resulted in a significant improvement in flavour and texture. The findings summary and comparison of sample A, sample B, sample C, and sample D.

Chapter 5: Discussion

The outcomes of the study are covered in this section in relation to the study's goals, which were to determine the gross chemical composition by proximate analysis, characterize the bioactive compounds, and evaluate panelist sensory evaluation. Overall acceptability of fortified biscuit measured on a hedonic scale. These elements were mentioned in the previous chapter, which covered the methodology of the study.

5.1 Proximate composition

Table 4.2 shows the chemical make-up of the reinforced biscuits. The moisture, total carbohydrate, crude fibre, ash, fat, and crude protein composition of Sample A, and Sample D were found to be considerably different.

5.1.1 Carbohydrate

Carbohydrate content were 65.44 %, 65.47 %, and 64.83 % in Samples B, C, and A, respectively, while it was 68.78 % in Sample A. This is to be expected, given that the amount of wheat flour in the recipe/formula was lowered and banana peel powder was substituted. The amount of carbohydrates decreased as PPF's share increased, but climbed as UBF and SPF's share did as well. This finding might be explained by the difference between PPF's low carbohydrate content and UBF's and SPF's high sugar content Ayo-Omogie and Iliyasu. (2019). The presence of wheat flour in Sample A can be linked to the high carbohydrate content.

5.1.2 Moisture

sample D having the highest figure (Table 4.2). This change in moisture indicates that adding banana peel powder to the biscuits increases the water content. These findings, though, were found to be greater than according to Aryani and Shintawati. (2017), who reported moisture content of biscuits containing 0%, 25%, 50%, and 75% banana peel flour to be 1.96 %, 1.51%, 1.31 %, and 1.08 %, respectively. Geographical differences and varietal differences could explain the disparity. Moisture content food is commonly utilized as a shelf-life indicator. The biscuits' higher moisture content clearly showed that they have a short shelf life.

5.1.3 Protein

Sample A had a protein value of 11.33 %, while sample D had a protein level of 10.2 %. These findings were similar to those of Gomes et al. (2016), who found protein content to be 8.9-10.8 g/100g in biscuit with 10% green banana peel powder. This is because banana peel flour is poor in protein, and the other components aren't high in protein. Furthermore, a reduction in the amount of wheat flour used to offer wheat protein in our biscuits could be due to a fall in protein content.

5.1.4 Crude fat

In sample C and sample D, the quantity of fat in biscuits with flour banana peels added is 16.70 % and 18.18 %, respectively, which is higher than sample A. This is similar to the findings of Oyeyinka et al. (2014) who found 2.98 %-4.30 % for fat, respectively. Fat is a key source of energy as well as a source of essential fatty acids. Many meals' fats content has a substantial impact on overall physical characteristics such as flavor, mouth feel, texture and look. The current investigation discovered that both sample B and, sample C had an appropriate amount of fat, which could be advantageous.

5.1.5 Ash content

Obtained results near the ash content biscuit code sample A (1.52%), sample B (1.46%), sample C (1.62%), and sample D (1.62%). (1.65 %). The nutritional evaluation that PPF and SPF are rich in iron, salt, magnesium, iron, and potassium is supported by the high ash content of the biscuit samples Torres et al. (2007). It's likely that the mineral content of flour banana peels is significant enough that the experimental findings reveal a significant ash level as well. The biological elements found in banana peels are thought to be responsible for the increased ash rates in biscuits. Mineral components that remain after the material has been burned to liberate the element carbon are referred to as ash content. The ash content can also be thought of as a non-volatile component left over from cremation and annealing organic molecules. The quantity of inorganic material that remains after the organic material in the food has been decomposed that is referred to as the ash content of the food. Ash content is not necessarily equal to mineral content since certain minerals are degraded during volatilization or interplay between constituents.

5.1.6 Crude fibre

The amount of crude fiber in flour biscuits with banana peels exceeds that in sample A, which includes no banana peel powder (0.60 %). Biscuits using 2%, 5%, and 10% flour banana peels had fiber levels of 0.65, 0.67, and 0.75 %, respectively. Because the amount of banana peel powder in sample D was higher than in sample A, this was to be expected. The addition of banana peel powder to wheat flour increases the insoluble fiber of the biscuits ($p<0.05$). The high fiber content of flour biscuits, combined with the possibility of include banana peels, makes for a nutritious supper. This biscuit is thought to be a snack that helps hypercholesterolemic people lower their blood cholesterol levels or maintain lipid and blood glucose balance. The combination of banana peels and flour cookies resulted in a little hard, gritty, and crumbly texture. The fiber content of flour banana peels is most likely to blame. The biscuits were a little rough, gritty, crumbly, and not as crispy as they could have been. This is presumably due to the fiber content of flour banana peels having a significant impact.

5.2 Antioxidant capacity in fortified biscuits

As shown in Table 4.3, the antioxidant capacity of common fruits was established using the DPPH test. DPPH became a stable free radical after accepting an electron or proton and scrounged from purple to yellow. A diamagnetic substance was identified when antioxidants were present Singh et al. (2015). Sample A (1.52267±0.00153mg/100 g) and sample D (mg/100 g) had different levels of DPPH activity. As a result, biscuits containing a lot of banana peel powder had more antioxidant capacity. The findings are similar to those of Ajibola et al. (2015), who discovered that increasing the amount of Moringa oleifera leaves and cocoa powder boosted the antioxidant capabilities of biscuits. According to Jan et al. (2015). mixing barley flour and wheat flour increased DPPH inhibition by 55.53 to 61.65%.

5.3 Bioactive compounds of fortified biscuits

Banana peels contain significant amounts of natural bioactive compounds that are beneficial to health and disease prevention. Flora and fauna contain polyphenols, flavonoids, and anthocyanins, which are bioactive compounds. As a result, it is vital to investigate and assess these compounds in connection to their sources.

5.3.1 Total polyphenol content (TPC)

Sample D (4.22867±0.0416 mg GAE/100g) showed a higher TPC value than samples A (4.147±0.005 mg GAE/100g) and B (4.15767±0.0162 mg GAE/100g) in this investigation. (Vasco et al., 2008) proposed categorizing phenolic content as low (100 mg GAE/100g), medium (100-500 mg GAE/100g), and high (>500 mg GAE/100g). As a result, sample D had a high polyphenol content, sample C had a medium polyphenol level, and the others had a low polyphenol concentration. Elhassaneen et al. (2016) found that incorporating 5% prickly peel and potato peel into biscuits increased the total phenolic content (TPC) of the biscuits when compared to the control (110.23 to 192.79 mg/100 g of sample).

5.3.2 Total flavonoid content (TFC)

Nearly all plant species include flavonoids, which are bioactive natural compounds. The most prevalent members of the family were catechins, flavonoids, flavones, and quercetin (Gadkari and Balaraman, 2015). TFC content varied from sample A (15.5313±0.0785 mg QE/g) to sample D (mg QE/g), according to the results. This result was quite similar to (Ahmed et al., 2022) TFC content of sample B (16.827±0.303 mg QE/g) and sample C (mg QE/g).

5.3.3 Total anthocyanin content (TAC)

The preponderance of the red, blue, and purple colors in fruits were caused by anthocyanins, water-soluble antioxidant pigments. In this investigation, the TAC of fruits differs from 8.5 to 13.41 mg/100g. Sample D (13.416±0.559 mg/100g) had more total anthocyanin content than sample A (mg/100g).

5.4 Sensory evaluation

5.4.1 Consumer characteristics

The male panelists accounted for 80% of the total, while the male panelists accounted for the remaining 20%. The panelists were between the ages of 20 and 28. Consumers prioritized educational attainment, with 80 percent pursuing a bachelor's degree and 20% pursuing a master's degree.

5.4.2 Sensory evaluation of fortified biscuits

Table 4.4 shows the sensory evaluation of the fortified biscuits prepared. The mean score for color, taste, smell, texture, and overall acceptability of biscuit samples is shown in Table 4.4. Each sample's mean hedonic scale scores for color, taste, smell, texture, and overall acceptability were found to be significantly different. Sample D's color and flavor scored lower on the hedonic scale than the other infused biscuit samples. The sensory characteristics of samples A and C revealed that the samples overall acceptability was slightly higher on the hedonic scale. Because sample D has the same hedonic scale as sample C, the results demonstrate that it is as acceptable. However, there was no discernible difference in odor or flavor.

5.4.3 Overall acceptability of fortified biscuits

The average hedonic scores of samples A, B, C, and D are displayed in Table 4.4. Despite the fact that sample D appeared to have a higher mean value, the results indicate that there is a significant difference ($p>0.05$) across samples A and C (7.60). This indicates because both Sample C and Sample D were approved by the panel.

5.5 Limitation of the current study

1. Due to a lack of time and resources, the study was incredibly hard to accomplish.
2. We employed a factor based on supposition that may or may not be correct to identify the wavelengths of TFC, TAC and TPC.
3. There are a variety of different bioactive compounds, but we only found three: TPC, TFC, and TAC.
4. Although GC-MS would be preferred for a better result, we used UV visible to measure bioactive compounds.
5. A microwave oven was used in this experiment. However, a new rotating gas oven would have been preferred for improved biscuit quality.

Chapter 6: Conclusions

The results of this study revealed that replacing banana peel powder with wheat flour raised the proportion of crude ash, crude fibre, bioactive components, and antioxidant capacity content in biscuits. Protein, fat, carbohydrate, and calorie composition of made biscuits showed significant differences. The prospective juror found that a 10% swap of banana peel powder biscuits was more satisfactory with all quality parameters in a sensory evaluation test. As a result, substituting wheat flour for banana peel powder will be much more nutritional and cost effective.

The physiological and nutritional specific properties of flour made from banana peel powder were investigated. According to the findings of this investigation, composites done through the following to 10% banana peel powder were necessary for the creation of biscuits. Beyond 10% banana peel powder blends, the colour of the biscuits darkens due to the maillard browning reaction, the inclusion of high fibre content, and baking process conditions. Banana peel powder could potentially be used to make a variety of bakery goods and useful food additives. This upregulation of AOA and bioactive chemicals was indicated as a marker of the existence of health-beneficial substances, which has a positive impact on environmental issues. It can also be turned into critical foods such as nutraceutical and rehabilitative functional food items. It also is a functional food because it contains a satisfactory limit of tannin yet after processing. Most often these human diseases are linked to free radical formation and metabolism, including cardiovascular disease, cancer, cataracts, age-related muscle degeneration, rheumatoid arthritis, and a variety of neurodegenerative disorders. Tannin has the ability to alter this structure and ensure resistance to a variety of disease.

Chapter 7: Recommendation and future perspectives

Biscuits are the most frequently consumed bakery products on the planet. Some of the reasons for their global appeal include their ready-to-eat nature, low cost, high nutritional content, variety of flavours, and long shelf life.

The abundance of bioactive chemicals, crude fibre, and antioxidant capacity, all of which contribute significantly in human health, as well as the existence of vitamins and minerals, has considered banana peel an important and nutritious source of nutrients. Its use of banana peel powder in biscuit manufacturing boosts nutritional quality while cutting production costs. This strategy of repurposing banana peels will help to decrease trash. It is now critical to disseminate the technique.

Recommendations for future research based on the findings of the development and evaluation of the biscuits with banana peel powder of the study include:

1.Should include the systematic evaluation studies for banana peel powder that has been manufactured.

2. Creating a product with high-value elements that may cause organoleptic alterations.

3. The current study demonstrates that banana peel has a higher content of bioactive chemicals, crude fibre, and anti-oxidants than other comparable fruits. As a result, additional research should be conducted.

4. The nutrient composition and physiochemical features of banana peel should be assessed. It is crucial to be aware about fruit waste and its potential as a source of food for the food sector.

5. This type of fortified bakery product could be further exploited if banana peel can be maintained better and products are made available to consumers across the year, meeting the body's need for bioactive chemicals.

6. The purpose of this study was to figure out the concentration of AOA and certain bioactive chemicals. Further research into the nutritional content and chemical profile of banana peels, as well as other areas, should be conducted.

7. Determining the shelf-life period using food analysis and various types of packaging techniques.

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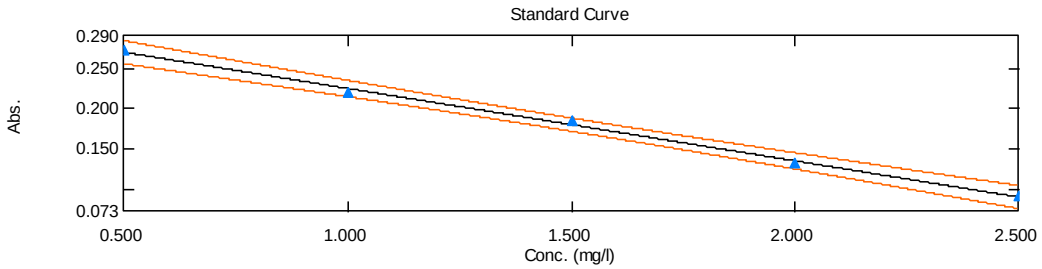
Appendix A: Standard curve & sample curve

Antioxidant capacity of banana biscuit

Standard table of Trolox:

	Sample ID	Type	Conc(ppm)	WL517.0
1	std1	Standard	0.500	0.272
2	std2	Standard	1.000	0.221
3	std4	Standard	1.500	0.185
4	std5	Standard	2.000	0.133
5	std6	Standard	2.500	0.092

Standard Curve:



y = - 0.0894539 x + 0.314536
r2 = 0.99735

One-way ANOVA: sample A, sample B, sample C, sample D

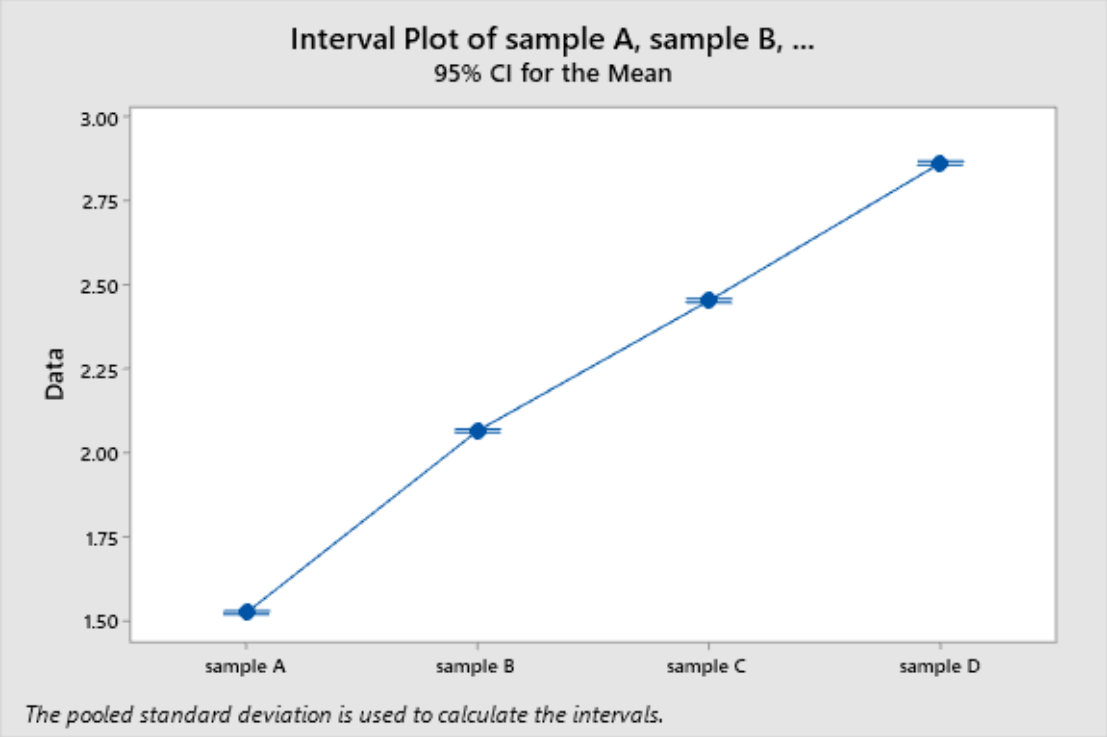
Means

Factor	N	Mean	StDev	95% CI
sample A	3	1.52267	0.00153	(1.51737, 1.52796)
sample B	3	2.06367	0.00451	(2.05837, 2.06896)
sample C	3	2.45033	0.00379	(2.44504, 2.45563)
sample D	3	2.85933	0.00513	(2.85404, 2.86463)

Grouping Information using the Fisher LSD method and 95% confidence

Factor	N	Mean	Grouping
sample D	3	2.85933	A
sample C	3	2.45033	B
sample B	3	2.06367	C
sample A	3	1.52267	D

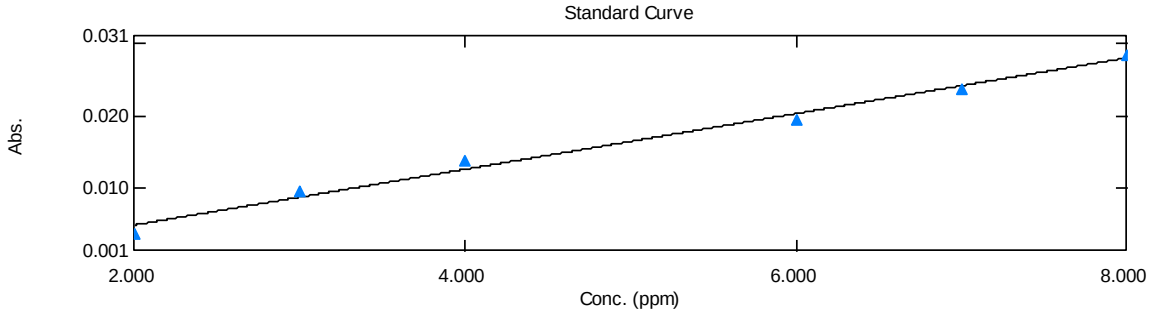
Means that do not share a letter are significantly different



TFC (Total flavonoid content)
Standard table of quercetin:

Id	Type	Conc(ppm)	WL415.0	Wgt.Factor
Std_1	Standard	2.000	0.004	1.000
Std_2	Standard	3.000	0.010	1.000
Std_3	Standard	4.000	0.014	1.000
Std_4	Standard	6.000	0.020	1.000
Std_5	Standard	7.000	0.024	1.000
Std_6	Standard	8.000	0.029	1.000

Standard curve:



y = 0.00385110 x - 0.00271158
r2 = 0.98868

One-way ANOVA: sample A, sample B, sample C, sample D

Means

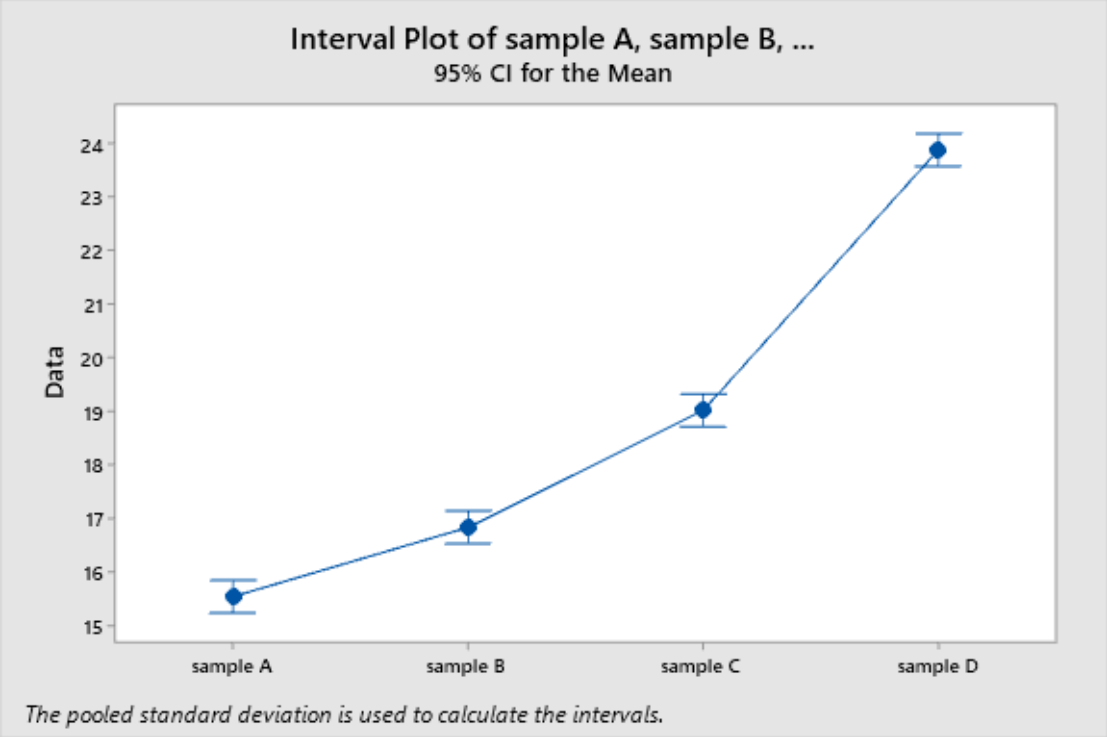
Factor	N	Mean	StDev	95% CI
sample A	3	15.5313	0.0785	(15.2268, 15.8358)
sample B	3	16.827	0.303	(16.522, 17.131)
sample C	3	19.013	0.331	(18.709, 19.318)
sample D	3	23.8790	0.0416	(23.5745, 24.1835)

Pooled StDev = 0.228724

Grouping Information using the Fisher LSD method and 95% confidence

Factor	N	Mean	Grouping
sample D	3	23.8790	A
sample C	3	19.013	B
sample B	3	16.827	C
sample A	3	15.5313	D

Means that do not share a letter are significantly different.

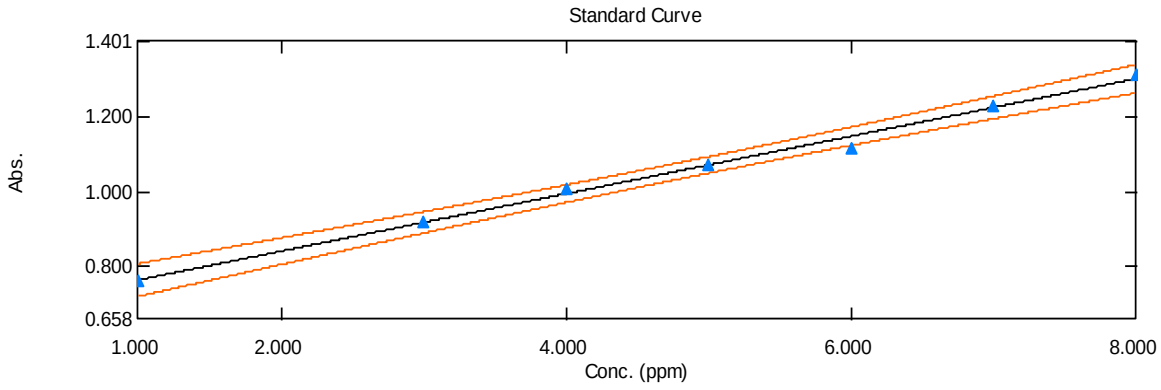


TPC (Total phenolic content)

Standard table of Gallic acid:

	Sample ID	Type	Conc(ppm)	WL760. 0	Wgt.Factor
1	STD1	Standard	1.000	0.763	1.000
2	STD2	Standard	2.000	0.780	1.000
3	STD3	Standard	3.000	0.920	1.000
4	STD4	Standard	4.000	1.007	1.000
5	STD5	Standard	5.000	1.074	1.000
6	STD6	Standard	6.000	1.115	1.000
7	STD7	Standard	7.000	1.230	1.000
8	STD8	Standard	8.000	1.314	1.000

Standard curve:



y = 0.0768527 x + 0.687090
r2 = 0.99301

One-way ANOVA: sample A, sample B, sample C, sample D

Means

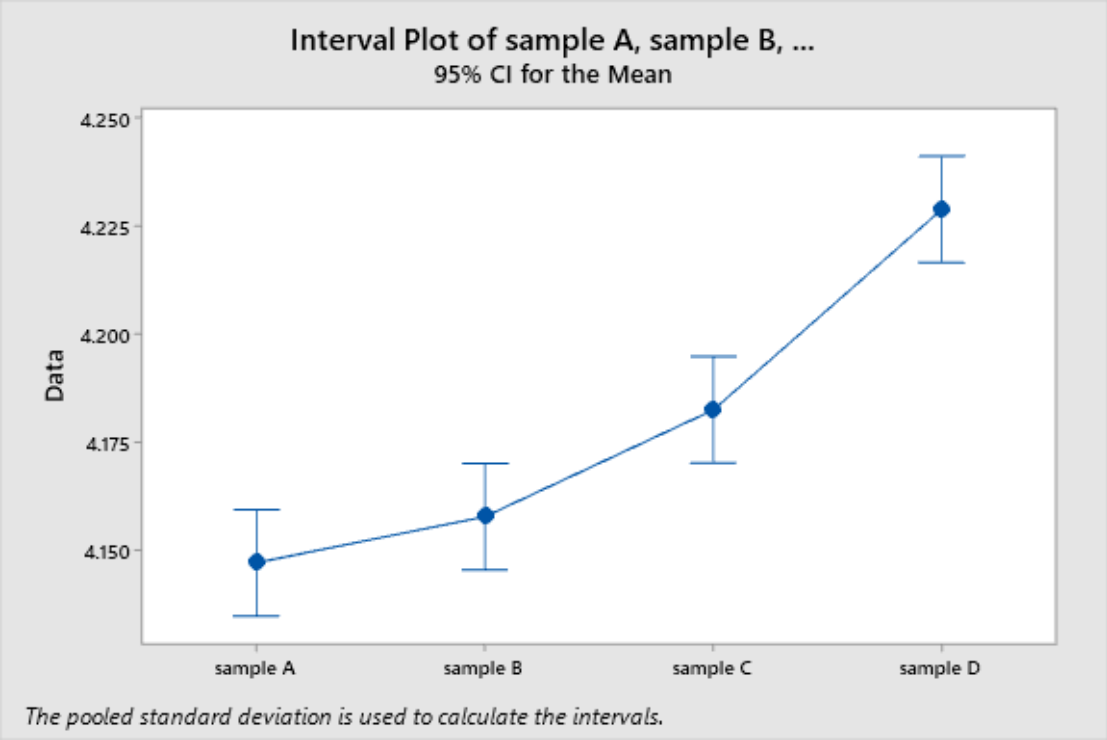
Factor	N	Mean	StDev	95% CI
sample A	3	4.14700	0.00500	(4.13469, 4.15931)
sample B	3	4.15767	0.01629	(4.14536, 4.16998)
sample C	3	4.18233	0.00586	(4.17002, 4.19464)
sample D	3	4.22867	0.00416	(4.21636, 4.24098)

Pooled StDev = 0.00924662

Grouping Information using the Fisher LSD method and 95% confidence

Factor	N	Mean	Grouping	
sample D	3	4.22867	A	
sample C	3	4.18233	B	
sample B	3	4.15767		C
sample A	3	4.14700		D

Means that do not share a letter are significantly different.



Total anthocyanin content:

One-way ANOVA: sample A, sample B, sample C, sample D

Means

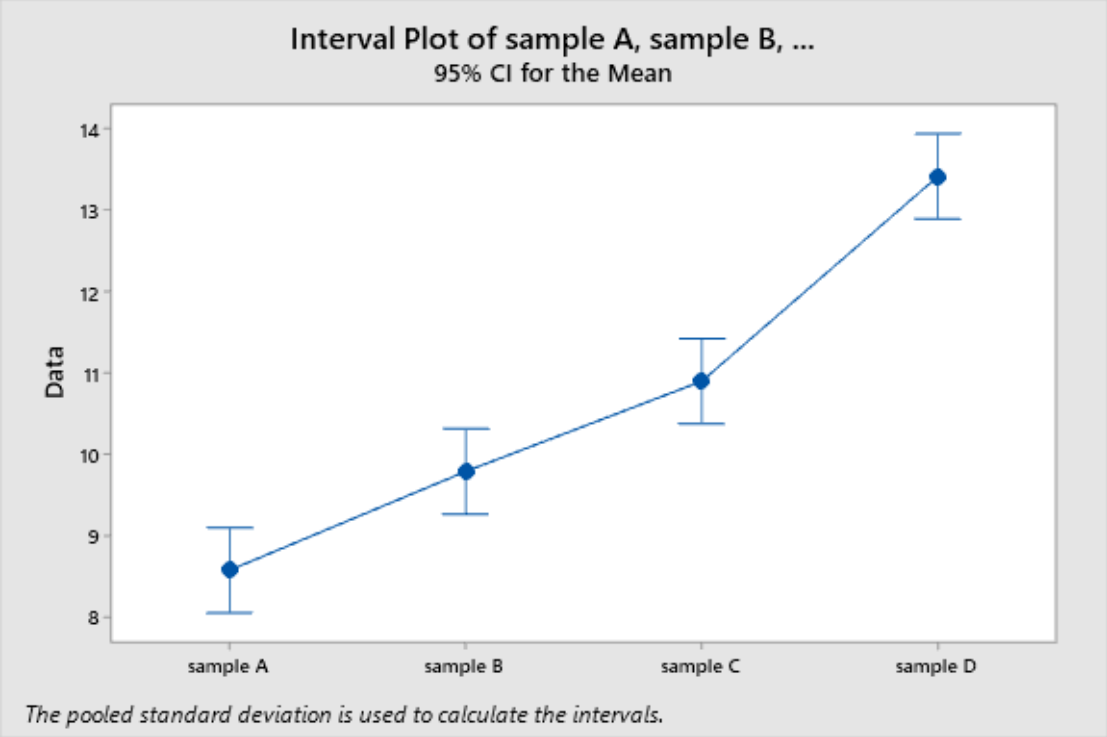
Factor	N	Mean	StDev	95% CI
sample 0	3	8.571	0.323	(8.046, 9.097)
sample 2	3	9.787	0.365	(9.261, 10.312)
sample 5	3	10.897	0.271	(10.371, 11.422)
sample 10	3	13.416	0.559	(12.891, 13.941)

Pooled StDev = 0.394686

Grouping Information using the Fisher LSD method and 95% confidence

Factor	N	Mean	Grouping
sample 10	3	13.416	A
sample 5	3	10.897	B
sample 2	3	9.787	C
sample 0	3	8.571	D

Means that do not share a letter are significantly different.



Over all acceptability:

One-way ANOVA: sample A, sample B, sample C, sample D

Means

Factor	N	Mean	StDev	95% CI
sample A	10	6.600	1.075	(5.938, 7.262)
sample B	10	6.200	0.919	(5.538, 6.862)
sample D	10	7.600	1.075	(6.938, 8.262)
sample C	10	7.000	1.054	(6.338, 7.662)

Pooled StDev = 1.03280

Crude fibre:
One-way ANOVA: sample A, sample B, sample C, sample D

Means

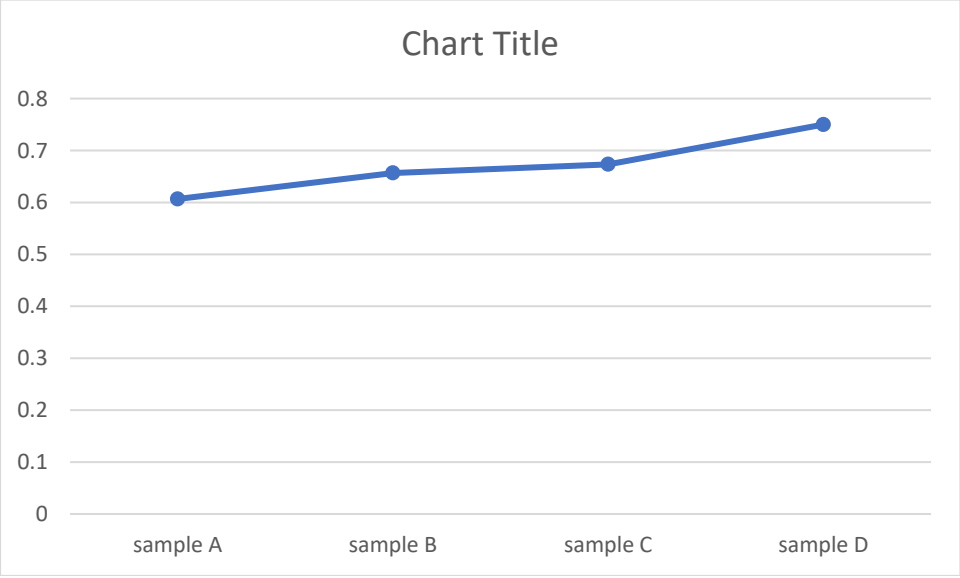
Factor	N	Mean	StDev	95% CI
sample A	3	0.60667	0.01528	(0.58004, 0.63329)
sample B	3	0.65667	0.01528	(0.63004, 0.68329)
sample C	3	0.6733	0.0208	(0.6467, 0.7000)
sample D	3	0.7500	0.0265	(0.7234, 0.7766)

Pooled StDev = 0.02

Grouping Information Using the Fisher LSD Method and 95% Confidence

Factor	N	Mean	Grouping	
sample D	3	0.7500	A	
sample C	3	0.6733	B	
sample B	3	0.65667	B	
sample A	3	0.60667		C

Means that do not share a letter are significantly different.



Appendix B: Questionnaire for hedonic test of biscuits

Sensory Evaluation Form
Consumer test for biscuits

Panellist No……. Sex…….

Age group (a) 20-30 (b) 30-40 (c) 45 and above

Time……... Date……....

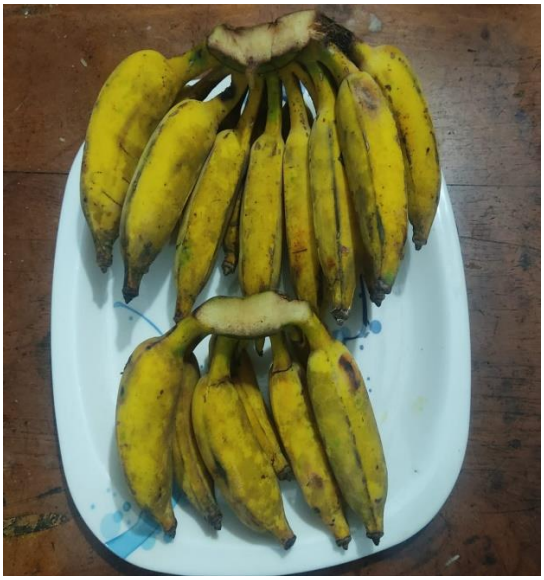
Education level
(a) Bachelor degree (b) Master's degree
(c) other specify…….....

Please taste each of the (4) coded products. Indicate how much you like or dislike each sample by checking the appropriate sample attribute and indicate your reference (1-9) in the column against each attribute. Put the appropriate number against each attribute.

9=Like extremely
8 = Like very much
7 = Like
6 = Like slightly
5 = Neither like nor dislike
4 = Dislike slightly
3 =Dislike moderately
2 = Dislike
1 = Dislike extremely

Attributes	**9 Like extremely**	**8 Like very much**	**7 Like**	**6 Like slightly**	**5 Neither like nor dislike**	**4 Dislike slightly**	**3 Dislike moderately**	**2 Dislike**	**1 Dislike extremely**
Texture									
Smell									
Taste									
Color									
Overall acceptability									

Appendix C: Picture gallery



Bangla Kola (*Musa sapientum*)

Washing of banana peel

Blanching

Drying

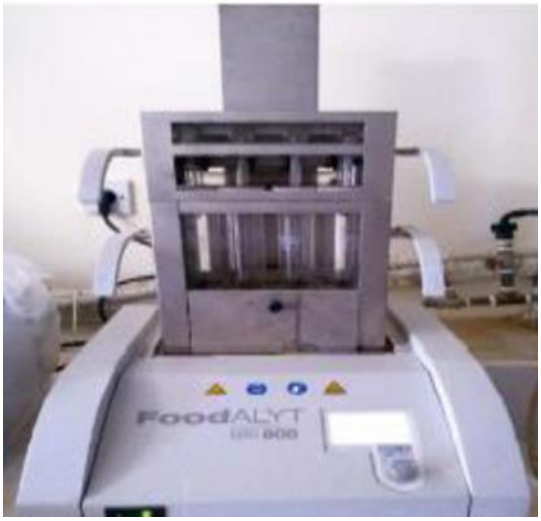
Raw fortified biscuits

Micro oven baking

Sensory evaluation

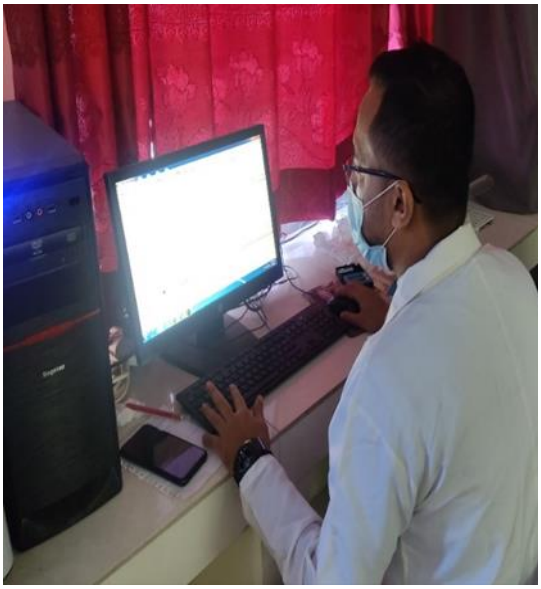
Sensory evaluation

Crude fibre determination



Protein digestion

Addition of Methanol



UV visible spectrophotometry

Brief biography

Md. Tanvir Ahmed received a GPA of 5 on the Secondary School Certificate Exam in 2011 and a GPA of 5 on the Higher Secondary Certificate Exam in 2013. He earned a B.Sc. (Hons.) in Food Science and Technology (BFST) with a CGPA of 3.52 from the Faculty of Food Science and Technology, Chattogram Veterinary and Animal Sciences University (CVASU) in Bangladesh. He is now an MS candidate in Food Chemistry and Quality Assurance at CVASU's Faculty of Food Science and Technology's Department of Applied Chemistry and Chemical Technology. He is very interested in working at a food testing lab doing analytical chemistry.