QUALITY EVALUATION OF NOVEL BISCUITS SUPPLIMENTED WITH WATERMELON (*Citrullus lanatus*) SEEDS AND MORINGA (*Moringa oleifera*) LEAVES POWDER

A thesis by-

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Roll No.: 0121/11

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A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Science in Applied Human Nutrition and Dietetics

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 aspects, and that all revisions required by the thesis examination committee have been made

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PLAGIARISM VERIFICATION

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Abbreviation

%	: Percentage
ºC	: Degree Celsius
µg	: Microgram
kg	: Kilogram
g	: gram
mg	: milligram
ppm	: parts per million
ml	: milliliter
DPPH	: 2,2-diphenyl-1-picrylhydrazyl
ANOVA	: Analysis of variance
CHO	: Carbohydrate
et al	: Et alii/ et aliae/et alia
Kcal	: kilocalorie
SD	: Standard deviation
MLP	: Moringa leaves Powder
WSP	: Watermelon seed powder
WSMLB	: Watermelon seeds moringa leaves biscuit

Abstract

Proteins, fibers and minerals are particularly abundant in watermelon seeds and moringa leaves. The current study's goal was to determine the effects of adding watermelon seeds and moringa leaves to whole wheat flour biscuits on the biscuits overall quality while taking into account the nutritional benefits of those powders. Watermelon seeds and moringa leaf powder were combined with whole wheat flour in three different ratios 10:90 (WSMLB1), 15:85 (WSMLB2), and 20:80 (WSMLB3) to create composite flours. The composite flours were used to make biscuits, which were then tested for physical characteristics, proximate composition, bioactive compounds, antioxidant activity, mineral content and sensory evaluation. The findings indicated that compared to whole wheat flour, biscuits made with watermelon seeds and moringa leaves had higher levels of protein, crude fat, ash, and crude fiber. The experimented biscuit contained 9.81-11.62% protein, 17.53-21.72% fat, 1.25-1.72% ash, 3.11-4.06% fiber, and 69.62-61.77% carbohydrates, respectively. When compared to the control, the prepared biscuit had greater levels of bioactive substances, total flavonoid content, and total phenolic content, ranging from 26.12 to 49.17 mg GAE/100 ml and 80.03 to 122.75 mg QE/100g, respectively and antioxidant activity increases 35.166 to 48.97 mg TE/100gm. Additionally, larger levels of the minerals (Fe, Mg, K, and Zn) were found in the supplemented biscuit (20.0-35.2 mg/gm, 0.50-0.91 mg/gm, 0.20-1.4 mg/gm, 0.45-0.65 mg/gm) as compared to the control. With the addition of powdered watermelon seed and moringa leaves for enrichment, the sensory characteristics were noted as being within the acceptable range. Consequently, the developed biscuit is highly nutrient-dense and can be recognized as functional food.

Keywords: Watermelon seed, Moringa leaves, biscuit, supplement

Chapter 1: Introduction

Biscuit (also known as cookies)- a cereal snack food has become very popular across the globe, especially among children. Numerous appealing qualities exist for biscuits, including a larger consumer base, a lengthy shelf life, and outstanding eating quality (Banureka *et al*., 2009). The fundamental component of biscuit production is wheat flour, a carbohydrate-based culinary raw material that is lacking in several nutrients. A blend of varied ratios of non-wheat flour from grains, roots, and tubers, with or without wheat flour, has been referred to as composite flour. Nowadays, as people are more concerned about their health and nutrition, eating only biscuits made of wheat may not be a good option. Instead, the general public is encouraged to consume functional and herbal foods (Kwak *et al*., 2001). To boost the nutritional content of biscuits, fortified or composite flour is commonly used in several nations (Hasmadi *et al*., 2014).As a result, manufacturers have recently changed their emphasis on the use of seeds and herbs in traditional meals like tea, juice, and snack chips by offering titles like "special diet foods," "medical foods," and "dietary supplements" in response to the rising trend of using herbal goods for wellness (Kwak *et al*., 2001).Functional foods contain components (either naturally occurring or added) that offer health advantages above and above the food's basic nutritional value, according to the Food and Drug Administration (Zhou *et al*., 2014). According to the FDA and other regulatory agencies, functional foods are not subject to the same regulations as conventional foods; yet, herbal components and oilseeds are used in functional meals must first have GRAS (Generally Recognized as Safe) approval (Robert *et al*., 2006). Various high protein foods have been produced industrially in various regions of the planet due to the growing recognition of the value of grain oilseeds in creating high protein diets that can meet the needs of the vulnerable population (Mooriya, 2003).

Watermelon (*Citrullus lanatus*) is a common fruit of cucurbitaceae family produced in the tropics, but its seeds are rarely used. The seeds have notable levels of protein (35%) and fat (50%) as well as magnesium, calcium, potassium, iron, phosphorus and zinc (Odibo *et al*., 2012) and with outstanding functional qualities that have been discovered to be useful in baking (EL-Adawy *et al*., 2001). Water melon seeds are well known for being very nutrient-dense; they are magnificent providers of fat, protein, B vitamins, minerals (including magnesium, potassium, phosphorus, sodium, iron, zinc, and manganese), and phytochemicals (Braide *et al*., 2012). Fat is the initial component responsible for tenderness, eatability, maintaining quality, grain, and texture of biscuit (O'brien, 2003). According to Nisbett *et al*. (1986), short dough biscuits with fat content over 20% dominate the biscuit industry.

A miracle plant or "tree of life," *Moringa oleifera* is praised for its medicinal applications and extraordinarily high nutritional value (Dhakar *et al*., 2020). This plant's has capacity to work for multiple purposes, including those of herbal biscuit goods, has been thoroughly investigated (Claughton *et al*., 1989). All nine essential amino acids are present in the leaves of the Moringa plant, which is unfamiliar within plant sources. Linoleic acid is also present in very high concentrations. Almost all vitamins, including vitamin A, vitamin B1 (which includes folic acid, pyridoxine, and nicotinic acid), vitamin C, vitamin D, and vitamin E, are also plentiful in moringa leaves (Feng *et al*., 2016). To improve the quality of biscuit and bread, some of the wheat flour can be replaced with moringa seed flour (Hedhili *et al*., 2021). This study is anticipated to establish the nutritional significance and familiarize consumers with the roasted watermelon seeds and moringa leaf enhanced biscuits.

1.1 Objectives

1. To develop biscuits with roasted watermelon seed and moringa leaves powder.
2. To examine the nutritional makeup, antioxidant levels, phytochemical and mineral content of formulated biscuits.
3. To evaluate consumer acceptance of produced biscuits.

Chapter 2: Review of Literature

2.1 Novel Biscuits

According to recent estimates, biscuit consumption is rising yearly and is primarily made of wheat flour (Melese and Keyata , 2022). Additionally, pomace, peels, and seeds make about 25% to 30% of the waste generated during the processing of fruits and vegetables, and they are incredibly underutilized (Nassar *et al*., 2008).On the other hand, these byproducts are significant sources of carbohydrates, minerals, organic acids, fiber, and phenolic chemicals, which offer a variety of nutritional sensory properties. Many fruits, such as watermelons (*Citrillus lanatus*) and oranges (*Citrus sinensis*), are only partially consumed. The fleshy parts are often juiced after being eaten raw, but the rinds, seeds, and pomace are frequently discarded.When watermelon juice is produced, for example, only about half of the fresh fruit's weight is converted into juice, leaving behind enormous volumes of residues (peel, pulp, and seeds) known as orange pomace that are packed with fiber and minerals.Particularly among baked goods, biscuits are a common ready-to-eat food in households. Due to their ready-to-eat status, variety of flavors, affordability, and most significantly, their capacity to provide the quick energy needed for activity, biscuits are well-liked bakery products that are consumed by a wide spectrum of communities (Ogo *et al*., 2021). In order to reduce waste, improve the nutritional and health benefits of biscuits, and lower the cost of biscuit production since these flours are relatively less expensive than the commonly used wheat flour, more flours that are obtained from these by-products should be used with the well-known wheat flour. This is due to the benefits that have been derived from composite flours, such as improved fiber, mineral and other nutritional content.

2.2 Antiquity and spread of watermelon

A significant horticultural crop, watermelon is cultivated worldwide in warm regions and is prized for its sweet and juicy fruit. While it is known that watermelon originated in Africa, its precise geographic origin and domestication history are unknown.The Kalahari Desert region, where the species can still be found in the wild in a variety of forms, is one likely gene center ,yet it has also been claimed that the Sahel region of Northern Africa is where it originated (Wasylikowa *et al*., 2004).

Around 81% of the world's total watermelon production comes from Asian countries (Assefa *et al*., 2020).

2.3 Taxonomical classification of watermelon

Kingdom : Plantae

Division : Magnoliophyta

Class : Magnoliopsida

Order : Cucurbitales

Family : Cucurbitaceae

Genus : *Citrullus*

Species : *Citrllus lanatus*

2.4 Nutritional significance of watermelon

Watermelon is exceptionally refreshing, high in water and nutrients, and has very few calories. Lower blood pressure, increased insulin sensitivity, and lessened muscle stiffness are just a few of the potential health advantages of this luscious melon. Watermelon's bioactive chemicals have been shown to have a various positive health impacts, like lowering the diabetes risk, obesity, cardiovascular disease, and other aging-related illnesses (Rao and Agarwal, 2000). Citrulline, a watermelon-derived non-essential amino acid necessary for the production of arginine, was discovered and purified (Mitsunori, 1930). To increase the level of arginine, watermelon serves as an essential dietary supplement. Citrulline supports watermelon's resistance to stressors like drought. The consumption of watermelon is increased by its hydrating flavor, high water content, and attractive red, yellow colors. Because of high lycopene, vitamin A, vitamin C, and antioxidant in watermelon, it can be regarded as a superior functional food (Rimando and Perkins-Veazie, 2005).

2.5 Watermelon seeds

While eating watermelon *(Citrullus lanatus*), the seeds are frequently thrown away. A well supply of fat, protein, fatty acids, vitamins, and minerals including potassium, zinc, magnesium, copper, etc. are present in watermelon seed. The potential for financial advantage of watermelon seed is growing daily due to the significant health benefits of the seed (Mariod *et al*., 2009). Due to its significant source of proteins, fat watermelon seeds are utilized to make a variety of protein, fat-based goods (El-Adway and Taha, 2011).Because seeds have a high concentration of protein, fat, and phytochemical compounds, they are used in baby food to help children develop their nutritional needs (Maynard, 2001). The seed is high in protein and fat content and advantageous functional characteristics make it a valuable ingredient in baking. Despite their small size, seeds contain vital nutrients like protein, fiber, vitamins, minerals, omega-3 fatty acids phytochemical compounds that prolong life by assisting the body in illness prevention and promoting a healthy, sound lifestyle (Omorayi and Dilworth, 2007). The watermelon seed is also used as a food component, such as flavoring and soup thickening agents.

2.6 Proximate composition of watermelon seeds

In a study on the chemical make-up of watermelon seeds, (Mehra *et al.*, 2015) found that the percentages of moisture, ash, protein, fat, fiber, and energy were, respectively, 3.575 %, 3.636%, 34.22%, 31.999%, 3.65%, and 529.23 Kcal/100g. According to the study, the watermelon seed had higher than average concentrations of K, Mg, Ca, and P and lower than average concentrations of Fe, Na, and Zn. There were 7599 ppm, 4496 ppm, 2477 ppm, 9249 ppm, 164 ppm, 74 ppm, and 75 ppm of K, Mg, Ca, P, Fe, Na, and Zn, respectively.The mineral content of watermelon seed extract and ash was calculated by Hannah and Krishnakumar (2015).According to the study, the watermelon seed ash sample was lower in Ca and P and higher in Fe than the seed extract sample.In watermelon seed extract, Fe, P, and Ca concentrations were 2.29 mg/g, 0.83 mg/g, and 29.63 mg/g, respectively. In an ash sample, the concentrations were 2.25 mg/g for Fe, 1.07 mg/g for P, and 45.14 mg/g for Ca. In order to determine the chemical characteristics of three different kinds of watermelon seeds (Charleston gray, Crimson sweet, and Black diamond), Tabiri *et al*. (2016) carried out an experiment. They discovered that the levels of calcium, magnesium, potassium, phosphorus, and copper in the three varieties of watermelon seeds were nearly identical. Three different varieties of seeds contained varied amounts of iron and zinc. The iron and zinc contents of the three varieties of seed were 3.71 mg, 2.72 mg, and 4.60 mg per 100g, respectively, and 3.71 mg, 0.81 mg, and 0.66 mg per 100g, respectively.According to the study, Black diamond watermelon seeds had a higher zinc level than the other two types of seeds, and Charleston gray seeds had a higher iron concentration.The mineral content of watermelon seed was examined by Jecob *et al*. (2015). They demonstrated that the iron level in the watermelon seed was higher than other minerals, at roughly 144.70 mg/100g. Approximately 22.73 mg, 21.05 mg, and 20.46 mg of manganese, zinc, and magnesium were present in the seed per 100g.

2.7 Functional property of watermelon seeds

Watermelon seed and rind chemical compositions and functional characteristics were assessed by Egbuonu (2015). In this study, it was assessed how much a material could absorb in terms of water absorption capacity, oil absorption capacity, foam capacity, emulsion stability. According to findings of this study, watermelon seed's functional characteristics were determined to be 116.30% for WAC, 123.50% for OAC, 21.5% for FC, 60.5% for FS, and.28% for ES, respectively.It said that the functional qualities of watermelon seeds had greater values than those of the rind, including 7.13% for WAC, 1.65% for OAC, and 5.65% for FC. 20.75% for FS in turn. Marie *et al*. (2015) did a study to determine the functional characteristics of four different types of watermelon seed powder. According to their report, the four different types of seed flour had functional properties that ranged from 130.23 to 254.42% for water absorption, 107.50 to 140.11% for oil absorption, 3.87 to 5.69% for foam capacity, 39.66 to 51.94% for foam stability, 23.48 to 29.99% for emulsion activity, and 11.53 to 14.67% for emulsion stability.

2.8 Phytochemical compound of watermelon seeds

Watermelon seed's phytochemical composition was examined by Mehra *et al*. (2015). They claimed that many phytochemical substances, including alkaloids, flavonoids, unprocessed phenol, and saponin, were present in watermelon seeds.The research found that the amounts of flavonoi,saponin, and crude alkaloid in watermelon seeds were, respectively, 4.22%, 0.041%, and 15.688%.It demonstrated that crude alkaloid levels in watermelon seed were higher than those of other phytochemicals.In an analytical investigation on the phytochemical components of two types of watermelon seeds in Limpopo, South Africa, In the two types of watermelon seed, the flavonoid concentration ranged from 0.015 to 0.347%, as reported by Mogotlane *et al*. (2018) research.

2.9 Nutritional benefit of watermelon seeds

2.9.1 Low in calorie

A few calories are found in watermelon seeds. Watermelon seeds only provide 23 calories per 4 grams.

2.9.2 Zinc

Zinc is the foundation of the nutrition in watermelon seeds. Watermelon seeds are a good source of zinc, which helps maintain your nervous system, enhance digestion, immunity, and cell growth.

2.9.3 Magnesium and iron

Magnesium is necessary for metabolic process. Magnesium-rich watermelon seeds aid in accelerating your body's metabolism. Iron found in watermelon seeds aids in the synthesis of hemoglobin in your blood.

2.9.4 Good fat

Monounsaturated and polyunsaturated fatty acids are examples of healthy fats. These healthy fats are crucial for decreasing your blood cholesterol levels and preventing heart attacks and strokes. The healthy lipids found in watermelon seeds are vital for excellent health.

2.10 Health benefit of watermelon seeds

2.10.1 Heart health

Due to their abundance in heart-healthy lipids, watermelon seeds are a fantastic source of nutrition. According to studies, swapping out bad saturated and trans fats with healthy unsaturated fats can lower the chance of developing heart disease. Watermelon seeds have a high magnesium concentration that supports heart health and controls blood pressure.Watermelon seeds are an excellent food for heart health due to their anti-inflammatory and antioxidant characteristics. Due to its ability to dilate blood arteries, melon seeds have positive effects on the heart.

2.10.2 Regulation of blood sugur

Among the advantages of watermelon seeds is their ability to control blood sugar levels by enhancing insulin sensitivity. The body benefits from the magnesium included in watermelon seeds by controlling how carbs are metabolized, which affects blood sugar levels.

2.10.3 Skin health

Because it contains a lot of antioxidants and vitamin C, watermelon seed oil is good for the skin. Melon seeds can help you get rid of acne, delay the onset of aging, and acquire smooth, radiant skin. Watermelon seeds' anti-inflammatory qualities and high magnesium content are good for your skin since they moisturize dry, dull skin.

2.10.4 Hair strength

As watermelon seeds include proteins, magnesium, iron, zinc, and copper, you can enjoy good hair. All of these important minerals support healthy hair growth and hair strength, reducing hair loss and damage.

2.10.5 Reduced risk of prostate cancer

Watermelon seeds contain lycopene, a chemical component. Lycopene has potent anti-cancer capabilities, and research indicates that it lowers the risk of prostate cancer.

2.10.6 Promote bone health

One of the main issues we have as we become older is weak bones. Low bone density and weak bones are symptoms of osteoporosis, which raises the risk of fractures. Regular use of dried watermelon seeds can delay the early degradation of your bones. One cup of these seeds provides more than 140% of your daily needs for magnesium, making them an exceptional source of the mineral. Copper, manganese, and potassium are also abundant in them. All of these nutrients help to strengthen your bones and increase their mineral density while also promoting bone health.

2.10.7 Boost energy and immunity

As a result of their high concentration of vitamins, watermelon seeds are a diet that gives you more energy. These seeds' vitamin B concentration strengthens the immune system and keeps the stomach full.

2.11 Antiquity of moringa

All varieties of Moringa are native to Asia, from which they have spread to various regions of the world, with warm countries such as Malaysia and many tropical nations. It is grown all over the plains and can reach heights of 6 to 12 meters. Temperatures between 20 and 30 degrees Celsius are no match for it.Moringa leaves are used in medicine for both medicinal and defensive purposes because it is thought that they have a range of phytonutrients. (Udikala *et al*., 2017).

Moringa is native to parts of the Indian subcontinent that are sub-Himalayan. This is one of the medium-sized perennial trees that grows quickly, is evergreen, and is deciduous roughly 10 to 12 meters tall. The bark has a whitish-grey color, and thick cork surrounds it. Bark on young shoots can be purple or greenish-white. Flowers have a lovely scent and are creamy white and yellowish. The mature fruit is a 20-45 cm long hanging capsule with 15 to 20 dark brown, spherical seeds, ranging in size from 1 to 1.2 cm (Mallenakuppe *et al*., 2015).

2.12 Taxonomical classification of moringa

Kingdom : Plantae
Division : Magnoliophyta
Class : Magnoliopsida
Order : Capparales
Family : Moringaceae
Genus : *Moringa*
Species : *Moringa oleifera*

2.13 Nutritional values of *Moringa oleifera* tree

The moisture content of dried moringa leaves were determined to be 72.39%, and they had a very high energy content (2625.25–79.30 Kcal/Kg).A significant amount of ash (9.89%), lipids (3.87%), protein (17.7)% crude fibers (12.58%), total carbohydrates (37.88%), free nitrogen extract (51.11%), cellulose (11.00%), hemicelluloses (10.24%), and lignin (2.41%) are also present in the composition.The leaves were discovered to be vitamin and mineral-rich. High concentrations of Mg^{2+}, Ca^{2+}, Zn^{2+}, K^+, and PO_3^- were present.When green or dried, the seeds are consumed (Berger *et al*., 1984). Many valuable substances, including protein, calcium, iron, vitamin A, ascorbic acid, and antioxidant substances like carotenoids, flavonoids, vitamin E, and phenol are found in the leaves and seeds of *Moringa olifera*. The presence of vitamins and minerals helps to strengthen the immune system against a wide range of ailments (Gopalakrishnan *et al.,* 2016). Numerous amino acids, including Arg, His, Trp, Phe, Thr, Leu, Met, Ile, Lys, and Val, are present in moringa leaves(Gopalakrishnan *et al*., 2016).

2.14 Medicinal properties of moringa

The Ayurvedic and Unani systems have recognized the tremendous therapeutic potential of moringa (Mughal *et al*., 1999).Almost each part of this plant, including the root, leaves, fruit, flowers, seeds, and seed oil, have been used in traditional medicine to cure various diseases

2.14.1 Antimicrobial and antihelmintic effect

The antibacterial activities of Moringa oleifera components were validated following the discovery of inhibitory action against a range of microorganisms. An inhibitory impact over a number of pathogenic bacteria, notably Staphylococcus aureus, Bacillus subtilis, Escherichia coli, and Pseudomonas aeruginosa, was observed in a recent study using Moringa oleifera water-soluble extracts (Saadabi and Abu Zaid , 2011). Spirochin and anthonine are two bioactive substances that are present in roots and have antibacterial activity. The *Moringa oleifera* blossom and leaves also have the ability to suppress parasitic worms; this has been shown in numerous research (Bhattacharya *et al*., 1982).

2.14.2 Anti asthmatic activity

It has long been known that an alkaloid from the moringa plant, which acts similarly to ephedrine, can be used to treat asthma. Bronchioles are relaxed by a moringine alkaloid. In a study to assess the effectiveness and safety of seed kernels for the management of asthmatic patients, the MO seed kernels also showed promise in the treatment of bronchial asthma. The research concluded that there was a significant improvement in concurrent respiratory function and a significant reduction in the intensity of asthma symptoms (Agrawal and Metha, 2008).

2.14.3 Analgesic activity

Numerous species of Moringa have been identified to have analgesic properties. In a study, fragile pod-like fruits from the Moringa concanensis plant were used, and the ethanolic extracts had a significant analgesic impact on test subjects. Additionally, alcoholic extract of Moringa leaves exhibits significant analgesic efficacy as demonstrated by hot plate and tail immersion methods (Sutar *et al.*, 2008).

2.14.5 Antidiabetic activity

Many medicinal plants have been researched for their capacity to treat diabetes. *Moringa oleifera* is another essential component of this group. In Wistar rats and Goto-Kakizaki (GK) rats, which are models for type 2 diabetes, Moringa leaves considerably lower blood glucose concentration (Nodong *et al.*, 2007). Quercetin-3-glycoside, rutin, kaempferol glycosides, and other polyphenols are among the powerful sources of polyphenols in moringa leaves.Thus, by the development of appropriate technology and obtaining anti-diabetic activity up to conventional medications, the potential antidiabetic activity of moringa can be exploited.

2.14.6 Antioxidant activity

Antioxidants are abundant in moringa. Aqueous preparations of moringa leaves, fruits, and seeds are said to have antioxidant effects (Singh *et al.*, 2009). In a study exposing the antioxidant properties of freeze-dried Moringa leaves from various extraction techniques, it was shown that methanol and ethanol extracts of Indian origin MO have the highest antioxidant activity, with 65.1 and 66.8%, respectively (Lalas and Taskins, 2002). Additionally, it was claimed that the main bioactive phenolic components quercetin and kaempferol are what give phenolics their antioxidant properties.Quercetin and kaempferol shown strong antioxidant effect on hepatocyte growth factor (HGF)-induced Met phosphorylation, with IC50 values of 12 and 6 M/L, respectively, in a different investigation (Labbe *et al.*, 2009).

2.14.7 Hepatoprotective activity

In numerous investigations, moringa has proven to have strong hepatoprotective effect. The ethanolic extracts of moringa leaves significantly reduced the liver damage that rats exposed to the antitubercular medications isoniazid (INH), rifampicin (RMP), and pyrazinamide (PZA) produced.In numerous investigations, MO has proven to have strong hepatoprotective effect. The ethanolic extracts of MO leaves significantly reduced the liver damage that rats exposed to the antitubercular medications isoniazid (INH), rifampicin (RMP), and pyrazinamide (PZA) produced (Pari and Kumar, 2002). Additionally, methanolic and chloroform extracts of MO leaves greatly reduced the liver damage that carbon tetracloride-induced in albino rats. The roots and blossoms of this plant, in addition to the leaves, have strong hepatoprotective qualities.The roots and blossoms of this plant, in addition to the leaves, have strong hepatoprotective qualities.Moringa blooms contain the well-known flavonoid quercetin, which may be the source of the plant's potent hepatoprotective benefits (Ruckmani *et al*., 1998).

2.14.8 Antitumor activity

Moringa has been identified as a strong anticancer plant, and various bioactive chemicals from moringa have been reported to exhibit noteworthy antitumor potential. Niazimicin, a thiocarbamate bioactive component from moringa leaves, was discovered to have strong anticancer potential.Additionally, niazimicin exhibits a reduction in the activation of the Epstein-Barr virus (EBV) caused by the tumor promoter teleocidinB-4 (Murakami *et al*., 1998). Another study looked at 12 trees that are utilized in traditional Bangladeshi medicine as possible sources of compounds that could fight cancer. In this study, tumor cell lines were used to investigate the plant extract's cytotoxicity against tumors using the MTT assay, hemolysis assay, sea urchin eggs assay, brine shrimp lethality assay, and sea urchin eggs assay. The research also showed that human multiple myeloma cell lines might be susceptible to the cytotoxic effects of MO leaf extract (Costa-Lotufo *et al*., 2005). In addition to the anticancer properties of the leaves, moringa seed extracts also contain antioxidant properties and influence the hepatic enzymes that break down carcinogens.

2.14.9 Antifertility activity

The moringa plant also exhibits important antifertility properties. In addition to having an antifertility impact after coitus in rats, the aqueous extract made from the root and bark of moringa also caused foetalresorption in late pregnancy.Additionally, the estrogenic, anti-estrogenic, progestational, and antiprogestational properties of an aqueous extract of MO roots were assessed.This extract has a number of negative effects on its antifertility activity (Sukla *et al*., 1988).

2.14.10 Cardiac and circulatory stimulant

It has been shown that MO can protect male Wister rats with low iron levels from developing hyperlipidemia. It was discovered that MO leaf extract causes significant changes in cardiovascular parameters during a study comparing it to antenolol (a selective 1 receptor antagonist drug used to treat cardiovascular diseases), on serum cholesterol level, serum triglyceride level, blood glucose level, heart weight, and body weight of adrenaline-induced rats. Using experimental animals, this study found that MO leaf extract was hypolipidemic, reducing body weight, heart weight, serum triglyceride level, and serum cholesterol level (Ara *et al*., 2008). When isoproterenol (ISP) produces myocardial infarction in male Wistar albino rats, MO also has cardioprotective benefits.

2.14.11 Antipyretic activity

The antipyretic effectiveness of the bioactive components of Moringa can be attributed to its anti-inflammatory effects. Using the yeast-induced hyperpyrexia method, a study was created to evaluate the antipyretic effects of ethanol, petroleum ether, solvent ether, and ethyl acetate extracts of MO seeds. During the trial, paracetamol was utilized as a control. Unsurprisingly, seeds' ethanol and ethyl acetate extracts significantly reduced rats' body temperatures (Hukkeri *et al*., 2006).

2.15 Conclusion

As consumer health awareness has grown, there has been a considerable a rise in interest in implementing active components such as Protein, phenolic, antioxidants, dietary fiber into ordinary foods such as Biscuit. For people who need to take advantage of these two great foods, this upgraded biscuit offers a wholesome and nutrient-dense option. While moringa leaf is an abundant supplier of minerals, protein, and other necessary nutrients, watermelon seed is widely known for its substantial amount of healthy fat, fiber, and antioxidants.

Chapter 3: Materials and Methods

3.1 Study area

The Applied Food Science and Nutrition Laboratory served as the study's location.The Animal Science laboratory performed the nutritional content analysis and the mineral analysis was done in the Laboratory of Physiology Biochemistry and Pharmacology, Chattogram Veterinary and Animal Sciences University, Chattogram. From 4 july 2023 to 23 September 2023, the experiment was carried out for a two-month period.

3.2 Sample collection

Watermelon seeds and moringa leaves have been purchased from a local market in Chattogram's Chawkbazar (Fultola). Wheat flour, sugar, salt, butter, milk, and baking powder were other components for the biscuit that were bought from the grocery store. The lab was looking for more stuff needed for the experiment.

3.3 Preparation of watermelon seeds powder: (Peter-Ikechukwu *et al*., 2018)

Selection of watermelon seeds

↓

Cleaning of watermelon seeds

↓

Sun drying

↓

Roasting for 10-15 minute at 70º C

↓

Collection of roasted watermelon seeds powder

↓

Grinding

↓

Watermelon seeds powder

Figure 3.1 Watermelon seeds powder

3.4 Preparation moringa leaves powder: (Emelike *et al*., 2005)

Selection of moringa leaves

↓

Cleaning of moringa leaves

↓

Sun drying

↓

Drying in oven 50º C for 12 hours

↓

Dried moringa leaves

↓

Grinding

↓

Dried moringa leaves powder

Figure 3.2 Moringa leaves powder

Table 3.1 Formulation of experimental biscuit

Ingredients	Control	WSMLB-2 10%	WSMLB-2 15%	WSMLB-3 20%
Wheat flour	100gm	90gm	85gm	80gm
Watermelon seeds powder	0gm	5gm	7.5gm	10gm
Moringa leaves powder	0gm	5gm	7.5gm	10gm
Powdered sugar	30gm	30gm	30gm	30gm
Salt	0.36gm	0.36gm	0.36gm	0.36gm
Butter	30gm	30gm	30gm	30gm
Baking powder	1.42gm	1.42gm	1.42gm	1.42gm
Milk	20ml	20ml	20ml	20ml

Note: Control (Biscuit developed from 100% wheat flour)

WSMLB 1 (Biscuit supplemented with 10% composite flour of watermelon seed, moringa leaf and 90% wheat flour)

WSMLB 2 (Biscuit supplemented with 15% composite flour of watermelon seed , moringa leaf and 85% wheat flour)

WSMLB 3 (Biscuit supplemented with 20% composite flour of watermelon Seed, moringa leaf and 80% wheat flour)

3.5 Method to prepare biscuit

Watermelon seed and moringa leaves biscuits were made using the traditional bakery technique. In order to manufacture dough, milk, sugar powder, and butter were first combined with a beater.After that, biscuits were made using all of the powdered ingredients, including wheat flour, WSP & MLP, regular salt and baking powder.

The mixture was mixed to create the dough for 15 minutes. On a platform, the dough was rolled up to a circumference of 1/4 inch using a wooden rolling pin. Round dough was cut into the desired shape for making biscuits, which were then baked for 15 minutes at 170°C. Until they were tested, the biscuits were then placed in an airtight container with a lid.

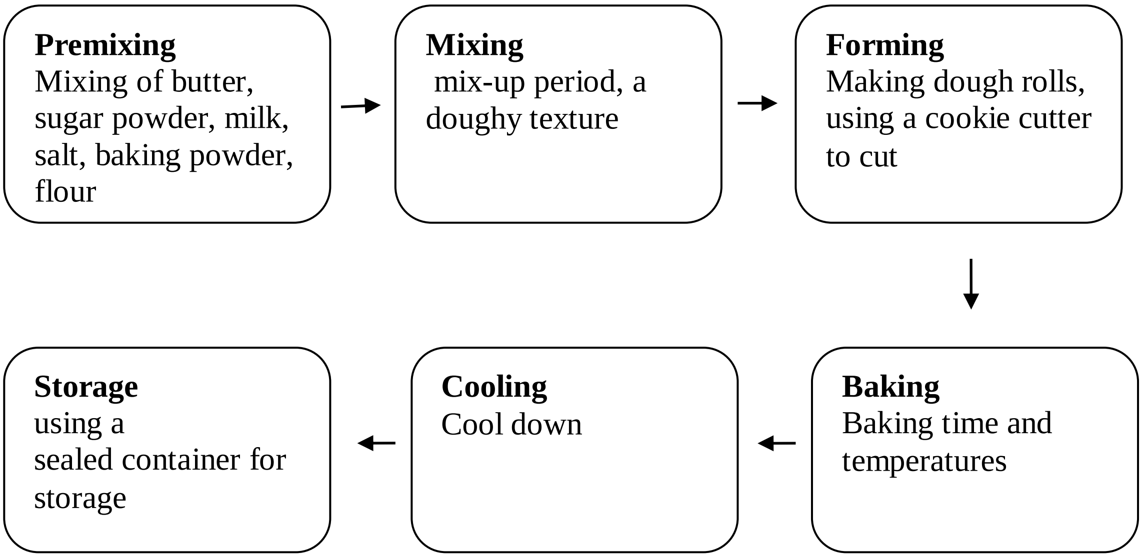

Figure 3.3 Biscuit Processing

Figure 3.4 Biscuit Processing pictorial step

3.6 Nutritional composition of WSML biscuits

3.6.1 Physical characteristics of WSML biscuits

Before and after baking, the diameter of the biscuits produced with watermelon seed and moringa leaves powder were measured at 4.8 cm and 4.10 cm, respectively. Biscuits weighed 7 g at the beginning and 9 g after baking.

With the final result being higher, it is clear from the data above that there has been a shift from the starting value to the final number.

3.6.2 Proximate analysis

Proximate analysis such as moisture, crude protein, crude fiber, ether extract or fat, ash, dry matter and carbohydrates amount were estimated in accordance with the guidelines established by the Association of Official Analytical Chemicals Method.The amount of carbohydrates was calculated by deducting the other nutritional components, such as protein, fat, moisture, ash, and fiber. A total of 5 samples, including biscuits made with pumpkin seed flour, were used for this analysis.

Moisture content

One of the key elements affecting food quality and shelf life is moisture.By heating 105ºC to a constant weight normal atmospheric pressure, the moisture content of biscuits is ascertained.

At first, a crucible of the proper size was accurately weighted. Reweighted after adding 10g of sample to the crucible. The crucible then put in a hot air oven set to 105ºC and warmed for 48 to 72 hours.From the oven, the crucible was removed. To cool down, insert a desiccator. Weighed was the crucible. It was carried out repeatedly until a reliable outcome was discovered.

The moisture percentages were computed as follows

$$\% \text{ Moisture} = \frac{\text{weight of dry sample} - \text{weight of dried matter}}{\text{weight of sample}} \times 100$$

Ether extract

Food samples are immersed in biological solvents (like methanol or chloroform) before the filtrates is separated.The filtration was divided into funnels, the final mixture was dried, and the amount of lipid was measured.The amount of crude fat in the sample was determined using a soxhlet device in accordance with AOAC (2016) recommendations. An exact weighting of a dry extraction flask was done in order to figure out the ether extract. The sample was then placed into the thimble in the amount of 2gm. The extractor's top was covered with fabric once the thimble was placed inside. Install the extractor and begin siphoning ether. Poured half of the previous amount of ether once more. The heater was turned on and let it boil for 6-8 hours at 40-60 degrees Celsius.After extraction was finished, the extraction flask was disassembled and dried in a water bath. The flask was heated at 100 degree celsius up to a constant weight. weighted the flask to measure the ether extract after cooling it in a desiccator.

Determine the ether extract percentages using the formula below

$$\text{\% Ether extract} = \frac{\text{Weight of the flask ether extract} - \text{Weight of the flask}}{\text{Weight of the sample}} \times 100$$

Crude protein analysis

Kjeldahl technique was used to determine the crude protein. To determine the crude protein content, a 0.5 gram sample of biscuit had been absorbed with sulfuric acid in the form of the digestion mixture. The digested material was diluted, the surplus acid was removed with alkali (40 percent NaOH), and then the ammonia that had been released was trapped in a 2% boric acid buffer. For titration, the collected distillate is contrasted with a typical N/10 HCl solution.

Determine the nitrogen percentage using the formula below

$$\text{\% crude protein} = \frac{A \times B \times 0.014}{W} \times 6.25 \times 100$$

Here,

A = Volume of Standard

B = Normality of Standard HCL solution

W = Weight of Sample

Crude fiber analysis

It can be calculated through digestion after a light acid solution (1.25% sulphuric acid for 30 minutes) and a weak alkali solution (1.25% NaOH over 30 minutes under equal volume) were used to heat up two grams of a nonfat sample. To calculate the crude fiber (2016), the AOAC technique was employed. After that, a muffle furnace was used to heat the leftover material to 550–600°C (whitish ash, 4-6 hours).

The crude fiber % is calculated as below

$$\% \text{ crude fiber} = \frac{W - W1}{W2} \times 100$$

Here,

W = Weight of crucible, crude fiber and ash

W1 = Weight of crucible and ash

W2 = Samples weight

Ash content analysis

Ash content refers to the remaining inorganic material after biological material has been degraded. Using this method, all organic material is ignited to get it to oxidize, and the quantity of the ash that is left over is computed. The ash content was determined by following AOAC (2000) methodology. In the beginning, the crucible was washed and dried in a hot air furnace. In a desiccator, the crucible was kept chilled and weighted. A sample of around 5gm was placed in a crucible. sample in crucible until there was no smoke produced.The sample was then put into a muffle furnace after cooling. White ash resulted from igniting the sample at 550–600 degree celsius for 6–8 hours. It was put into the desiccators after being allowed to cool in the furnace at 150 degree celsius. The sample was cold and very slightly warm when it was weighed.

Calculate the ash percentage as follow

$$\% \text{ of ash} = \frac{\text{Weight of crucible and ash} - \text{Weight of crucible}}{\text{Weight of sample}} \times 100$$

Determination of total carbohydrate

The variation among the Nitrogen Free Extractive (NFE) and the carbs content was used to compute its departure from 100 from the sum of the other components.

The following equation utilized to estimate total carbohydrate

% CHO = 100% - % (Protein + Fat + Fiber + Ash + Moisture content)

Energy estimation

The calories and the proportions of protein, fat, and carbs in all meals were determined using the equation below

Energy = (Protein × 4.2) + (Fat × 9.2) + (Charbohydrate × 4.1)

3.6.3 Mineral content analysis

Nitric-perchloric digestion was performed on minerals (P, Mg, Zn, Fe, and Na) in temperature-controlled digestion blocks to ascertain their composition. The chromogen in the reagent interacts with the significant mineral to produce a soluble, colored chemical agent that can be evaluated by passing a certain wavelength of radiation into it. It was possible to determine the concentration of the important mineral from the standard curve of absorbance vs. concentration.

The Traditional approach for Mineral analysis (Robert and Jerrad, 2017) was used to ascertain the minerals present in the biscuits.

3.7 Analysis of bioactive compounds

3.7.1 Extract preparation

Samples were made with various changes to the Zhang and Hamauzu (2004) method in order to identify the bioactive ingredient in the extracts.10g of the samples were added to 40 mL of 60% methanol (CH3OH) and homogenized for 2 min using a homogenizer (Model: HG-15A, Daihan Scientific Co. Ltd., Korea). The mixture then incubated at 20°C for 45 minutes. After that, the mix had been spun (416G, Gyrozen, Korea) at 4000 rpm for 10 minutes. After centrifugation, the mix went through filtering using Whatman No. 4 filter paper. The extract was then used as a working solution for measuring the TPC, TFC, and DPPH radical scavenging activities after being allowed to stand in darkened circumstances at 4°C. Since the stock answer was made just before analyzing a new batch, it worked well.

3.7.2 Total phenolic compounds

Using the Folin-Ciocalteu Phenol reagent, Da Silva et al. (2011) estimated the total phenolic content (TPC). 8.5 ml of distilled water, 0.5 ml of the Folin-Ciocalteu Phenol reagent, and 0.5 ml of the extract were combined. One milliliter of a 35% sodium carbonate solution was put in after the mixture had been allowed to sit at ambient temperature for five minutes. After being vortexed, the resulting mixture was let sit at ambient temperature over 20 minutes. A UV-Vis spectrophotometer (Shimadzu, UV-1800, Japan) that was set to detect absorption at 765 nm was used to take the readings. Deionized water was used to create the blank instead of the sample. To create a calibration curve, standardized Gallic acid solutions of different strengths were read against a blank. Milligrams of gallic acid equivalents per 100 grams (mg GAE/100) were used to measure TPC.

3.7.3 Total flavonoid contents

To determine the amount of flavonoids, mix 0.5 ml of the extract with 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminum chloride (AlCl3), 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water. Next, let the mixt sit at ambient temperature up to 40 minutes. In a UV-Vis spectrophotometer (Shimadzu, UV-1800, Japan), the absorption of the mixture was determined at 415 nm using deionized water as a blank. In terms of mg QE/100 g of quercetin equivalent, the data were presented.

3.8 Antioxidant activity

The extract's antioxidant activity has been assessed using the 2,2-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging method explained by Adiletta et al. (2018). First, 1.4 ml of DPPH radical methanolic solution (0.1 mM in methanol) and 100 l of extracts were mixed.The DPPH sample was dissolved at a concentration of 0.0039 g in 100 ml of methanol, and the solutions were then left in unlighted place up to 31 min. As reaction progressed, color of the solution changed from yellow to purple as the concentration of the complex 2,2,2-diphenyl-2-picrylhydrazyl (DPPH) of solution decreased, as determined in UV-Vis spectrophotometer (Shimadzu, UV-1800, Japan) at 517 nm. Water (Acontrol) was used to provide the blank position of the sample, and the absorbance was calculated. The DPPH radical in a sample was identified.

The source of the sample DPPH radical was discovered in:

% Antioxidant activity = $(Abs_{control} - Abs_{sample}) / Abs_{control} \times 100$

Here,

$Abs_{control}$ = The absorbance of control at the initial time

Abs_{sample} = The absorbance of the sample

3.9 Orgnoleptic Analysis

Twenty panelists with an equal number of men and women conducted the analysis at the CVASU. A group of experts conducted the test. The panelists were instructed to choose the Biscuit formulation with the highest level of acceptability out of four available options. Every WSMLB sample undergone randomized coding. The white porcelain dishes utilised for serving WSMLB to the judges were given alphabetic codes at random. WSMLB's 4 samples and a sensory evaluation worksheet with a 7-point hedonic scale were provided to the judges. The criteria applied were

Table 3.2 : Scale of standard for sensory evaluation

Category	Score
Like extremely	7
Like moderately	6
Like slightly	5
Neither like or dislike	4
Dislike slightly	3
Dislike moderately	2
Dislike extremely	1

Each of four biscuit samples received a score from the participants based on its appearance, flavor, texture, crispiness, and general acceptability.

3.10 Statistical Analysis

To do statistical analysis, Minitab Version 21. was employed. The results obtained were subjected to a one-way analysis of variance (ANOVA). The level of significance for Turky's test is (p 0.05), and To ascertain whether there were statistically significant variations between the samples, it was used in statistical software.

3.11 Cost Analysis

The price of the biscuits made with watermelon seeds and moringa leaves powder was calculated using the total cost of the ingredients.

The price per biscuit pack was computed and the amount was displayed in taka.

Chapter 4: Result

4.1 The Experimental Biscuits Nutrient Content

The nutritional value of the various experimental biscuits is displayed in Table 4.1. Control sample had higher dry matter (98.91%), and carbohydrate (71.80%) than other supplemented sample. On the other hand ash (1.72%), moisture (3.64), fiber (4.06%), protein (11.62%) and fat (21.72%) are high in WSMLB 3.

Table 4.1 Nutrient content of watermelon seed and moringa leaves biscuits

Parameter	Control	WSMLB 1 (10%)	WSMLB 2 (15%)	WSMLB 3 (20%)	1-ANOVA P Value
Dry matter(%)	98.81 ± 0.01^{a}	97.70 ± 0.02^{b}	98.23 ± 0.04^{a}	96.34 ± 0.02^{c}	0.001
Ash(%)	1.08 ± 0.02^{d}	1.25 ± 0.04^{c}	1.34 ± 0.01^{b}	1.72 ± 0.03^{a}	0.001
Moisture(%)	1.19 ± 0.02^{d}	2.30 ± 0.01^{b}	1.76 ± 0.02^{c}	3.64 ± 0.03^{a}	0.001
Crude fiber(%)	1.37 ± 0.03^{c}	3.11 ± 0.03^{b}	4.07 ± 0.01^{a}	4.11 ± 0.02^{a}	0.001
Crude protein(%)	9.44 ± 0.03^{d}	9.81 ± 0.02^{c}	10.54 ± 0.03^{b}	11.62 ± 0.02^{a}	0.001
Ether extract(fat)(%)	16.48 ± 0.03^{d}	17.53 ± 0.01^{c}	19.08 ± 0.02^{b}	21.72 ± 0.01^{a}	0.001
CHO(%)	71.80 ± 0.01^{a}	69.62 ± 0.02^{b}	66.77 ± 0.02^{c}	61.77 ± 0.01^{d}	0.001

Legends: Mean ±SD and value in the same row with same superscripts are not statistically significant ($p<0.05$)

4.1.8 Energy

The WSMLB 3 sample had the highest energy content, with 500.12 kcal, while the control sample had the lowest at 484.74 kcal.

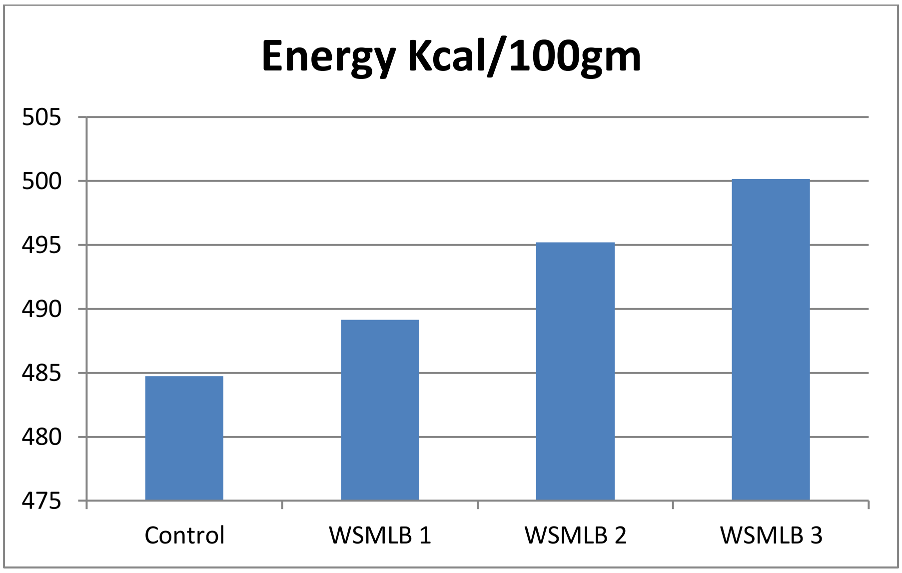

Figure 4.1 Energy content comparison of several biscuit formulas

4.2 Bio-active composition of watermelon seeds and moringa leaves biscuit

The results for the bioactive components TPC, TFC, and TAC are shown in Table 4.2. The values of each sample were found to be significantly different. When compared to the control, WSMLB 1 and WAMLB 2, WSMLB 3 had the greatest levels of total phenolic, flavonoids compound, and antioxidant capacity.

Table 4.2: Bioactive composition of watermelon seeds and moringa leaves biscuit

Parameter	Control	WSMLB1 (10%)	WSMLB2 (15%)	WSMLB3 (20%)	1-ANOVA P Value
TPC (mg GAE/100ml)	19.19 ± 0.02^{d}	26.12 ± 0.04^{c}	33.62 ± 0.03^{b}	49.17 ± 0.02^{a}	0.001
TFC (Mg QE/100g)	66.67 ± 0.01^{d}	80.03 ± 0.04^{c}	116.17 ± 0.02^{b}	122.75 ± 0.02^{a}	0.001
Antioxidant Activity (mg TE/100ml)	25.89 ± 0.03^{d}	35.16 ± 0.02^{c}	44.11 ± 0.03^{b}	48.97 ± 0.02^{a}	0.001

Legends : The difference between the Mean ±SD in the same row and for each treatment with a distinct superscript is significant at the $P<0.05$ level.

4.3 Mineral content of watermelon seeds and moringa leaves biscuit

The macromineral content of watermelon seed moringa leaves formulated biscuits, where the highest amount was observed biscuits supplemented with 20% watermelon seed and moringa leaves biscuit (Table 4.3). Overall, the mineral content of the watermelon seed and moringa leaf-containing biscuit samples was considerably greater than that of the control.

Table 4.3: Micromineral content of watermelon seed and moringa leaves biscuit

Parameter	Control	WSMLB1 (10%)	WSMLB2 (15%)	WSMLB3 (20%)	1- ANOVA P Value
Iron (µg/gm)	20.06 ± 0.01^{d}	31.50 ± 0.02^{c}	34.61 ± 0.03^{b}	35.21 ± 0.02^{a}	0.001
Magnesium (mg/gm)	0.33 ± 0.02^{d}	0.51 ± 0.02^{c}	0.64 ± 0.03^{b}	0.91 ± 0.03^{a}	0.001
Potassium (mmo/gm)	0.1 ± 0.01^{c}	0.2 ± 0.04^{b}	0.31 ± 0.03^{b}	1.41 ± 0.03^{a}	0.035
Zinc (ppm/gm)	0.14 ± 0.02^{c}	0.5 ± 0.04^{b}	0.6 ± 0.03^{a}	0.6 ± 0.03^{a}	0.01

Legends: Note: Mean ±SD and value in the same row with same superscripts are not statistically significant ($p<0.05$)

4.4 Sensory evaluation

The sensory characteristics of control biscuits have a mean of 6.9 for color, 6.1 for texture, 6.9 for flavor, 6.5 for appearance, 6.01 for crispiness, and 6.7 for overall acceptability, as shown in Table 4.4.

The control sample obtained higher approval scores due to its improved look, color, flavor, crispiness, and general acceptability.

Table 4.4: Watermelon seed and moringa leaf biscuits hedonic sensory score.

Parameter	Control	WSMB1 (10%)	WSMB2 (15%)	WSMB3 (15%)	1-ANOVA P Value
Appearance	6.5 ± 0.2^{a}	5.2 ± 0.4^{c}	6.1 ± 0.5^{b}	6.3 ± 0.14^{ab}	0.01
Color	6.9 ± 0.2^{a}	6.7 ± 0.2^{a}	5.5 ± 0.5^{ab}	5.3 ± 0.2^{b}	0.01
Texture	6.1 ± 0.5^{a}	6.3 ± 0.4^{a}	6.3 ± 0.3^{a}	6.5 ± 0.1^{a}	0.8
Crispness	6.01 ± 0.2^{a}	6.4 ± 0.4^{a}	6.7 ± 0.2^{a}	6.5 ± 0.3^{a}	0.6
Flavour	6.9 ± 0.2^{a}	6.9 ± 0.2^{a}	6.1 ± 0.5^{b}	6.2 ± 0.4^{b}	0.4
Overall Acceptability	6.7 ± 0.3^{a}	6.3 ± 0.3^{a}	6.1 ± 0.5^{b}	6.2 ± 0.2^{b}	0.2

Legends: Mean ±SD and value in the same row with same superscripts are not statistically significant (<0.05)

4.5 Cost analysis

To create biscuits with watermelon seed and moringa leaf powder, the components were obtained from the grocery shop, as well as from other stores and online. The cost of all the raw materials that were acquired as well as the cost of processing and packaging were then used to determine the price of the produced product. To estimate the cost of producing 100g of WSMLB 3 (20% watermelon seed and moringa leaves biscuits) and 100g of the control sample, Table 4.5 lists the price of the components per kg or liter. These prices are also taken into account in grams or milliliters.

Table 4.5: Cost analysis

Raw material	TK	Quantity used	Cost in tk (100gmof WSMLB3)	Cost in tk (100 gm of control)
Wheat flour	75/kg	100gm (control) 80gm (WSMB3)	6 tk(WSMB3)	7.5tk(control)
Watermelon seed	400tk/500gm	10gm	8tk	-
Moringa leaves	60tk/kg	10gm	1tk	-
Sugar	120/kg	30gm	3.1tk	3.1tk
Butter	220tk/200gm	30gm	33 tk	33 tk
Baking powder	60tk/50gm	1.6gm	1tk	1tk
milk	50tk/500ml	20ml	2tk	2tk
Total			53.5 tk	46.6tk
Processing cost @ 15% of the cost of raw materials			9.09tk	7.5tk
Packaging cost			1.5tk	1.5tk
Total cost			64.09tk	55.6tk

According to Table 4.5, the overall manufacturing cost for 100gm WSMLB 3 was 64.09Tk, whereas the cost for the control was 55.6 Tk.

Chapter 5: Discussion

5.1 Biscuits Proximate analysis

Table 4.1 provides the proximate composition of the control and enhanced biscuits. The table shows that as the concentration of watermelon seeds and powdered moringa leaves grew, the quantity of moisture, ash, crude fiber, crude fat, and protein increased while the content of carbs declined.

Table 4.1 displays the results of the analysis of the biscuits' moisture content. The control biscuit's moisture content was measured to be at the lowest (1.19%) and the WSMLB3's moisture content to be at the highest (3.64%). This outcome is consistent with the findings Riaz and Wahab (2021), who found that cookies made with Moringa leaves powder had moisture contents 4.08%. Peter-Ikechukwu *et al*. (2018) added that while utilizing 20% watermelon seeds powder as a supplement in biscuit production, the moisture content was around 6.25% .Which means moisture content increase with the higher ratio of watermelon seeds incorporation. Moisture content has an impact on storage life and is also used as an identity standard (Nielsen and Bradley, 2010). Comparing the moisture level of biscuits to that of shelf-stable goods, which is reported to be less than 5%, the observed moisture content of the supplemented biscuit is acceptable. This shows that biscuits can be stored for a longer amount of time. Due to the low water absorptivity, the supplemented biscuit had a low moisture content (Throat *et al*., 2017)

Ash content of the biscuits increased as the amount of powdered moringa leaves and watermelon seeds supplementation increased; the control sample had the lowest value (1.08%), while WSMLB 3 had the greatest value (1.72%). Because there are more moringa leaves and watermelon seed powder, the biscuit's ash level has increased, indicating that there are more minerals in the biscuit. According to Dachana *et al*. (2010), the ash content of cookies increased from 0.9 to 1.5% while the amount of powdered moringa leaves increased from 0 to 10%. A comparable outcome was also attained by Peter-Ikechukwu *et al*. (2018) from biscuits made of 20% watermelon seeds where the ash content was 2.49%. The ash content is increasing with the higher ratio of watermelon seed and moringa leaves powder this is because both seeds and leaves are higher in calcium, iron, potassium, magnesium, sulphur and phosphorus (Akubor, 1998), (Anjorin *et al*.,2010).

In comparison to other biscuit samples, WSMLB 3 contained the most crude fiber (4.11%), whereas WAMLB 1 had the least (1.37%). There was a significant difference ($p<0.05$) between samples. The amount of dietary fiber in the biscuits was within the acceptable range of 5g per 100g of dry matter (FAO/WHO, 1994). This result is in line with research by Ubbor and Akobundu (2009), who discovered that cookies produced with 10% watermelon seed powder had moisture contents of 3.92%. This finding was supported by a study by Alam *et al*. (2014) that produced biscuits using moringa leaves in a variety of ratios and discovered that 10% of the sample contained 3.0% fiber. According to Chugh *et al*. (2013), dietary fiber has a preventive effect against a number of diseases including colon cancer, appendicitis, hemorrhoids, constipation, and diabetes mellitus.

In comparison to the control , samples of biscuits containing moringa leaves and watermelon seeds had a greater crude protein content (11.62%). Within the samples, there were significant differences ($p< 0.05$), however the protein concentration rose as the level of substitution rose.The study by Ubbor and Akobundu (2009), who made biscuits with watermelon seed in various ratios found (13.34%) crude protein, which was corroborated with this conclusion. According to Riaz A. and Wahab (2021), the crude protein content for a 10% sample of biscuits made with moringa leaf powder as a supplement was around (9.11%). As watermelon seeds and moringa leaves contain an acceptable amino acid profile for nourishment, the biscuit contain a high protein level (Jack, 1972; Gopalakrishnan *et al*., 2016). Protein functions as hormones, enzymes, and maintains the body's acid-base and electrolyte equilibrium (Adeola and Ohizua, 2018).

Table 4.1 provides information on the crude fat or ether extract concentration of the control and enriched biscuits. The control biscuits had the lowest crude fat level at 16.48%. The largest percentage of total fat was discovered to be in WSMLB 3's, which made up 20% of the mixture. In biscuits fortified with moringa flour, a similar range of crude fat of 23.76% -25.44% was also discovered (Riaz and Wahab, 2021). Kausar *et al*. (2019), who developed cookies supplemented with watermelon seeds, also noted comparable results. The presence of sterols, tocopherols, and monounsaturated fatty acids with a high MUFA/SFA ratio in moringa leaves is what causes the rise in fat content (Leone *at al.*, 2016) and high lipid (52.34%) content in watermelon seeds (Ramazan *et al.*, 2012).

WSMLB 3 contained the least amount of carbohydrates (61.77%), whereas the control had the greatest percentage (71.70%). Within the flour samples, there were significant differences ($p<0.05$). The carbohydrate content of composite flours biscuit dropped as replacement increased. The results are in line with the research by Peter-Ikechukwu *et al*. (2018) and Riaz and Wahab (2021).

5.2 Bio-active compound and antioxidant activity analysis

Table 4.2 shows the outcome of adding watermelon seeds and moringa leaves' bioactive and antioxidant characteristics to cookies. Additionally, the supplemented samples showed significant differences ($p<0.05$) in TPC, TFC, and antioxidant activity. This observation was similar with the research study by Fapetu *et al*., (2017), which included the addition of moringa leaves to wheat flour to make samples of supplement biscuits. Additionally, cookies coated with powdered watermelon seeds showed higher TPC, TFC, and antioxidant activity, according to ERTAŞ and ASLAN (2020). The levels of phenolic and flavonoid compounds in flours made from so-called high-value seeds (buckwheat, quinoa, sorghum, teff, and cucurbit seeds) are much greater when compared to whole-grain cereal flours (ranging from 1000 to as much as 4500 mg/kg) (Hager *et al*., 2012). Additionally, the research effort demonstrated that the supplemented biscuits had significant TPC, TFC, and antioxidant activity due to the addition of more powdered watermelon seeds and moringa leaves .Plants produce a variety of phytochemicals, including phenolic acids and flavonoids, which serve as a major defense against the damaging effects of reactive oxygen species and UV radiation. Veggies, fruits, nuts, and cereal seeds, especially flour products, are the principal sources of these chemicals in the human diet (Oghabei and Prakash, 2019). Phenolics, including flavone derivatives, have been successfully examined for other biological features such as antibacterial and anticancer activities. Phenolics are mostly known for their antioxidant and anti-inflammatory actions. Research has demonstrated that consuming foods high in these chemicals can help avoid several chronic illnesses, such as type 2 diabetes and cardiovascular disorders (Dias *et al.*, 2021).

5.3 Mineral analysis

In table 4.3, the micromineral content of the supplemented WSMLB and the control are shown.

Table 4.3's data on the biscuits' iron content revealed a substantial rise ($p < 0.05$) in the enriched biscuits. Riaz *et al*. (2022) also noted an increase in the iron content of cookies that had been treated with powdered moringa leaves. The addition of 10% of the leaf powder caused the authors to note an increase in the amount of leaf powder per 100g of cookies from 1.62 to 2.78 mg. In biscuits made with watermelon seed and peel powder, Ertas and Aslan (2020) also observed an increase in iron content.

The magnesium content of the biscuit was significantly ($p < 0.05$) raised by the addition of powdered watermelon seeds and moringa leaves to the biscuit mixer. Riaz *et al*. (2022) who created biscuits using moringa leaf powder and wheat flour saw similar results of an increase in magnesium content (51.28-74.67 mg/100g of biscuit). Ertas and Aslan (2020) also noted an increase in Mg content with the addition of more seeds and peels to biscuits made with watermelon seed and peel powder. Magnesium plays a role in the synthesis of proteins as well as the development of teeth and bones. Additionally, it supports muscular function, the transmission of nerve impulses, and the release of energy from muscle glycogen (Sharif *et al.*, 2009).

According to statistical analysis, the addition of powdered watermelon seeds and moringa leaves to the biscuit increased the potassium level (Table 4.3). By regulating the balance of acids, potassium and sodium help to keep the fluid balance. Additionally, it functions as a component of the body's enzyme systems (Sharif *et al.*, 2009). Our findings on the potassium level of the biscuits are similar with those of Tessera *et al*. (2015). The authors made biscuits and reported that the potassium content of wheat and moringa leaf biscuits, respectively, was 112.05-144.44mg/100g. Additionally, when more watermelon seeds and peels were added to biscuits, Ertas and Aslan (2020) also observed an increase in K content.

The addition of powdered watermelon seeds and moringa leaves to the biscuit mixer considerably increased the zinc content of the biscuit. Our findings on the biscuits' zinc content is in contrast from those of Tessera et al. (2015) and Riaz *et al*. (2022). The Zinc level of wheat and moringa leaf biscuits, which the authors manufactured, was reported to be 1.06-m1.85g/100g. As we include both watermelon seeds and moringa leaves in the biscuit dough, this variation may be the cause of the disparity. Additionally, Ertas and Aslan (2020) noticed a rise in zinc content when additional watermelon seeds and peels were put to biscuits.

5.4 Sensory analysis

The visual perception of a food product is its color. The taste and flavor of a product are significantly influenced by how color is perceived. In Table 4.4, the color rating for biscuits is provided. The control biscuits received the highest color score from the panelists, which gradually fell, while the biscuits supplemented with 10% powdered moringa leaves and watermelon seeds received the lowest color score. The color of the biscuit with the powdered moringa leaves had a greenish appearance. Although the sensory score was lower when the amount of moringa leaves and watermelon seeds powder was gradually increased,but the cookies were not rejected because of the deeper colors of the biscuits. Golden brown to brownish green is the color change in the biscuit (Figure 3.5). Our findings for both the control and moringa-enriched biscuits' color scores are consistent with those of Riaz and Wahab (2021), who reported a color score of biscuits decreased with moringa leaf powder added.

WSMLB 3's texture was good with values ranging from 6.1 to 6.5. Composite biscuit samples with increased substitution had a strong, good texture. Given that fats are required to lubricate, weaken, or shorten the structure of food components and to generate a food product with positive textural properties, this discovery may be the result of the presence of fat from the composite biscuit recipe. (Mamat and Hill, 2014).

The biscuits supplemented with 10% Moringa leaves and watermelon seeds powder earned the lowest score (6.2) for overall acceptance, while the control biscuits received the highest score (6.7). The current study's results are in agreement with those of Riaz and Wahab (2021), who employed a 9-point hedonic scale and reported overall acceptability scores of 7.28 to 6.21 for control and 10% moringa leaf powder cookies and Peter-Ikechukwu *et al*. (2018) also found 7.75 to 6.05 acceptebility scores on 9-point hedonic score for control and 10% watermelon seeds powder biscuit. The study's findings indicated a modest level of customer acceptance for the biscuits' 20% level of enrichment. This suggests that in the future, functional foods supplemented with watermelon seeds and moringa leaves may be well-liked and consumed.

Chapter 6: Conclusion

It can be inferred from the results of the powdered watermelon seeds and moringa leaves can be used as functional ingredients in food items. The study also showed that biscuits manufactured from composite flour blends have better proximate composition , bioactive compounds, antioxidant activity and mineral content than those prepared only from wheat flour, allowing for up to 20% substitution of roasted watermelon seed and moringa leaves biscuit. Additionally, the biscuit's sensory evaluations are also inside the permitted range. As the biscuit (20%) contain higher levels of protein, crude fiber, ash, bioactive compound and mineral (Fe,Zn,K,Mg) than biscuits made entirely of wheat flourt could have the potential to be a useful food, especially for people with heart disease, diabetes, obesity, and celiac disease. The biscuit also has lower levels carbohydrates than biscuits made entirely of wheat flour. Biscuits prepared from such composite flour could help in combating protein energy malnutrition.

Chapter 7: Recommendation and future prospective

Both watermelon seeds and moringa leaves have high nutrient value. All supplemented sample showed better result compared to control, however 20% of watermelon seeds and moringa leaves biscuit are recommended for better nutrition. This parameter could have a great impact on human health. The following suggestions and options for more research are provided based on the results of the current investigation.

1. Other seeds that may be purchased in stores, such as pumkin seed, nigella, linseed, kidney bean etc , ought to also be the subject of similar research.
2. To raise the value, aromatic flavors might be added.
3. Further research should be done to identify the biscuits vitamin, mineral and amino acid profiles because the biscuits' protein and ash content is high.
4. The results are valuable from a therapeutic point of view, since they are important from a medical standpoint.

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Appendix A: Questionnaire for sensory test of biscuit

Name of the Taster: ……………… Date: ……….

Please taste these samples and check how much you like or dislike each one on four sensory attributes such as color, flavor, texture and overall acceptability. Use the appropriate scale to show your attitude by checking at the point that best describe your sense and feeling about the sample please give a reason for this attribute. Remember you are the only one who can tell what you like. An honest expression of your personal feeling will help us. For Taste/Flavor/Mouth feel/Appearance/Overall Acceptability.

The scale is arranged such that; Like extremely =9, Like very much =8, Like moderately =7, Like slightly=6, Neither like nor dislike =5, Dislike slightly =4, Dislike moderately =3, Dislike very much =2, and Dislike extremely =1.

Appendix B: Procedure of making WSMLB supplemented biscuit

Watermelon seed powder

Moringa leaves powder

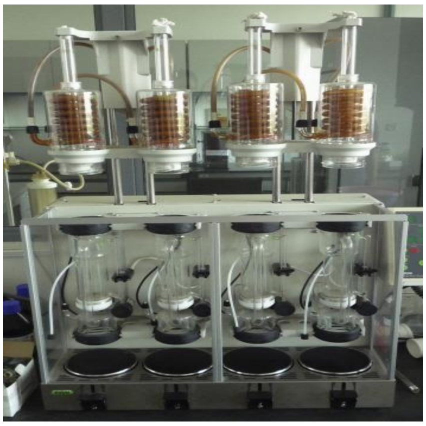
Weighted ingredient

Dough

Baking

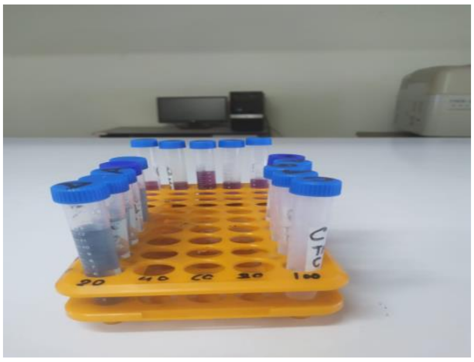
Final product

Appendix C: Pictures of analysis



Protein determination **Fat determination**

Crude fiber determination **Bioactive sample preparation**



Sample preparation **UV spectrophotometer**

Brief biography

Arpa Mohajan passed the Secondary School Certificate Examination in 2012 from Lakshmipur Govt. Girls' High School, and then Higher Secondary Certificate Examination in 2014 from Lakshmipur Govt. College. She obtained her B.Sc. (Honors) in Food Science and Technology from the Faculty of Food Science and Technology at 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, Chattogram Veterinary and Animal Sciences University (CVASU). She has an immense interest to work in improving the health status of people through proper guidance and suggestions and to create awareness amongpeople about food safety and nutrition.