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Shapla Das

August 2022

Nutritional Evaluation of Developed Supplementary Food (Biscuit) Incorporating Pumpkin Seed Flour for Enrichment

Shapla Das

Roll no: 0219/07

Registration No: 768

Session: July-December, 2021

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

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August, 2022

DEDICATION

Each Challenging work needs self-efforts as well as guidance of elders specially those who are very close to heart and understanding. So, I dedicated my MS research work to my beloved

Family Members

& respected

Teachers.

Acknowledgement

I would like to express my gratitude to **The Almighty** from my deepest sense of heart, whose blessing has enabled me to complete the thesis for the degree of **Master of Science (MS)** in **Applied Human Nutrition and Dietetics** under the **Dept. of Applied Food Science and Nutrition,** Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh.

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Last, but not the least, I am ever indebted to my beloved parents, brother-sisters, friends and well wishers for their moral support, sacrifice, blessing and encouragement to complete my research work successfully.

The Author
August 2022

PLAGIARISM VERIFICATION

Title of Thesis: Nutritional Evaluation of Developed Supplementary Food (Biscuit) Incorporating Pumpkin Seed Flour for Enrichment.

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This is to report that as per the check 18% of the content of the above thesis is stated to be plagiarized and is preferable as per plagiarism policy and instructions issued from CASR, Chattogram Veterinary and Animal Sciences University. The report has been sent to the coordinator, CASR, Chattogram Veterinary and Animal Sciences University.

The thesis may be considered for the evaluation.

……

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List of Abbreviation

%	: Percentage
ANOVA	: Analysis of variance
AOAC	: Association of Official Analytical Chemists
°C	: Degree Celsius
DPPH	: 2, 2-diphenyl-1-picrylhydrazyl
et al	: Et alii/ et aliae/ et alia
etc	: Et cetera
PSB	: Pumpkin Seed Biscuit
gm	: Gram
mg	: Milligram
ppm	: Parts per million
SD	: Standard deviation
TE	: Trolox equivalent

Abstract

There are many initiatives aimed at raising the consumption of locally produced foods in an effort to combat malnutrition; however, consumers need better access to information on the nutritional quality of these foods in order to increase consumer intake. This study aimed to find ways to use pumpkin seeds, which are usually thrown away, in the development of value added food products namely biscuits, standardize to enrich nutritive value of developed product. The seeds were ground into flour to make these pumpkin-seed-flour-based biscuits. These biscuits were produced with 90:10, 80:20, and 70:30 ratios of refined wheat flour to pumpkin seed flour. Physical properties, proximate analysis, antioxidant activity, and sensory attributes have been evaluated for the biscuits. On a 7-point hedonic scale of sensory evaluation, the panelists accepted the pumpkin seed flour based biscuit. The study also reveals that biscuit with 30% pumpkinseed flour supplementation had better nutritional profile. Consequently, the developed biscuit is highly nutrient-dense and can be used to prevent malnutrition as well as can be used as a supplementary food product for all age's people especially for school going children.

Key words: Biscuits, Nutrients, PSB, Supplement, Flour.

Chapter 1: Introduction

A fit and active lifestyle with nutritious food is necessary for a person to be healthy and well-nourished. Today's society pays significant values for malnutrition. Malnutrition can take place in several forms, with under nutrition being the most common type in developing nations (Liz, 2002). Micronutrient malnutrition is mostly caused by protein, vitamin, and mineral deficits in Bangladesh. The abundance of animal - based food protein is insufficient in developing nations to fulfill the rapidly increasing population's daily recommended protein intake. Therefore, it is necessary to intensify current research efforts to evaluate the nutritional characteristics of food and the potential for utilizing protein and other essential nutrients from locally grown food crops that are not being used effectively (Enujiugha and Ayodele, 2003). Using low-cost, high-protein, and fiber rich foods selectively is necessary along with underutilized foods that are rich in micronutrients and will enhance the protein and minerals in the diet or methods that will increase the availability of nutrients in common foods to increase their nutritional quality (Norfezah *et al.*, 2011). Currently, the center of attention and considerable effort has been in the development of food stuffs using leftovers or underutilized agricultural by products with the aim of increasing the nutrient content of cereal flours in terms of protein, minerals, vitamins, and dietary fiber specifically. Oilseeds and oilseed meals are also the significant sources of protein in the diets of the majority of people in developing countries that have not yet been fully utilized for enhancing human diets, aside from cereals and pulses .Researchers in various countries have been focusing on the issue of providing affordable, well-balanced protein supplements using oilseeds and oilseed meals to combat malnutrition in underdeveloped nations (Sarwar, 2013). The use of high protein oilseed flour to enhance the nutrients content of the products made with wheat flour has been considered significantly (Hoover, 1979).

Pumpkin is a year-round crop that grows well in hot, humid climates. It is a member of the family Cucurbitaceae and genus Cucurbita. In the world, there are three common varieties of pumpkin: Cucurbita pepo, Cucurbita maxima, and Cucurbita moschata (Hussain *et al.*, 2021; Mukherjee *et al.*, 2021). The size, color, and shape of pumpkins can differ considerably. It comes in either orange or yellow in terms of color. In addition, some varieties come in shades of brown, white, red, gray, dark to

light green and brown (Hernández *et al*., 2021). Pumpkin seeds and pulp are found in the thick shell. Fruit pulp has a golden-yellow to orange color due to its composition. Pepitas, or pumpkin seeds one of this oilseeds, are tiny, flat, palatable, and green seeds. These seeds are rich in protein, polyunsaturated fatty acid**s,** vitamins, antioxidants and micronutrients (Murkovic, 1996). Additionally, high quality and plentiful phytochemical sterols found in pumpkin seed extract have positive effects on the immune system, reproductive health, and other areas of health (Martins and Ferreira, 2017; Bučko *et al*., 2015). Although the pumpkin's seeds are its most vital component, they are typically wasted.

Pumpkin seed flour's appealing greenish color and nutty flavor make it acceptable to use it to make new food products that are nutrient adequate (Patel, 2013). So, it is a valuable addition to food products to combat malnutrition among children in Bangladesh.

Demand from consumers for high-quality food products with flavor, safety, convenience, and nutrition has increased. As a result, nutrition has become a new tract in the process of developing food products. Also due to rising consumer demand for quick-to-eat, wholesome foods, the biscuit industry is rapidly expanding. Biscuits are a familiar food item consumed by a diverse population because of its wide variety of flavors, long shelf life, and comparatively low price. However limited research has been carried out to develop nutrient enrich product using pumpkin seed in order to prevent nutritional deficiencies and health issues among the population of Bangladesh. Therefore, the current study was conducted to produce food products named biscuits containing pumpkin seeds for nutritional enhancement and to analyze the chemical composition and sensory attributes of supplemented biscuits.

1.1 Objectives

1. To formulate and standardize the pumpkinseed flour incorporated biscuits.
2. To analyze the nutritional composition, antioxidant and phytochemical properties of pumpkin
3. To evaluate the consumer acceptance of the pumpkin seed flour incorporated biscuits.

Chapter 2: Review of Literature

2.1 Antiquity and Spread of pumpkin

Cucurbita is indigenous to South America (Yadav *et al*., 2010) and has been widely grown for more than ten thousand years in North America and more than five hundrad years in Europe (Paris, 1996) . Prior to its spread to Asia and Europe, the pumpkin was introduced to Chile and Argentina (Yadav *et al*., 2010). They are cultivated in nearly every region of the world, regardless of height. Pumpkin is a fruit-vegetable native to the Western hemisphere, with different varieties growing in North America, Europe, Australia and in some Asian countries (Chomicki *et al*., 2020). In addition to Malaysia and Indonesia, pumpkin has been cultivated in tropical Asia (Tindall, 1983).

2.2 Taxonomy of pumpkin (George, 2014)

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Cucurbitales

Family: Cucurbitaceae

Genus: *Cucurbita spp.*L.

Species: *C.maxima*
C.mixta
C.moschata
C.pepo

2.3 Cultivation of pumpkin all around the world

Pumpkins (*Cucurbita spp*.) are a substantial economic crop in the Cucurbita family. They are a common source of nutrition and an important source of cultivars for Cucurbitaceous (FAOSTAT 2017).During the hot season, pumpkin plants thrive in nutrient-rich, well-drained soil. The fruits of the pumpkin are heat sensitive and highly perishable under warmer climates (Chomicki *et al*., 2020). The pumpkin and squash are cultivated on around 3 thousand acres globally, yielding 27,832 metric tons. China produces approximately 58% of the world's pumpkins, followed by India with 20%, Ukraine with 4%, and Russia with 4%. In tropical areas such as Mali, Thailand, Malawi, Malaysia, Djibouti, Barbados, Antigua and Barbuda, and Dominica, there is a chance to increase the production and consumption of pumpkin due to its low production (FAOSTAT, 2017).

2.4 Pumpkin production scenario in Bangladesh

In Bangladesh, pumpkin is a commonly cultivated vegetable crop. It is cultivated both as a commercial crop and in home gardens. Although it is usually planted by laying beds, pumpkin planting on trellises and rooftops is prevalent in Bangladesh. Pumpkins can be easily preserved for approximately 4 to 5 months. Huge quantities of pumpkins are grown annually in Bangladesh owing to the fact that it is a very productive, nutrient-rich and storage-friendly crop.

According to Faostat, pumpkin output reached 635 kt in Bangladesh in 2019. This is a 5.80% increase over the previous year. All the high for pumpkin cultivation in Bangladesh was 635 kt in 2019 and the all-time low was 80.2 kt in 1962. Bangladesh is placed sixth among the 105 countries in terms of the rate of interest on cultivation of pumpkins (FAOSTAT, 2022).

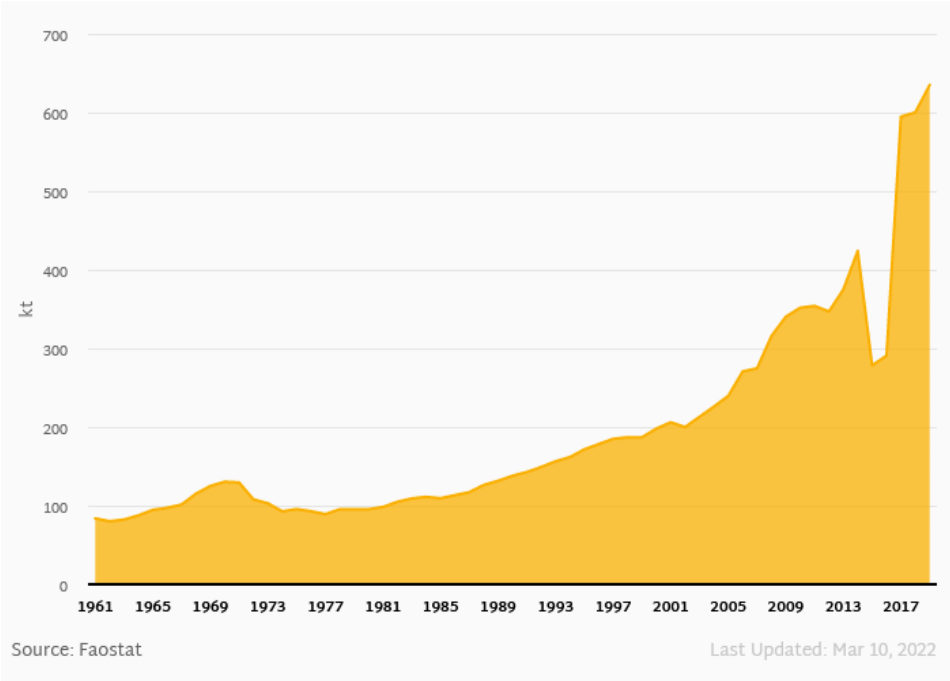

Figure 2.1: Pumpkin cultivation in Bangladesh(FAOSTAT, 2022)

Calabaza **Speckled Hound**

Butternut Squash **Baromashi**

Figure 2.2: Different types of pumpkin found in South Asian Countries

2.5 The Nutritional Significance of Pumpkin Parts

Pumpkin is regarded as a valuable vegetable with therapeutic and culinary benefits. Pumpkin pulp, peel, and seeds are the richest sources of phyto chemicals such as polyphenols and flavonoid content, as well as possessing antioxidant activity.

2.5.1 Plant

Cucurbita maxima are an evergreen diclinomonoecious herb. The plant's radial system reaches a thickness of 1.8 m, but the majority of it is within the first 60 cm. The stems are rough and frequently angular, with a propensity to grow roots in the nodes. There are creeping stems with 10 to 30 m long internodes and semi-erect stems with short internodes. The leaves of Cucurbita maxima are wide, sub orbicular, slightly curved, single-layered, and sub orbicular. In Sub-Saharan Africa, Asia, Korea, the Pacific Islands, India, and Bangladesh, pumpkin leaves (Cucurbita spp.) are frequently consumed and considered healthy and useful vegetables (Salama, 2006).

2.5.2 Flowers

The flowers are yellow, mostly solitary, and sometimes arranged in fascicles for the males. The male flowers have lengthy peduncles, three stamens, free filaments, conniving linear anthers, and one monothecae. The female flowers have a short peduncle, an inferior, oblong or unipolar ovary, 3-6 multi ovulated placentas, a shirt-shaped stigma, and 3-5 lobes. During entomophily pollination, the stigma stays open and active for 12 hours (Salama, 2006).

2.5.3 Fruit

The fruit is an indehiscent berry (unilocular with many seeds) of varying size and color. It contains an interior chamber containing fibers and seeds. Pumpkin pulp is beneficial to human consumption because it contains vitamins, antioxidants including carotenoids, lutein, and zeaxanthins, and mineral (Hussain *et al*., 2021) (Hashash *et al*., 2017)

2.5.4 Seeds

Seeds are large, flat, and oval shaped with a pointed end. Their weights range from from 50 to 250 mg, and they come from both little and large fruits (Salama, 2006). The large size makes for a great reserve, which helps the seeds germinate and re grow.

In mature seeds, the endosperm is no longer able to perform its biological function. The developing embryo fills up all of the space in the seed coat, while the cotyledons store reserves.
Germination is most successful between 25 and 30°C, and it is inhaled at temperatures below 15°C (Salama , 2006).

2.6 Significance of Pumpkin Seeds

Due to the nutritional composition and health-protecting properties of its seeds, pumpkin has achieved considerable popularity in recent years. Pumpkin seeds are utilized for therapeutic purposes across the world because of their abundance of nutrients and medicinal benefits (Kundu *et al*., 2014). Pumpkin seeds are frequently consumed as a snack after being roasted and salted. Because of their significance to human nutrition, proteins are frequently used as dietary additives. Animal protein is the healthiest and most complete protein source. As animal protein is prohibitively expensive, most people in the world depend on vegetable protein as their primary source of protein in diets.

Most studies in recent years have focused on the role of plant proteins in the development of new food products and their incorporation into existing diets. There is as much tryptophan in pumpkin seed protein as is present in a glass of milk (Mohammed, 2004). Albumins and prolamins are found in smaller quantities in storage than salt-soluble globulins and glutelins. To this day, pumpkin is rarely utilized in food industry, although it remains a popular ingredient in baked goods, soups, and pies all around the world. Pumpkin seeds, after being hulled or partially hulled, can be roasted and enjoyed as a snack. To make pumpkin seeds suitable for human consumption, they should always be removed from the pumpkin's flesh, coated

in a generally salty sauce, spread out on a baking sheet, and baked at a low temperature for a long time.

The pumpkin seeds have recently gained popularity for their health-promoting and nutritional qualities. The seed is high in protein and has pharmacological effects like those of an Antidiabetic, an anti-fungal, and an anti-bacterial and anti-inflammatory (Hussain *et al*., 2021). It has been discovered that the seeds have antioxidant properties (Pongjanta *et al*., 2006). Flavor may be enhanced by proteins in one of four ways: bound molecules, absorption, breakdown products, or chemical processes. The Cucurbita plant seeds have many positive attributes, including their low cost and easy availability. Social and economic progress is delayed severely by malnutrition, especially among young children. A growing number of people are starting to pay greater attention to the value of traditionally wasted agricultural products. Without a doubt, this type of use would lead to the development of a wide variety of innovative food items and the utilization of available resources.

2.7 Medicinal and Pharmacological Values of pumpkin Seed

For ages, humans have consumed plants and herbs as medicine. Several research studies have focused on the popular appeal of pumpkin and pumpkin seeds as food and therapeutic interventions in herbal medicine for a range of ailments.

2.7.1 Cancer-Fighting Effects

According to National Institutes of Health, extracts from pumpkin seeds have been used for generations to treat kidney, bladder, and prostate issues in alternative medicine. Substances like cucurbit, which are responsible for the effects, slow the proliferation of cancer cells.

Approximately 40-50% of prostate, breast, and colon cancer cell growth was inhibited in experimental research (Murkhovic *et al*., 1996). This may be related to the presence of phytoestrogens (estrogen-like compounds) in pumpkin seeds. Compounds such as lignin’s and flavones modify the expression of genes primarily involved in breast and prostate cancer prevention and treatment.

2.7.2 Skin Nourishment and Repair

Both pumpkin seeds and pumpkin seed oil have beneficial effects on the skin. According to International Journal of Science and Research, carotenoids, the chemicals responsible for the orange color, have anti-aging properties. They mop up the free radicals that lead to skin aging. Collagen formation is boosted by the vitamin A and vitamin C found in the seeds. Collagen aids in the recovery from wounds and helps maintain skin smooth and supple.

Both omega-3 and beta-carotene can be found in the oil. Strong anti-inflammatory effects can be attained by consuming these substances. Acne, blisters, and chronic inflammation of the skin can all be alleviated by applying pumpkin seed oil topically (Al-Noor, 2017). When rubbed, applied as a scrub, or consumed, it protects against bacterial and fungal illnesses.

2.7.3 Could stop heart and blood vessel diseases

Several studies on animals show that eating pumpkin seeds is good for the health of the heart. Total cholesterol levels went down significantly in people who ate a lot of fat. Studies also show that levels of inflammatory markers like nitric oxide have gone down.

Adding pumpkin seeds to your diet can stop cholesterol from building up and keep blood vessels from getting hard (Murkhovic *et al.,* 1996). This keeps heart problems like coronary artery disease, stroke, and so on away from happening.

2.7.4 Enhanced Prostate Health

There is significant evidence that pumpkin seeds can help prevent prostate cancer. Minerals like zinc may be stored in the prostate. Problems like testosterone-related prostate growth (hyperplasia) can be avoided by consuming these minerals (Gossell *et al*., 2006). These seeds and oil have been demonstrated to reduce prostate enlargement because of the high zinc content (Yadav *et al*., 2010). Oil-free pumpkin

seeds may support in benign prostatic hyperplasia management. In male individuals, urine retention could be reduced by treating hyperplasia. Urinary tract infections would be less likely to occur as a result of this (Leibbrand *et al*., 2019).

2.7.5 Help to Treat Infections in the Urinary Tract (UTIs)

Oil from pumpkin seeds has been used to help men with urinary problems. It accomplishes so by suppressing concerns like prostate hypertrophy. Clinical tests have shown that this oil doesn't cause any problems. National Institutes of Health declared that large doses of 500–1000 mg/day did not cause any negative side effects (Nishimura *et al.*, 2014).

2.7.6 Reduce hair loss

Seeds are abundant in omega-3 fatty acids. In conjunction with other micronutrients, these fatty acids improve the texture of dry, brittle hair. Another component that stimulates the creation of hair proteins is zinc. Zinc is in plenty in pumpkin seeds.

In balding men, twenty-four weeks of treatment with pumpkin seed oil enhanced hair growth by approximately 40%. This is possible because the seeds contain active molecules known as phytosterols. They inhibit the enzymes (proteins) responsible for hair protein degradation and hair loss (Kanedi *et al*., 2017).

2.7.7 Act as Antidiabetic

Cucurbita ficifolia is a plant has the natural source of the insulin mediator D-chiro-inositol (D-CI), which serves as an anti-hyperglycemic to lower blood glucose levels in Type-2 diabetic patients (Nawirska, *et al*., 2016).

2.7.8 Promote mental growth of children

Healthy omega-6 and omega-9 fatty acids, phytosterols, and vitamins E and K are present in raw pumpkin seed (Hashash *et al*., 2017). Linoleic acid, which is present in

pumpkin seed, enhances brain health and skin elasticity. Pumpkin seed's oleic acid reduces "bad" cholesterol, promoting heart and liver health (Yadav, *et al*., 2010).

2.8 Supplementary foods

A balanced diet is the most effective method for preventing nutrient deficits and maintaining excellent health.

Changes due to aging and the presence of other disorders can make this process complicated.

Therefore, supplementing the diet with functional foods that include an increased amount of a specific vitamin or bioactive chemical can promote good health and reduce the likelihood of disease.

2.8.1 Supplementary foods from plant origin

A wide variety of plant-based diets and plant-based physiologically active chemicals have been studied for their potential to promote health and prevent disease. Nevertheless, only a few of these have significant clinical evidence to support their beneficial health effects. Even fewer have met the even higher threshold of "significant scientific agreement" needed by the FDA for approval of a health claim, as will be elaborated upon below (Grubben and Denton, 2014).

2.9 Utilizations of pumpkin as Ready-to-Eat Meals

Ready-to-eat (RTE) meals are gaining popularity among consumers due to their simplicity of storage and preparation, as well as customer desirability factors such as cost, attractive appearance, and flavor. Ready-to-eat items are defined as “any food for consumption without any more cooking or preparing.” (Brennan *et al.*, 2013)

This description applies to both open and pre-packaged ready-to-eat food, regardless of whether the ready-to-eat meal is hot or cold. The term "further warming or cooking" does not include consumer food preparation activities such as basic cleaning, slicing, chopping, portioning, marinating, and even storage (Assous *et al*.,

2014). Therefore, processing pumpkin fruits into easily transportable and stockpilable foods, such as juices, jelly, purees, jams, pickles, and even dried foods, could overcome these challenges and boost the marketability of pumpkin fruits .The cultivation of pumpkins for their edible seeds appears to be a developing industry .The marketplaces for ready-to-eat (RTE) foods, such as dried and roasted pumpkin seeds, have garnered a great deal of interest recently (Brennan *et al.*, 2013)

Pumpkin dishes have a naturally sweet and appealing flavor, and the addition of pumpkins increases the beta-carotene content of foods. As the snack market expands, producers have been encouraged to create new products that include a variety of ingredients to enhance the aesthetic and nutritional content of their goods (Norfezah *et al.*, 2011). Customers are interested in the color, flavor, texture, and nutritional content of fresh-cut vegetable and fruit products (Barrett *et al.*, 2010).

Chapter 3: Materials and Methods

This chapter describes the materials and methods used in the process of data collection as well as appropriate formulae for data analysis and presentation.

3.1 Study Area

This study was conducted in Chattogram and lasted for six months from the beginning of March to the end of August 2022.

Pumpkin seed flour and biscuit formulations were developed in the Applied Food Science and Nutrition Laboratory, Department of Applied Food Science and Nutrition, Faculty of Food Science and Technology. And the nutritional content analysis was carried out in the laboratory of Animal Science and Nutrition, Department of Animal Science and Nutrition and in the Laboratory of Physiology Biochemistry and Pharmacology, Department of Physiology and Biochemistry, Faculty of Veterinary Medicine, Chattogram Veterinary and Animal Sciences University, Chattogram.

3.2 Experimental design

The sample was collected from Rangamati Hill Tract area. After collected the samples, it was used to make powder from or pumpkin seed's flour. Then, pumpkin seed biscuits were made out of powder. Then the four different biscuit such as Control (without pumpkin seed flour supplementation), 10% Pumpkin Seed Biscuits (PSB 1), 20% Pumpkin Seed Biscuits (PSB 2), 30% Pumpkin Seed Biscuits (PSB 3) were prepared according to the formulation of Table 3.6.

Pumpkin seed flour and all type biscuits sample were taken for analysis of proximate composition (dry matter, moisture, ash, crude fat, crude protein, crude fiber, and carbohydrate), micro and macro nutritional composition, antioxidant activity and consumer acceptability of each product category.

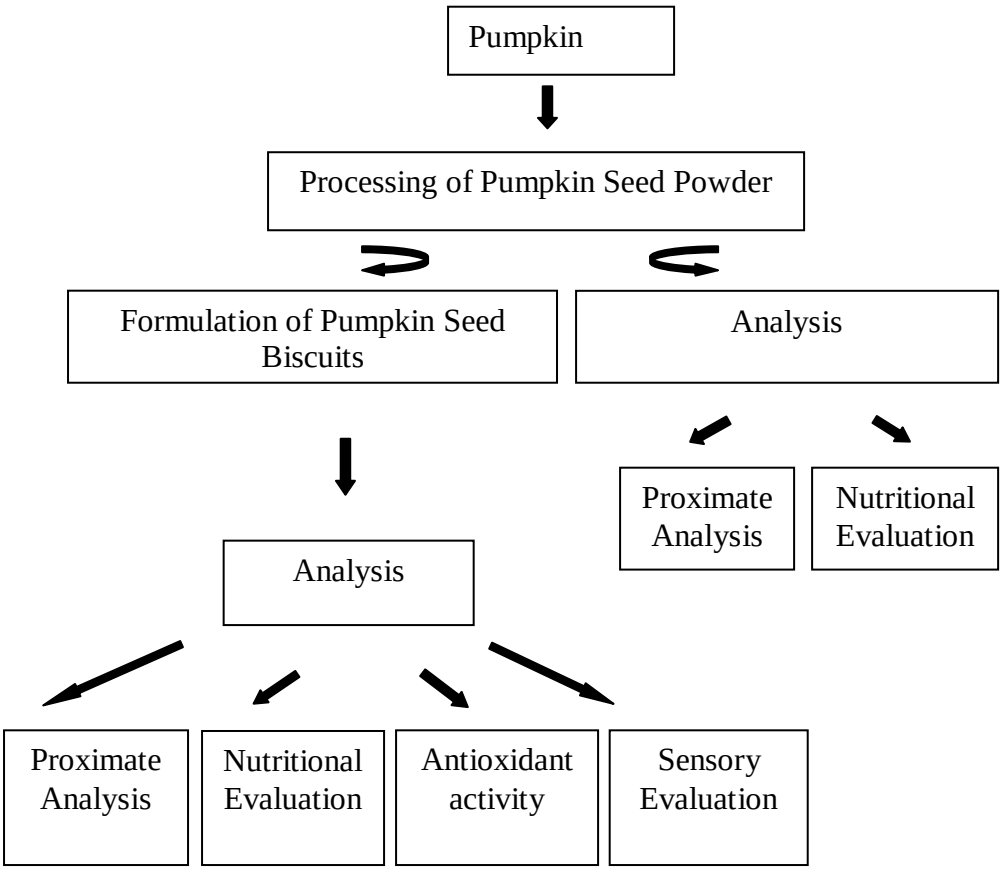

Figure 3.1 Design of the experiment

3.3 Collection

Cucurbita maxima, also referred to as "*Mistikumra*" in Bangladesh, was collected from an experimental plot situated at district of Rangamati, Bangladesh. After collection whole pumpkins were thoroughly washed with clean water and dried. Using a stainless-steel knife, the pumpkins were then cut into halves, then, the fibrous content and seeds were scooped out. Pumpkin seeds were meticulously selected for their freshness.

Other biscuit formulation ingredients such as wheat flour, sugar, baking powder, milk and butter were purchased from the local store. Other necessary chemicals for the experiment were obtained from the laboratory ‘stockpiles.

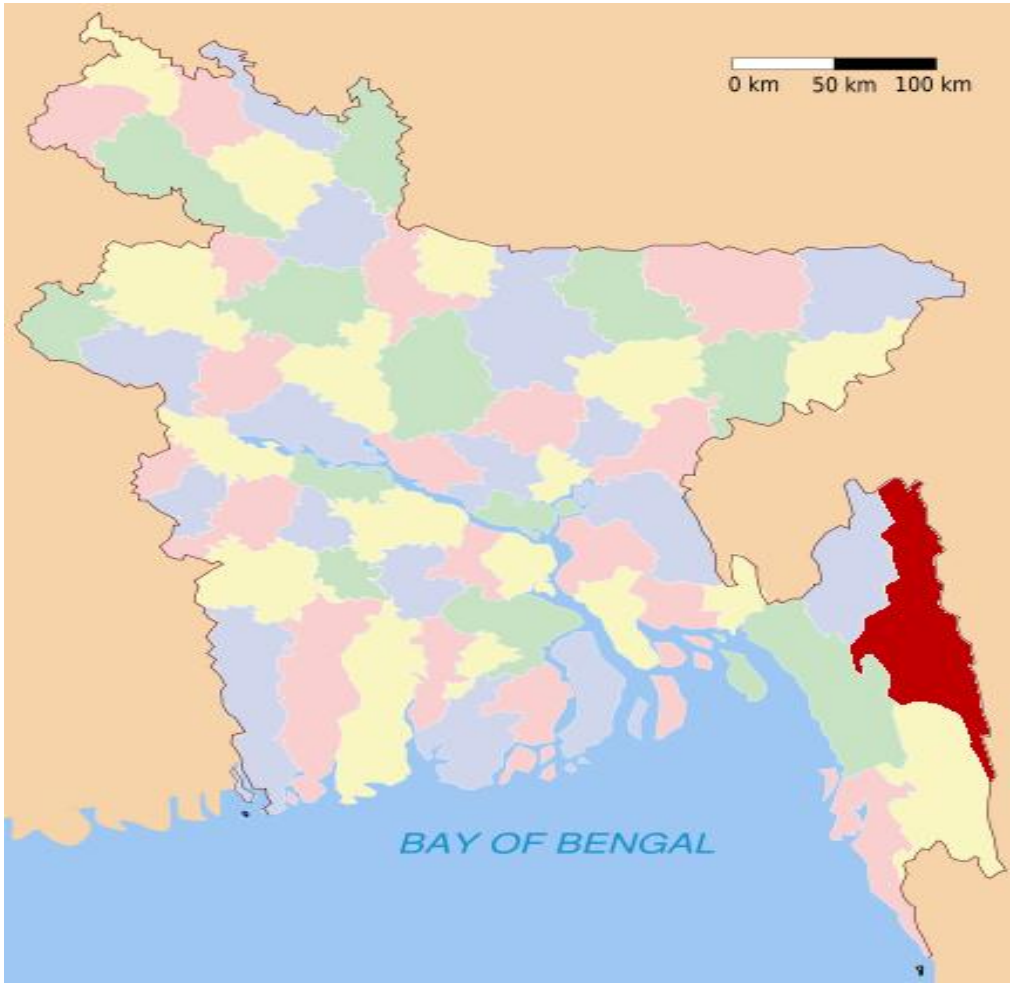

Figure 3.2: Sample Collection Area

3.4 Preparation of Pumpkin Seed flour:

Selection of pumpkin seeds

↓

Cleaning of pumpkin seeds

↓

Sun drying

↓

Shelling of pumpkin seeds

↓

Roasting for 15-20 mins at 75 ° C

↓

Powdered

↓

Pumpkin Seed Flour

Figure3.3: Processing of pumpkin seed flour

Figure3.4: pumpkin seed flour

Table 3.1 Formulation of experimental biscuit

Ingredients	***Treatment**			
	Control	**Pumpkin Seed Biscuits (10%) (PSB1)**	**Pumpkin Seed Biscuits (20%) (PSB 2)**	**Pumpkin Seed Biscuits (30%) (PSB 3)**
Refined wheat flour	100gm	90gm	80gm	70gm
Pumpkin Seed flour	0gm	10gm	20gm	30gm
Powdered sugar	40gm	40gm	40gm	40gm
Salt	1pinch	1pinch	1pinch	1pinch
Milk	20ml	20ml	20ml	20ml
Butter	50gm	50gm	50gm	50gm
Baking Powder	¼ tea spoon	¼ tea spoon	¼ tea spoon	¼ tea spoon

3.5 Method used to prepare biscuits

The conventional bakery method was used to make Pumpkin seed biscuits. First, a beater was used to mix milk, sugar powder, and butter to make cream. Then, all of the powdered ingredients such as wheat flour, PSP, common salt, baking powder were mixed together to make biscuits.

For 5 minutes, the mixture was stirred together to make the dough. With a wooden rolling pin, the dough was flattened on a platform to ¼ inch thickness. Biscuits in the shape of interest were cut out of round dough and baked at 170°C for 15 minutes. The biscuits were then put in an air tight jar with a lid until they were tested.

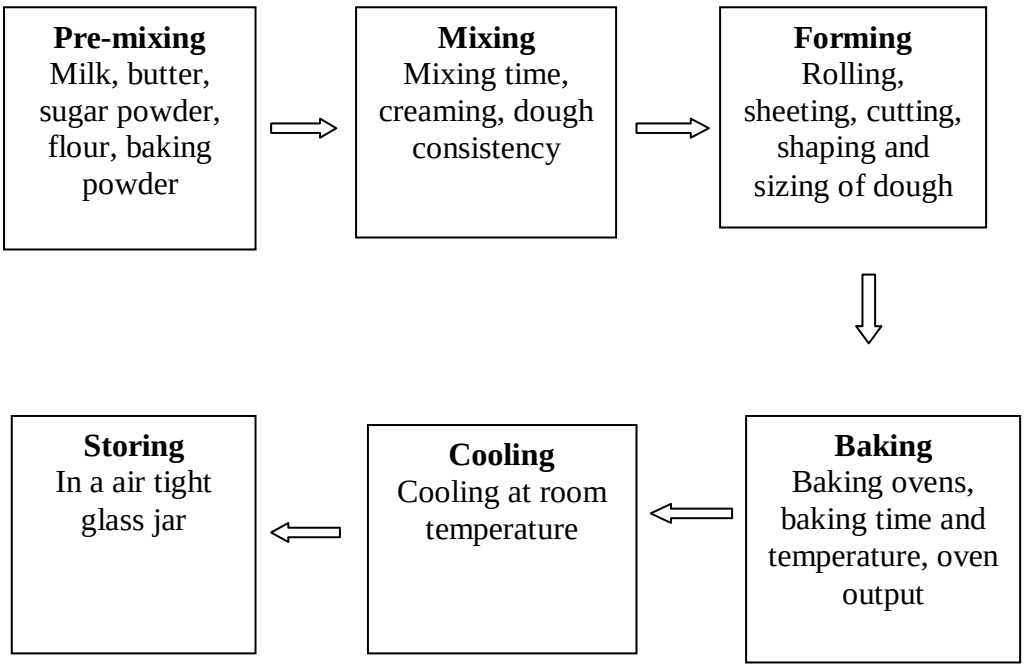

Figure 3.5: Processing steps for pumpkin seed biscuits

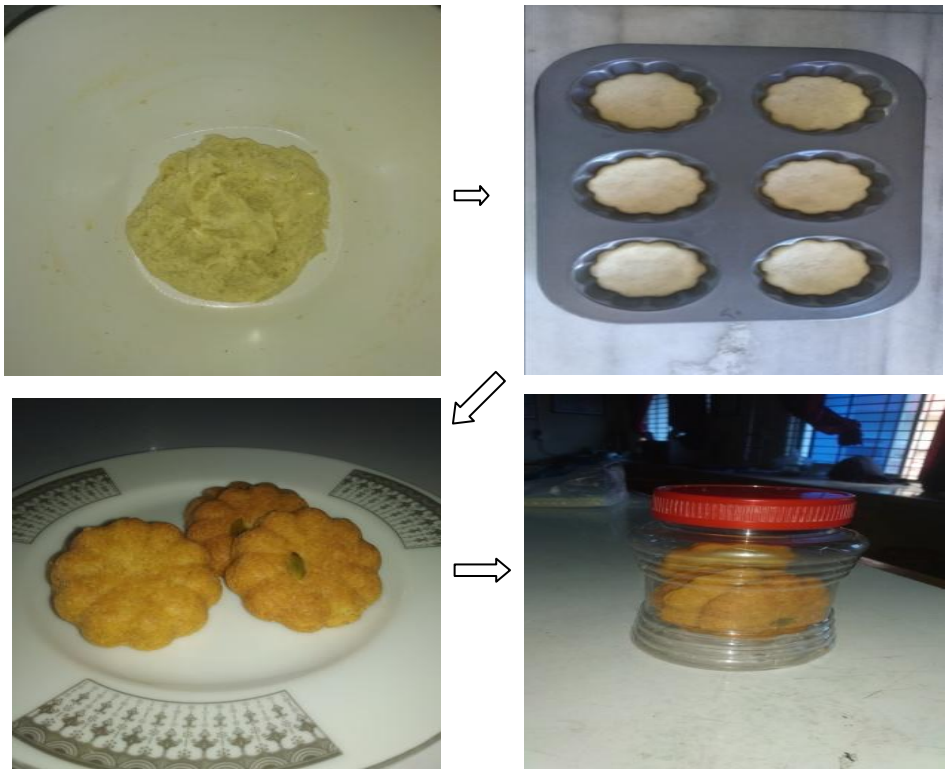

Figure 3.6: Pictorial steps for processing PSB sample

3.6 Nutritional evaluation of developed biscuits

3.6.1 Physical characteristics of developed biscuits

The diameter of the biscuits made with pumpkin seed flour was measured at 4.5cm before baking and at 4.7cm after baking. The cookies' starting weight was 12gm and their ending weight was 10 gm. From the data presented above, it is clear that there is a change from starting to end value, with the end result being higher.

3.6.2 Proximate Analysis

Proximate analysis such as moisture, crude protein, crude fiber, ether extract or fat, ash, and dry matter and carbohydrate content was determined according to the Association of Official Analytical Chemicals Method. The carbohydrate content was determined by subtracting the other food value i.e. protein, fat, moisture, ash and fiber. With pumpkin seed flour and formulated biscuits, total of 5 samples were used for this analysis.

Moisture content analysis:

The moisture content of biscuits is determined by heating 105℃ to a constant weight normal atmospheric pressure. Moisture is one of the most important factor influencing food quality and storability.

Procedure:

Accurately weighed a crucible of appropriate size. Added 10g sample to the crucible and reweighted. Placed the crucible in a hot air oven at 105℃ and dried for 48-72 hours. The crucible was taken out of the oven. Put in a desiccator to cool down. The crucible was weighed. It was done on a regular basis until a constant result was found.

Calculation: The percentages of moisture were calculated as follow:

$$\% \text{ Moisture} = \frac{\text{Weight of Fresh Sample} - \text{Weight of Dry Sample}}{\text{Weight of Fresh Sample}} \times 100$$

Figure 3.7: Moisture content Analysis

Ether Extract analysis:

Organic solvents (such methanol or chloroform) are used to dissolve food samples before the filtrate is separate the filtration was spilt into funnels, drying the final mixture produced the extracts, and the fat content was calculated. Utilizing a soxhlet apparatus, the samples amount of crude fat was examined in line with AOAC (2016) guidelines. To determined ether extract, first a dry extraction flask carefully was weighted. Then 2gm of sample was transferred into the thimble. Placed the thimble into extractor and closed the top with cotton. Fit the extractor and pour ether up to siphoning. Again half of the ether poured in previous amount. Switched on the heater and continued boiling at 40℃ to 60℃ for 6-8 hours. After completing extraction dismantled the extraction flask and dried on water bath. Placed the flask in a hot air oven and heat at 100℃ upto constant weight. Cooled the flask in a desiccators and weighted to measure the ether extract.

Calculation:

Calculate the nitrogen percentages as follow:

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

Crude Protein analysis:

The crude protein was determined by kjeldahl method. To determined crude protein approximately 0.5 gm biscuit sample was digested with H_2SO_4 in presence of digestion mixture. The digested sample was distilled after neutralizing excess acid with alkali (40% NaOH) and the released ammonia was trapped in 2% boric acid solution. The collected distillate is titrated against standard N/10 HCl solution. The percentage nitrogen is determined and crude protein is calculated by using following formula:

Calculation:

Calculate the nitrogen percentages as follow:

% Crude Protein = $\frac{A \times B \times 0.014}{W} \times 6.25 \times 100$

Where,

A= Volume of Standard

B= Normality of standard HCl solution

W= Weight of the sample

Crude Fiber analysis:

Two gm of fat-free sample were boiled in a weak acid solution (1.25% H2SO4) for 30 minutes, followed by a weak alkali solution (1.25% NaOH) for 30 minutes at constant volume, and then subtracting ash from the residue obtained, it can be computed through digestion. The AOAC method was used to determine the crude fiber (2016). The residual material was then heated to 550–600°C (white ash, 4-6 hours) in a muffle furnace.

Calculation:

Calculation of the crude fiber percentage as follows:

Crude fiber % = $\frac{w - w1}{w2} \times 100$

Here, W= Weight of crucible, crude fiber and ash

W1=Weight of crucible and ash

W2= Weight of sample

Ash content analysis:

Ash content is the inorganic residue remaining after destruction of organic matter. This method performs oxidation of all organic matter by incineration and determination the weight of weight of remaining ash. Ash content was determined by methods of AOAC (2000).

Procedure:

At first the crucible were washed and dried in hot air oven. The crucible was cool in a desiccators and weighed. About 5gm of sample was taken in a crucible. Burned the sample with crucible up to no smoke. Then the sample was cooled and transferred into muffle furnace. The sample was ignited at 550-600℃ for 6-8 hours until white ash. The furnace was allowed to cool at 150℃ and transferred it into the desiccators. Then weighed the sample while it is mild warm.

Calculation:

Calculate the Ash percentages as follow:

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

Figure 3.8: Ash content analysis

Determination of total carbohydrate:

By estimating the difference between the Nitrogen Free Extractive and the carbohydrate content (NFE), it was specified as the deviation from 100 from the sum of the other proximate components.

Calculation:

So, it was estimated using the following equation:

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

Estimation of Energy:

Using the following equation, the amount of protein, fat, and carbs in each food, as well as the number of calories it has, were figured out.

Energy = (Protein x 4.1) + (Fat x 9.2) + (Carbohydrate x 4.1)

3.6.3 Mineral content analysis

To determine minerals (Ca, P, Mg, Cl, Cu, Zn, Fe, K, and Na) underwent a nitric-perchloric digestion in temperature-controlled digestion blocks.

The chromogen in the reagent reacts with the mineral of significance to make a soluble, colored chemical agent that can be analyzed by letting a certain wavelength of light pass through it. From the standard curve of absorbance vs. concentration, the concentration of the mineral of significance was found.

To determine the minerals content of the biscuits, Traditional method for mineral analysis (Robert and Jerrad, 2017) was followed.

3.6.4 Determination of Antioxidant capacity by DPPH scavenging method

Extract preparation

1 g of sample was collected in a felcon tube. After adding 10 ml of 100 percent methanol, the mixture was left for 72 hours. Repetitive straining was done after a period of 4 hours, then after 72 hours, a methanoic extract was found in the supernatant. With a few minimal changes to the method reported by Azlim *et al*. (2010), the dpph scavenging activity was used to assess the antioxidant susceptibility of the extracts.

Procedure

In order to make a methanoic DPPH solution, 6 mg of DPPH was dissolved in 100 mL of pure methanol. Then, 1 ml of methanoic extract was mixed with 2 ml of DPPH solution.

The mixture was then gently stirred and left to rest for 30 minutes at room temperature in the dark. Using a UV-VIS spectrophotometer, the absorbance at wavelength 517nm was recorded (UV-2600, Shimadzu Corporation, and USA). As a blank, methanol was utilized, and the control was obtained by mixing 1 mL of methanol with 2 mL of DPPH solution. Discussed in the following functioned as the standard, while methanol acted as the blank (Akther, 2020). The fall in intensity of the samples relative to the DPPH standard solution functioned as a benchmark for the movement of scavenging. Antioxidant capacity computed based on the DPPH free radical scavenging motion of extracts using the following equation.

$$\text{Inhibition \%} = \frac{Blank\ Absorbance - Sample\ Absorbance}{Blank\ Absorbance} \times 100$$

Using TEAC composition (Trolox equivalent antioxidant mobility), which was also applied as the standard, the calibration standard curve was constructed. The values were provided as mg/100 g of Trolox equivalents (TE) per gram of powder, based on dry weight (DW).

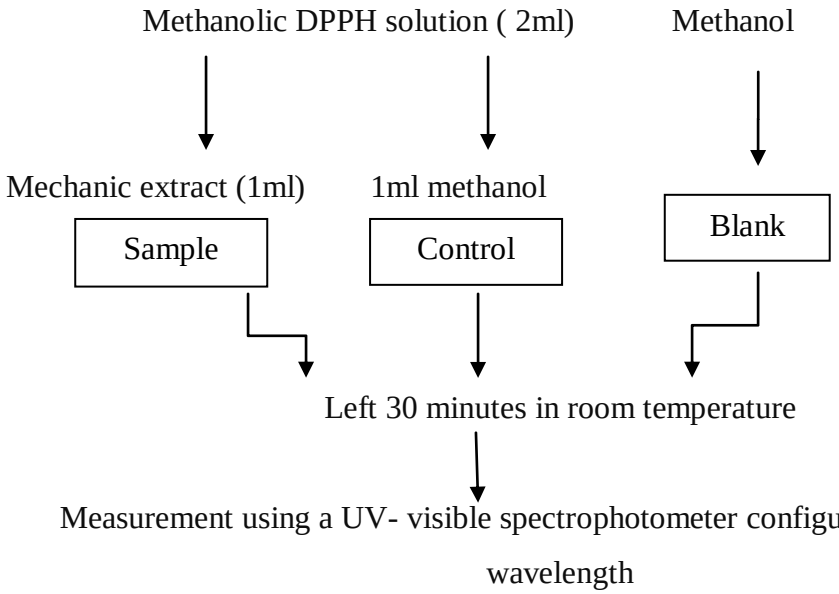

Figure 3.9: Determination of antioxidant capacity

3.6.5 Sensory Analysis

The analysis was conducted in the CVASU by twenty panelists with equal male and female participants. The test was conducted by expert panel. There were four formulations of Biscuit, and panelists were asked to select the one with the highest degree of acceptability.

Each sample of PSB was randomly coded. Alphabetic codes were assigned randomly to white ceramic plates that were used to serve the PSB to the panelists. The panelists were given four PSB samples and a sensory rating questionnaire with a 7-point hedonic scale. The rankings used were

Table 3.2: Scale of Ranks

Score	**Ranks**
7	Like extremely
6	Like moderately
5	Like slightly
4	Neither like nor dislike
3	Dislike slightly
2	Dislike moderately and
1	Dislike extremely

The participants were instructed to score each of the four samples of biscuit based on color, smell, flavor, texture, crispiness and overall acceptability. In order to prevent cross-reactions, the panelists were given water to drink in between judging the biscuit samples.

3.6.6 Statistical Analysis

The software Minitab Version 14.0 was used to do statistical analyses. On the collected data, a one-way analysis of variance was done (ANOVA). Tukey's test was used statistical software to see if there were statistically significant differences between the samples of biscuits; the level of significance is ($p < 0.05$).

3.6.7 Cost Analysis

The total cost of the ingredients was used to calculate the pumpkin seed powder biscuit cost.

The amount was shown in taka, and the price per biscuit pack was calculated.

Chapter 4: Result

4.1 Proximate compositions of pumpkin seed

The estimated data for proximate analysis on the pumpkin seed are represented in **Table 4.1**. The experimental pumpkin seeds were selected for the proximate analysis in which moisture, protein, fat, fiber, and ash value had been calculated and the mean value of the analysis are 36.40% crude protein, 48.15% ether extract, 4.40% ash, 13.30 % crude fiber, 3.00% moisture and 97.00% dry matter respectively.

The result indicates that pumpkin seed contain a large amount of crude protein, crude fiber and fat.

Table 4.1: Proximate composition of pumpkin seed

Nutrients	Mean Value (%)
Dry Matter	97.00%
Moisture	3.00%
Crude Fiber	13.30%
Ash	4.40%
Ether Extract/Fat	48.15%
Crude Protein	36.40%

4.2 Minerals content of pumpkin seed

Figure 4.1 is represent that contained the mineral content of pumpkin seed**.** The result showed that pumpkin seed contains 2.2mg/100gm calcium, 42.12mg/100gm magnesium, 134.4mg/100gm sodium, 204.02mg/100gm potassium, 27.83mg/100gm phosphorus, 18.71mg/100gm zinc, 4.01mg/100gm iron and 9.4mg/100gm copper. So,

it is also can revel that pumpkin seed is a rich source of potassium, phosphorus, zinc and iron.

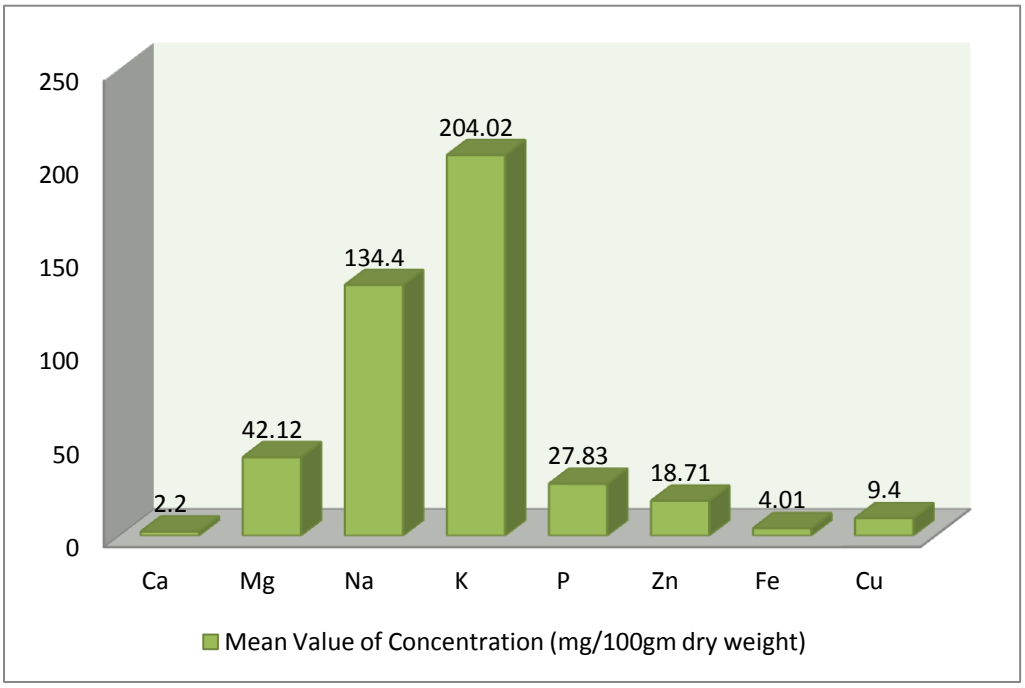

Figure 4.1 Mineral Compositions of Pumpkin Seed

4.3 Nutrient Content of the Experimental Biscuits

The Nutrient Content of the different Experimental Biscuits shown in **Table 4.2.**

4.3.1 Moisture

It was determined that the moisture content of the control sample was 0.64%, which was lower than the moisture content of the PSF1, PSF2 and the moisture content of the PSF3 respectively. So, it is cleared that the moisture content of the biscuits is in increased amount with the higher ratio of pumpkin seed flour incorporation in biscuits.

4.3.2 Crude Protein

From **Table 4.2** the control sample's crude protein content (9.94%) was substantially lower than that of the PSB 1 (10.15%), PSB 2(10.95%) and PSB 3 (12.05%). So, it

can be said that, the biscuits containing higher amount of pumpkin seed obtained in this experiment are healthier than the control sample since this biscuits contained higher amount of crude protein

4.3.3 Ether extract

Ether extract or fat content was higher in the PSB 3(34.54%) than in the PSB 2(31.17%), PSB 1(26.95%) followed by the control sample (26.34%).

4.3.4 Fiber

Fiber content of PSB 3 (3.55%) and PSB 2's fiber content (2.82%) were considerably higher than the PSB 1 (1.95%) and control (1.24%). So, the control had the lowest and the 30% pumpkin seed flour biscuits contained the highest fiber.

4.3.5 Ash

From table 4.2 the ash concentration of the control sample was likewise lower than that of the PSB 1 and PSB 2 and PSB 3, respectively.

4.3.6 Dry Matter

The control contained a higher amount of dry matter compared to the PSB 3, which contained 97.56%, followed by the PSB 2, which contained 97.75% and PSB 1, which contained 97.44%.

4.3.7 Energy

The control sample had the lowest energy content, 514.86 kcal, whereas the PSB 3 had the highest energy content which was 578.49 kcal.

From the results, it was concluded that the supplementation of pumpkin seed

flour in biscuits was found to significantly ($p<0.05$) increased in nutrient content like crude protein, fat, fiber, carbohydrate and ash as compared to the control biscuits.

Table 4.2 Proximate composition of Experimental Biscuits

Parameters	Control	PSB 1	PSB 2	PSB 3	* P Value
Dry Matter	99.34±0.03[a]	97.44±0.05[d]	97.75±0.09[b]	97.56±0.02[c]	<0.001
Moisture	0.64±0.02[d]	2.55±0.02[a]	2.25±0.07[c]	2.50±0.02[b]	<0.001
Crude Fiber	1.24±0.03[d]	1.95±0.02 [c]	2.82±0.22[b]	3.55±0.02 [a]	<0.001
Ash	1.24±0.03[d]	1.36±0.02 [c]	1.65±0.05[b]	1.85±0.02 [a]	0.01
Ether Extract	26.34±0.03[d]	26.95±0.02[c]	31.17±0.77[b]	33.54±0.02 [a]	<0.001
Crude Protein	9.94±0.03[d]	10.15±0.02 [c]	10.95±0.34[b]	12.05±0.02 [a]	<0.001

Mean±SD in the same row and in each treatment with different superscripts differ significantly at $P<0.05$ level

*Control - (0% supplementation), PSB 1- (10% supplementation), PSB 2- (20% supplementation), PSB 3- (30% supplementation)

4.4 Mineral content of Experimental biscuits

The mineral content of the control and PSB 1, PSB 2 and PSB 3 content biscuits samples is shown in Table 4.3. The macro mineral such as calcium, magnesium, phosphorus, sodium, chloride, potassium content of pumpkin seed containing biscuits were significantly ($p<0.05$) higher compared to control, where highest amount was

observed in PSB3 (Table 4.3). In case of other trace mineral such as, Iron and Zinc the value was also significantly ($p<0.05$) higher in PSB3 sample and lower in control sample.

On the other hand, copper content of supplemented biscuits had no significant difference ($p>0.05$) compared to control.

Table 4.3 Mineral content of experimental biscuits

Mineral	Control	PSB 1	PSB 2	PSB 3	*P Value
Ca mg/dl	0.04 ± 0.30^{d}	0.14 ± 0.30^{c}	0.35 ± 0.31^{b}	1.34 ± 0.14^{a}	0.006
Mg mg/dl	0.14 ± 0.20^{d}	0.25 ± 0.12^{c}	0.55 ± 0.12^{b}	1.76 ± 0.11^{a}	<0.001
P mg/dl	1.85 ± 0.20^{c}	2.20 ± 0.53^{b}	2.85 ± 0.25^{a}	2.85 ± 0.31^{a}	0.035
Na Mmol/L	5.14 ± 0.25^{d}	7.24 ± 0.30^{c}	9.84 ± 0.23^{b}	10.983 ± 0.30^{a}	<0.001
K mmol/L	0.24 ± 0.23^{d}	0.35 ± 0.27^{c}	0.56 ± 0.31^{b}	0.94 ± 0.27^{a}	0.018
Cl mmol/L	3.24 ± 0.03^{d}	4.14 ± 0.37^{c}	5.85 ± 0.29^{b}	7.95 ± 0.32^{a}	<0.001
Cu Ppm	0.11 ± 0.12	0.18 ± 0.78	0.20 ± 0.15	0.30 ± 0.14	0.554
Fe µg/dl	0.05 ± 0.00^{c}	0.20 ± 0.00^{c}	0.35 ± 0.31^{b}	0.66 ± 0.02^{a}	0.01
Zn Ppm	0.09 ± 0.00^{d}	0.31 ± 0.15^{c}	0.66 ± 0.00^{b}	0.99 ± 0.00^{a}	<0.001

Mean±SD in the same row and in each treatment with different superscripts differ significantly at $P<0.05$ level

*Control - (0% supplementation), PSB 1- (10% supplementation), PSB 2- (20% supplementation), PSB 3- (30% supplementation)

4.5 Total Antioxidant Activity (TAA)

The total antioxidant activity value of experimental biscuits is represented in **Table 4.4**. From the table it was observed that, PSB 3 (43.78%) had more Antioxidant Activity than the sample PSB 2 (39.31%) and PSB 1 (37.54%). The mean score of TAA reveled that, there is a significant difference ($p<0.05$) in case of all the biscuits with pumpkin seed flour supplementation. Thus, total antioxidant activity was found to be highest in biscuits containing higher amount of pumpkin seed flour, followed by biscuits with lower pumpkin seed flour.

Table 4.4: Total Antioxidant Activity Value of Experimental Biscuits Samples

Sample	Total Antioxidant Activity (TAA) (mg TE/gram)	*P Value
PSB 1	37.54 ± 0.006^{c}	<0.001
PSB 2	39.31 ± 0.006^{b}	
PSB 3	43.78 ± 0.006^{a}	

Mean±SD in the same column and in each treatment with different superscripts differ significantly at $P<0.05$ level

* PSB 1- (10% supplementation), PSB 2- (20% supplementation), PSB 3- (30% supplementation)

4.5 Sensory Evaluation

The sensory attributes of different types of biscuits samples had significant difference ($p<0.05$) in appearance, color, flavor, crispiness and overall acceptability. Table 4.5 shows that the sensory criteria of biscuits coded with PSB 3 have mean 6.7 for color, 6.7 for texture, 6.8 for flavor, 6.6 for appearance, and 6.3 for crispiness and 6.8 for overall acceptability.

Due to the enhanced appearance, color, flavor, crispiness and overall acceptability of the PSB 3 sample, the other samples received lower acceptability scores.

Table 4.5: Hedonic sensory score for experimental biscuits

Sample	Appearance	Color	Texture	Crispiness	Flavor	Overall Acceptability
Control	5.4±0.16[c]	5.5±0.15[b]	6.6 ±0.09	5.7±0.08[c]	5.5±0.16[b]	5.6±0.12[b]
PSB 1	6.4±0.14[d]	6.3±0.17[d]	6.6±0.07	6.0±0.08[c]	6.3±0.15[d]	6.3±0.18[d]
PSB 2	6.5±0.15[b]	6.4±0.12[c]	6.6±0.03	6.0±0.06[b]	6.5±0.11[c]	6.5±0.17[c]
PSB 3	6.6±0.16[a]	6.7±0.18[a]	6.7±0.09	6.3±0.11[a]	6.8±0.17[a]	6.8±0.17[a]
***P Value**	<0.001	<0.001	0.555	0.01	<0.001	<0.001

Mean±SD in the same column and in each treatment with different superscripts differ significantly at P<0.05 level.

*Control - (0% supplementation), PSB 1- (10% supplementation), PSB 2- (20% supplementation), PSB 3- (30% supplementation).

4.6 Cost Analysis

To developed pumpkin seed powder incorporate biscuits, the ingredients for formulation of biscuits dough were purchased from the local market as well as from super shop and from the tribal area of Rangamati. So, the developed product's price was calculated from the total cost of purchased raw ingredients and cost of processing and packaging. **Table 4.6** indicate the price of the ingredients per Kg or liter, which also considered in gm or ml to predict the cost of developing 175gm of PSB 3 (30%Pumpkin Seed Biscuits) and 175 gm of the control sample.

Table 4.6: Cost Analysis

Raw Materials	Tk/kg/L	Cost in Tk/175gm developed product	
		PSB 3	Control
Pumpkin Seed	500/kg	15	0
Wheat Flour	70/kg	4.9	7
Sugar	90/kg	3.6	3.6
Butter	150/250gm	30	30
Baking Powder	58/50gm	1.5	1.5
Milk	80L	1.6	1.6
Sub Total		56	45.61
Processing Cost	@ 15% of raw material	8.49	8.49
Packaging cost	1.91 Tk./pkt		
Total Production Cost		67.00	54.10

Table 4.6 shown that, to developed 175gm PSB 3 the total production cost was 67Tk and for control, cost was 54.10Tk.

Chapter 5: Discussion

5.1 Proximate analysis of pumpkin seed

According to the findings of present study in Table 4.1, the seed had a moisture content of 3.00%, which was lower than the moisture contents of pumpkin (*Cucurbitapepo L*) seed 5% (Aaliyu *et al*., 2012) and the moisture content of pumpkin seed 5.66% (Kwiri *et al.*, 2014) respectively. So, the experimental pumpkin (*Cucurbita maxima*) seed had the lower moisture content then the comparable samples which give it a higher storage ability.

The fiber content of the experimental pumpkin seeds was 13.30%, which is comparatively higher than the seeds of (*Cucurbitapepo L*) have fiber content 1% (Aaliyu *et al*., 2012).
Although fiber-rich foods are known to stretch the internal surface of the colon, easing waste passing and therefore acting as an efficient anti-constipation aid, it also lowers the blood cholesterol level and lowers the risk of many malignancies.

In terms of protein content, the protein content of the seed was estimated to be 36.40% which demonstrates that the seed can act as a source of protein considering the level of protein inadequacy in the locality. This is comparatively higher with the protein content (27.48%) of the seeds of pumpkin of C.popo species from the findings of research work by Aaliyu et al., (2012).

The ether extract or fat content of the seed was between 48.00% to 49.00%; hence, it might be categorized as an oil seed, similar to peanuts, melons, etc. Fats are necessary because they supply the body with the greatest amount of energy (Oluyemi, 2006).

The ash content of the seed was observed 4.40 %. It is comparatively lower to results of pumpkin seed's ash content of *C.popo* species 5.50% (Aaliyu *et al*., 2012). The sample's percentage ash conveys a sense of its own inorganic composition, by which the mineral composition could be determined. Samples with high ash content should

contain a substantial amount of various mineral elements that must accelerate metabolic processes and promote growth and development.

5.2 Mineral content analysis of pumpkin seed

From the **Figure 4.1** the most abundant element in the seed in our study were potassium 204.0mg/100gm,, Zinc 18.71mg/100gm. and iron4.01mg/100gm.

It suggests that having a lot of potassium in the body made the body use iron better (Adeyeye, 2002). Iron is required by the organism for the creation of oxygen transport proteins, specifically hemoglobin and myoglobin, as well as the formation of heme enzymes (Abbaspour *et al*., 2014).

The concentration of sodium in pumpkin seed was 134.4mg/100gm; this element is required by the body to regulate blood pressure and blood volume. In the pumpkin seeds 2.2mg/100gm calcium was found. Calcium aid in better sleep and helps regulate nutrients get in and out of cells. The pumpkin seed contain also high amount of phosphorus concentration 27.83mg/100gm, respectively. These results are comparable to the results by Aaliyu *et al*., (2012) who reported that pumpkin seeds are a good source of minerals especially potassium, calcium, sodium, magnesium, phosphorus, zinc and iron.

5.3 Proximate composition of Experimental biscuits

In **Table 4.2** the proximate composition analysis findings are demonstrated. There are significance difference ($p<0.05$) among the biscuits samples in terms of moisture, crude fiber, crude protein, ether extract, ash and enery content..

In terms of moisture content the PSB 1 sample has the significantly ($p<0.05$) higher moisture content than the control and the PSB 2 and PSB 3 samples which mean value of moisture content are 0.64%, 2.25% and 2.50% respectively. The lower moisture content indicates that the samples made with lower amount of pumpkin seed flour have more storability than the others.

From the findings of **Table 4.2**, higher ash content was observed in the sample PSB 3 (1.85%) compared to control (1.24%), PSB 1() and PSB 2(). A significance difference($p<0.05$) excised among the samples this compares well with the research work by (Aminuddin *et al.*, 2019) who reported that the biscuits supplementation with pumpkin seed flour also had a higher ash content of 1.65% for formulation 1 and 0.59 % for formulation 2.

Considering the fat content or ether extract there was a significance difference ($p<0.05$) in between the pumpkin seed incorporated biscuits and the control sample. The experimental biscuits sample PSB 3 made with the higher amount of pumpkin seed powder had the higher amount of fat content 33.54% than respective lower amount of pumpkin seed incorporated biscuits sample PSB 2 (31.17%) and PSB 1 (26.95%) where the control sample has lowest fat content 26.34%. This results are confirm research by Kumari and Sindu (2019), in which the fat content of biscuits with pumpkin seed flour is also higher according to the higher amount of pumpkin seed incorporation. Fat provides energy to human body, protects organs, promotes cell growth, regulates cholesterol and blood pressure and aids in food absorption.

The crude protein content was seen to be higher in pumpkin seed containing biscuits samples than the control sample (9.94%).This observation agreed to the study by Aminuddin *et al*. (2019), who prepared biscuit with pumpkin seed cookies in different ratios . Higher protein in dietary intake for child and other aged people gropu is necessary as protein act as a building block for the muscle growth and development and to boost up the immune health.

With regards to the fiber content, there is significance difference among the samples supplemented with pumpkin seed powder had a higher amount of fiber content 1.95, 2.82 and 3.55 compared to the control sample 1.24% This findings are agreed with the study worked by Kumari and Sindu (2019).

5.4 Mineral Analysis of Experimental biscuits

The mineral composition of the experimental PSB samples and control samples is displayed in the **Table 4.3**. There was significance difference ($p<0.05$) among the biscuits samples with the minerals Ca, Mg, K, P, Na, Cl, Fe and Zn. Besides no significant difference ($p>0.05$) among the biscuits samples with the mineral Cu, .

The results demonstrated that supplemented biscuits contained more calcium than their control counterparts. PSB 1 had the highest Ca concentration in biscuits with 10.14mg/dl, followed by PSB 2 with 0.35mg/dl, PSB 3 with 1.34mg/dl, and the control sample (refined wheat flour) with 0.04 mg/dl.

From **Table 4.3** the zinc content of PSB samples were in increased amount compared to the control with a significance different ($p<0.05$). Kanwal *et al* (2015) also analyzed the zinc content of biscuits enriched with 33% pumpkin seed flour and observed that these contained 3.11 mg/100gm, which was greater than the control biscuits prepared from refined flour, which contained 0.96 mg/100gm.
With the addition of pumpkin seed flour, the zinc content of cake and cookies increased significantly.

The results indicate that fortified biscuits had higher magnesium with significance difference ($p<0.05$). PSB 1 had the greatest Mg concentration in biscuits (0.25 mg/dl), followed by PSB 2 (0.55 mg/dl), PSB 3 (1.76 mg/dl), and the reference sample with no supplementation with th concentration 0.14 mg/dl, which also agreed by the research work by Kumari and Sindhu (2019).

The sodium and chloride content of the control was relatively lower than the biscuits with supplementation with a significant difference ($p<0.05$). Sodium and chloride are necessary to transport nutrients and absorb them keep blood pressure in balance & maintain the proper fluid flow.

The iron content of the PSB samples are also higher and significantly different ($p<0.05$) than the control. Kanwal *et al* .(2015) researched the iron content of biscuits

fortified with 33% pumpkin seed flour and found that biscuits contained 2.28 mg/100gm.

Control biscuits had 0.364 mg/100gm, which was less than the fortified biscuits. So, with the addition of pumpkin seed flour, the iron level of all the biscuits increased dramatically.

In view of phosphorus, potassium and copper content, the findings showed that these minerals content of the biscuits was increased significantly after being supplemented compared the control sample without any supplementation which also reveled by the research work conducted by Aminuddin *et al*., (2019).

5.5 Antioxidant Activity Analysis

The results of the DPPH test indicate that the pumpkin seed powder incorpored biscuits extract functioned as effective free redical scavenged with the increased ratio of pumpkin seed powder incorporation (**Table 4.4**). And there was a significance different($p<0.05$) for total antioxidant activity among the supplemented samples. This observation agreed with the research work in which pumpkin seed also added to the wheat flour to make supplement biscuits samples by Kaur and Sharma (2017).

5.6 Sensory Analysis

The sensory attributes of different types of biscuits samples had significant difference ($p<0.05$) in appearance, color, flavor and overall acceptability.

The mean values of the individual's attributes of sensory analysis shows that sample PSB 3 was mostly accepted by the panelists. The present findings is similar to the research work by Kaur and Sharma (2017) who observed lower acceptability scores of control compared to pumpkin seed powder containing biscuits in term of appearance, color and nutty flavor.

Chapter 6: Conclusions

The research showed that fruit and vegetable seeds, a byproduct found in food processing facilities, may be utilized in culinary purpose. Using pumpkin seed flour to create new biscuit varieties was a pleasure. From this study, according to proximate analysis, the 30% pumpkin seed flour supplemented biscuit was a rich supply of crude fiber, protein, and fat, as well as a high source of ash. It is also observed that, the biscuit rich in phytochemicals such as antioxidants and bioactive compounds. The nutritional values were determined to be satisfactory, hence leading to the improvement of nutritional profile. In the sensory evaluation there was significant difference ($p<0.05$) in all sensory attributes and evaluation showed that biscuits with 30% pumpkin seed flour supplementation preferred compared to others biscuits with different supplementation. Moreover, the biscuits making technique used in this study was easy and affordable so it would be acceptable by the consumer .Thus it can be concluded that 30% pumpkin seeds supplementation can be used for developed nutrient enriched biscuits which will be a healthy dietary supplement for malnourished people especially for women and school going children.

This study also indicates a promising opportunity for the growers, processors, and consumers of Bangladesh to earn from the processing of pumpkin seed into biscuits. It should also be mentioned that the sale of international-standard biscuits of the highest quality can earn foreign currency, thus supporting the economy of Bangladesh.

Chapter 7: Recommendations and future perspectives

More than half of individuals in Bangladesh are malnourished. In this situation, this enriched biscuit, could be a great way to get nutrients and in rural and tribal areas of the country. It also has a better market value and is quicker to sell. Modern food companies can use the process of making food on a medium or large scale.

Based on the current investigation, the following ideas and possibilities for further research are given.

i) The current research could be done again with large number sample to back up the results of the experiments.

ii) People who are struggling financially will benefit from it from an economic point of view.

iii) Similar studies should be done on other seeds that can be bought in stores, like nigella, linseed, kidney bean, and others.

iv) Modern packaging and storage conditions will be made for pumpkin seed supplemented biscuits for store the quality of product.

v) Aromatic flavors can be added to increase the value.

vi) From therapeutic point of view, the findings will be helpful because they have medical significance.

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Appendix: Photo Gallery

Development of Biscuits





Chemical analysis

Sensory Evaluation

Biography

Shapla Das, daughter of Pradip Das and Krishna Das, passed the Secondary School Certificate Examination in 2011 from Belaichari Model High School, Belaichari and then Higher Secondary Certificate Examination in 2013 from Rangamati Government College, Rangamati. 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 among people about food safety and nutrition.