

DEVELOPMENT AND QUALITY EVALUATION OF THERAPEUTIC BISCUITS FROM RED KIDNEY BEAN, POTATO FLOUR AND STEVIA LEAF

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A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Science in Food Processing and Engineering

> Department of Food Processing and Engineering Faculty of Food Science & Technology Chattogram Veterinary and Animal Sciences University, Chattogram -4225, Bangladesh

> > **DECEMBER 2021**

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December 2021

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

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DECEMBER 2021

DEDICATED TO MY RESPECTED AND BELOVED PARENTS AND TEACHERS

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December, 2021

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List of abbreviation

Abbreviation	Elaboration		
RKBP	Red kidney bean powder		
PF	Potato flour		
WF	Wheat flour		
LDL	Low density lipoprotein		
HDL	High density lipoprotein		
DAE	Department of Agricultural Extension		
USDA	United States Department of Agriculture		
FDA	Food and Drug Administration		
EFSA	European Food Safety Authority		
CVD	Cardio vascular disease		
NaCl	Sodium chloride		
QE	Quercetin equivalent		
TE	Trolox equivalent		
TPC	Total phenolic contents		
TFC	Total flavonoid contents		
DPPH	2,2-diphenyl-hydrazyl-hydrate		
ANOVA	A one way analysis of variance		
SD	Standard deviation		

Abstract

The aim of the research was to create a healthy therapeutic biscuit and analyze its nutritional facts. In this study red kidney bean and potato were dried and ground into powder. The therapeutic biscuits were prepared by incorporating different levels of red kidney bean powder and potato flour. Stevia leaf powder was added in substitution of sugar. The therapeutic biscuits were investigated for their physic-chemical, sensory and bioactive compound properties. Chemical analysis showed that protein, fat and ash contents are higher in formulated biscuits than control biscuit. In physical analysis it was shown that therapeutic biscuits having more weight than control. Spread ratio was found the highest in control. Sensory evaluation showed that there is no significance difference (p<0.05) between control biscuit and sample with 10% potato flour, 5% red kidney bean powder and 85% wheat flour. Therapeutic biscuit is nutritionally acceptable and capable of meeting daily nutritional requirements, much as commercially available biscuits.

Keywords: Therapeutic biscuit, proximate analysis, bioactive compounds, red kidney bean powder, potato flour

Chapter 1: Introduction

Developing new food products is a complicated process requiring knowledge of ingredients, processing, techniques, packaging materials, legislation and consumer demands and preference. Food industries are now focusing on consumer health issues. Furthermore, people are concerned about foods high in fat, cholesterol, and sugar due to the possibility of being affected by various diseases such as coronary heart disease, obesity, hypertension, various cancers, gall bladder diseases, and so on (Ding and Malik, 2008). Excess fat and sugar consumption causes increasing of body weight that is a risk factor for cardiovascular disease in and of itself, as well as a source of other risk factors such as hypertension, dyslipidemia, and type 2 diabetes (Eyre et al., 2004). High-glycemic diets have also been linked to several types of cancer in several studies (Eyre et al., 2004, Bonovas et al., 2004, Ding and Malik, 2008). Because of these concerning conditions people are increasingly interested in foods that are not high in fat and sugar (Akoh, 1995).

Considering all these condition the new therapeutic biscuit was made upon the availability, uniqueness, reduction of wastage, nutrition of raw material.

We know that micronutrients found in legumes include iron, zinc, vitamins A and E. Kidney bean (*Phaseolus vulgaris*) is chosen as a raw material to prepare biscuits which are one of Bangladesh's underutilized legumes. Kidney bean is a herbaceous annual plant that is grown for its edible dry seed. It is also high in phenolic content, which has varying levels of antioxidant activity. The health benefits, such as the prevention of cancer, heart disease and Alzheimer's disease, have been attributed in part to the presence of antioxidants in kidney beans, particularly polyphenols. As a result, eating kidney beans is beneficial to one's health. Kidney bean powder can be used in biscuits and is a low-cost food item for Bangladeshis on a tight budget. Biscuits prepared with red kidney bean (RKB) powder can increase the protein and ash content, as well as other beneficial bioactive compounds (Shehzad et al., 2015).

Another main component is Potato. It is the tuber of the cultivated plant *Solanum tuberosum*, which belongs to the Solanaceae family. It is now one of Bangladesh's staple foods. Potatoes can be used as a natural therapy for constipation and the prevention of hemorrhoids. Potatoes are high in potassium, which helps to control and reduce blood pressure. Potato tubers contain antioxidant properties that protect the

body from cancer and cardiovascular disease due to the presence of polyphenols (Sawicka et al., 2018). Potato flour is increasingly widely utilized in the food industry, particularly in the baking industry for making bread and cookies. In order to keep bread fresh, potato flour is used in the baking process. It also has a unique, pleasant flavor and improves toasting characteristics. The amount of potato flour in the bread that is commonly recognized. It is also good in crackers, pastries, yeast-raised doughnuts, and cakes and cake mixes ((Kulkarni et al., 1996).

Finally, the component Stevia is a non-nutritive sweetener. This means it has almost no calories. Stevia is used as a healthy substitute for added sugar in a variety of foods and beverages. In recent days people are health conscious. In case of diabetes, stevia do not increase blood sugar levels. Stevia leaf powder may help manage cholesterol (Goyal et al, 2010).

Aims and Objectives

- i. To produce therapeutic biscuit that will be healthy option to people.
- **ii.** To determine nutritional profile of the developed product.

Chapter 2: Literature Review

2.1 Biscuit

Biscuit is an important processed food in human diet and is usually eaten by all classes of people. It is low moisture containing flour based bakery product which is also available to us in the form of confectionery (Masoodi et al., 2012). A biscuit is a flour-based baked food product. Biscuits today can be savory or sweet, but the majority is small and flat, measuring roughly 5 cm (2.0 in) in diameter. Biscuits should be made with wheat flour that has low flour content (8-9.5%). (Borla et al, 2004). Sweet biscuits are generally baked with wheat flour or oats and sweetened with sugar or honey, and are typically eaten as a snack item. Chocolate, fruit, jam, nuts, ginger, and other fillings may be used in some varieties, and they can even be used to sandwich additional fillings. Biscuits are an ideal food because of their nutritional benefits, palatability, compactness, and convenience (Rana et al., 2020). Because biscuits have lower moisture content than cakes and bread, they are more resistant to microbial decomposition and have a longer shelf life. Biscuits have long been one of the most popular and appealing foods due to its nutritional, sensory, and textural features, as well as their ready-to-eat convenience and low cost (Pabitra et al., 2018).

2.1.1 Types of biscuit

It's becoming easier to distinguish between "types" of biscuits based on how they're created, so here's a chart showing the four basic processes and examples of the sorts of biscuits that may be made using these methods.

2.1.1.1 Rubbed in Biscuits

These varieties are primarily based on pastries, and the term refers to the rubbing in of fat into the flour, which is done when producing short crust pastry. This is accomplished by placing flour in a big mixing bowl, adding cut-up butter, and kneading the two together with your fingertips until the result resembles breadcrumbs. The trick to preparing these types of biscuits (as with all pastry) is not to overwork the gluten in the flour; otherwise, the biscuits would be tough instead of crumbly.

2.1.1.2 Creamed Biscuits

The word alludes to the process of combining fat and sugar in the preparation of cakes. Place softened butter in a large mixing bowl, add the sugar, and beat the two together with a wooden spoon or electric whisk until the mixture is well combined,

light, and fluffy. Wet components, such as eggs or milk, are then incorporated in before the flour or other dry ingredients are added, depending on the type of biscuit being created. Small spoonfuls of dough are deposited onto baking sheets, widely spaced apart, because this dough is often very soft.

2.1.1.3 Whisked Biscuits

The name alludes to how the egg content is handled. Egg whites are whisked until firm. Egg yolks/whole eggs are mixed together with the sugar until the mixture thickens and lightens. These biscuits can be as simple as whipped egg whites with sugar in the shape of light as air meringues to more substantial biscuits like coconut macaroons.

2.1.1.4 Melted Biscuits

The name comes to the method of preparing the ingredients. In most cases, liquid sweeteners like nectar, golden (corn) syrup, or molasses are used in the recipes. All of the sweeteners (including sugar) are blended with the fat content in a large skillet and slowly melted and stirred until well integrated. Because the remaining ingredients are frequently added to the melted ones, most of the mixing takes place in the saucepan rather than in a mixing bowl.

2.2 Therapeutic food

Therapeutic foods are dietary supplements that are created for certain, typically nutritional, therapeutic objectives. Therapeutic foods are commonly used to feed malnourished children in an emergency or to enhance the diets of those who have unique nutritional needs, such as the elderly. Ready-to-Use Therapeutic Food (RUTF) is a combination of nutrients intended for the treatment of severe acute malnutrition without consequences (Vijay, 2018). Ready-to-use therapeutic foods (RUTF) are high energy, fortified, ready-to-eat foods suitable for the treatment of children with severe acute malnutrition. These foods should be soft or crushable and should be easy for young children to eat without any preparation. At least half of the proteins contained in the foods should come from milk products (FAO, 2010)

2.2.1 Ideal characteristics of ready to use therapeutic food

According to Vijay (2018), this food should be calorie dense, high in proteins, mineral and vitamins. It should not required any additional preparation or processing before consumption and will be easy to use. RUTF must be highly palatable with good taste and appearance.

2.3 Main components of therapeutic biscuit

2.3.1 Red kidney bean

Kidney beans are a type of common bean (Phaseolus vulgaris), a Central American and Mexican legume. Throughout the world, the common bean is an essential food crop and a key source of protein. According to Shweta et al (2013), high fiber dietary intake can significantly reduce the incidence of diabetes and reduce glycemic index value of snacks. It is helpful to one's health to consume kidney beans. Kidney bean powder is a low-cost food item for Bangladeshis on a restricted budget that can be used in biscuits. Biscuits supplemented with red kidney bean (RKB) powder can boost protein, ash, and other useful bioactive elements.

Kidney beans are very good for the health. It is a good choice for asthma and diabetes sufferers. They help in boosting the immune system. They are a great source of vitamins, minerals, proteins, dietary fiber and iron. Regular consumption of kidney beans is beneficial to the hair and skin. These legumes are tasty and nutritious. They contribute to the proper functioning of the nervous system and brain (Shehzad et al. 2015)

2.3.1.1 Nutrition value of kidney bean

Red kidney bean provides 333 kcal per 100 gram.

Component	Amount
Carbohydrate	60g
Protein	24g
Fat	0.8g
Sodium	24mg
Potassium	1406mg
Calcium	0.14mg
Iron	0.45mg
Magnesium	0.35mg
Vitamin B6	0.20mg
Vitamin B1	0.608mg
Vitamin C	0.07mg

Table 2.1 Nutritional value of kidney bean per 100gm

*Source: United States Department of Agriculture (USDA)

2.3.1.2 Health benefits of kidney bean

These particular Kidney beans are high in manganese and have anti-oxidant properties. The antioxidant defense in the mitochondria reduces free radicals. Kidney beans are also high in vitamin K, which is important for protecting body cells from oxidative stress and lowering cancer risk. Including kidney beans in our diet can help us avoid colon cancer.

High blood sugar levels can increase the risk of chronic conditions like heart stroke. It is crucial to keep in diet this amount moderate and add kidney beans, which are also high in protein and fiber. Kidney beans have a low glycemic index, which means they can assist diabetics control their blood sugar levels.

Kidney beans are a high-protein food that can be included in a weight-loss regimen. Beans are high in protein, complex carbohydrate and fiber, and they assist to keep blood sugar levels in check in the body. Diabetics and insulin-resistant people can consume these healthy beans. As the kidney bean stabilizes blood sugar levels, the pancreas releases less insulin. This mechanism aids in fat storage in the body, allowing for the maintenance of a healthy weight.

Kidney beans are high in protein and carbohydrate, but they're also high in antioxidants. They contain anti-ageing qualities and are proven to reduce cell damage and improve longevity. By using kidney beans in our diet, we can lessen the difficulties that bad foods cause to our bodies and skin.

Kidney beans contain a lot of zinc. As a result, including kidney beans in your diet on a regular basis can help you maintain good skin. Acne is caused by an increase in sebaceous gland activity, which is responsible for sweat production. Zinc, which is contained in kidney beans, can be used to treat acne. As a result, certain glands are able to operate properly. Folic acid, which is found in kidney beans, is also useful, as it aids in the production of healthy skin cells. The newly formed skin cells are quite beneficial in reducing acne breakouts and clearing the pores on the skin. Because kidney beans are an excellent source of zinc, eating a bowl full of them every day is beneficial to eye health. Regular consumption of dietary fiber in meals can help to reduce blood cholesterol levels in the body. Bean fiber forms a connection with bile acid in the colon, which is favorable to cholesterol production. Acid availability is reduced in this manner. As a result, the risk of excessive blood pressure is reduced.

Regular consumption of kidney beans, which are high in soluble fiber, lean protein, potassium, zinc and magnesium can help to maintain a healthy immune system. Individuals' blood pressure can be readily managed by consuming dietary fiber, which helps to avoid osteoporosis.

Amino acid, fatty acid, neurotransmitter synthesis, gluconeogenesis, histamine synthesis, hemoglobin creationare all maintained by kidney beans. As a result, eating kidney beans can help keeping skin young and healthy. Regular consumption of these nutritious kidney beans, which are high in fiber and protein can provide complete nourishment.

Kidney beans are high in Vitamin B1, which aids in the creation of acetylcholine, which is necessary for proper brain function. Kidney beans are also beneficial to the healthy functioning of the brain, since they help to improve memory and focus. Incorporating kidney beans into one's diet can also help to prevent dementia and Alzheimer's disease.

Kidney bean boosts immune system's performance. Iron, manganese, magnesium, vitamins, proteins, and fiber are all abundant in kidney beans. Manganese in kidney beans is good for the body's metabolism since it helps to break down nutrients and produce energy.

The glycemic index of kidney bean is quite low, resulting in a balanced blood sugar level in the body. By eating kidney beans in meals, can lower one's risk of acquiring diabetes.

The magnesium in black kidney beans acts as a bronchodilator, allowing air to flow freely into and out of the lungs. In studies, low magnesium levels have been associated to asthma.

2.3.2 Potato flour

Potato (*Solanumtuberosum L.*) cultivation began in Peru between 8000 and 5000 BC. Potatoes are now one of the world's top five crops. Bangladesh is the world's seventh largest producer of tubers. According to the Department of Agricultural Extension, it produced a record high of 1.09 croretonnes in 2019 (DAE). Potato flour was created as a gluten-free substitute for wheat flour. Also, as a low-cost ingredient to compensate for the occasional high price and scarcity of baking flour. There are two varieties of potato flour commercially available. One is Potato granules that are fine powder granules and another Potato flakes that comes in pieces and crumbs.

According to Seevaratnam et al. (2012) Potato flour with negligible fat content, high dietary fiber, high vitamins, a good amount of minerals and 6-12% protein content can be substitute for wheat flour in the preparation of biscuits. This also helps in lowering the gluten level and prevent from Coeliac disease. The sensory properties of biscuits and other baked goods are improved when potato flour is used as a complementing vegetable or as a snack item. Furthermore, potatoes contribute significantly to the industries and are cost-effective to use in biscuit nutritive value of a meal because they showed higher manufacturing.

2.3.2.1 Nutritional value of Potato Flour

Potato flour provides 357 kcal per 100 gram. It is high in vitamin B6 and minerals, particularly iron, magnesium, calcium, potassium, and zinc.

Component	Amount
Carbohydrate	83g
Protein	7g
Fat	0.3g
Sodium	55mg
Potassium	1001mg
Vitamin B6	0.4mg
Vitamin C	0.06mg
Magnesium	0.16mg
Iron	0.07mg
Calcium	0.06mg

 Table 2.2 Nutritional value of commercial potato flour per 100gm

*Source: United States Department of Agriculture (USDA)

2.3.2.2 Function

In baked goods, potato flour has several functions. Potato flour draws and retains water, which contributes in the production of moist yeast bread and rolls. It works as thickener and increases the viscosity of many food systems like sauces and gravies. This flour provides a pale yellow color and sweet earthy flavor. It also increases shelf life stability and decreases dough development time.

2.3.2.3 Application

Potato flour may be used to make potato mash, snack meals, extruded foods, sweets and other bakery items, weaning foods, and infant foods, among other things (Karuna et al., 1996). Potato flour can be used to make potato breads, rolls, cookies, dumplings, cakes, and other desserts, among other things. Breads and rolls produced with potato flour partially replaced for wheat flour have a distinct potato taste, are lighter and moister, have a bigger loaf volume, and have a lighter and fluffier texture. When working with potato flour the following considerations should be taken into account like for baked goods in general, wheat flour should be substituted up to 25% with potato flour for optimal results and for yeast leavened products, 15% of wheat flour with potato flour for optimal results. Higher concentrations of potato flour cause dough to develop gummy consistency. Potato flour, in addition to other gluten-free flours such as rice flour can be used to make gluten-free items.

2.3.3 Stevia

Stevia, the leaves of the plant *Stevia rebaudiana*, a member of the chrysanthemum family, a subset of the Asteraceae family (ragweed family), has piqued scientific attention as a sugar alternative. (Michaud et al., 2002; Bell and Sears, 2003). Steviol glycosides, the active chemicals in stevia, are 150-200 times sweeter than table sugar. It is heat resistant, pH stable, and non-fermentable (Franceschi et al., 2001). Stevia contains no calories because the body is unable to metabolize its glycosides. The use of zero-calorie sweeteners in baked items may have a considerable influence on the finished product's softness, color, and flavor (Mariotti and Alamprese, 2012). Artificial sweeteners do not participate in the Maillard process or caramelization, hence the final dish is lighter in color after cooking (Gallagher et al., 2003; Lin et al., 2010).Due to the fact that stevia is 200 to 300 times sweeter than table sugar. To provide the same amount of sweetness as other mainstream sweeteners, it typically requires about 20% of the land and far less water.

There are eight glycosides in stevia. These are the sweet components extracted and purified from stevia leaves. These glycosides arestevioside, rebaudiosides A, C, D, E, and F, steviolbioside and dulcoside A.The most abundant of these components arestevioside and rebaudioside A (reb A). Stevioside is found in varying concentrations in stevia leaves, ranging from 1% to 18%.

2.3.3.1 Market name of stevia

Some of the typical market names for stevia sweeteners areEnliten, PureVia, Rebiana, Stevia, Steviacane, Stevia Extract In The Raw and SweetLeaf.

2.3.3.2 Nutrition value of Stevia

Stevia contains no carbohydrate, protein, fat and fiber. Thus it gives no calorie. Source: United States Department of Agriculture (USDA)

2.3.3.3 Health benefits of Stevia

According to research stevia sweetener do not add calories or carbohydrates to the diet. They have no effect on blood glucose or insulin sensitivity. This allows diabetics to eat a wider range of foods while adhering to a balanced diet plan.

Researchers examined the effects of stevia on metabolic outcomes to the effects of placebos in five randomized controlled trials. According to the study, stevia had little to no effect on blood glucose, insulin levels, blood pressure, or body weight.

People with Type 2 diabetes reported significant reductions in blood glucose and glucagon response after consuming stevia in one of these investigations. Glucagon is a hormone that controls blood glucose levels, and diabetics' secretion mechanisms are commonly faulty. Glucagon levels drop when blood glucose levels rise. As a consequence, the glucose level is controlled.

Obesity and overweight are caused by a variety of factors, including physical inactivity and increased consumption of high-fat, high-sugar meals. Added sugars make for 16 percent of total calories eaten in the American diet, according to research. Weight gain and poor blood glucose management have been linked to this. Stevia is sugar-free sweetener with very few calories. It may be used as part of a well-balanced diet to help you lose weight while maintaining taste.

Stevia contains a variety of sterols and antioxidant compounds, including kaempferol. In trials, kaempferol has been proven to lower the risk of pancreatic cancer by 23%.

Blood arteries dilate when particular glycosides in stevia extract are consumed. They can also aid in the excretion of salt and the production of urine. In a 2003 study, stevia was discovered to have the capacity to help lower blood pressure. The stevia plant may have cardiac tonic qualities, according to the studies. Cardio tonic exercises help to balance blood pressure and control pulse. Recent study, however, has discovered that stevia has no effect on blood pressure. More study is needed to confirm the health advantages of stevia.

Foods and beverages containing stevia can help cut down on the calories eaten by unwanted sweets in children's meals. Thousands of things, ranging from salad dressings to snack bars, are now available on the market that includes ethically produced stevia. This accessibility allows children to consume sweet foods and beverages without adding calories as they transition to a lower sugar diet. Overconsumption of sweets and calories has been linked to obesity and cardiovascular disease (CVD).

2.3.3.4 Side effects of Stevia

Sugar alcohol is present in some stevia products. Bloating, stomach cramps, nausea, and diarrhea are common symptoms of sugar alcohol sensitivity, while one form of sugar alcohol, erythritol, is less likely to cause symptoms than others.

Stevia, as long as it is well purified and used in moderation, has no negative side effects and can be ingested without concern.

2.3.3.5 Uses of Stevia

For diabetics, stevia is harmless because it has no effect on blood sugar levels. Other artificial sweeteners have neurological and renal negative effects, while Stevia does not. In addition to its many other applications, stevia has antifungal and antibacterial properties. It is safe to use in herbal medications, tonics for diabetes patients, and everyday goods like mouthwashes and toothpastes. An upset stomach can be soothed with mild Stevia leaf tea (Goyal et al.,2010).

Chapter 3: Materials and method

3.1 Study period and Study area

The research work was conducted from January 2020 to December 2020. Raw materials were collected from local market of Chattogram. Experimental procedures were done in Department of Food Processing and Engineering, Department of Fishing & Post-Harvest Technology at Chattogram Veterinary and Animal Sciences University.

3.2 Preparation of materials

3.2.1 Preparation of potato flour

Potatoes were cleaned with fresh water to remove dirt and mud. Then potatoes were chopped into thin slice and blanched with hot water at 100°C for 5 minutes. Potato slices were dried in cabinet dryer at 80±2°C for 8 hours. After cooling at room temperature, the dried potato slices were ground into powder in a grinder and stored at room temperature in an airtight box.

3.2.2 Preparation of red kidney bean powder

Beans were peeled and blanched with hot water at 100° C for 5 minutes. After water drainage beans were dried in cabinet dryer at $80\pm2^{\circ}$ C for 8 hours. After cooling, the beans were ground in powder and stored in an airtight box.

3.2.3 Formulation of therapeutic biscuits

A new type of therapeutic biscuit was formulated by combining potato flour and red kidney bean powder with whole wheat flour. In addition, dry stevia leaf powder was used as a sugar substitute. The formulations are shown in Table 3.1

Ingredients	Quantity (g)			
	S1 (Control)	S2	S 3	S4
Wheat flour	100	85	75	70
Potato flour	-	10	15	20
Red kidney bean	-	05	10	10
powder				
Fat	40	40	40	40
(hydrogenated)				
Salt (NaCl)	0.5	0.5	0.5	0.5
Egg	30	30	30	30
Baking Powder	1.5	1.5	1.5	1.5
Sugar	50	-	-	-
Stevia powder	-	1.5	1.5	1.5
Milk powder	5	5	5	5
Ammonium	0.5	0.5	0.5	0.5
bicarbonate				

Table 3.1 Formulation of therapeutic biscuits

Here

S1 (Control) = Biscuit with 100% wheat flour

S2= Biscuit with 85% wheat flour, 10% potato flour, 5% red kidney bean powder S3= Biscuit with 75% wheat flour, 15% potato flour, 10% red kidney bean powder S4= Biscuit with 70% wheat flour, 20% potato flour, 10% red kidney bean powder

3.2.4 Preparation of biscuits

Initially, the wheat flour, red kidney bean powder, potato flour, stevia powder and other ingredients were accurately measured in an electric weigh machine and mixed. Butter and eggs were added into the dry ingredients the dough was formed accurately. The dough was then kneaded and rolled to a uniform thickness of 3mm. The biscuits were cut out with round biscuits cutter of 3.1cm diameter. Then the biscuits were baked at 220°C for 12 minutes, cooled to ambient temperature and stored.

3.3 Physical properties

The diameter of biscuits was measured by setting six biscuits edge to edge on a scale, rotating them 90°, and measuring the diameter (cm) of six biscuits again, and then taking the average value. The thickness of six biscuits was measured by stacking them on top of each other and taking the average thickness (cm). With the use of a digital weighing scale, the weight (g) of biscuits was calculated as the average of the values of six individual biscuits. The spread ratio was computed by dividing the average diameter of the biscuits by the average thickness of the biscuits.

3.4 Proximate analysis

The moisture content of formulated biscuit was determined using the standard method of AOAC, 2012. The protein, fat, fiber and ash content of therapeutic biscuit made from potato flour, red kidney bean powder and stevia leaf were analyzed on dry weight basis according to Association of Official Analytical Chemists (AOAC, 2012). Triplicate analysis of all samples was conducted.

3.4.1 Moisture content determination

Moisture content was determined by laboratory drying oven. First of all samples were grinded into small pieces. Empty crucibles were cleaned and kept into hot air oven for sterilization. After cooling the crucible weight were taken. Then 3g sample were put into empty crucible and placed in the chamber of hot air oven. The time was set for overnight (12hrs) at 105°C. After taking them out crucibles were put into desiccator for cooling. Final weight of samples with crucibles was measured.

Formula for determination of moisture content

 $Moisture content(\%) = \frac{(Weight of wet material - Weight of dry material)}{Weight of wet material} \times 100$

3.4.2 Crude protein determination

Protein content was determined by micro kjeldhal apparatus method. At first samples were taken into small pieces and 0.3g sample were taken in the digestion tube. Then 4g catalyst (A mixture of 72gm potassium sulfate and 8 gm of copper sulfate) and 5ml conc. H_2SO_4 were added into digestion tube. Digestion tubes were placed in the digestion unit and digested for 30minutes. After completion of digestion tubes were cooled at room temperature 30minutes. 25ml distilled water were added in the

digestion tube. Then 10ml mixed indicator were taken in the conical flask of distillation unit. Then 25ml NaOH (white pipe) and distilled water (black pipe) were taken below the pipe of distillation unit. Finally, after the distillation samples were titrated with 0.2N HCl.

Formula for determination of protein:

Nitrogen(%) = $\frac{\text{ml of titrant} \times \text{Strength of HCl}(0.2\text{N}) \times \text{Equivalent of Nitrogen}(0.014) \times 100}{\text{weight of sample}}$

Percentage of protein = $\%N \times 5.85$ (for plant origin)

3.4.3 Crude fat determination

Fat content was determined by soxlhet apparatus. Samples were grinded into small pieces and empty beakers were kept in hot air oven for sterilization. Chiller 1 and then chiller 2 were switched on. When the temperature was got below 12°C then the work was started. Weight of empty beakers was taken and beakers were marked. 2g samples with the help of foil paper were taken and put them into thimble paper. Thimble paper under the magnetic holder by magnetic ring was set and lifted it up. In the marked beaker 70ml Diethyl ether was taken. Beakers were screwed with solvent under the condenser and the stopcock was opened in vertical position. The thimble was lowered into the beaker and the extraction beaker was placed in burner by lifting lever handle. Then switchwas on, heated to boiling (100°C) and waited for 20minutes for boiling. Thimbles were lifted up and waited for 20minutes to close the reflux stopcock. Again 10-15minutes needed to evaporate solvents. After evaporation lever was lifted up and switch was turned off. Extraction beakers were taken to put at hot air oven at 105°C for 30minutes. Then beakers were put into desiccator to cool down. After cooling down the weight of extraction beakers were measured.

Formula for determination of fat content

$$Fat(\%) = \frac{Weight of fat}{Weight of sample} \times 100$$

3.4.4 Crude fiber determination

Fiber content was determined by raw fiber extractor. At first weight of crucibles were measured and 1g samples were taken into the crucible which was denoted by F_0 . Then filter crucible with the fiber analyzer was set. Each sample with 150ml Sulfuric acid (1.25%) was boiled for 30minutes. After this they were kept for few minutes for cooling. Then each sample was washed by using 30ml hot distilled water for three times. Again each sample with 150ml Sodium Hydroxide (1.25%) was boiled for 30minutes. Then kept them few minutes for cooling. Again each sample was washed with 30ml hot distilled water for three times. After that each sample was washed with 30ml normal distill water for one time. Finally each sample was washed by 25ml Acetone in condenser chamber for three times. Samples were kept at hot air oven for 1hr at 105°C. Crucibles were kept in desiccator for cooling and the sample weight was taken which was denoted by F_1 . Then samples were kept in muffle furnace at 550°C for 3hrs. Again they were kept in desiccator and the final weight was taken denoted by F_2 .

Formula for determination of fiber content:

Fiber Content =
$$\frac{F_1 - F_2}{F_0} \times 100$$

3.4.5 Ash Content Determination

Ash content was determined by muffle furnace. At first samples were grinded into small pieces. Empty crucibles were cleaned and kept in hot air oven for sterilization. After completing the sterilization crucibles were cooled and weighed. 3gram samples were taken into crucible and were placed them into the chamber of muffle furnace. The time was set for 5hrs at 550°C. Then samples were kept into desiccator for cooling. After cooling final weight were measured with crucible.

Formula for determination of ash percentage:

Ash (%) =
$$\frac{\text{Ash weight}}{\text{Sample weight}} \times 100$$

3.4.6 Carbohydrate Content Determination

The available carbohydrate content was determined by subtracting the sum of the values of moisture, ash, protein and fat from 100 (per 100gm) (AOAC, 2012).

Percentage of Carbohydrate = 100 - (Moisture % + Ash% + Protein% + Fat%)

3.5 Sensory evaluation

The sensory evaluation of the biscuits was performed by 20 semi-trained pannel of judges drawn from Chattogram Veterinary and Animal Sciences University, Chattogram. All panelists were regular consumers of biscuits and were familiar with sensory quality attributes of biscuits. The evaluation was conducted using the nine-point hedonic scale ranging from 1 (disliked extremely) to 9 (liked extremely). The samples were coded with three digit random numbers and presented in identical containers. Questionnaire for entering scores and potable water for mouth rinsing between tasting were made available to the panelists. Each sample was rated for appearance, taste, texture, crispiness, sweetness and overall acceptability.

3.6 Bio-active compound determination

3.6.1 Total phenol content

Total phenol content of Therapeutic biscuit extract was determined by Folin-ciocalteu method as described by (Wojdylo et al., 2007).

3.6.1.1 Preparation of standard gallic acid solution

About 10 mg of gallic acid was dissolved into 10ml of distilled water to make the stock solution. The concentration of the solution is 1mg/ml. Different concentrated solutions (2ppm, 4ppm, 8ppm, 16ppm, 32ppm) were prepared through serial dilution of this stock solution.



Figure 3.1: Standard curve of Gallic Acid

3.6.1.2 Procedure

1ml of sample extract or standard of different concentrations were mixed with 1.5ml of 10times diluted Folin-ciocalteu reagent. Then incubated at room temperature for 3 minutes and 1.5ml of sodium carbonate (75g/L) solution was added. Then this solution was left for about an hour at room temperature for the incubation process. The absorbance was measured with a Shimadzu UV-VIS-2600 spectrophotometer against the blank solution. The blank solution contains all the reagents without standard or sample extract. The gallic acid standard curve was used to determine the total phenol content of the sample and expressed as mg of Gallic acid equivalent (GAE) per gm of dried sample. All determinants were performed in triplicate (n=3).

3.6.2 Total flavonoids content

Flavonoids content was measured by the aluminum chloride colorimetric method as described by Shah and Hossain (2014).

3.6.2.1Preparation of standard quercetin solution

About 10 mg of quercetin acid was dissolved into 10 ml of water. This is called the stock solution and the concentration of the solution in 1 mg/ml. Different concentrated solutions (6ppm, 12ppm, 24ppm, 48ppm, 96ppm) were prepared through serial dilution method.



Figure 3.2: Standard curve of Quercetin

3.6.2.2Procedure

At first, 0.9815 g of potassium acetate was dissolved in 10 ml water to prepare 1M potassium acetate solution. 10% Aluminum chloride solution was prepared by dissolving 1g of AlCl₃ in 10ml of water. About 0.5ml of sample or standard at different concentration solution was taken in a test tube. Then 5ml of 95% ethanol was given. After that 0.1ml of 10% aluminum chloride and 0.1 ml of 1M potassium acetate were added. After that 2.8ml distilled water was added. The mixture of the solution was incubated at room temperature for 30 minutes to complete the reaction. The absorbance of the mixture was at 415nm against the quercetin standard curve. The result was expressed as mg of quercetin equivalent (QE) per gram of dried weight. All determinations were performed in triplicate (n=3).

3.6.3Anti-oxidant activity

The antioxidant activity of formulated therapeutic biscuits and ascorbic acid were determined on the basis of radical scavenging capacity of the DPPH (2, 2- Diphenyl-1- picrylhydrazyl) stable free radical. DPPH radical scavenging abilities of the crude methanolic extracts of the fruit samples were determined by the method described by (Nariya et al., 2013)

Different concentrations (2ppm, 4ppm, 8ppm, 16ppm, 32ppm) of sample extract solution and ascorbic acid were prepared with methanol.1 ml of solutions of different concentrations was made with methanol and taken in a test tube. Then 2ml of DPPH solution was added. The test tubes were kept at room temperature in dark for about 30minutes. Then the absorbance was read at 517nm in the UV-visible spectrophotometer. Same concentrations of ascorbic acid solutions were also prepared and used as the standard in this method.

The differences between the sample and standard solution were determined and expressed as

% of scavenging of DPPH = $(A_0 - A_s)/A_0 \times 100$

Where A_0 is the absorbance of DPPH alone and A_s is the absorbance of sample or standard with DPPH solution of different concentrations.

3.7 Statistical analysis

The data obtained from the experiments were statistically analyzed for analysis of variance (ANOVA) and consequently Fisher's Least Significant Difference (LSD) method was used to determine significant differences among the various samples in triplicate. Data were analyzed using the software Minitab version 20 at the 0.05 level.

Chapter 4: Results

4.1 Proximate analysis of formulated therapeutic biscuits

The results of proximate analysis on control and sample biscuits made with red kidney powder and potato flour as substitution to wheat flour have been presented in Table 4.2. A one-way analysis of variance (ANOVA) was carried out for the biscuit samples. The study has revealed that biscuits with high amount red kidney bean powder and potato flour have higher amount of protein content than that of control which is made of 100% wheat flour. Significant difference found among Control and the treated sample at P<0.05. No significant difference was found in fat content. Significant difference found in case of moisture content and carbohydrate content at P<0.05.

Parameter	Sample code of biscuits			
	S1	S2	S3	S4
Moisture (%)	3.67 ± 1.20^{a}	3.33±0.34 ^a	3.88±1.01 ^a	3.29 ± 0.26^{a}
Ash (%)	1.85 ± 0.64^{b}	2.45 ± 0.57^{a}	2.66 ± 0.67^{a}	2.72 ± 0.49^{a}
Fat (%)	21.51±3.42 ^a	22.92±5.52 ^a	23.12±3.71 ^a	24.86±2.05 ^a
Protein (%)	17.77±1.28 ^b	22.93±0.82 ^a	23.21±0.473 ^a	23.48±0.473 ^a
Carbohydrate (%)	55.20±0.08 ^a	48.16±0.06 ^b	46.46±0.06 ^c	44.69 ± 0.09^{d}
Fiber (%)	0.4 ± 0.28^{b}	0.49±0.01 ^a	0.51±0.13 ^a	$0.54{\pm}0.04^{a}$
Energy (kcal/100g)	$485.47 \pm 0.14^{\circ}$	490.64 ± 0.06^{b}	$486.76 \pm 0.28^{\circ}$	496.42±0.08 ^a

 Table 4.1 Effect of wheat flour replacement on proximate analysis of formulated

 biscuit

(*Values in the table represent mean \pm SD and the presence of different superscript along a row indicates a significant difference and the same superscripts are not significantly different at P<0.05)

The result shows that the moisture content of formulated biscuits ranges between 3.29 and 3.88 percent and there is no significant change among values. The values are followed by S1 (3.67 %), S2 (3.33 %), S3 (3.88 %) and S4 (3.29%). The ash content is then determined to be S1 (1.85%), S2 (2.45%), S3 (2.66%), and S4 (2.72%). There is no discernible difference in the fat content of four biscuit samples. However, the fat content in S4 (24.86 %) sample is higher than in S1 (control) which is (21.51%). There is no significant difference in protein content between samples made with potato flour, red kidney bean powder, and stevia. S4 has a higher percentage (23.48

%) and S1 has a lower percentage (17.77 percent). There is no significant difference in fiber content between the three samples except the control. Fiber content of S1 (0.4%), S2 (0.49%), S3 (0.51%), and S4 (0.54%) respectively. Carbohydrate content in control S1 is highest (55.20 %) and lowest in sample S4 (44.69%). Sample S4 shows higher amount of energy that is 496.42kcal/100g and lower amount in S1 (485.47kcal/100g), S3 (486.76kcal/100g).

4.2 Physical properties of formulated therapeutic biscuits

The results of physical properties like weight, diameter, thickness and spread ration are shown in Table 4.1. The study shows significant difference in weight among all samples. There is no significant difference in diameter of sample S2, S3, S4. In case of thickness and spread ratio no significant difference is found between S3 and S4 sample. For this study significant difference level (P<0.05) was used.

 Table 4.2 Effect of wheat flour replacement on Physical properties of formulated

 biscuit

Sample	Weight(g)	Diameter(D)	Thickness(T)	Spread
code		in cm	in cm	ratio(D/T)
S1	3.29 ± 0.05^{d}	3.70±0.04 ^a	0.62±0.04 ^c	5.97±0.01 ^a
\$2	4.73±0.04 ^a	3.34 ± 0.04^{b}	0.71 ± 0.05^{b}	4.70 ± 0.10^{b}
S3	3.98±0.03 ^c	3.30±0.03 ^b	0.79±0.02 ^a	4.18±0.02 ^c
S4	4.20 ± 0.03^{b}	3.30 ± 0.04^{b}	0.79 ± 0.03^{a}	$4.18 \pm 0.01^{\circ}$

(*Values in the table represent mean \pm SD and the presence of different superscript along a column indicates a significant difference and the same superscripts are not significantly different at P<0.05)

The study found that the weight of the sample biscuits (S2, S3, S4) was higher than the weight of the control (S1) made entirely of wheat flour. The weights ranged from 3.29g to 4.73g. S2 sample has the highest value, whereas S1 (control) has the lowest. The diameter spans from 3.30cm and 3.70cm. S1 sample has a higher value of 3.70cm, while S3, S4 samples have a lower value of 3.30cm. Furthermore, biscuit thickness varies from 0.62cm to 0.79cm. Samples S3 and S4 had the greatest values, whereas sample S1 has the lowest. Finally, spread ratios range from 4.18 to 5.97, with S1 (control) having the highest value of 5.97, followed by S2 (4.70), S3 (4.18), and S4 (4.18).

4.3 Hedonic sensory score of formulated therapeutic biscuits

The sensory evaluation scores of biscuit sample have been shown in Table 4.3. Statistical analyses were performed using the MINITAB (version 20). Difference in sensory scores was detected using one-way analysis of variance (ANOVA). A significant level of (P<0.05) was used for the study.

Parameter	Sample code of biscuits			
	S1	S2	S3	S4
Appearance	8.75±0.44 ^a	8.15±0.75 ^b	8.20±0.78 ^b	8.05 ± 0.83^{b}
Texture	8.60±0.50 ^a	8.25±0.55 ^b	8.55±0.51 ^{ab}	8.25±0.64 ^b
Taste	8.70±0.47 ^a	8.30±0.66 ^a	7.55±0.95 ^b	7.05±0.51 ^c
Crispiness	8.70±0.47 ^a	8.50±0.64 ^a	8.00±0.56 ^b	7.90±0.64 ^b
Sweetness	8.55±0.51 ^a	8.40±0.68 ^a	$7.80{\pm}0.77^{b}$	7.10±0.79 ^c
Overall	8.65±0.49 ^a	8.45±0.51 ^a	7.65 ± 0.75^{b}	7.10±0.91 ^c
acceptability				

Table 4.3 Sensory evaluation of formulated biscuits

(*Values in the table represent mean \pm SD and the presence of different superscript along a row indicates a significant difference and the same superscripts are not significantly different at P<0.05)

There is no significant difference between the control S1 and sample S2 in case of crispiness, sweetness and overall acceptability. Biscuit sample S2 was made with 85g wheat flour, 10g potato flour, 5g red kidney bean powder and 1.5g stevia powder. Even the sweetness acceptability of control made with sugar and S2 sample made with stevia powder is same. Furthermore, there is no significant difference between control S1 and sample S2.

4.4 Bio-active compounds of therapeutic biscuits:

Bio-active compounds of formulated biscuits are shown in Table 4.4. Here significant difference is found in the values of total flavonoids content (TFC), total phenol contents (TPC) and anti oxidant capacity. The highest value is found in S4 sample in each parameter.

Sample code	TFC (mg	TPC (mg	Anti oxidant capacity						
	QE/100g)	GAE/100g)	(mg TE/100g)						
S 1	37.60±0.01 ^d	$0.33 {\pm} 0.06^{d}$	$1.77{\pm}0.007^{d}$						
S2	42.06 ± 0.02^{c}	1.50±0.04 ^c	$3.27 \pm 0.002^{\circ}$						
S 3	47.30±0.01 ^b	1.78±0.03 ^b	3.30±0.001 ^b						
S4	50.72 ± 0.08^{a}	3.24 ± 0.05^{a}	3.32±0.001 ^a						

Table 4.4 Effect of wheat flour replacement on bio-active compound of formulated biscuits

(*Values in the table represent mean \pm SD and the presence of different superscript along a column indicates a significant difference and the same superscripts are not significantly different at P<0.05.)

The result shows that total flavonoids content, total phenol content, the anti oxidant capacity values are found highest in S4 biscuit sample and lowest in control S1 sample. Total flavonoids content varies between 37.60mg to 50.72mg. Highest amount is found in biscuit sample S4 (50.72mg) and lowest in control S1 (37.60mg). Total phenol content is highest in S4 sample that is 3.24mg and lowest in control that is 0.33mg. The folin-ciocalteu technique was used to determine the total phenolic content of the samples in this study. Sample S4 has the highest antioxidant capacity among all samples.

Chapter 5: Discussion

Four types of biscuits were manufactured, one of which was the control and was made entirely of wheat flour and sugar. The other three samples were made with wheat flour and varying amounts of potato flour and red kidney bean powder. In place of sugar, stevia leaf powder was utilized.

Proximate Analysis

Jothi et al (2014) analyzed the nutrient of gluten free composite flour biscuit where potato flour was incorporated. The authors reported the composition of gluten free biscuit as moisture 3.46-3.64%, ash 2.21-2.93%, fat 14.11-14.22%, carbohydrate 70.02-73.43% and fiber 1.82-2.36%. In another research, Mukta et al (2020) analyzed biscuits fortified with RKB powder. Authors reported that Protein (13.23-20.80%) was increased and carbohydrate (63.89-72.08%) was decreased with the increasing amount of RKB powder. Rana et al (2020) in studies stated that ash content of biscuits increased slightly with the reduction of fat and sugar but decreased with the increasing of polydextrose and stevia in the formulation.

Physical properties

According to Khaliduzzaman et al. (2010), increasing the level of potato flour replacement up to 25% results in a slight increase in biscuit thickness. However, as the substitution level of potato flour increases in the baked samples, the diameter and spread ratio of biscuits decrease, which may be due to the higher water holding capacity of potato flour. In another study Inyang et al. (2018) found similar result that is the diameter and thickness of the biscuits containing red kidney bean flour showed lower diameter but higher thickness than the control biscuit and biscuit made from 75% wheat and 25% acha flour. Maximum diameter was 4.40cm in sample made with 75% wheat flour and 25% acha flour, 25% acha flour and 25% kidney bean flour. In case of thickness highest (0.78cm) in sample made with 75% wheat flour and 25% kidney bean flour. Minimum thickness (0.69cm) was in sample 75% wheat flour and 25% acha flour. Spread ratio is calculated from diameter and thickness. Biscuits with higher spread ratio values are considered to be more desirable than those with lower values. According to Zucco et al. (2011) and Tiwari et al. (2011), for the blend

containing legume flour, the increasing number of hydrophilic sites available due to increased protein content competes for the limited free water in the dough thereby affecting the spread ratio

Sensory Evaluation

Mukta et al (2020) analyzed the sensory evaluation of biscuits fortified with RKB powder. According to the study biscuits formulated with more amount of RKB powder did not get satisfactory score in sensory evaluation. Color and aroma depends on reducing sugar and amino acids (protein content) as they are responsible for the Maillard reaction that gives desirable brown color and flavor of foods.

Bio-active compound

According to Mukta et al (2020), biscuits incorporated with red kidney bean increase the bio-active compound amount. The study found TPC content (11.19-12.50mg GAE/g) was increased with the increasing amount of RKB.

Chapter 6: Conclusion

People nowadays are more concerned about their health and are looking for natural alternatives that are both pleasant and nutritious. After studying the quality evaluation we can say that this therapeutic biscuit can be a healthy choice for those people after completing the biscuit formulation and numerous parameter examinations. For a long time, protein and energy enriched diets have been utilized to prevent malnutrition. As a result, health-conscious people and diabetics are looking for a natural option that would provide them with no calories but the taste of food. This therapeutic biscuit is made with potato flour, which is a wheat flour substitute. Baked items with potato flour have a nice appearance and texture. It contains red kidney bean powder, which is readily available and reasonably priced. It also has high protein content. Furthermore, stevia is being used as a sugar substitute. Stevia is a sweetener leaf with no calories. In a proximate analysis, the proportions of fat, protein, ash, and carbohydrate are acceptable. Formulated biscuits have more bioactive ingredients. The general acceptability of the sugar biscuit and the formed biscuit with PF, RKBP, and stevia was found to be the same during sensory evaluation. According to various researchers, Therapeutic foods contain more protein and fat. In our study it is shown that our formulated biscuits are rich in protein and fat. As red kidney bean has good amount of protein which reflects our formulated biscuit made with RKBP. As a result, this could play a significant part in reducing our country's malnutrition.

Chapter 7: Recommendation and future perspective

Red kidney bean and potato production and consumption are always high on a global and national scale due to a variety of factors. The presence of bioactive compounds, which play an important role in human health, as well as other proximate parameters, is the most important of these factors.

The current study is being carried out to look into the formulation and quality (physical properties, nutritional, bioactive compounds, sensory) evaluation of Therapeutic biscuit made from red kidney bean powder, potato flour, and stevia. The establishment of a small-scale processing unit at the grower level could use bean and potato for biscuit processing, which will be useful for obtaining this product during the off season. Ultimately, it reduces red kidney bean and potato postharvest losses and generates income for growers. The following recommendation is based on the formulation and quality evaluation of Therapeutic biscuit:

- A proper method for producing red kidney bean powder and potato flour, as well as storing them properly for use during the off season, should be evaluated.
- The rate of stevia production should be increased and used in commercial food.
- A new food processing method based on red kidney bean powder, potato flour, and stevia should be investigated.
- There should be a greater emphasis on not wasting red kidney beans and potatoes during the season. And it has the potential to be a food industry scope.

It is necessary to promote scientific methods of postharvest handling, primary processing, and preservation of red kidney bean and potato so that raw materials are available to consumers and entrepreneurs all year to avoid waste. To develop new healthy products, the establishment of a red kidney bean, potato, and stevia processing industry should be considered. For the betterment of these new products, modern packaging and storage conditions would be developed.

References

- Akoh, C.C. 1995. Lipid based fat substitutes. Food Science and Nutrition, 35(5), 405-430. Doi.org/10.1080/10408399509527707.
- AOAC. 2012. Official methods of analysis. 15th ed. Washington DC: Association of Official Analytical Chemists.
- Bell, S.J. and Sears, B. 2003. Low glycemic load diets: impact on obesity and chronic diseases. Critical Reviews in Food Science and Nutrition, 43(4), 357 – 377. doi.org/10.1080/10408690390826554.
- Bonovas, S., Filioussi, K. and Tsantes, A. 2004. Diabetes mellitus and risk of prostate cancer: a meta – analysis . Diabetalogia, 47(6), 1071 – 1078. doi.org/10.1007/s00125-004-1415-6.
- Borla OP, Motta EL, Saiz AI, Fritz R. 2004. Quality parameters and baking performance of commercial gluten flours. LWT-Food Science and Technology. 37(7):723-9..
- Ding Eric L, Malik Vasanti S. 2008. Convergence of obesity and high glycemic diet on compounding diabetes and cardiovascular risks in modernizing China: An emerging public health dilemma. Globalization and health, 4:4
- Eyre, H., Kahn, R., Robertson, R.M., Clark, N.G., Doyle, C., Hong, Y., Gansler, T., Glynn, T., Smith, R.A., Taubert, K. and Thun, M.J. 2004. Preventing cancer, cardiovascular disease and diabetes: a common agenda for the American Cancer Society, the American Diabetes Association and the American Heart Association. Circulation, 109(25), 3244-3255. doi.org/10.1161/01.CIR.0000133321.00456.00.
- Food and Agriculture Organization (FAO) data base. 2010. The state of food insecurity in the world. Rome, Italy.
- Franceschi, S., Dal Maso, L. and Augustin, L. 2001. Dietary Glycemic Load and Colorectal Cancer Risk. Annals of Oncology, 12(2), 173 – 178. Doi.org/10.1023/A:1008304128577.
- Gallagher, E., O'Brien, C.M., Scannell, A.G.M. and Arendt, E.K. (2003). Evaluation of sugar replacers in short dough biscuit production. Journal of Food Engineering, 56(2 3), 261–263. Doi.org/10.1016/S0260-8774(02)00267-4

- Goyal S.K., Samsher & R.K. Goyal. 2010. Stevia (Stevia rebaudiana) a biosweetener: a review. International Journal of Food Science and Nutrition. 61(1): 1-10.
- Inyang, U.E., Daniel, E.A. and Bello, F.A. 2018. Production and Quality Evaluation of Functional Biscuits from Whole wheat flour supplement with Acha (Fonio) and Kidney Bean Flours. Asian Journal of Agriculture and Food Sciences, 6, 193-201
- Johry Pragya, Samsher, G.R. Singh, B.R. Singh, Vaishali and Suresh Chandra. 2016. Development of cookies from potato flour and their quality evaluation. South Asian Journal of Food Technology and Environment. 2(1): 309-312.
- Jothi, J.S., Hashem, S., Rana, M. R., Rahman M. R. T. and Shams-Ud-Din, M., 2014, Effect of Gluten-Free Composite Flour on Physico-Chemical and Sensory Properties of Cracker Biscuits, Sci. Res. 6 (3): 521-530.
- Karuna D. Kulkarni, Noel Govinden, and Dilip Kulkarni. 1996. Production and use of raw potato flour in Mauritian traditional foods. Food and Nutrition Bulletin, vol. 17(2).
- Khaliduzzaman M, Shams-Ud-Din M, Islam M N. 2010. Studies on the preparation of chapatti and biscuit supplemented with potato flour. Bangladesh Agricultural University 8:153-160.
- Kulkarni KD, Govindan N, Kulkarni O. Production and use of potato flour in Mauritian traditional foods. Food nutrition bulletin. 1996; 72:162-167.
- Lin, S.D., Lee, C.C., Mau, J.L., Lin, L.Y. and Chiou, SY. (2010). Effect of erythritol on quality characteristics of reduced - calorie Danish cookies. Journal of Food Quality, 33(1), 14 - 26. Doi.org/10.1111/j.1745-4557.2010.00307.x
- Mariotti, M. and Alamprese, C. 2012. About the use of different sweeteners in baked goods. Influence on the mechanical and rheological properties of the doughs.
 LWT Food Science and Technology, 48(1), 9-15. Doi.org/10.1016/j.lwt.2012.03.001.
- Masoodi L., Dr Aeri Vidhu, Bashir Khalid. 2012. Fortification of biscuit with flaxseed: Biscuit production and quality evaluation. Journal of Environmental Science, Toxicology And Food Technology. 1(5), 6–9.
- Michaud, D.S., Liu, S. and Giovannucci, E. 2002. Dietary Sugar, Glycemic Load and Pancreatic Cancer risk in a Prospective Study. Journal of the National Cancer Institute, 94(17). 1293 - 1300. doi.org/10.1093/jnci/94.17.1293

- Mukta Roy, Sheikh Mohammad Nasirul Haque, Rana Das, Manobendro Sarker, Md.
 Azmain Al Faik, Shagor Sarkar. 2020. Evaluation of Physicochemical
 Properties and Antioxidant Activity of Wheat-Red Kidney bean biscuits.
 World Journal of Engineering and Technology. 8,689-699.
- Nariya Pankaj B., Bhalodia Nayan R., Shukla Vinay J., Acharya Rabinarayan and Nariya Mukesh B. 2013. In vitro evaluation of antioxidant activity of Cordia dichotoma (Forst f.) bark. US National Library of Medicine National Institute of Health. 34(1): 124-128. Doi: 10.4103/0974-8520.115451.
- Pabitra Chandra Das, Md Suman Rana, Md Saifullah, Md Nazrul Islam, 2018, Development of composite biscuits supplementing with potato or corn flour, Fundamental and applied agriculture, Vol 3(2), pp. 453-459.
- Rana, M.S., Das, P.C., Yeasmin, F. and Islam, M.N., 2020, Effect of Polydextrose and stevia on quality characteristics of low-calorie biscuits, Article on food research 4(6).
- Sawicka B, Noaema AH, Marczak BK, Skiba D. 2018. Potato as a medicine plant. University of life sciences in Lublin. Doi: 10.13140/RG.2.2.17540.6336
- Shah MD, Hossain MA. 2014. Total flavonoid content and biochemical screening of the leaves of tropical endemic medicinal plant *Merremia borneensis*. Arabian Journal of Chemistry. 7(6): 1034-38.
- Shehzad Aamir, Umer Masood Chander, Mian Kamran Sharif, Allah Rakha, Anam Ansari and Muhammad Zuhair Shuja. 2015, Nutritional, functional and health promoting attributes of red kidney beans; A review, Pakistan Journal of Food Science, Volume 25, Issue 4, Pages 235-246.
- Shweta Sharma, Seema Rana, Charu Katare, Tara Pendharkar, GBKS Prasad (2013). Evaluation of Fiber Enriched Biscuits as a Healthy Snack.
- Seevaratnam Vasantharuba, P. Banumathi, M.R. Premalatha, S.P. Sundaram, T. Arumagam, 2012, Studies on the preparation of biscuits incorporated with potato flour, World Journal of Dairy & Food Sciences 7(1):79-84
- Tiwari, B. K., Brennan, C. S., Jaganmohan, R., Surabi, A and Alagusundaram, K. 2011. Utilization of pigeon pea (Cajanus cajan L.) by–product in biscuit manufacture. LWT – Food Science and Technology, vol. 44, no. 6, pp. 1533-1537.
- United States Department of Agriculture (USDA) National Nutritional Database for Standard reference.

- Vijay D Wagh (2018). Ready to Use Therapeutic Food formulation & packaging for malnutrition: an Overview. Nutrition & Food Science International Journal. 2018; 4(5): 555648. DOI: 10.19080/NFSIJ.2018.04.555648.
- Wojdylo A, Oszmianski J, Czemerys R. 2007. Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chemistry. 105(3): 940-49.
- Zucco, F., Borsuk, Y. and Arntfield, S. D. 2011. Physical and nutritional evaluation of wheat cookies supplemented with pulse flour of different particle sizes. LWT – Food Science and Technology, vol. 44, no. 10, pp. 2070-2076.

Appendix A: Sensory evaluation test (Therapeutic biscuit)

Name of Tester.....

Date.....

Please taste these samples and check how much you like or dislike each one on four sensory attributes such as appearance, texture, taste, crispiness, sweetness and overall acceptability. Use the appropriate scale to show your attitude by checking at the point that best describes you're feeling about the sample please give a reason for this attitude remember you are the only one who can tell what you like. An honest expression of your personal feeling will help me.

Hedonic Attributes	Appearance			Texture			Taste				Crispiness				Sweetness				Overall Acceptability					
	Sample			Sample				Sample			Sample				Sample				Sample					
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Like extremely																								
Like very much																								
Like moderately																								
Like slightly																								
Neither like nor dislike																								
Dislike slightly																								
Dislike moderately																								
Dislike very much																								
Dislike extremely																								

Extra comments on each sample if any:

N.B. Overall scale used: 9= like extremely; 8=like very much, 7= like moderately; 6= like slightly; 5= neither like nor dislike; 4= dislike slightly; 3= dislike moderately;2= dislike very much; 1= dislike extremely

Appendix B: Panelist work for sensory evaluation

Sensory panelist doesn't do it-

- Eat, drink or smoke within 90 minutes prior to test
- Use gum, mints etc. flavored items within 30minutes
- Wear perfume, cologne and fragrance item during test
- Talk and comment during evaluation
- Taste if you have a cold
- Share the product with others
- Taste if you have a lot of prior knowledge about the manufacturing which you may dislike

Sensory panelist should-

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

Remember

- No food entry is so poorly done that it is not worthy of an encouraging comment.
- No food entry is so well done that some improvement may not be made

Appendix C (a): Pictorial view of raw material



Figure C (a): Red kidney bean powder, potato flour and stevia powder

Appendix C (b): Biscuit Preparation



Appendix C (c): Proximate analysis



Fig C (i): Determination of lipid content





Fig: Extracted ash from biscuit

Fig C (ii): Determination of ash content





Fig: Samples after moisture removal

Fig C (iii): Determination of moisture content





Fig: Extracted fiber from biscuit

Fig C (iv): Determination of fiber content



Fig: Digestion of samples



Fig: Distillation of samples



Fig: Titration of samples

Fig C (v): Determination of protein content

Appendix C (d): Analysis of bio-active compound



Fig: Preparation of extract



Fig: Analysis of bio-active compound

Fig C (d): Determination of bio-active compound

Brief biography

Tanjida Islam has passed the Secondary School Certificate (SSC) Examinations in 2011 with Grade Point Average (GPA) 5.00 followed by Higher Secondary Certificate (HSC) Examination in 2013 with GPA 4.70. She received the B.Sc. (Hon's) in Food Science and Technology in 2019 from Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh. Now, she is a candidate for the degree of MS in Food Processing and Engineering under the Department of Food Processing and Engineering, CVASU.

She has immense interest to work in food processing, preservation and development of modified food products, functional food product development and nutritional value analysis, quality control and quality assurance regarding food, new techniques to measure food quality, taste and flavor, control of unit operation in food processing and instrumental food analysis with various instruments.