FORMULATION OF GLUTEN-FREE CAKE FOR GLUTEN INTOLERANT INDIVIDUALS AND EVALUATION OF NUTRITIONAL QUALITY

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Roll No.: 0119/05

Registration No.: 00663

Session: January-June (2019-2020)

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

Department of Applied Food Science and Nutrition

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DEDICATATION

I Dedicated My Small Piece of Work

To My Beloved Family Members and Respected Teachers

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December, 2020

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Abbreviations

%	:Percentage
&	:And
ANOVA	:Analysis of variance
AOAC	:Association of Official Analytical Chemists
CD	:Celiac disease
℃	:Degree Celsius
CHO	:Carbohydrate
dl	:Deciliter
GSE	:Gluten sensitive enteropathy
et al	:Et alii/ et aliae/ et alia
etc	:Et cetera
gm	:Gram
DH	:Dermatitis herpetiformis
NCGS	:Non-celiac gluten sensitivity
mg	:Miligram
IBS	:Irritable bowel syndrome
GFD	:Gluten free diet
LDL	:Low density lipoprotein
AACC	:American Association of Cereal Chemist
ml	:Millilitre
cm	:Centimeter
SD	:Standard Deviation
SPSS	:Statistical Package for Social Science
AGA	:Antigliadin antibodies

Abstract

Now a days, gluten intolerance is a common health problem among modern people. About 6% to 7% of U.S. population may be intolerance to gluten that means about 20 million people in the United States alone could have the condition. This study was carried out to produce gluten free cake from gluten-free flours for gluten intolerant individuals. Cake samples were prepared from rice, oat, almond flours and mixture of rice, oat, almond and corn flours. Cake made of 100% wheat flour was prepared for comparison. Proximate composition, mineral contents, baking quality and sensory properties of cake were evaluated. Proximate analysis were evaluated according to Association of Official Analytical Chemist (AOAC) methods. Minerals content were determined by using a biochemical analyzer (Humalyzer 3000). Specific volume of cake was calculated according to the method described in American Association of Cereal Chemists (A.A.C.C.-2000). Sensory evaluation was done using 7 point hedonic scale. A remarkable improvement in protein, minerals (Na, K, P) and crude fibre were found in cakes prepared from rice flour, oat flour and almond flour as compared with control cake. Protein and crude fibre was higher (11%) in almond flour cake. Crude fibre was higher (1.79%) in both rice and almond flour cake. Cake that was produced from rice flour had the lowest (7.7%) protein content. Highest K (37.83 mg/dl), Mg (1.10 mg/dl) and Fe (0.08 mg/dl) were found in almond flour cake. Baking performance showed that all cake samples had lower volume and specific volume than control. Sensory evaluation of cakes indicated that all samples were acceptable to the consumer but cake which contained almond flour had superior sensory characteristics and nutritional value. From this study we can say that, we are successful to formulate a new gluten-free food products that might be helpful for gluten intolerant individuals to improve their gluten-free lifestyle. Further research is needed to evaluate bioactive compounds, antioxidant properties and shelf-life of the products.

Key words: rice flour, oat flour, almond flour, wheat flour, proximate, minerals, sensory, gluten intolerance patients, cakes.

Chapter 1: Introduction

Gluten intolerance is a clinical entity induced by the ingestion of gluten present in wheat, rye, and barley leading to serious damage to small intestinal mucosa differentiated by inflammation, lymphocytic infiltration, villous flattening, and crypt hyperplasia. Diarrhoea, abdominal pain, bloating and weight loss are typical gastrointestinal symptoms of gluten intolerance. However, it is much more common in women than men (Casella et al., 2018).

International studies proved that the gluten sensitivity is one of the most common lifelong disorders, which concern 1% of the European population (Mustalahti et al., 2010). According to Singh et al. (2018) this number is increasing day by day. They found that the presence of the gluten sensitivity in global is 1.4% based on serological tests and 0.7% based on biopsy.

A strict gluten-free diet is the only validated treatment to restore epithelium integrity and eliminate risk of complications. Zorzi et al. (2020) wrote in their research that predictably between 2020 and 2027 the demand for gluten-free products will be increased by 9.2%, in which the gluten-free bakery products will be in the highest ratio. On the other hand, the gluten-free diet is getting more popular and lots of people start to follow this eating habits without having the food intolerance. It is generally accepted that the gluten-free diet is low in fiber and micronutrients such as vitamins and minerals and gluten-free products have a lower protein content than their counterparts made with wheat flour (Mazzeo et al., 2015).

Formulation of gluten-free bakery products is still a challenge both for baking industries and food technologist. The baking industry should use new ingredients, technologies or methods to make more and better quality products for the gluten sensitive consumers (Biro et al., 2019). Replacing gluten functionally has been a challenge and the absence of gluten causes weak cohesion and elastic dough which results in a crumbling texture, poor color, and low specific volume in cake. Hence, during the last few years, numerous studies have been attempted for improving the physical properties of gluten-free foods, especially baked and fermented foods, by using the many ingredients and additives which could mimic the property of gluten. Gluten-free starches are used as gelling, thickening, adhesion, moisture-retention, stabilizing, film forming, texturizing, and anti-staling ingredients in absence of gluten, where the extent of these properties varies depending on the starch source. In gluten-free products, starch is incorporated into the food formulation to improve baking characteristics such as the specific volume, color, and crumb structure and texture. Corn, rice, buckwheat, waxy high amylose oat, potato, quinoa, sorghum, tapioca, and amaranth have been used as conventional sources of starch, whereas acorn, arrowroot, banana, black beans, breadfruit, chestnut, chickpea, cow pea, kudzu, lentils, lotus, mung bean, navy bean, pinto bean, sago, taro, white yam, yam, yellow pea have been used as unconventional sources of starch (Mazzeo et al., 2015) . Some alternative source of suitable nutrients for celiac sufferers is found in soybean and its derivatives. When gluten-free flour is mixed to form batter, it does not continuous phase or batter structure and consequently fails to produce good quality cake. A gluten-free product is not only essential for managing signs and symptoms of gluten intolerant but also other medical conditions associated with gluten. A gluten-free diet is also popular among people who haven't been diagnosed with a gluten-related medical condition. The claimed benefits of the diet are improved health, weight loss and increased energy, but more research is needed. Development of gluten-free cake for gluten intolerant individuals was done in this study. This will help the consumer for a safe food for consumption and gives clear idea to the person associated with this business about the production of new product for the consumers. The knowledge emerged from the study will improve the branch of food science and nutrition.

In this study, a new product was prepared for gluten intolerant individuals so that they can add a qualityful and nutritive food item in their gluten-free diet. Therefore, this study is designed to study the effect of using some different gluten-free flour (rice, oat and almond flour) and mixture of rice, oat, almond and corn flour on the rheological properties of cake quality.

Aim and Objectives of the Study

The study aim is to formulate gluten-free cake for gluten intolerant individuals by using some gluten-free flour (rice, oats, almond) and mixture of rice, oats, almond and corn flour. The objectives of the study include:

- To evaluate the baking quality of prepared gluten-free cakes and acceptability of the cakes
- To evaluate the proximate compositions and minerals content of gluten-free cake

Chapter 2: Review of Literature

2.1 Overview of Gluten

Gluten is a complex mixture of hundreds of related but distinct proteins, mainly gliadin and glutenin. It naturally found in some grains including wheat, barley, and rye. It provides no essential nutrients. Gluten is heat stable and has the capacity to act as a binding and extending agent and is commonly used as an additive in processed foods for improved texture, moisture retention and flavor. Gliadin contains peptide sequences that are highly resistant to gastric, pancreatic, and intestinal proteolytic digestion in the gastrointestinal tract. The average daily intake in a Western diet is thought to be 5-20 gm/day and has been implicated in several disorders. Gluten is among the most complex protein networks and plays an important role in determining the rheological dough properties (Jessica et al., 2016).

2.1.1 Health Benefits of Gluten

Gluten is most often associated with wheat and wheat-containing foods that are numerous in our food supply. Negative media attention on wheat and gluten has caused some people to doubt its place in a healthful diet. There is little published research to support these claims; in fact published research suggests the opposite.

In a 2017 study of over 100,000 participants without celiac disease, researchers found no association between long-term dietary gluten consumption and risk of heart disease (Lebwohl et al., 2017). In fact, the findings also suggested that non-celiac individuals who avoid gluten may increase their risk of heart disease, due to the insufficient consumption of whole grains.

Many studies have linked whole grain consumption with improved health outcomes. For example, people who intake highest amount of whole grains including wheat (2-3 servings daily) compared with groups who eating the lowest amounts (less than 2 servings daily) were found to have significantly lower rates of heart disease and stroke, development of type 2 diabetes, and deaths from all causes (Liu et al., 1999).

Gluten may also act as a prebiotic, feeding the “good” bacteria in our bodies. Arabinoxylan oligosaccharide is a prebiotic derived from wheat bran that has been shown to stimulate the activity of bifidobacteria in the colon. These bacteria are normally found in gut of a healthy human. Changes in their amount or activity have been associated with gastrointestinal diseases including inflammatory bowel disease, colorectal cancer, and irritable bowel syndrome (Tojo et al., 2014).

2.1.2 Adverse Health Effect of Gluten

Gluten can cause adverse side effects in certain individuals. Some people react differently to gluten, where the body senses it as a toxin, causing one’s immune cells to overreact and attack it. If an unknowingly gluten sensitive person eating gluten continuously, this creates an adverse health effect. The side effects can range from mild effect such as fatigue, bloating, alternating constipation and diarrhea to severe effect such as unintentional weight loss, malnutrition, intestinal damage.

Due to gluten intake, following disorders can occur-

- Celiac disease, an autoimmune disorder of the small intestine caused by exposure to gluten in the genetically predisposed individuals (Laurin et al., 2002; Hamer, 2005). Estimates suggest that 1 in 133 Americans has celiac disease, or about 1% of the population, but about 83% of them are undiagnosed or misdiagnosed with other conditions (Riddle et al., 2012). Research shows that people with celiac disease also have a slightly higher risk of osteoporosis and anemia (due to malabsorption of calcium and iron, respectively), infertility, nerve disorders and in rare cases cancer (Freemen et al., 2012).

- Non-celiac gluten sensitivity, also referred to as gluten intolerance, an intolerance to gluten that has similar symptoms as seen with celiac disease, but without the accompanying elevated levels of antibodies and intestinal damage. There is not a diagnostic test for gluten intolerance but is determined by persistent symptoms and a negative diagnostic of celiac test.

- Wheat allergy, an allergy to one or more of the proteins (albumin, gluten, gliadin, globulin) found in wheat, diagnosed with positive immunoglobulin E blood tests and a food challenge. Compare this with celiac disease, that is a single intolerance to gluten. Symptoms of wheat allergy range from mild to severe and may include swelling or itching of the mouth or throat, itchy eyes, breathing difficulties, nausea, diarrhoea, cramps, and anaphylaxis. People who test negative for this condition still may have gluten sensitivity. This condition is most often seen in children, which most outgrow by adulthood.

- Dermatitis herpetiformis (DH), a skin rash that results from the intake of gluten. It is an autoimmune response which exhibits itself as a persistent red itchy skin rash that may produce blisters and bumps. Although people with celiac disease may have DH, the reverse is not always true (Wang et al., 2021).

It is important to note that gluten is a problem only for those who react negatively to it, or test positive for celiac disease. Most people can and have eaten gluten most of their lives, without any adverse side effects.

2.2 Importance of Developing Gluten-free Products

The only treatment of people who are intolerant to gluten is to follow a gluten-free diet (Rubio-Tapia et al., 2013). Complete avoidance of gluten enables the intestine to heal, and the nutritional deficiencies and other symptoms to resolve (Dhankar, 2013). A strict maintenance of gluten-free diet also reduces the risk of developing many of the serious long-term complications related to untreated celiac disease. However, following a gluten-free diet is not so easy, as it not only involves eliminating gluten-containing grains and all products that contain them, which requires constant vigilance, but there is also a sense of social isolation and pressure that accompanies the process (Bauman et al., 2008). Since most of the breads, biscuits, pasta, cakes, cookies, breakfast cereals, bagels, soups are made of wheat, avoidance of all these foods indicates a complete change in life style which might not be possible for all. Due to all these reasons, the demand for gluten-free products is increasing day by day. That's why, Food scientists and food producers should give more attention to produce more gluten-free food products and their availability in the market.

2.3 Specific Considerations in the Development of Gluten-free Food Products

Preparation of gluten-free products is a big challenge to the manufacturers with the main challenge of finding suitable alternatives for gluten. The main protein fractions of gluten such as glutenin and gliadin play an important role in baking characteristics because they are responsible for water absorption capacity, cohesivity, viscosity, and elasticity of dough. Hence, removal of gluten is a major problem especially for bakers in terms of quality (Gallagher et al., 2004). Apart from this, other challenges faced by the developers are safety of the product, its acceptability and affordability and being in line with the guidelines approved by FDA (Food and Drug Administration). So, keeping all these points in mind, some specific considerations need to be followed by the manufacturers when developing any gluten-free products. Some considerations are given below:

i. **Avoidance of gluten-containing sources:** The first consideration in the preparation of gluten-free product includes the elimination of any food or food ingredient that contains gluten, as celiac disease is triggered by the ingestion of gluten or its protein fraction such as gliadin in wheat, hordeinss in barley, secalins in rye etc. (Moreno et al., 2014).

ii. **Utilization of alternative sources:** Due to the avoidance of gluten-containing cereals which are the major protein sources in the diet, the alternative gluten-free and protein rich sources such as rice, corn, amaranth, millet, sorghum, lentil, soybean, almonds, pumpkin seeds, arrowroot, potato etc. have to be considered during the manufacturing of gluten-free food products (Moreno et al.,2014; Alajaji et al., 2006 ; Arab at al., 2010; Tosh et al., 2013; Green et al., 2007).

iii. **Assurance of sensory characteristics:** Gluten is the main structure-forming protein in flour which is responsible for the elasticity of dough and also contributes to the desired appearance and crumb structure of many products especially the bakery products (Gallagher et al., 2004). So, one of the main challenge during development of gluten-free product is to ensuring that the product has desired texture as well as mouth feel as the gluten-containing product. To improve textural properties of gluten-free products some gluten alternate components such as starch, plant proteins, animal proteins, hydrocolloids such as gums, pectins, hydroxymethylcellulose, xanthan gums etc. could be use (Moreira et al., 2013; Mahmoud et al., 2013).

iv. **Nutritional value of gluten-free products:** Patients who are following a strict gluten-free diet often suffering from various nutritional deficiencies. A study reported that, signs of vitamin deficiency in celiac patients adhering to gluten-free diet (Hallert et al.). Another study showed that, adults with celiac disease who are following a strict gluten-free diet have significantly lower weight, body mass index, fat and lean body mass (Ciacci et al.). Strict adherence to a gluten-free diet also reduces the fiber consumption in celiac patients. So, a gluten-free product must have good nutritional value.

v. **Should meet recommended dietary allowances (RDA) requirement:** RDA is defined as "the average daily dietary nutrient intake level sufficient to meet the nutrient requirement of nearly 97%–98% healthy individuals in a particular life age" (Shrilakshmi, 2012). Fulfilling the RDA helps to provide necessary nutrients to the body and helps to prevent adult-diet related chronic diseases, such as cardiovascular disease, cancer and osteoporosis. It has been seen that gluten-free diet therapy has often low content of vitamins and minerals such as vitamin B, vitamin D, calcium, iron, zinc, and magnesium, as well as fiber (Wierdsma et al., 2013). When developing a gluten-free product it is important to ensure that nutritional profile of the product should be enough to maintain the RDA of a gluten intolerant individual.

vi. **Should be cost effective:** Various studies and market surveys have shown that, gluten-free diet adds a lifelong economic burden to the patients because of the unavailability of gluten-free products in the market. The prices of gluten-free products much higher as compared to the non gluten-free products. However, to avoid any economic burden on the patients, the gluten-free food products should be cost effective (Lee et al., 2007).

vii. **Should follow the FDA guidelines:** During the development of gluten-free products for gluten intolerant and celiac disease patient, manufacturer must be follow the rules and regulations that are set by Food and Drug Administration (FDA).

2.4 Difference Between Gluten Intolerance and Celiac Disease

Gluten intolerance and celiac disease are different. People with celiac disease have an autoimmune response to gluten. This means their bodies try to fight against gluten as if it was a virus. This reaction causes inflammation and damage to their digestive tracts. Gluten intolerance is not an autoimmune disease. An inflammation can occur after ingestion of gluten in gluten intolerance. Celiac disease is the result of an abnormal gene. People with celiac disease also have high levels of certain antibodies in their blood, which are substances that fight gluten. Gluten Intolerance and celiac disease cause a lot of the same symptoms. But people with gluten intolerance don’t have an abnormal gene or antibodies in their blood.

2.5 Overview of Gluten Intolerance

Gluten Intolerance, also known as Non-celiac gluten sensitivity (NCGS), is a reaction to gluten leading to intestinal and extra-intestinal manifestations that are not mediated by an allergic or immunologic response (Casella et al., 2018). Anyone can have a gluten intolerance but it’s more common in women. Some people are born with a gluten intolerance, others develop it later in their life. Research suggests that about 6% of the

U.S. population is gluten intolerant. It's more common than celiac disease, which affects about 1% of the population.

2.5.1 Signs and Symptoms of Gluten Intolerance

After consuming gluten, gluten intolerant individuals may experience the following signs and symptoms for several hours or days :

Intestinal disturbances includes-

- Abdominal pain.
- Diarrhoea
- Nausea and vomiting
- Body mass loss
- Bloating or gas.

Cutaneous disturbances includes-

- Erythema
- Eczema

General disturbances includes-

- Headache
- Bone and joint pain
- Muscle contractions
- Numbness of hands and feet
- Chronic tiredness

Haematological problem include-

- Anaemia

Behavioural problems includes-

- Disturbance in attention
- Depression
- Hyperactivity
- Ataxia

Dental problem include-

- Chronic ulcerative stomatitis

Many people with gluten intolerance also have irritable bowel syndrome (IBS) (Casella et al., 2018).

2.5.2 Diagnosis of Gluten Intolerance

There are no laboratory markers specific to gluten intolerance. It is still a major limitation of clinical studies, making the differential diagnosis with other gluten-related disorders difficult. The only known antibodies observed in the gluten intolerant patients are IgG antigliadin antibodies (IgG-AGA) which, unfortunately, occur in only a half of the patients (Volta et al., 2012)

However, the standards of food challenge tests in gluten intolerant patients have not been yet developed, therefore the researchers use the protocols adopted in the diagnostics of adverse reactions to food. The healthcare provider carefully reviews the symptoms and medical history of patients. If they suspect, patients have a gluten intolerance, these are the next steps to confirm the diagnosis:

- **Step 1:** The patients eat a gluten containing diet for about six weeks. During this time, healthcare provider performs blood tests and skin tests to rule out a wheat allergy or celiac disease. This isn't a gluten intolerance test.
- **Step 2:** If patients don't have a wheat allergy or celiac disease, the healthcare provider will ask the patients to exclude gluten from their diet for at least six

weeks. Keep a thorough record of patients symptoms during this time, noting which (if any) symptoms improve.

- **Step 3:** If patients symptoms do improve while they were on a gluten-free diet, then patients gradually reintroduce gluten back into their diet. If symptoms return, then it diagnosed that, the patients likely have a gluten intolerance (Catassi et al.,2013)

2.5.3 Treatment of Gluten Intolerance

There's no cure for gluten intolerance. But most people find relief from symptoms by following a gluten-free diet. Probiotics can be added into a gluten free diet because probiotics help to increase the good bacteria in gut. They may reduce symptoms of bloating, gas or constipation. Some research suggests that taking certain enzymes may help the patients to digest gluten. But experts are still investigating this treatment. Before taking any enzymes, patients should talk to healthcare provider (Catassi et al., 2013).

2.6 Overview of Celiac Disease

Celiac disease (gluten sensitive enteropathy), sometimes called sprue or coeliac, is a long-term autoimmune disorder that primarily affects the small intestine triggered by dietary gluten in genetically susceptible persons.

Celiac disease is now considered to be one of the most common chronic conditions affecting mankind and occurs in about 1% of the general population in the United States (Sylvia, 2014).

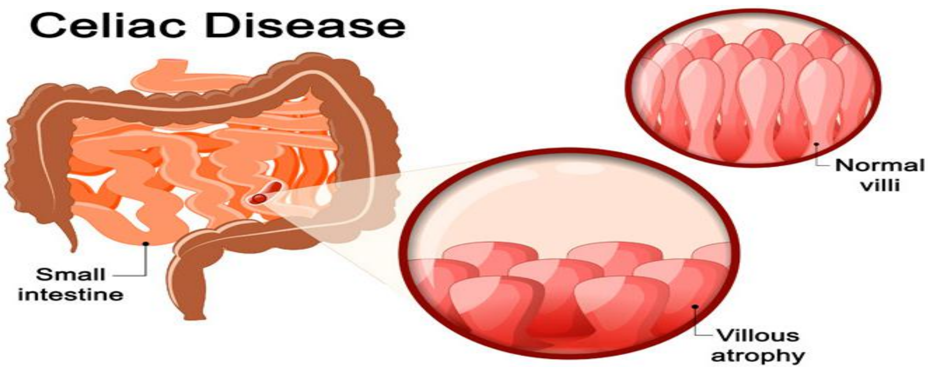

Figure 2.1: Affected villi of celiac disease patient due to gluten intake

In some people who are exposed to gluten in their diet, an enzyme called tissue transglutaminase changes the gluten into a chemical that causes an immune response, leading to inflammation of the lining of the small intestine. The normal finger like projections(villi) that make up the lining of the intestine are blunted and destroyed, preventing the normal absorption of nutrients from the diet. In children, malabsorption can affect the growth and development, in addition to the symptoms seen in adults.

2.6.1 Signs and Symptoms of Celiac Disease

The signs and symptoms of celiac disease can vary greatly and differ in children and adults. Generalized symptoms for adults include:

- Headache
- Fatigue
- Weight loss
- Hair loss
- Joint pain
- Heart palpitations
- Shortness of breath

- Iron deficiency anemia

Gastrointestinal problems include-

- Diarrhoea
- Constipation
- Bloating
- Reflux (heartburn)
- Abdominal pain
- Increased gas after eating
- Bulky or lose stools
- Irritable bowel syndrome

Skin problems includes-

- Itchy rash on elbows, knees
- Blistering rash

Nervous system disturbances includes-

- Numbness and tingling of feet and hands
- Difficulty with balance
- Depression
- Anxiety
- Behavioral changes

Problems in reproductive system includes-

- Infertility
- Still birth
- Recurrent abortion
- Preterm birth
- Low birth weight
- Cesarean section
- Endometriosis
- Pelvic pain
- Severe menstrual cramps
- Missed menstrual periods

Problems in mouth includes-

- Dental enamel problems
- Aphthous ulcers (canker sores)

Structural problems includes-

- Osteopenia
- Osteoporosis (fractured or thin bones)
- Other bone deficiencies
- Fibromyalgia
- Joint pain

Children with celiac disease are more likely than adults to have digestive problems, including:

- Nausea and vomiting
- Chronic diarrhea
- Swollen belly
- Constipation
- Gas
- Pale, foul-smelling stools

The inability to absorb nutrients might result in:

- Failure to thrive for infants
- Damage to tooth enamel
- Delayed growth
- Weight loss
- Anemia
- Diabetes type 1
- Down syndrome
- Short stature
- Delayed puberty
- Neurological symptoms, including attention-deficit/hyperactivity disorder (ADHD), learning disabilities, headaches, lack of muscle coordination and seizures (Sylvia, 2014).

2.6.2 Risk Factors of Celiac Disease

Celiac disease can affect anyone. However, it tends to be more common in people who have:

- A family member with celiac disease or dermatitis herpetiformis
- Type 1 diabetes mellitus
- Down syndrome or Turner syndrome
- Autoimmune thyroid disease
- Microscopic colitis (lymphocytic or collagenous colitis)
- Addison's disease
- Rheumatoid arthritis (Sylvia, 2014).

2.6.3 Complications of celiac disease

Untreated, celiac disease can cause the following complications-

- **Malnutrition:** Due to the destruction of small intestine, it can't absorb enough nutrients. Malnutrition can lead to anemia and weight loss. In children, malnutrition can cause slow growth and short stature.
- **Loss of calcium and bone density:** Malabsorption of calcium and vitamin D can lead to the softening of bone (osteomalacia or rickets) in children and loss of bone density (osteoporosis) in adults.
- **Infertility and miscarriage:** Malabsorption of calcium and vitamin D can contribute to reproductive issues such as infertility, miscarriage, preterm birth, low birth weight.
- **Lactose intolerance:** Damage of small intestine might cause the abdominal pain and diarrhea after eating or drinking dairy products that contain lactose.

- **Cancer:** People with celiac disease who don't maintain a gluten-free diet have a greater risk of developing several forms of cancer, including intestinal lymphoma and small bowel cancer.
- **Neurological problems:** Some people with celiac disease may develop neurological problems such as seizures or peripheral neuropathy (disease of the nerves that lead to the hands and feet).

In children, celiac disease can also lead to failure to thrive, delayed puberty, weight loss, irritability and dental enamel defects, anemia, arthritis and epilepsy (Sylvia, 2014).

2.6.4 Diagnosis of Celiac disease

Many people with celiac disease don't know they have it. Two blood tests can help diagnose it:

- **Serology testing:** It looks for antibodies in patient's blood. Elevated levels of certain antibody proteins indicate an immune reaction to gluten (Leffler et al., 2010).
- **Genetic testing:** Genetic testing for human leukocyte antigens (HLA-DQ2 and HLA-DQ8) can be used to rule out celiac disease.

It's important to be tested for celiac disease before trying a gluten-free diet. Eliminating gluten from patient's diet might make the results of blood tests appear normal. If the results of these tests indicate celiac disease, doctor will likely order one of the following tests:

- **Endoscopy.** This test uses a long tube with a tiny camera that's put into patient's mouth and passed down into the throat (upper endoscopy). The camera enables doctor to view patient's small intestine and take a small tissue sample (biopsy) to analyze for damage to the villi.
- **Capsule endoscopy.** This test uses a tiny wireless camera to take pictures of patient's entire small intestine. The camera sits inside a vitamin-sized capsule,

which is swallowed by patient. As the capsule travels through digestive tract, the camera takes thousands of pictures that are transmitted to a recorder (Leffler et al., 2010).

2.6.5 Treatment of Celiac Disease

A strict, lifelong gluten-free diet (GFD) has been considered the only effective treatment for celiac disease (Ciacci C et al., 2015). Celiac disease patients must avoid the food and drinks that are made from the following gluten containing grains-

- Barley
- Bran
- Bulgur
- Couscous
- Durum
- Farro
- Farina
- Graham flour
- Kamut
- Orzo
- Malt
- Rye
- Semolina
- Spelt (a form of wheat)
- Triticale

- Wheat

Celiac patients should be avoided packaged foods unless they're labeled as gluten-free or have no gluten-containing ingredients, including emulsifiers and stabilizers that can contain gluten. In addition to cereals, pastas and baked goods such as breads, cakes, pies and cookies, other packaged foods that can contain gluten include-

- Beers
- Candies
- Gravies
- Imitation meats or seafood
- Processed luncheon meats
- Salad dressings and sauces, including soy sauce
- Seasoned snack foods, such as tortilla and potato chips
- Self-basting poultry
- Soups

Following basic foods are allowed in a gluten-free diet-

- Eggs
- Fresh meats, fish and poultry that aren't breaded, batter-coated or marinated
- Fruits
- Lentils
- Most dairy products
- Nuts

- Potatoes
- Vegetables
- Wine and distilled liquors, ciders and spirits

Grains and starches allowed in a gluten-free diet include:

- Amaranth
- Arrowroot
- Buckwheat
- Corn
- Cornmeal
- Flax
- Millet
- Montina flour
- Gluten-free flours (rice, soy, corn, potato, bean)
- Pure corn tortillas
- Quinoa
- Rice
- Sorghum
- Soybeans
- Tapioca

2.7 Gluten Free Flour

Gluten free flour is a term that is applied to flours that are made of non-gluten containing products. There are many kinds of gluten free flours available at supermarkets these days, along with many "all purpose" gluten free flour blends that are designed to be an easy to use replacement for wheat flour.

A variety of healthy, gluten-free alternatives to regular or wheat flour exist for people with celiac disease, non-celiac gluten sensitivity or those avoiding gluten for other reasons. Some gluten-free flours have more nutrients than others, making them healthier choices to include in diet.

In this study we used rice flour, oat flour, almond flour and the mixture of rice, oat, almond and corn flour to make gluten free cake.

Rice flour is a common gluten free flour that is widely used in different baking products instead of wheat flour. It is an excellent choice for those suffering from gluten intolerance and autoimmune conditions, such as celiac. It contains a healthy amount of insoluble fibre that helps to improve cardiovascular health, manage blood sugar level and help with digestion. Rice flour is rich in calcium, and this rice flour nutrition makes it an excellent food choice to maintain bone and skeletal health. White rice flour (100gm) contains 9.2 mg of Choline, which prevents the buildup of fat and cholesterol in the liver. Due to its high mineral density, especially Zinc, rice flour can give the body's natural immune response a boost. Rice flour also contains a healthy amount of protein.

Oat flour is made by grinding whole-grain oats. It gives baked goods more flavor than all-purpose flour and results in a chewier, crumblier texture. Baking with oat flour will likely make the end product more moist. Due to its lack of gluten, some ingredients will need to be adjusted to create light and fluffy baked goods. Oat has a well-balanced nutritional composition. It is a good source of carbohydrates and quality protein with good amino acid balance. Oat contains high percentage of oat lipids especially unsaturated fatty acid, minerals, vitamins and phytochemicals (Head et al., 2010). Oats contain a type of soluble fiber called beta-glucan, which has numerous health benefits. This fiber can help lower “bad” LDL cholesterol, as well as blood sugar and insulin levels. Oat flour also rich in other nutrients like protein, magnesium, phosphorus, B-vitamins and the antioxidant group avenanthramides (Lin Nie et al., 2006)

Almond flour is a popular and healthy alternative for people with gluten intolerance and celiac disease. It is rich in manganese that helps the body properly clot blood, allowing it to heal after injuries. Almond flour is also rich in magnesium, which can help to control blood sugar levels. It contains monounsaturated fat that control the body’s cholesterol level. Almond flour is rich in vitamin E and other antioxidants, which help to reduce the risk of serious health conditions like cancer, diabetes, stroke, and heart disease (Kamil et al., 2012)

Corn flour is gluten free and is a great alternative to wheat flour. It contain dietary fibre that helps to reduce the level of LDL cholesterol in the blood which can cause heart-related diseases when accumulated in the body. It also reduce the constipation. Magnesium and potassium are essential minerals contained in cornflour which help to improve bone mineral density. Corn flour helps to reduce the risk of cardiovascular diseases such as high blood pressure, stroke and other related coronary artery diseases because it is scientifically considered a low-sodium diet. Corn flour is enriched with vitamins B's such as vitamin B1, thiamin and B3, niacin and the importance of these vitamins is to elevate the cognitive functions (Siyuan et al., 2018).

2.8 Previous Research on Gluten-free Cake

Ahmed et al. (2012) formulated gluten-free cakes by using sorghum flour, gelatinized corn flour, germinated rice flour, rice flour and their blends that are suitable for celiac patients. Cake made of 100% wheat flour was prepared for comparison. In this study, xanthan gum was used at level of 0.5% to improve the quality of cakes. Chemical composition, dough rheological properties, starch gelatinization, starch crystallinity, samples spectra by Fourier Transform Infra- Red (FT-IR), color attributes, stalling rate, baking quality and sensory properties of cake were evaluated in this study. A remarkable improvement in minerals such as Ca, P, K and Fe and also crude fat and crude fibre was found in Sorghum flour and gelatinized corn flour cake as compared to control cake. All cake samples had lower volume and specific volume than control. Sensory evaluation indicated that all cake samples were acceptable suitable for celiac disease patient but sample which contained sorghum flour, gelatinized corn flour and germinated rice had superior sensory characteristics and nutritional value.

At another study Ammar et al.(2013), they produced gluten free cake for celiac patients which were prepared from cassava, pumpkin, potato flours and their mixture of (1:1:1). Chemical composition and amylogram characteristics of investigated flours were determined. Prepared cake samples were evaluated for their water activity, proximate chemical analysis, physical characteristics (weight, volume and specific volume), color parameters, staling rate and sensory characteristics. All cake samples had good nutritional value and functional characteristics nearest to the control cake (wheat flour cake) sample. But cassava cake had best sensory characteristics than others. In this study they proved that, it is feasible to produce gluten free cake samples from cassava, pumpkin, potato flours and their mixture (1:1:1) for celiac patients.

Atef et al. (2011) formulated gluten-free cake from faba bean and cowpea flours. In this study, the used raw and germinated beans and cowpea flours in ratios of 25%, 50%, 75% and 100% to prepare gluten-free cake and also used 100% wheat flour for control cake. The rheological properties of flour dough and product quality properties (physical, chemical, protein content, color and sensory characteristics) resulted from wheat flour, raw and germinated of either beans or cowpea flours were examined in cake manufacturing. In this study they observed that, in case of raw and germinated flours the water absorption, dough development time (DDT) and dough weakening were increased whereas mixing tolerance index (MTI) and dough stability were decreased. On the other hand, mixing tolerance index (MTI) values was increased in case of germinated legumes flour. From baking properties, color and sensory evaluation test it was proved that the samples were providing good quality and can be used as a substitute of wheat flour.

Other study (Kirbas et al., 2019) showed that, gluten-free cake was produced by using different fibre sources such as apple pomace powder, carrot pomace powder and orange pomace powder on batter rheology and quality characteristics of rice flour. Gluten-free cake batters were formulated by replacing different amounts of rice flour (0,5,10 & 15%) with apple, carrot and orange pomace powder. A control cake also produced from rice flour that contained no pomace powder. The flow behaviors and viscoelastic properties of dietary fibre-enriched cake batters were evaluated. In this study result showed that, apparent viscosity, elastic modulus and viscous of the batter increased with increasing pomace content. Cakes that were contained 5% orange pomace powder had similar volume to the control cake and also received highest consumer acceptability. From this study it is considered that orange pomace powder can be used to produce gluten-free cakes with high percentage of dietary fibre.

Chapter 3: Materials and Methods

3.1 Study Period and Study Area

The research work was conducted for a period of five months from July 2020 to November 2020. Experimental procedures were carried out in the laboratory of the Department of Applied Food Science and Nutrition, Department of Physiology, Biochemistry and Pharmacology, Poultry Research and Training Center (PRTC) at Chattogram Veterinary and Animal Sciences University, Bangladesh.

3.2 Sample Collection and Sample Preparation

Rice flour, corn flour, wheat flour, milk, sugar, baking powder, baking soda, eggs were purchased from the supershop called The Basket, Khulshi, Chittagong. Almonds and oats were purchased from Khulshi Mart supershop which was located at Khulshi area of Chittagong city. The almonds with husk and oats were sun dried for 1 day and then finely blended with a electric blender to form almond flour and oat flour. All flours were stored in air tight containers.

3.3 Preparation of Gluten-free Cake

To prepare gluten-free cake, we followed Ahmed et al., 2012, with some modification. All ingredients were weighed: Flour 150 gm, Sugar 120 gm, whole egg 190 gm, milk 60 ml, baking powder 6 gm, baking soda 2 gm, sunflower oil 60 ml, vanilla essence 2 gm. Four cake formulations were prepared (Rice flour, oat flour, almond flour and mixture of rice, oat, almond and corn flour). In mixture flour cake we use 50 gm rice flour, 40 gm oat flour, 30 gm almond flour and 30 gm corn flour (total 150 gm flour). Also a control cake were prepared with wheat flour.

For the preparation of cake, at first flour, baking powder and baking soda were mixed. Then eggs, sugar, milk, oil and vanilla essence were whipped for 6 minutes by a mixer at high speed. Flour mixture was added gradually to the egg- milk mixture and mixed gently. Then 350 gm of batter was poured in baking pans and placed in a preheated oven and baked at 180 ̊C for 35 min. After baking, cakes were removed from the pans and left to cool for 30 min at room temperature and placed in coded plastic zipper bags to prevent drying.

3.4 Proximate Analysis of Gluten-free Cake

Moisture, protein, fat and ash contents of gluten-free cake samples were measured in triplicate according to AOAC methods. The moisture was measured by oven drying at 105 ̊C to constant weight (AOAC, 2016). The crude protein content was measured by the Kjeldahl procedure (6.25×N). Total lipid was extracted by the AOAC (2016) method using the Soxhlet apparatus. Ash was measured gravimetrically in a muffle furnace by heating at 550 ̊C to constant weight (AOAC, 2003).

3.4.1 Moisture/Water

At first weight of empty crucibles were dried and 5gm of sample was placed on it. Then the crucible was placed in an air oven (thermostatically controlled) and dried at temperature of 105 ̊C for 24 hrs. After drying, the crucible was removed from the oven and cooled in desiccator. It was then weighed with cover glass. The crucible was again placed in the oven, dried for 30 minutes, took out of the dryer, cooled in desiccator and weighed. Drying, cooling and weighing were repeated until the two consecutive weights were same. From these weights, the percentage of moisture in food samples was calculated as follows:

$$\% \text{ Moisture} = \frac{Loss\ of\ weight\ of\ sample}{Initial\ weight\ of\ sample} \times 100$$

3.4.2 Crude Protein

Reagents used: Concentrated H_2SO_4 (98% pure), Digestion mixture ($CuSO_4$: K_2SO_4 =1:9), 4% Boric acid solution, NaOH (35%), mixed indicator solution (Bromocresol green + methyl red), Standard HCl (0.2N).

For estimation of protein, the steps were followed:

Digestion: 0.3g sample, 4g digestion mixture and 5 ml H_2SO_4 was taken in a kjeldahl digestion flask. It was heated at 320 ̊C for 30 mints in a kjeldahl digestion and distillation apparatus. The digestion was completed when the color of the substance was pale yellow.

Distillation: After digestion 25 ml water, 25 ml 35% NaOH and glass blitz were added to kjeldahl flask which containing about 10 ml 4% boric acid and 2-3 drops mixed indicator. Cooled tube and receiving solution were placed into the distillation unit. 25 ml of 35% NaOH was automatically filled into the tube. The distillation process takes place for 3 minutes. The receiving solution turned green at the end of the process.

Titration: The solution collected was titrated with 0.2N HCl solution and titer value was recorded.

Calculation: The calculation of the percent of protein in the sample using protein factor 6.25.

$$\% \text{ Nitrogen} = \frac{ml\ of\ titrant \times Normality\ of\ acid \times meq.\ of\ Nitrogen}{Weight\ of\ sample\ (gm)} \times 100$$

Where,

Normality of acid = 0.2N

meq. of N_2 = 0.014

% Crude Protein = % Nitrogen × 6.25

3.4.3 Ash

The ash content of the samples were determined by the standard AOAC method (AOAC, 2003). In this method, an empty crucible was cleaned properly and dried in a hot air oven. It was placed in desiccators and cooled then the weight was recorded. 3 gm of the sample was weighed and placed in the crucible. It was allowed to burn upto no smoke. The crucible was cooled and transferred to the muffle furnace at 550℃ for 5 hours. The process ends when formation of white ash accomplished. It was cooled at 150°C and then placed to desiccator. When it cooled to mild warm the weight was recorded. Ash content was calculated using the following formula.

Percentage of Ash=$W-W_1/W_2 \times 100$

Where,

W= weight of the crucible with ash

W_1= weight of the empty crucible

W_2= weight of the sample

3.4.4 Crude Fiber

Crude fiber was determined according to AOAC method (2005). At first 2 gm of the sample was weighed and then taken into a beaker. Then 125ml 0f 1.25% sulfuric acid solution and 3-4 drops of n-octanol were added into the same beaker. N-octanol was using as an antifoaming agent. The beaker was boiled for 30 minutes at constant volume. After that, the sample was washed three times to remove the acid. After washing 125ml of 1.25% sodium hydroxide and 3-5 drops of antifoam were added. It was again boiled for another 30 minutes at constant volume. The mixture was filtrated and again washed the residue like before. It was washed again with 1% HCL solution in order to remove the acid. Then the residue was dried in a hot air oven at 105°C until a constant weight was found out. It was placed in a desiccator for cooling and the weight was recorded. Finally, the residue was burned up to smoke and ignited in the muffle furnace at 550-660°C for about 3-4 hours until that turned into white ash. The ash particles were weighed and calculated to determine the crude fiber content of the sample.

Percentage of Crude Fiber=$W-W_1/W_2 \times 100$

Where,

W= weight of crucible containing crude fiber and ash

W1= weight of crucible containing ash

W= weight of the sample

3.4.5 Crude Fat

The dried sample remaining after moisture determination was transferred to a thimble and plugged the top of the thimble with a wad of fat free cotton. The thimble was dropped into the fat extraction tube attached to a Soxhlet flask. Approximately 75ml or more of anhydrous ether was poured into a flask. The top of the fat extraction tube was attached to the condenser. The sample was extracted for 16 hrs or longer on a water bath at 80℃. At the end of the extraction period, the thimble was removed from the apparatus and distilled off most of the ether by allowing it or collected in Soxhlet tube. The ether was poured off when the tube was nearly full. When the ether reached a small volume, it was poured into a small, dry beaker through a small funnel containing a plug of cotton. The flask was rinsed and filtered thoroughly, using ether. The ether was evaporated on a steam bath at low heat; it was then dried at 100℃ for 1hr, cooled and weighed. The difference in the weights gave the ether soluble material present in the sample.

The presence of fat was expressed as follows:

$$\text{Crude Fat} = \frac{Loss\ of\ ether\ soluble\ materials}{Weight\ of\ sample} \times 100$$

3.4.6 Carbohydrate Content

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). Hence it was calculated using the formula below:

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

3.5 Determination of Mineral Content

This method involves the extraction of minerals from the organic food matrix by digestion through wet digestion. The mineral contents in the digested compounds was determined by spectrophotometer (Humalyzer 3000®). Commercially available biochemical kit (Randox) was used for biochemical assay. The whole procedure was done in the Postgraduate Research lab under the Dept of Physiology, Biochemistry and Pharmacology at Chittagong Veterinary and Animal Sciences University. All the analyses were done in triplicates and expressed in mg/dl.

Apparatus: Beaker, Measuring pipets, Volumetric flask, Analytical balance, Heating mantle or hot plate, Filter paper, Whatman® No. 541

Required Reagent: Nitric acid and Perchloric acid

Procedure: One (01) g of dry sample was weighted in a conical flask. For dried samples, 7.5 ml conc. HNO_3, and 2.5 ml conc. $HClO_4$ in the ratio of 2:1 was prepared. For wet sample, 5 ml HNO_3 and 1 ml $HClO_4$ was added (HNO_3: $HClO_4$ = 5:1). Then the flask was placed in a hot plate at 200W for 1-2 hours until full digestion. After digestion, it was cooled at room temperature. Then transferred the digested samples into 100 ml volumetric flask and diluted up to 100 mark with Deionized water and mixed well. Later, the solution was filtered through Whatman® filter paper No. 1 and transfer to Eppendorf Tube for mineral quantification (Schoenfeld et al., 1964; Prince et al., 2003; Chauhan et al., 1969; AACC, 2000).

3.5.1 Determination of Sodium (Na)

Sodium is precipitated as a triple salt with magnesium and Uranyl acetate. The excess of uranyl ions are reacted with ferrocyanide in an acidic medium to develop a brownish colour. The intensity of the colour produced is inversely proportional to the concentration of sodium in the sample (Schoenfeld, 1964).

Procedure:

Table 3.1: Sodium (Na) determination

Step 1: Precipitation

	Pipette into cuvette	
	Blank	**Standard**
Precipitating Reagent(L1)	1.0 ml	1.0 ml
Sodium Standard	20 µl	-
Sample	-	20 µl

Mix well and let stand at R.T. for 5 mins. With shaking well intermittently. Centrifuge at 2500 to 3000 RPM to obtain a clear supernatant.

Step 2: Color Development

	Pipette into cuvette		
	Blank	**Standard**	**Sample**
Acid Reagent(L2)	1.0 ml	1.0 ml	1.0 ml
Supernatant from step 1	-	20 µl	20 µl
Precipitating Reagent(L1)	20 µl	-	-
Colour Reagent(L3)	100 µl	100 µl	100 µl

Calculations:

Sodium in mmol / L = $\frac{\text{(A)blank} - \text{(A)sample}}{\text{(A)blank} - \text{(A)standard}}$ × Standard conc. (mmol / L)

3.5.2 Determination of Potassium (K)

Principle: Sodium tetraphenyl boron reacts with potassium to produce a fine turbidity of potassium tetraphenyl boron. The intensity of turbidity is directly proportional to the concentration of potassium in the sample (Schoenfeld, 1964).

Procedure:

Table 3.2: Potassium (K) determination

	Pipette into cuvette		
	Blank	**Standard**	**Sample**
Sample	-	-	0.02ml
Deionized water	0.02ml	-	-
Standard	-	0.02ml	-
K^+ Reagent	1.0ml	1.0ml	1.0ml

Calculation:

Potassium in (mmol / L) = $\frac{\text{(A)sample}}{\text{(A)standard}}$ × Standard conc. (mmol / L)

3.5.3 Determination of Calcium (Ca)

Principle: Calcium ions form a violet complex with O-Cresolphthalein complexone in an alkaline medium (Prince et al., 2003).

Procedure:

Table 3.3: Calcium (Ca) determination

	Pipette into cuvette		
	Reagent blank SO	**Standard SI**	**Sample**
Sample	-	-	25µl
Distilled water	25 µl	-	-
Standard	-	25 µl	-
Working Reagent	1.0ml	1.0ml	1.0ml

Calculation:

Concentration in mg / dl = $\frac{(A)sample}{(A)standard}$ × Standard conc. (mg/dl)

3.5.4 Determination of Magnesium (Mg)

Principle: The method is based on the specific binding of calmagite, a metallochromic indicator and magnesium at alkaline pH with the resulting shift in the absorption wavelength of the complex. The intensity of the chromophore formed is proportional to the concentration of magnesium in the sample (Burtis et al., 2012).

Procedure:

Table 3.4: Magnesium (Mg) determination

	Pipette into cuvette		
	Blank	**CAL. Standard**	**Sample**
Sample	-	-	10µl
CAL. Standard	-	10µL	-
R1. Reagent	1.0ml	1.0ml	1.0ml

Calculation:

Magnesium (mg /dl) = $\frac{\text{(A)sample}}{\text{(A)standard}} \times$ Standard conc. (mg/dl)

3.5.5 Determination of Phosphorus (P)

Principle: Inorganic phosphate reacts with ammonium molybdate in the presence of sulfuric acid to form a phosphomolybdic complex which is measured at 340nm (Burtis et al., 2012).

Procedure:

Table 3.5: Phosphorus (P) determination

	Pipette into cuvette		
	Blank	**CAL. Standard**	**Sample**
Sample	-	-	10µl
CAL. Standard	-	10µl	-
R1. Reagent	1.0ml	1.0ml	1.0ml

Calculation:

Phosphorus concentration (mg / dl) = $\frac{\text{(A)sample}}{\text{(A) Standard}}$ × Standard conc. (mg/dl)

3.5.6 Determination of Iron (Fe)

Principle: The iron is dissociated from transferring-iron complex in weakly acid medium. Liberated iron is reduced into the bivalent form by means of ascorbic acid. Ferrous ions give with FerroZine a colored complex. The intensity of the color formed is proportional to the iron concentration in the sample (Elizabeth, 1992).

Procedure:

Table 3.6: Iron (Fe) determination

	Pipette into cuvette		
	Blank	**Standard**	**Sample**
Sample	-	-	100µl
Standard	-	100µl	-
Reagent	1.0ml	1.0ml	1.0ml

Calculations:

Iron in µg / dl= $\frac{\text{(A)sample} - \text{(A)sample blank}}{\text{(A)standard}}$ × Standard conc. (mg/dl)

3.6 Baking Quality of Cake

Volume(cm^3) and weight (gm) of five cake samples were recorded. Specific volume (gm/cm^3) was calculated as the ratio between the volume of the cake and its weight according to the method described in AACC (2000).

3.7 Sensory Evaluation

Prepared cake samples were subjected to sensory evaluation by 20 panelists. The cake samples were coded with four digit numbers and presented in a randomized order. The panelists were asked to evaluate each cake for color, odor, softness, taste, crumb texture, and overall acceptability. Each panelist scored samples independently and recorded the scores on the sheets provided. A 7 point hedonic scale were used. The scale was arranged such that : Dislike very much=1, Dislike moderately= 2, Dislike slightly= 3, Neither like or dislike= 4, Like slightly= 5, like moderately= 6, Like very much=7 (Ostermann-Porcel et al., 2020; Akosua et al., 2015).

3.8 Statistical Analysis

Data (proximate composition, minerals content and sensory evaluation) were determined and stored in Microsoft Excel 2013 spread sheet to evaluate statistical analysis. All samples were in three replicates. Descriptive statistics (mean, standard deviation) were done for proximate composition, mineral contents and sensory evaluation of all cake samples. Data were sorted, coded and recorded in IBM SPSS Statistics 26. After that statistical analysis were conducted. Proximate composition, mineral content and sensory evaluation data were analyzed by using One-way ANOVA procedures to assess significant level of variation at 95% confidence interval. Post hoc "Tukey" test was conducted to identify the variation within the sample groups. The statistical analysis was conducted for at 5% level of significant ($P < 0.05$).

Chapter 4: Result

4.1 Proximate Analysis of Cakes

Data presented in Table 4.1 showed and compared the proximate composition of five types of cakes such as rice flour cake, oat flour cake, almond flour cake, mixture flour(rice, oat, almond and corn) cake and wheat flour cake. Wheat flour cake had the highest moisture content (32.18 ± 0.11)% whereas mixture flour cake had the lowest moisture content (21.57 ± 0.24)%. Protein content was higher (11.00 ± 0.22)% in almond flour cake and lower (7.70 ± 0.12)% in rice flour cake. Mixture flour cake had the highest carbohydrate content (51.86 ± 0.31)% and almond flour cake had the lowest carbohydrate content (30.93 ± 0.27)%. The highest value of crude fibre (1.79 ± 0.23)% and (1.79 ± 0.66)% was found in both rice and almond flour cake respectively and lowest (1.05 ± 0.07)% in wheat flour cake. Almond flour cake had the highest content of crude fat (23.57 ± 0.17)% and ash (1.87 ± 0.21)% whereas rice flour had the lowest (10.66 ± 0.20)% and.(0.96 ± 0.13)% respectively. Descriptive statistics and Post hoc Tukey test in One-way ANOVA procedures were conducted to analyze data statistically at 5% level of significance.

Table 4.1: Proximate composition of cake samples

Cake samples	Moisture (%)	Protein (%)	Crude Fiber (%)	Crude Fat (%)	Ash (%)	CHO (%)
Rice Flour Cake	31.19 ± 0.15^{b}	7.70 ± 0.12^{d}	1.79 ± 0.23^{a}	10.66 ± 0.20^{e}	0.96 ± 0.13^{c}	47.70 ± 0.21^{c}
Oat Flour Cake	23.93 ± 0.06^{d}	9.36 ± 0.07^{b}	1.45 ± 0.21^{ab}	12.57 ± 0.44^{d}	1.35 ± 0.24^{b}	51.34 ± 0.25^{b}
Almond Flour Cake	30.84 ± 0.09^{c}	11.00 ± 0.22^{a}	1.79 ± 0.66^{a}	23.57 ± 0.17^{a}	1.87 ± 0.21^{a}	30.93 ± 0.27^{e}
Mixture Flour Cake	21.57 ± 0.24^{e}	8.31 ± 0.28^{c}	1.18 ± 0.16^{ab}	15.98 ± 0.13^{b}	1.10 ± 0.07^{bc}	51.86 ± 0.31^{a}
Wheat Flour Cake	32.18 ± 0.11^{a}	8.75 ± 0.40^{c}	1.05 ± 0.07^{b}	14.84 ± 0.48^{c}	1.07 ± 0.09^{bc}	42.11 ± 0.17^{d}

Legends: All values in the table showed (ME±SD) of data, where ME= Mean and SD= Standard Deviation, superscripts a, b, c,d,e denotes significant difference ($p \leq 0.05$) among samples.

4.2 Mineral Contents of Cakes

Table 4.2 showed the mineral contents of five cake samples. Sodium and Phosphorus were higher in oats flour cake whereas Sodium was lower in mixture flour cake and Phosphorus was lower in wheat flour cake. Potassium, Magnesium and Iron were higher in almond flour cake whereas potassium lower in wheat flour cake, magnesium lower in rice flour cake and iron lower in mixture flour cake. Calcium was higher in wheat flour cake and lower in oat flour cake.

The highest Sodium, Potassium, Calcium, Magnesium, Phosphorus and Iron were (723.64 ± 0.13) mg/dl, (37.83± 0.39) mg/dl, (6.72 ± 0.05) mg/dl, (1.10 ± 0.06) mg/dl, (0.78 ± 0.05) mg/dl, (0.0835± .001) mg/dl respectively and the lowest values were (334.83 ± 0.27) mg/dl, (5.41± 0.20) mg/dl, (4.22 ± 0.05) mg/dl, (0.52 ± 0.05) mg/dl, (0.41 ± 0.08) mg/dl, (0.0508± .001) mg/dl respectively

Table 4.2: Minerals content of cake samples

Minerals (mg/dl)	Rice Flour Cake	Oat Flour Cake	Almond Flour Cake	Mixture Flour Cake	Wheat Flour Cake
Na	613.82 ± 0.31^{c}	723.64 ± 0.13^{a}	631.74 ± 0.08^{b}	334.83 ± 0.27^{e}	352.85 ± 0.12^{d}
K	9.18 ± 0.50^{c}	10.84 ± 0.49^{b}	37.83 ± 0.39^{a}	10.81 ± 0.48^{b}	5.41 ± 0.20^{d}
Ca	4.79 ± 0.05^{c}	4.22 ± 0.05^{d}	5.62 ± 0.04^{b}	4.80 ± 0.05^{c}	6.72 ± 0.05^{a}
Mg	0.52 ± 0.05^{d}	0.61 ± 0.05^{cd}	1.10 ± 0.06^{a}	0.69 ± 0.03^{c}	0.79 ± 0.05^{b}
P	0.70 ± 0.05^{a}	0.78 ± 0.05^{a}	0.60 ± 0.04^{b}	0.52 ± 0.07^{b}	0.41 ± 0.08^{c}
Fe	$0.0626 \pm .009^{c}$	$0.0518 \pm .002^{d}$	$0.0835 \pm .001^{a}$	$0.0508 \pm .001^{e}$	$0.0784 \pm .001^{b}$

Legends: All values in the table showed (ME±SD) of data, where ME= Mean and SD= Standard Deviation, superscripts a, b, c,d,e denotes significant difference ($p \leq 0.05$) among samples.

Figure 4.1 showed that oat flour cake had highest Na content and mixture flour had lowest.

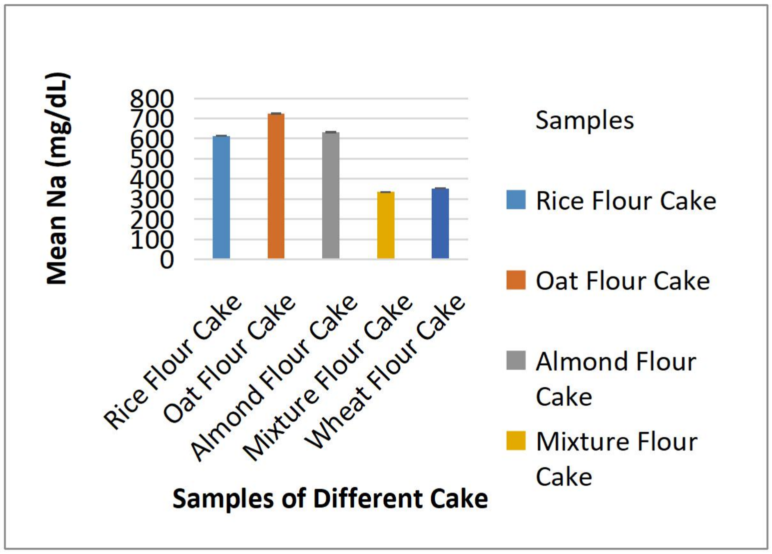

Fig 4.1: Comparative presentation of Na content of all cake samples

Figure 4.2 showed that K was highest in almond flour cake and lowest in wheat flour cake.

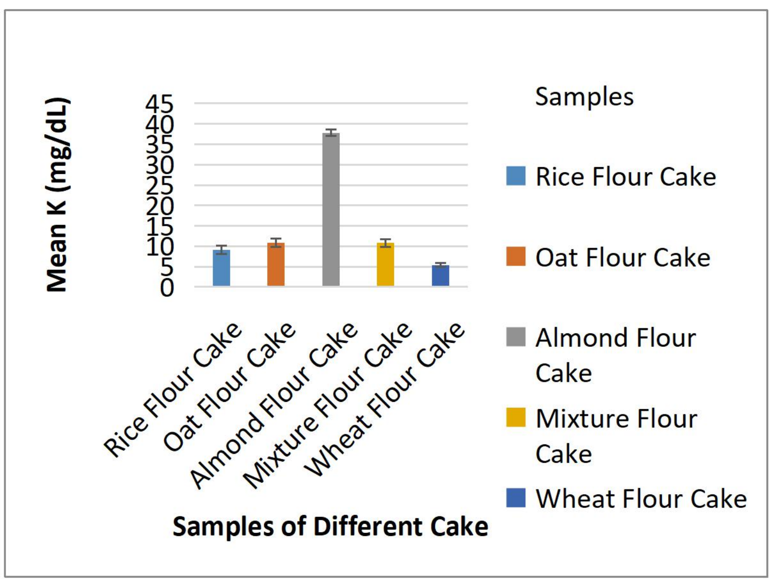

Fig 4.2: Comparative presentation of K content of all cake samples

Figure 4.3 showed that Ca was highest in wheat flour cake and lowest in cake produced from oat flour.

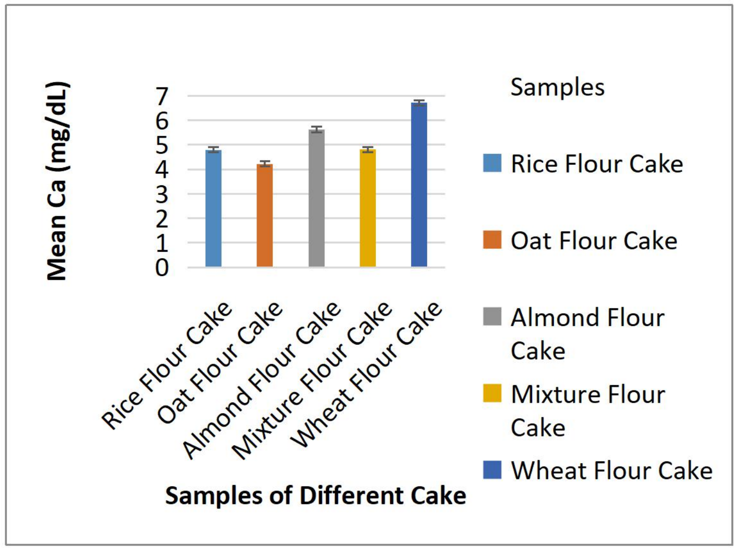

Fig 4.3: Comparative presentation of Ca content of all cake samples

Figure 4.4 showed, Cake produced from almond flour had highest Mg content than other cake samples.

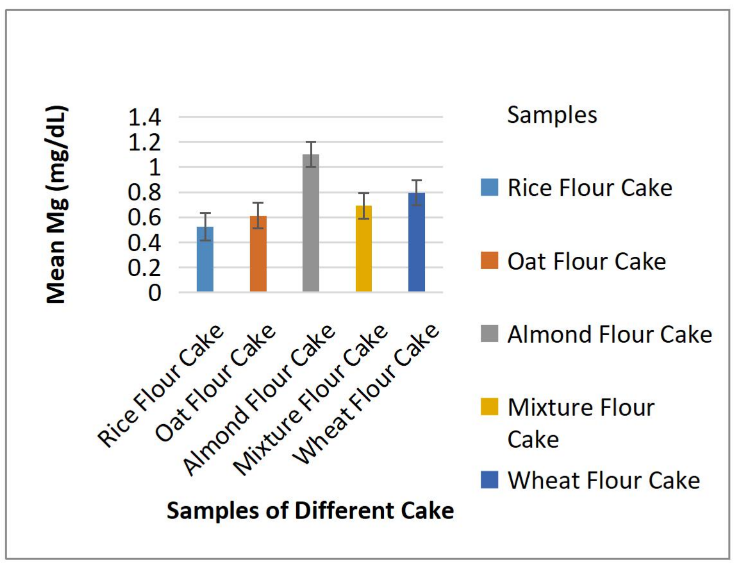

Fig 4.4: Comparative presentation of Mg content of all cake samples

Figure 4.5 showed that, P was highest in oat flour cake and lowest in wheat flour cake.

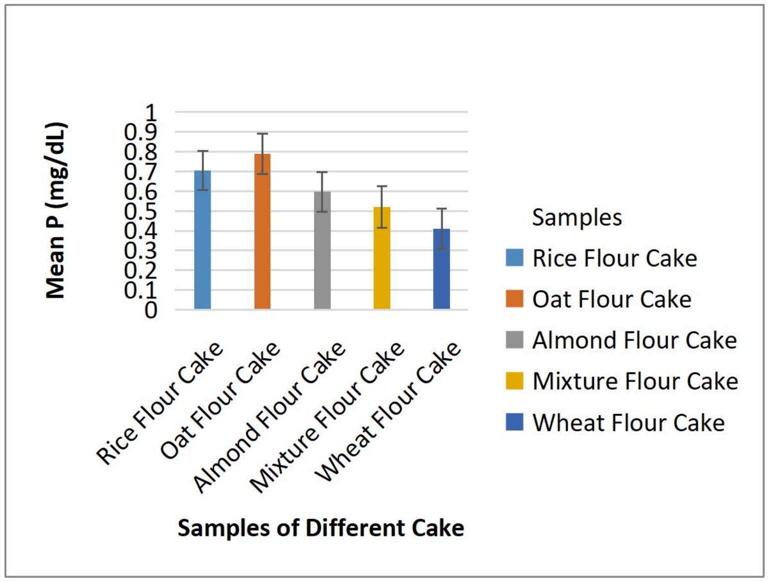

Fig 4.5: Comparative presentation of P content of all cake samples

Figure 4.6 showed that, Almond flour cake had the highest Fe content and mixture flour cake had the lowest Fe content.

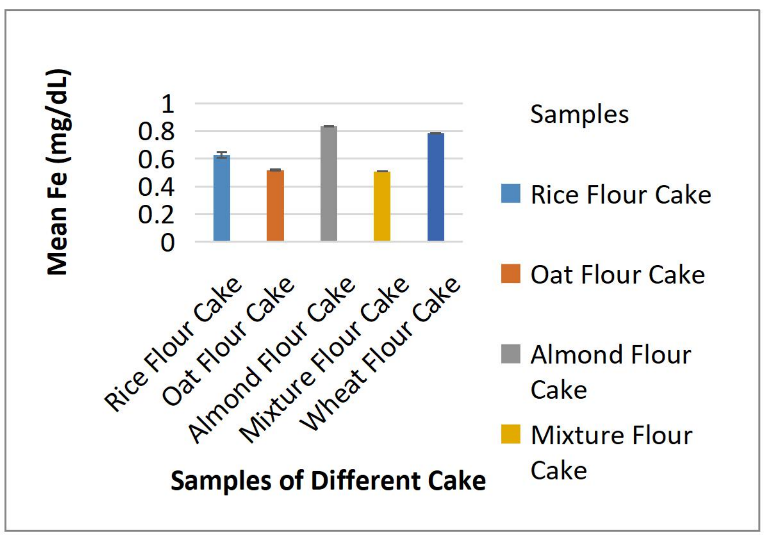

Fig 4.6: Comparative presentation of Fe content of all cake samples

4.3 Baking Quality of Cakes

The physical characteristics of the produced cakes were presented in Table 4.3. Volume of the cake produced from wheat flour (control) (726.75 cm^3) was highest compared with other cake samples, while weight of the cake produced from oat flour and mixture flour (340gm) were highest compared with other samples. From the same table, cake produced from wheat flour had the highest specific volume (2.17cm^3/gm) than other cake samples. On the other hand, oat flour cake had lowest specific volume (1.52cm^3/gm) compared with other cakes.

Table 4.3: Baking quality of cake samples

Cake samples	Baking Quality		
	Weight (gm)	Volume(cm^3)	Specific volume (cm^3/gm)
Rice flour cake	330	549.45	1.67
Oat flour cake	340	516.15	1.52
Almond flour cake	325	532.8	1.64
Mixture flour cake	340	532.8	1.57
Wheat flour cake	335	726.75	2.17

4.4 Sensory Characteristics of Cake

The sensory attributes of cakes produced from rice flour, oat flour, almond flour, mixture flour and wheat flour were evaluated for color, odor, softness, taste, crumb texture and overall acceptability in table 4.4. In table, the highest score for color, taste, crumb texture and overall acceptability were recorded (6.70±0.571), (6.15±0.813), (6.05±0.945) and (6.30±0.733) respectively in case of wheat flour cake, highest score for odor was

(6.00±0.973) in mixture flour cake and highest score for softness was (6.60±0.681) in almond flour cake.

On the other hand, the lowest score for color, odor, taste and overall acceptability were recorded (5.40±1.392), (5.15±1.040), (5.40±1.273) and (5.40±1.14) respectively for oat flour cake. For softness and crumb texture, lowest value were (4.90±1.210) and (5.55±1.317) respectively for mixture flour cake.

Table 4.4: Sensory quality of cake samples

Sensory attributes	Rice Flour Cake	Oat Flour Cake	Almond Flour Cake	Mixture Flour Cake	Wheat Flour Cake
Color	6.05 ± 0.759^{c}	5.40 ± 1.392^{bc}	6.25 ± 1.251^{abc}	5.65 ± 1.226^{ab}	6.70 ± 0.571^{a}
Odor	5.45 ± 1.191^{b}	5.15 ± 1.040^{ab}	5.50 ± 1.606^{ab}	6.00 ± 0.973^{a}	6.00 ± 0.795^{a}
Softness	5.10 ± 1.334^{b}	5.00 ± 1.214^{b}	6.60 ± 0.681^{a}	4.90 ± 1.210^{a}	6.45 ± 0.605^{a}
Taste	5.50 ± 1.192^{a}	5.40 ± 1.273^{a}	6.10 ± 1.021^{a}	5.55 ± 1.317^{a}	6.15 ± 0.813^{a}
Crumb texture	5.60 ± 1.095^{a}	5.65 ± 1.182^{a}	6.00 ± 1.556^{a}	5.55 ± 1.317^{a}	6.05 ± 0.945^{a}
Overall accept-ability	5.60 ± 1.14^{b}	5.40 ± 1.14^{ab}	6.00 ± 1.338^{ab}	5.60 ± 1.392^{ab}	6.30 ± 0.733^{a}

Legends: All values in the table showed (ME±SD) of data, where ME= Mean and SD= Standard Deviation, superscripts a, b, c denotes significant difference ($p\leq0.05$) among samples.

Chapter 5: Discussion

5.1 Proximate Analysis of Cakes

The moisture content of the cake samples were slightly higher than that of other study report (Ahmed et al., 2012). Moisture content plays a significant role in establishing proper conditions for the preservation, storability, packaging and shipping of food products (Maur et al., 2017). It's essential for meeting compositional specifications and standards of identity, as well as computing the nutritional value by expressing analytical results on uniform dry or moist basis. The protein content of the cake samples were close to the other studies (Ahmed et al., 2012) and (Ammar et al., 2013). The ash content of the cakes were higher than the report of other study (Ammar et al., 2013) but slightly close to the another study (Ahmed et al., 2012). Fiber is an important component in preventing overweight, constipation, diabetes, an increase of serum cholesterol, risk of heart diseases, breast and colon cancer, hypertension, etc. The crude fibre content of all cake samples were slightly close to other study (Ahmed et al., 2012). Fat is a concentrated source of energy which is stored in the body as reserves to be used when energy supply is required by the body. Generally, fat helps in the protection of the internal organs such as heart, kidney, lungs and subcutaneous tissues of the skin. The crude fat content of cakes were found higher than that of the results of other study (Ahmed et al., 2012). The most abundant nutrient was found to be the carbohydrate.The carbohydrate content of cake samples were lower than the other study (Ahmed et al., 2012). From the results of the proximate analysis established, the gluten-free cake samples will be the suitable food item for gluten intolerant individuals.

5.2 Minerals Content of Cake Samples

Minerals play an important role in maintaining proper function and good health in the human body. Inadequate intake of minerals in the diet is often associated with an increased susceptibility to infectious diseases due to the weakening of the immune system. Table 4.2 showed the minerals content of five cake samples. Sodium and Phosphorus were higher in oats flour cake whereas Sodium was lower in mixture flour cake and Phosphorus was lower in wheat flour cake. Potassium, Magnesium and Iron were higher in almond flour cake whereas potassium lower in wheat flour cake, magnesium lower in rice flour cake and iron lower in mixture flour cake. Calcium was higher in wheat flour cake and lower in oat flour cake. In this study, all gluten free cakes are good source of minerals than control (wheat flour) cake. In the study, the mineral content of all gluten-free cake samples and control cake sample varied significantly. The mineral content of all cake samples were slightly close to other study (Ahmed et al., 2012).

5.3 Baking Quality of Cakes

Baking quality of cake depends on the weight, volume and specific volume of cake. In this study, almond flour cake had the lowest weight than other samples. Volume and specific volume were higher in wheat flour cake. Gluten plays an important role in the baking quality of cake. In gluten-free cake, hydrocolloids such as pectin, guar gum and xanthan gum are added to naturally gluten-free flours to mimic the viscoelastic properties of gluten and to improve the structure, sensory attributes and shelf-life of the cakes (Moore et al.,2006; Lazaridou et al., 2007). But in this study, no hydrocolloids were added to gluten-free cake samples to evaluate the actual baking quality of the gluten-free cakes. In this study, rice flour cake had the highest volume and specific volume than other gluten-free cake samples and oat flour cake had the lowest.

5.4 Sensory Characteristics of Cake

The sensory attributes of cakes produced from rice flour, oat flour, almond flour, mixture flour and wheat flour were evaluated for color, odor, softness, taste, crumb texture and overall acceptability.as shown in Table 4.4 in comparison to cake sample prepared from 100% wheat flour as control samples. All cake samples were acceptable to the consumer. Data in Table 4.4 show that there are significant differences ($p \leq 0.05$) among the different prepared cake samples. Almond flour cake was the best in all sensory properties nearest to wheat flour cake except odor where mixture flour cake had a highest sensory score of odor that was more than the wheat flour cake. From this study we can say that, there is no significant difference between almond flour cake and wheat flour cake.

Chapter 6: Conclusion

This study concludes that the formulation of gluten-free cakes from gluten-free flours is an exceptional value-added product and is also seen to have acceptability with respect to all parameters like color, odor, softness, taste and crumb texture. The nutrient profile of the product was also appealing from health point of view. Rice flour cake had the highest moisture content which was near to the control (wheat flour) cake and mixture flour cake had the lowest. Protein, crude fat and ash content were higher in almond flour cake than wheat flour cake. Rice flour and almond flour cake had the highest crude fat content than control cake. Carbohydrate was highest in both oat flour and mixture flour cake. Sodium and phosphorus were higher in oat flour cake. Potassium, magnesium and iron were higher in almond flour cake. From the result it could be concluded that, all the cake samples which are produced from rice, oat, almond flours and mixture of these flour are suitable for gluten hypersensitive individuals. They have good nutritional value and also have good sensory characteristics nearest to cake sample made from wheat flour (100%). The sample which was made from almond flour seems to be superior for sensory characteristics and nutritional value but there were no big significance differences among the samples.

In conclusion, we can say that our product development was successful. Our results indicate that there were no huge differences between the products in all attributes and this provides a free choice to the bakery industry as to whose production is worthy from a technological or economical point of view. The formulated cake can be a new product for gluten intolerant individuals. The knowledge emerged from this study will improve the branch of food science and nutrition. Further research is needed to investigate bioactive compounds, antioxidant properties and the shelf-life of the products.

Chapter 7: Recommendations and Future Perspectives

Now a days, many people are suffering from gluten intolerance and celiac disease. A gluten-free product is not only essential for managing signs and symptoms of gluten intolerance but also other medical conditions associated with gluten. A gluten-free diet is also popular among people who haven't been diagnosed with a gluten-related medical condition. The claimed benefits of diet are improved health, weight loss and increased energy but more research is needed.

Present study is conducted to investigate the formulation and quality (baking, proximate, minerals, sensory) evaluation of gluten-free cakes from gluten-free flour such as rice, oat, almond flour and mixture of these flour. On the basis of present investigation, the following suggestions and prospects are made for the further research work.

- The present studies may be repeated for confirmation of the experimental findings.
- The physicochemical characteristics, bioactive compounds and antioxidant capacity of the cake extracts should be evaluated.
- This study can also be repeated with addition of hydrocolloids such as pectin, guar gum, xanthan gum etc. to improve structure, sensory attributes and self-life of these products.
- Such types of research should be done for other gluten free flours like okara, sorghum, arrowroot, potato, soy etc.
- Gluten-free cakes should be made commercially for gluten intolerant individuals.

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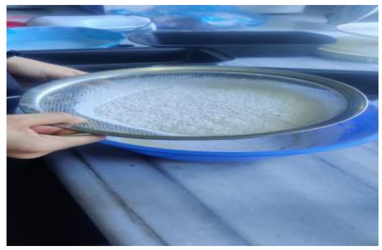
Appendices

Appendix A: Photo Gallery

Appendix A1: Pictorial Presentation of Preparation of Gluten-free Cakes

Weighing of ingredients

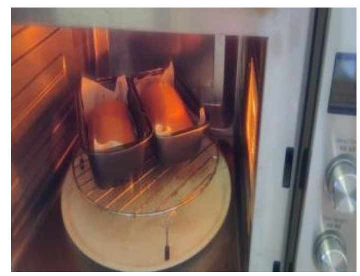


Adding all ingredients

Mixing

Greasing the pan

Weighing the batter



Baking

Cooling of cake

Storing

Rice flour cake

Oat flour cake

Almond flour cake

Mixture flour cake

Wheat flour cake

Sensory Evaluation

Appendix A2: Pictures of Laboratory Work

Sampling for moisture determination

Sampling for ash determination

Crude fat determination

Crude fibre determination

Crude protein determination

Minerals determination

Appendix B: Hedonic Rating Test For Gluten-free Cake

Date: Sample code:

Instruction:

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

Colour

Dislike extremely Neutral Like extremely

Odour

Dislike extremely Neutral Like extremely

Softness

Dislike extremely Neutral Like extremely

Taste

Dislike extremely Neutral Like extremely

Crumb texture

Dislike extremely Neutral Like extremely

Overall acceptability

Dislike extremely Neutral Like extremely

Comment (if any)

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Brief Biography

Sumiya Akter passed Dakhil examination in 2010 and Higher Secondary Certificate Examination in 2012. She obtained her B.Sc. (Hon's) 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, Faculty of Food Science and Technology, Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh. She has an immense interest in exploration on clinical nutrition and dietetics to improve the health of people through proper guidelines and suggestions with a vision of improving the overall nutritional status of Bangladesh.