EFFECTS OF SPINACH (*Spinacia oleracea*) POWDER ON THE PHYSICAL, PHYSIOCHEMICAL AND SENSORY QUALITIES OF BISCUITS

A Thesis

By

ARMIN CHOWDHURY RUMPA

Examination Roll No. 1605569

Admission Semester: July-December, 2016

MASTER OF SCIENCE (MS)

IN

FOOD ENGINEERING AND TECHNOLOGY

DEPARTMENT OF FOOD ENGINEERING AND TECHNOLOGY

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY DINAJPUR

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June 2018

DEDICATED

TO

MY BELOVED

PARENTS

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The Author

Abstract

Low-calorie and high-fiber vegetable powder supplemented biscuits have a great demand among the biscuit consumers. Therefore, an attempt was undertaken to prepare composite biscuit supplementing wheat flour by spinach powder. A multistoried universal cabinet dryer, developed in the laboratory of Food Engineering and Technology department, was used to dry raw spinach at a temperature of 55-60 ^{0}C and powder was prepared by grinding and sieving through 300 MSI mesh. The powder was found to be rich in protein, fiber, fat and ash but low in carbohydrate and energy compared to wheat flour. Three biscuit samples were prepared by supplementing wheat flour at the rate of 3%, 5% and 7% those were coded as F_1, F_2, and F_3, respectively. Further, one control sample was prepared to compare quality of the proposed samples. Physical, physiochemical and sensory quality of the samples were evaluated. Spinach powder incorporation increased weight, diameter and density of the biscuits and decreased thickness, spread ratio and energy value compared to sample C. Lower values of moisture content 3.26%, 3.15% and 3.12% and fat content 14.67%, 14.57% and 14.74% were obtained in case of F_1, F_2 and F_3 sample than sample C (moisture content 3.85% and fat 20.12%). Spinach powder enhanced protein, fiber, ash, and carbohydrate content (from 6.53%, 1.16%, 1.54%, and 66.72% up to 10.18%, 1.93%, 3.7% and 69.75% respectively) in F_1, F_2 and F_3 samples. The biscuits were found to be rich in essential minerals like calcium, iron and phosphorus ranged from 123.67 to 261.49 mg/ 100g, 1.20 to 5.19 mg/ 100g and 61.46 to 112.87mg/ 100g respectively. Furthermore, ANOVA analysis showed significant differences in sensory attributes (color, flavor, texture, taste and overall acceptability) of the biscuits samples. The preference values for all the attributes are higher for control sample. Among the composite biscuit samples F_1 (supplemented with 3% spinach powder) obtained higher scores for color, flavor, texture, taste and overall acceptability (7.2, 7.13, 7.0, 7.2 and 7.73 respectively). The current study revealed higher amount of nutrition in all the composite biscuits with suitable formulae and biscuit with 3% spinach powder (F_1) was mostly preferred by the consumers.

ABBREVIATIONS

ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemist
^{0}C	Degree centigrade
cm	Centimeter
DMRT	Duncan's Multiple Range Test
et al.	And others
FAO	Food and Agriculture Organization
Fig	Figure
g	Gram
ha	Hectare
Kg	Kilogram
mg	Milligram
SE	Standard Error
SAS	Statistical Analysis System
%	Percentage

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CHAPTER I

INTRODUCTION

Spinach (*Spinach oleracea*) is an edible flowering plant in the family of Amaranthaceae. Spinach is an annual plant having medicinal property native to central and southwestern Asia. It was first cultivated by the Arabs. The Persians cultivated it about 2000 years ago (Bakhru, 2001). It was cultivated for the sake of its succulent leaves and was introduced in Europe in the 15th century. Spinach has a high nutritional value and is extremely rich in antioxidants. Spinach contains different carotenoids like lutein, ß-carotene, violaxanthin and neoxanthin. The high concentrations of vitamins like A, E, C, and K and also folic acid, oxalic acid and minerals were present in the spinach (Kavitha *et al.*, 2013).

Spinach is an excellent source of folate and has been shown to inhibit DNA synthesis in proliferating human gastric adenocarcinoma cells (Dias, 2012). Bags of frozen chopped spinach are more convenient to use than block kinds, and this mild-flavored vegetable can be added to soups, pasta dishes, and casseroles. It contains more than a dozen individual flavonoid compounds, which work together as cancer-fighting antioxidants (Settaluri *et al.,* 2015).

A registered dietitian nutritionist Megan Ware said that, “Eating spinach is beneficial for maintaining healthy skin, hair and strong bones, as well as helping in digestion, lowering the risk for heart disease and improving blood glucose control in diabetics”. Spinach is rich in chlorophyll. Chlorophyll rich plants are a safe and effective alternative treatment for aliments such as high blood pressure, obesity, diabetes, gastritis, ulcers, pancreas and liver problems, osteomyelitis, asthma, eczema, hemorrhoids, skin problems, fatigue, anemia, halitosis, body odor, and constipation (Wigmore, 1984).

Spinach is one of the foods with ample iron and a very good source of vitamin K (Hanif, 2006). It is employed as a food medicine in anemia with satisfactory results (Mukherjee, 1983). So, spinach is called as "Life Protective Food". Spinach can be also called as QUEEN OF GREENS which gives us gift of natural health (Tewani, 2016).

A large amount of confectionary product is being produced overall the world to meet the demand of customers. Biscuits are one of those. Baking technique is probably the earliest and oldest of all other techniques and is still going steady over food processing field. Bakery products have played a key role in the development of mankind, being a principal source of convenience, variety and a healthy nutrition component to modern lifestyles. Bakery biscuits are very popular, ready-to-eat, convenient, inexpensive and an important product in human diet. These are usually eaten with tea and used as a weaning food for infants. It is also used for school going children who are often underweight. It may be used as a nutrient supplement during emergency situation (Baljeet *et al.*, 2010). Not only long shelf-life of biscuits makes large scale of production and distribution possible but also good eating quality makes biscuits more attractive for protein fortification and other nutritional improvements (Hooda and Jood, 2005).

The primary ingredient used for the biscuits is wheat flour which is deficient in several nutrients including vitamins, minerals as well as dietary fiber (Ayo and Nkama, 2003). It also lacks essential amino acids such as lysine, tryptophan (Awan*et al.*, 1991). Hence, partial replacement of wheat flour with other ingredients to make functional food in high demand (Sanchez, 2010). Spinach powder contains high nutritional value than wheat flour.

Presently, foods are not intended to only satisfy hunger. It should provide necessary nutrients for human to prevent some nutrition related diseases and improve physical and mental well-being (Neothlings *et al.*, 2007; Takachi *et al.*, 2008). Supplementation of foods is our current interest because of increasing nutritional awareness among consumers.

In this study spinach powder was incorporated at various levels with wheat flour and biscuits were prepared with the following objectives:

1. To know the effect of spinach powder on physical quality of composite biscuits.
2. To evaluate physiochemical compositions and sensory qualities of prepared biscuits.
3. To find the suitable formulation for quality composite biscuits.

CHAPTER II

REVIEW OF LITERATURE

2.1 History of spinach

Spinach originated in Persia (now Iran) where it was known as *aspanakh*. It made its way to China in the 7th century. Spinach arrived in Europe in the 15th century. In fact, spinach was known as "the Spanish vegetable" in England. In the 16th century, spinach became the favorite vegetable of the Italian Renaissance and dishes prepared on a bed of spinach are referred to as '*la Florentine'*.

North Americans began growing spinach in the early 19th century. In the 20th century, spinach was popularized all over the world (Anonymous).

2.2 Production of Spinach

California is the leading state for spinach production, where 10,521 ha spinach was harvested in 2014. Arizona, Texas, and New Jersey follow with 3,237, 607, and 526 ha harvested, respectively. In order to meet demands, production of spinach has risen as well in U.S. Harvested acres of spinach have raised from 13,937 ha in 2012 to 15,191 ha in 2014. Spinach production in the U.S. was valued over $250 million in 2014 (USDA, 2015).

2.3 Commercial Importance of Spinach

The economic importance of spinach, especially fresh-market spinach, continues to grow in the U.S. as well as globally. In 2014, the annual per capita consumption of fresh market spinach was 0.77 kg per person in the U.S, and has been as high as 1.27 kg (Naeve, 2014). Spinach is grown commercially for fresh market, frozen, and canned products. Fresh market spinach may be sold as clipped, bagged "baby leaf" spinach or in bunches, and spinach produced for fresh market made up 90% of all spinach production in the U.S. in 2014 (Naeve, 2015).

2.4 Importance of spinach as a food

Joseph *et al.* (1998) studied that feeding young rats a freeze-dried aqueous spinach extract and observing changes related to mental function as they aged, showed that a diet with spinach extracts retarded age-related decline the most. Although two groups of rats had been fed other diets high in antioxidants (strawberry extracts or vitamin E supplements), the beneficial effects were most pronounced in the spinach-fed group.

Duma *et al.* (2014) researched the content of phytochemicals (chlorophyll a and b, total carotenoids, lutein, vitamin C) inspinach (*Spinaciaoleracea*).It was found out that spinach are the good source of chlorophyll.

Tewani *et al.* (2016) suggested that, the chief chemical constituents of spinach are Essential Amino Acid, Iron, Vitamin A and folic acid. Eating fresh spinach daily supplies the required quantity of iron, vitamin A and Folic acid. Leaves contain almost all the vitamins and are the richest source of carotenoids, beta-carotene, lutein and carotene from which we get vitamin A. They contain also almost all the minerals and are one of the richest sources of iron. In fact, leafy vegetables, taken with cereals, improve the protein quality of the latter, by providing the amino acid components of protein which are deficient in cereals.

Fico *et al.* (2000) suggested that skin and stomach cancers in laboratory animals were reduced by a diet with spinach extracts. The study also conducted that spinach has strong antioxidant activity and high levels of antioxidant compounds such as phenolics and carotenoids.

Hedges & Lister (2007) reported that ascorbic acid in baby spinach is a well-known antioxidant and enzyme co-factor with many roles in human health. Epidemiological and laboratory studies have also shown that normal spinach, spinach extracts and spinach compounds may delay or retard age-related loss of brain function, reduce the extent of post-ischaemic stroke damage to the brain, and protect against cancer through various different mechanisms.

Teddy & James (2008) said one of the major health benefits attributed to two major compounds in baby spinach, lutein and zeaxanthin, is that of protection against eye diseases such as macular degeneration and the gradual loss of central vision associated with old age.

Davey *et al.* (2000) suggested that glutathione, an extremely important endogenous antioxidant synthesised within the body, is relatively rare in foods but is found in spinach. One of its major functions is to protect DNA from oxidation but it also detoxifies carcinogens, boosts the immune system, supports liver health and reduces inflammation. The enzyme glutathione, which is reductase dependent, is usually reduced back to ascorbic acid and α-lipoicacid which are vital antioxidants largely synthesised in the body but also present in some foods. Glutathione is a cysteine-containing peptide found in most forms of aerobic life.

2.5 Role of spinach on diseases/ disorders

Spinach (*spinach oleracea*) has high potential as a leafy vegetable in the preparation of different dishes and treatment of various diseases due to its nutritional potentials.

Dias (2012) suggested that vegetables are essential for well-balanced diets since they supply vitamins, minerals, dietary fiber, and phytochemicals. In the daily diet vegetables have been strongly associated with improvement of gastrointestinal health, good vision, and reduced risk of heart disease, stroke, chronic diseases such as diabetes, and some forms of cancer. Some phytochemicals of vegetables are strong antioxidants and are thought to reduce the risk of chronic disease by protecting against free radical damage, by modifying metabolic activation and detoxification of carcinogens. All the vegetables give protection to humans against chronic diseases. Each vegetable contains a unique combination of phytonutriceuticals.

Kopsell *et al.* (2006) investigated the correlation between the risk of prostate cancer and the intake of vegetable, including baby spinach, broccoli, cauliflower, cabbage, Brussels sprouts, mustard greens, turnip greens, collards and kale. Of all the vegetables, only baby spinach was found to provide significant protection against aggressive prostate cancer, defined as Stage III or IV prostate cancer with a Gleason score of at least 7. Serum carotenoid concentrations as well as macular pigment optical density (a possible predictor of macular degeneration) increased with an increase in the consumption or intake of spinach carotenoids. Neoxanthin from spinach inhibited the proliferation of prostate cancer.

Spinach vegetable is a valuable source of high grade iron. After its absorption in the system, the formation of hemoglobin and red blood cells take place. It is thus highly beneficial building up the blood and in the prevention and treatment of anemia.Spinach contains more vitamin A than most other green vegetables. This vitamin promotes growth and health, specially the health of (rods and cons) the eyes. So, it is an effective food remedy for the prevention and treatment of night blindness.

Spinach is also a rich source of calcium and other alkaline elements which are essential for keeping the tissues clean and for preserving the alkalinity of the blood. This popular green leafy vegetable possesses mucus clearing property and helps control respiratory diseases. It is an extremely rich source of antioxidants and anticancer compounds. It contains about four times more beta carotene and three times more lutein than broccoli.

Spinach also reduces type 2 diabetes risks due to their high magnesium content. Chewing raw spinach leaves cures pyorrhea. Spinach juice is especially strengthening to the teeth and gums because of its high concentration of alkaline minerals. As the richest source of folic acid, spinach is a very valuable food during pregnancy and lactation. Megaloblastic anemia of pregnancy occurs because the mother is deficient in folic acid. Spinach is also good source of nutrition for nursing or lactating mothers and improves the quality of their milk. It increases the secretion and discharge of urine. It can be safely given in cystitis, nephritis and scanty urination due to dehydration (Bakhru, 2001&2014)**.**

2.6 Possible health benefits of spinach

Megan Ware (2017) said that spinach is a nutrient rich plant with many possible health benefits. Spinach has the following possible health benefits:

Diabetes management

Spinach contains an antioxidant known as alpha-lipoic acid, which has been shown to lower glucose levels, increase insulin sensitivity, and prevent oxidative stress-induced changes in patients with diabetes. Studies on alpha-lipoic acid have also shown decreases in peripheral neuropathy and autonomic neuropathy in diabetics.

Cancer prevention

Spinach and other green vegetables contain chlorophyll, which has been shown to be effective at blocking the carcinogenic effects of heterocyclic amines, which are generated when grilling foods at a high temperature.

Asthma prevention

The risks for developing asthma are lower in people who consume a high amount of certain nutrients. One of these nutrients is beta-carotene, of which spinach is an excellent source. Apricots, broccoli, cantaloupe, pumpkin, and carrots are also rich sources of beta-carotene.

Lowering blood pressure

Due to its high potassium content, spinach is recommended for people with high blood pressure. It can help reduce the effects of sodium in the body. A low potassium intake may be just as big of a risk factor for developing high blood pressure as a high sodium intake.

Bone health

Low intakes of vitamin K have been associated with a higher risk of bone fracture. Adequate vitamin K consumption is important for good health, as it acts as a modifier of bone matrix proteins, improves calcium absorption, and may reduce urinary excretion of calcium.

Promotes regularity

Spinach is high in fiber and water, both of which help to prevent constipation and promote a healthy digestive tract.

Healthy skin and hair

Spinach is high in vitamin A, which is necessary for sebum production to keep hair moisturized. Vitamin A is also necessary for the growth of all bodily tissues, including skin and hair. Spinach and other leafy greens high in vitamin C are imperative for the building and maintenance of collagen, which provides structure to skin and hair. Iron deficiency is a common cause of hair loss, which may be prevented by an adequate intake of iron-rich foods, like spinach.

2.7 Nutritional composition of spinach

Karmakar *et al.* (2013) analyzed the chemical composition, such as water, ash, iron and total vitamin C content, of 28 green leafy vegetables in his investigation. The water content of the leafy vegetables varied between 83.8 to 95.5 g/100 g fresh vegetable sample. The ash content of the samples varied between 8.0 to 22.6 g /100 g of dry vegetable powder. The iron content varied from 11.8 to 78.2 mg/100 g of dry sample and the total vitamin C content varied from 191.5 to 21.6 mg/100 g of fresh sample. These findings conclusively suggest that the locally available leafy vegetables are good source of water, minerals, iron and vitamin C. The water content of spinach is analyzed as 95.5 ± 0.2 g/100g and the ascorbic acid is 41.2 ± 1.4 mg/100g in fresh samples. The ash content is18.75 ± 0.3 g/100g and the iron content is 41.65 ± 0.4 g/100g in dry powder samples.

Balfour *et al.* (2014) studied the extruded fortified corn snack which was developed by single type screw extruder using Corn Meal, Oat Meal and Whey Protein Concentrate. Spinach (at the ratio of 3%, 5% and 7%) and mint leaves were used for flavoring and seasoning. Physico-chemical and sensory analysis of the samples were estimated. During storage it was observed that moisture content of the sample showed slight increase whereas there was a slight decrease in all the other proximate analysis. But it was detected that all the proximate parameters were increased from the control sample to the different treatments.

Verma *et al.*, (2012) reported that the protein and iron content of dried vegetables (spinach, mint and carrot) mathri i.e. 7.44 g and 5.37 mg was higher as compared to fresh vegetables mathri.

Kavitha *et al.* (2013) found that raw fresh spinach is a good source of vegetable protein (11.10%) and fiber (21.38%). Other proximate parameter studied,include moisture (36.8%), Ash (6.96), Carbohydrate (20.28%) and fat (3.47%). Mineral magnesium content in raw spinach was found to be abundant (205.10mg/100g). The powder obtained from the shade dried spinach leaf contain rich source of vegetable protein (19.10%) and other parameter includes moisture(11.17%), ash(5.57%), carbohydrate(41.49%), fat (7.11%) and fiber(15.48 %).

Sies (1997) evaluated that spinach is high in vitamin K, an essential nutrient that contributes to bone health. It is one of the most nutrient-dense foods in existence, low in calories and high in vitamins.

Lomnitski*et al.* (2003) said that,spinach is a rich source of antioxidants, especially high in vitamins A, C, E, K, beta-carotene, selenium and omega-3 fatty acids as well as rich in lutein and zeaxanthin. Spinach contains microelements such as potassium, calcium, magnesium, manganese, zinc and others. Spinach extracts have several beneficial effects, such as anticancer, anti-aging and protecting of central nervous system.

Lampe (1999) investigated that spinach has high potassium content and low sodium content.This mineral composition is very beneficial for patients with high blood pressure, as potassium lowers blood pressure while sodium raises it. Spinach is an excellent source of other antioxidant nutrients such as vitamin C, vitamin E, β-carotene, manganese, zinc and selenium, which help reduce the risk of numerous health problems associated with oxidative stress.

2.7.1 Proximate composition of spinach

Kavitha *et al.* (2013) evaluated the proximate composition of spinach which is given below:

Table 2.7.1 Proximate composition of spinach

Nutrients in percent	Fresh/Raw leaf	Shade dried leaf powder
Moisture	36.8	11.17
Protein	11.10	19.10
Fiber	21.38	15.48
Fat	3.42	7.11
Ash	6.96	5.57
Carbohydrate	20.25	41.49

2.7.2 Amino acid content of spinach

Tewani *et al.* (2016) suggested that spinach contains following essential amino acids:

Table 2.7.2 Amino acid content of spinach

Amino acids	g /100g
Arginine	6.4
Histidine	2.8
Isoleucine	5.4
Leucine	8.0
Lysine	7.6
Methionine	2.0
Phenylalanine	5.4
Theronine	3.4
Tryptophen	1.3
Valine	5.0

2.8 Formulation of supplemented biscuits

Kumari *et al.* (1996) prepared a supplementary food in the form of biscuits using wheat, soy and sugar at a proportion of (70:10:20). The energy contents of 100 gm biscuits were 37 and 456 KCal respectively, while protein contents were 13.o and 9.0 gm respectively.

Krishna *et al.,* (1996) suggested supplementary food developed from wheat, sugar and defatted soy flour. They studied the food for proximate composition, viscosity and protein quality. They prepared biscuits and it contained 4.6% moisture, 9.0% protein, 17.45% fat, 2.85 ash, 0.09% fibre and 64.9% carbohydrates.

Awan *et al.* (1995) used mothbean flour at levels of 10, 15, 20 and 25% was applied in whole wheat flour for the production of biscuits to improve the quantity and quality of protein, mineral content and fiber. The protein content in biscuits increased from 6.97% (100% white flour)to 9.48% in composite flour containing 25% mothbean flour. Similarly, crude fiber and mineral contents increased from 0.28 to 1.36% and 0.46 to 0.91 % respectively. Physical studies exhibited a decrease in the width and spread factor and an increase in the thickness of the biscuits with rising levels of supplementation. The sensory evaluation showed that the quality score decreased with increasing levels of supplementation. However, in case of 10% and 15% supplemented biscuits, the score remained at fairly good level for all the parameters. Net protein utilization, protein efficiency ratio, biological value and feed efficiency value increased with higher levels of supplementation. A decrease in digestibility was observed as a result of various treatments. Storage of biscuits for 4 weeks did not remarkably affect the quality of the product.

Rosli *et al.* (2012) worked on effect of wheat flour biscuit incorporating Oyster mushroom (*Peurotus sajor-caju*) by adding 2%, 4% and 6% level. They found that moisture content 2.1% to 2.9%, fat content 21% to 24% and protein content 7.2% to 10.10% respectively.

Ibrahim *et al.* (2014) reported, effect of incorporating mushroom powder (*Peurotus florida*) in wheat flour biscuit with nut and found that their protein content 8.9% to 10.2%, fat content 21.64% to 22.57% and ash content 1.68% to 1.99% when adding 2%, 4% and 6% mushroom powder respectively. Fiber content was found 2.2% to 2.96% and total carbohydrate was present in range of 66.2% to 64.2%.

Prodhan *et al.* (2015) work on quality evaluation of mushroom (*Peurotus sajor-caju*) enriched biscuit. They found 8.5% protein is present in 2% mushroom biscuit, 9.46% % protein is present in 5% mushroom biscuit and 10.4% protein is present in 10% mushroom biscuit. The amount of fat is 20%, 21.7% and 22.2% respectively. Here, fiber in control sample is 0.3%, where 2% mushroom biscuit contains 2.4% fiber, 5% mushroom biscuit contains 2.8% fiber and 10% mushroom biscuit contains 3.2% fiber. Ash content is ranged from 1.56%, 1.8% and 2.1% respectively.

Roy *et al.* (2004) work on effect of mushroom powder on the baking properties of sweet biscuits by adding 2%, 5% and 10% mushroom powder. They found moisture content range from 2.15% to 3.30%, protein content 8.70% to 10.10%, fat content 22.90% to 23.93% and ash content 1.6% to 2.39% when adding 2%, 5% and 10% mushroom powder respectively. Total carbohydrate was present in range of 61.71% to 63.55%.

Hozova *et al.* (1995) analyzed the biscuits prepared from amaranth flour and tested for microbiological quality and sensory characteristics during 6 month storage at 20^0 C and 62% RH. They found that the biscuits were satisfactory and the presence of microorganisms is not detected in biscuits during first 4 month of storage.

Foda *et al.* (1984) accomplished an experiment on low fat soy flour (LFSF) mixed with wheat flour and prepared biscuits. Dough and prepared biscuits were analyzed for several characteristics (farinograph data of dough, chemical composition and organoleptic quality of biscuits). Farinograph data were observed as absorption ratio, dough stability, arrival time, dough development time, mixing tolerance index. Organoleptic parameters were examined as appearance, tenderness, color, flavor, chemical composition. The chemical composition was moisture 2.24-6.97%, fat 9.43-10.45%, protein 11.43-17.4% and ash 0.49-1.9% LFSF markedly increased the nutritional value without adversely affecting quality.

2.9 Formulation of another supplemented food products

Angella *et al.* (1999) reported that supplementation of other minor ingredients for enhancement of nutritional benefits has been practiced the world over. Soy and flax seed are extensively used for enhancement of protein content and fiber and $\omega-3$ fatty acids respectively in foods. Whole wheat flour was substituted with 25% and 50% of flaxseed flour (or 12.5% and 25% of the total flour) in a master mix to evaluate banana bread and peanut butter and observed that acceptable bread and butter cookies were produced without affecting characteristics such as flavor, texture, and mouth feel.

Singh *et al.* (2007) suggested that green leafy vegetables are an important part of daily diet. Bathua (*Chenopodium album* Linn.) leaves, rich in micronutrients were selected for dehydration. Leaves were tray dried at 50-60^{o}C for three to four hours till the moisture reached to 6-7 per cent. These dehydrated leaves were incorporated at 3-15 percent levels in two conventional foods namely green gram dal and paratha. Proximate composition, iron content and carotene content of leaves and products were analyzed. Results showed that dehydrated leaves were rich source of protein, carbohydrate and ash.

Iron and carotene contents of dehydrated bathua leaves (27.48mg/100g and 14826 μg/100g, respectively) were 6-8 times higher than fresh leaves. Green gram dal and paratha incorporated with 7 and 5 per cent dehydrated bathua leaves were liked most. Iron content of green gram dal (8.8mg/100g) and paratha incorporated with dehydrated bathua leaves was higher than their respective control. In comparison to control enriched paratha (4255.66±0.6 μg/100g) and green gram dal (984±1.8μg/100g) had many fold greater carotene content.

Bottcher *et al.*(1995) studied the possibility of developing flour suitable for biscuits manufacture. Baking trials were carried out in which rye, corn flour were substituted for wheat flour at 10-30%, thus reducing protein content, Addition of rye flour to wheat flour did not produce biscuits of acceptable quality. Addition of rice corn flour to wheat flour resulted is a suitable flour for biscuits manufacture. Granulation had an important influence on dough and baking properties.

Galla *et al.* (2017) researched with spinach supplemented biscuits with a percentage of 5%, 10% and 15% incorporation of spinach powder. They dried spinach (*Spinacia oleracea L.*) leaves in a cabinet tray dryer at 55 °C and ground to pass through 220 μm mesh to obtain spinach powder (SP). Fresh spinach leaves yielded 6.5% of SP possessing 28.70% protein, 8.8% crude fiber. The powder was rich in essential minerals like calcium (1336 mg/100 g), iron (30 mg/100 g) and phosphorous (336 mg/100 g). Biscuits were prepared and evaluated for their nutritional, textural, sensory quality and sorption behavior. Textural quality revealed that hardness and breaking strengths increased with increased addition of SP. Sensory studies of biscuits showed that 5% supplementation of spinach powder was more acceptable.

Ranjana *et al.* (1998) analyzed the physical, chemical and sensory properties of biscuits prepared from wheat flour with 0-50% replacement by defatted soybean flour. They observed that thickness of biscuits was increased. Sensory properties dictated that there was no substantial adverse effect on overall quality that the replacement of 20% defatted soy flour in biscuits formulation.

Shrestha *et al.* (2002) produced kinema, which was prepared by natural fermentation of soybeans and used for making biscuits. *Bacillus* strains, only tentatively identified, were isolated from the traditional product and used for making kinema in the laboratory. Kinema prepared by both methods was dried and ground to fine flour. Full-fat and low-fat soyflours were also prepared. Soy and kinema flours were incorporated at the 15% level into biscuit formulation. Protein content of all products was more than 17%. There was little difference in the proximate composition of the four types of biscuits prepared except for biscuits supplemented with low-fat soyflour. Kinema supplementation resulted in decreased hardness but increased weight and spread ratio in fortified biscuits. Evaluation of sensory characteristics showed greater acceptance of kinema-supplemented biscuits in comparison with full-fat soyflour-supplemented biscuits.

Ullah *et al.* (2016) studied the effect of alfalfa seed flour supplementation on the quality characteristics of refined wheat flour-based biscuits. The proximate composition of refined wheat flour and alfalfa seed flour was determined. Refined wheat flour contained 12.43% moisture, 11.52% crude protein, 1.61% crude fat, 0.71% crude fiber, 1.43% ash and 70.83% nitrogen free extract, while alfalfa seed flour contained 5.79%, 29.49%, 12.71%, 5.53%, 4.80% and 41.73% moisture, crude protein, crude fat, crude fiber, ash and nitrogen free extract correspondingly. Alfalfa seed flour at 5%, 10%, 15% and 20%

supplementation levels was incorporated in refined wheat flour to produce composite flour. The biscuits prepared were subjected to quality evaluation. Physical analysis of biscuits disclosed that supplementation of alfalfa seed flour decreased the width from 47.25 to 42 mm and the spread factor from 62.7 to 53.12, while it increased the thickness from 7.53 to 8.10 mm. Supplementation of refined wheat flour–based biscuits with alfalfa seed flour at different inclusion levels significantly ($p < 0.05$) increased the crude protein content from 10.19% to 15.30%, the crude fiber content from 0.73% to 1.62%, the crude fat content from 17.46% to 21.59% and the ash content from 1.37% to 1.92%, whereas it decreased the moisture content from 3.57% to 3.26% and the nitrogen free extract from 66.90% to 59.32%. The effect of supplementation on the mineral contents of biscuits was also significant ($p < 0.05$). Potassium, magnesium, calcium, iron and zinc contents increased from 105.30, 14.65, 43.91, 3.74 and 0.94 to 145.00, 26.64, 79.60, 7.93 and 1.60 mg/100 g, respectively. Sensory evaluation revealed that the quality score of biscuits in terms of color, taste, texture and overall acceptability decreased with increased supplementation. The present research work confirmed that a maximum of 10% alfalfa seed flour supplementation in refined wheat flour could produce acceptable biscuits with an appropriate nutritional profile.

Mohammed *et al.* (2016) evaluated the proximate analysis and amino acid profile carried for biscuit flour and whey protein. The gluten quantity and quality was tested for biscuit flour and biscuit flour-whey mixture with different concentration 0, 5, 10 and 15% whey. The results of the proximate analysis showed that there was no difference between protein 11.3% and carbohydrate (74.87%) for biscuit flour and protein (11.7%), carbohydrate (74.47%) for whey. The moisture content of biscuit flour was 10.97% which was higher than whey (5.47%) with highly significant difference at level of ($p>0.05$). The fat and ash contents of Biscuit flour were significantly ($p>0.05$) lower than the other one. The biscuit flour had lower content in essential amino acids especially limiting amino acid (Lysine) compared to whey protein. The gluten quantity and quality was affected by supplementation with whey and decreased with increased the concentration of whey. The overall quality of biscuits made from mixture showed high acceptability, Biscuit flour blended with 10% spray-dried whey showed best biscuit.

Hung *et al.* (2012) applied the freeze- and heat-drying methods to dry fresh vegetables (carrot, taro, tomato, red beetroot and eggplant) grown in Vietnam and then the total phenolic and flavonoid compounds extracted by alcoholic and alkaline-hydrolysis methods were evaluated to determine the effects of the drying methods on the bioactive compounds of the vegetables. Furthermore, they also investigated the correlations between the content of bioactive compounds and their antioxidant capacity in this study. The results show that phenolic and flavonoid compounds were mainly located in free form in the vegetables which was easily extracted by alcoholic solvent. A high temperature in the heat-drying method in sample preparation significantly reduced total free and bound phenolics, total free and bound flavonoids and their antioxidant capacity. The antioxidant capacity of the extracts highly correlated with free phenolic compounds (r2 = 0.8936) and free flavonoid compounds (r2 = 0.6682). In contrast, the antioxidant capacity of the extract did not correlate with the bound phenolic and flavonoid compounds (r2 = 0.0124 and r2 = 0.0854, respectively).

Shalini *et al.* (2005) studied the supplementation of fenugreek flour in biscuits at 10% level in biscuits was found to be acceptable with good puffing, increased contents of protein, dietary fiber, calcium and iron. However expansion in biscuits was found to be lower than the control. Biscuits prepared by supplementation with fenugreek flour at 10% level resulted in products with enhanced mineral contents of calcium and iron and they were found to be organoleptically acceptable.

Tyagi *et al.* (2007) studied the nutritional, sensory and textural characteristics of defatted mustard flour fortified biscuits to optimize the mustard flour supplement in the blend for making biscuits.The wheat flour was replaced by defatted mustard flour at 5, 10, 15 and 20% incorporation levels in biscuit preparation. The protein content of mustard flour biscuit increased nearly 2.5 times as a result of mustard flour incorporation, coupled with reduction in fat and an increase in fiber content. Sensory evaluation results exposed that the sample containing 15% defatted mustard flour scored highest in most of the attributes including overall acceptability. Textural characteristics of all dough and biscuit upto 15% supplement of defatted mustard flour were similar while at 20% level, the values were significantly different. In the study incorporation of 15% defatted mustard flour gave desirable results.

2.10 Sensory evaluation of biscuits

Awan *et al.* (1995) used mothbean flour at levels of 10, 15, 20 and 25% was applied in whole wheat flour for the production of biscuits to improve the quantity and quality of protein, mineral content and fiber. The sensory evaluation showed that the quality score decreased with increasing levels of supplementation. However, in case of 10% and 15% supplemented biscuits, the score remained at fairly good level for all the parameters. A decrease in digestibility was observed as a result of various treatments. Storage of biscuits for 4 weeks did not remarkably affect the quality of the product.

Mohammed *et al.* (2016) evaluated the proximate analysis and amino acid profile carried for biscuit flour and whey protein. The gluten quantity and quality was tested for biscuit flour and biscuit flour-whey mixture with different concentration 0%, 5%, 10% and 15% whey.The values obtained increased from 3.7 (control) to the highest score of 7.46 (15% replacement). A significant difference ($p \leq 0.05$) was observed in score of color between biscuits made from the control and those from whey powder-supplemented flours. Increasing the levels of supplementation with whey powder also resulted in a significant increase in the score of aroma of biscuits. The values obtained were 4.01 (control), 5.31 (5% substitution), 6.53 (10% substitution) and 6.53 (15% substitution). The score of texture was increased with increasing level of whey powder supplementation with significant difference at a level of ($p \leq 0.05$). The values obtained were 1.99 (control), 4.5 (5% substitution) 6.16 (10% substitution), and 6.35 (15% substitution). The score of taste was increased with increasing whey powder levels in the substituted flours with significant difference at a level of ($p \leq 0.05$). The values obtained were increased from 4.09 (control) to the higher value of 6.53 (10% replacement). The sensory evaluation of biscuits showed that there was a significant difference in the overall acceptability of biscuits made by different levels of whey powder supplementation. Biscuits made from wheat flour supplemented by 10% whey powder showed the best panelist's scores for overall acceptability of the biscuits produced.

Ullah *et al.* (2016) studied the effect of alfalfa seed flour supplementation on the quality characteristics of refined wheat flour-based biscuits. Alfalfa seed flour at 5%, 10%, 15% and 20% supplementation levels was incorporated in refined wheat flour to produce composite flour. Sensory evaluation revealed that the quality score of biscuits in terms of color, taste, texture and overall acceptability decreased with increased supplementation. The present research work confirmed that a maximum of 10% alfalfa seed flour supplementation in refined wheat flour could produce acceptable biscuits with an appropriate nutritional profile.

Galla *et al.* (2017) researched with spinach supplemented biscuits with a percentage of 5%, 10% and 15% incorporation of spinach powder. Biscuits were prepared and evaluated for their nutritional, textural, sensory quality and sorption behavior. Textural quality revealed that hardness and breaking strengths increased with increased addition of SP. Sensory studies of biscuits showed that 5% supplementation of spinach powder was more acceptable.

Tyagi *et al.* (2007) studied the nutritional, sensory and textural characteristics of defatted mustard flour fortified biscuits. The wheat flour was replaced by defatted mustard flour at 5, 10, 15 and 20% incorporation levels in biscuit preparation. Sensory evaluation results exposed that the sample containing 15% defatted mustard flour scored highest in most of the attributes including overall acceptability. Textural characteristics of all dough and biscuit up to 15% supplement of defatted mustard flour were similar while at 20% level, the values were significantly different. In the study incorporation of 15% defatted mustard flour gave desirable results.

Ranjana *et al.* (1998) analyzed the physical, chemical and sensory properties of biscuits prepared from wheat flour with 0-50% replacement by defatted soybean flour. They observed that thickness of biscuits was increased. Sensory properties dictated that there was no substantial adverse effect on overall quality that the replacement of 20% defatted soy flour in biscuits formulation.

CHAPTER III

MATERIALS AND METHODS

The experiment was conducted in the laboratory of the Department of Food Engineering and Technology, under the Faculty of Engineering, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh.

3.1 Materials

Fresh sample of spinach (*Spinacia oleracea*) leaves were procured from the local market of Dinajpur. Wheat flour (TEER brand), sugar, baking powder, soybean oil, milk powder, egg and dalda were also collected from the local market of Dinajpur. The chemicals and reagents were used from laboratory stocks. High density polyethylene bags were used for package and storage of the samples. Other necessary items such as knife, tissue paper, wood sheet, scale etc were collected from local market and laboratory stocks.

(a) **Spinach stems** **(b) Spinach leaves**

Fig 3.1: Photographic view of raw spinach

3.2 Preparation of spinach powder

Washing

The collected spinach samples were washed thoroughly in running tap water to remove undesirable dirt, soil and dust. Blanching was avoided due to undesirable color and increased losses of soluble solids.

Sorting

Healthy leaves were sorted after washing in running water. The stems were sorted separately.

Cutting

The leaves and stems were then chopped with a hand knife into 3cm length.

Drying

The samples were spread on trays to remove excess water. Then the samples were dried at a temperature of 55 to 60ºC in a lab scale multistoried universal cabinet dryer for 3-4 hours to achieve 8-10 percent moisture content.

Multistoried universal cabinet dryer used for drying of spinach is given below:

Fig 3.2: Multistoried universal cabinet dryer used for drying of spinach.

Grinding

The dried samples were blended in a blender machine to get spinach powder.

Sieving

The powder was sieved at (300 MIC) mesh.

Storing

The fine powder was stored in polyethylene bags for further use.

The flow diagram for preparing of spinach powder is given below:

Receiving raw spinach

↓

Washing

↓

Cutting

↓

Spreading on trays

↓

Drying in multistoried universal cabinet dryer (55-60^{0}C)

↓

Grinding in blender

↓

Sieving in 300 MSI mesh

↓

Packing in HDPE bags

↓

Storage at room temperature

Fig 3.2.1: Flow diagram of preparation of spinach powder.

3.3 Preparation of biscuits

Control biscuits (C) and spinach powder supplemented biscuits (F_1, F_2 and F_3) were prepared according to the method reported in the literature with some modifications (Tyagi *et al.*, 2007).

3.3.1 Trial operation

Wheat flour, powdered sugar, baking powder, milk powder, egg white and dalda weighed accurately. The spinach powder was incorporated at different levels of percentage. Spinach powder was added at 15% level with wheat flour. Baking powder was used as a leavening agent. At first the dalda was heated and kneaded instantly with wheat flour. Then other ingredients were mixed thoroughly to make adequate dough and kept for a while. Then the dough was rolled to a sheet of 3cm thickness. The sheet was cut into circular moulds of 3cm diameter and baked in the oven at 160^0C for 30 minutes, cooled to ambient temperature and allowed for sensory evaluation taste. The results were not acceptable. It was bitter in taste and very dark in color. Then the biscuits were prepared with 12% level with wheat flour. The same procedure was followed for preparing the biscuits. After the sensory evaluation taste the results were also not acceptable. It was also bitter in taste, dark in color and hard also. Then to change the formulation 10% spinach powder added with wheat flour and biscuits were prepared. There was not desirable change compared with previous biscuits.

The formulations of trialed biscuits are given in following table.

Table 3.3.1 Formulations of trialed biscuits

Ingredients (gm)	15% SP	12% SP	10% SP
Wheat flour	25	28	30
Spinach powder	15	12	10
Sugar	20	20	20
Dalda	16	16	16
Milk powder	2	2	2
Baking powder	1	1	1
Salt	0.5	0.5	0.5
Egg	20	20	20

N. B. SP means spinach powder.

3.3.2 Preparation of desired biscuits

From the above circumstances dough was prepared at a low ratio of spinach powder and the levels were 3%, 5% and 7%. Wheat flour, powdered sugar, baking powder, milk powder, egg white and dalda weighed accurately as indicated in below. The antioxidant rich spinach powder was incorporated at 0%, 3%, 5% and 7% levels with wheat flour to provide extra health benefit and different taste in biscuits and the biscuits are named as C, F_1, F_2 and F_3respectively. Baking powder was used as a leavening agent. At first the dalda was heated and kneaded instantly with wheat flour. Then other ingredients were mixed thoroughly to make adequate dough and kept for a while. Then the dough was rolled to a sheet of 3cm thickness. The sheet was cut into circular moulds of 3cm diameter and baked in the oven at 160^0C for 30 minutes, cooled to ambient temperature and packed in plastic bags. Then the bags were sealed and stored at room temperature (25-30) ^{0}C for further analysis.

The formulations of desired biscuits are given in following table.

Table 3.3.2 Formulations of desired biscuits

Ingredients (gm)	C	F_1	F_2	F_3
Wheat flour	40	37	35	33
Spinach powder	0	3	5	7
Sugar	20	20	20	20
Dalda	16	16	16	16
Milk powder	2	2	2	2
Baking powder	1	1	1	1
Salt	0.5	0.5	0.5	0.5
Egg	20	20	20	20

The flow diagram for preparing of spinach biscuits is given below:

Preparation of composite flour
(0%, 3%, 5% and 7% level of spinach powder)
↓
Premixing (dalda and powdered sugar)
↓
Mixing (milk powder and baking powder)
↓
Kneading
↓
Baking at 160^0C for 30 minutes
↓
Cooling to room temperature
↓
Packaging (HDPE)
↓
Storage at ambient temperature

Fig 3.3.3: Flow diagram of preparation of spinach biscuits.

3.4 Physical Analysis

The prepared biscuits were analyzed for thickness, spread-ratio, volume, and density. All of these data were presented as average value. Weight (g) of four individual biscuits was measured with the help of digital weighing balance and height (cm) by stacking four biscuits on top of each other.

3.4.1 Spread-ratio

Spread-ratio was calculated by dividing the average value of diameter by average value of thickness.

Spread Ratio (SR) = D/T

Where,

D=Diameter of biscuits (cm);

T=Thickness of biscuits (cm).

3.4.2 Volume

Volume of biscuits was calculated using the formula:

$$\text{Volume (cm}^3\text{)} = \frac{\pi \mathbf{D}^2}{4} \times T$$

Where,

D= average diameter of biscuits (cm);

T = average thickness of biscuits (cm).

3.4.3 Density

Density was obtained by the following method used by Srivastava*et al.* (2012).

Density (g/cm^3) = {Weight(g) /Volume(cm^3)}.

3.5 Chemical Analysis

All the prepared biscuits and the control biscuit were analyzed for their moisture, ash, fat, protein and total carbohydrate content. All the determinations were done in triplicate and the results were expressed as average value.

3.5.1 Determination of Moisture Content

The moisture content was determined by using the standard protocols as mentioned by the Association of Official Analytical Chemists (AOAC, 2005).

At first, the weight of empty previously dried crucible (1hr at 100^0C) with cover was taken and 5 gm of sample was placed on it. Then the crucible was placed in a Hot Air Oven and dried at 105^0C for 24 hours. After drying the crucible was removed from the oven and cooled in desiccators. It was weighed. The crucible was again placed in the oven, dried for 15 minutes, taken out of the drier, cooled in desiccators and weighed. Drying, cooling and weighing were repeated until 2 consecutive weights were same, for accuracy at least 3 samples were dried in the oven. The loss of weight was calculated as percent moisture. The percent moisture content was calculated by applying the following equation:

$$\% \text{ Moisture Content} = [(W_1 - W_2)/W] \times 100$$

Where,

W= Weight of sample.

W_1= Weight of sample with crucible.

W_2= Weight of dried sample with crucible.

The moisture free samples were then used in order to determine the crude protein, lipid and ash content.

3.5.2 Determination of Ash Content

AOAC method (2005) was used to determine the total ash content as described below:

Muffle furnace, desiccators and an electronic balance were used to determine the ash content of the fish sample. The moisture free samples were taken in porcelain basin made crucible and weighed. Thereafter the ash content was measured by igniting the samples in a muffle furnace at a temperature of 550 °C for 6 hours. The samples were then cooled in desiccators. The average weight of each sample of the remaining material was taken as percentage. Ash content of the sample was then calculated by using the following formula:

$$\% \text{ ash} = \frac{\text{Weight of crucible with ash (g)} - \text{Weight of empty crucible (g)}}{\text{Weight of sample (g)}} \times 100$$

3.5.3 Determination of protein content

The protein content was determined by the method of AOAC (2000) with some modification called Kjeldahl method using kjeldhal apparatus. In this case, total nitrogen content was determined by digesting the sample with concentrated sulphuric acid (H_2SO_4), in presence of digestion mixture into boric acid as explained in detail below:

3.5.3.1 Digestion

Sample (1g), selenium powder (1g), $CuSO_4$ (0.1g), K_2SO_4 (10g)were taken into a volumetric flask. Then 25ml of H_2SO_4 (conc.) was added. After that the volumetric flask was heated at 100^{o}C for 3 hours and cooled for 20 minutes at room temperature.

3.5.3.2 Distillation

After digestion 300ml of distilled water and 125ml of 40% NaOH were added to the volumetric flask. 250ml of 4% boric acid solution and 2-3 drops mixed indicator were taken in a conical flask. The volumetric flask was connected with one end of the condenser and the conical flask was connected with other end. The volumetric flask was heated continuously until the conical flask was filled to 150ml.

3.5.3.3 Titration

The conical flask was disconnected and was taken for titration. Titrated against 0.2 N of H_2SO_4 solution. The end point was indicated by orange color.

The total nitrogen value was then calculated by using the following formula:

$$\% \text{ Nitrogen} = \frac{\text{ml. of titrant used} \times \text{normality of titrant} \times \text{milli equivalent weight of Nitrogen}}{\text{Weight of the sample (g)}} \times 100$$

The amount of crude protein was then calculated by multiplying the % of total nitrogen with the Protein conversion factor 6.25, which is generally used in calculating the protein content.

3.5.4 Determination of Fat Content

AOAC method (2000) was used to determine the crude fat content as described below:

1.0 g of sample was taken into the thimble and plugged with cotton. The thimble was attached to the Soxhlet apparatus which was attached with round bottom flask containing 200ml of petroleum benzene. The petroleum benzene was filled into a weighted conical flask. The fat was extracted for 6 hours. After that benzene was evaporated until the conical flask was completely dried and then cooled in desiccators. Then the weight is taken.

The fat content was then determined by using following formula:

$$\% \text{ Fat Content} = [(W_1 - W_2)/W] \times 100$$

Where,

W= Weight of sample.

W_1= Weight of evaporated flask with sample.

W_2= Weight of empty flask.

3.5.5 Determination of Fiber content

The fiber content was determined by the method of Ayesha *et al.* (2002) with some modification. Fat free (2 gm) sample was taken in a 500 ml beaker and added 200 ml 0.255 N H_2SO_4then boiled for 30 min. After that the mixture was filtered with muslin filter cloth and residue was washed with hot water until free from acid. Then residue was transferred into beaker and 0.313 N 200 ml NaOH was added and boiled for 30 minutes. After that the mixture was filtered with muslin filter cloth and residue was washed with hot water until free from alkali and then washed with alcohol and diethyl ether. Then it was transferred into crucible and dried at 105^0C for overnight. Then crucible was heated in a muffle furnace at 600^0C for 3-5 hours. After that cooled and weighed. The crude fiber was obtained by the following formula:

$$\% \text{ Crude Fiber} = [(W_1 - W_2)/W] \times 100$$

Where,

W= Weight of sample.

W_1= Weight of crucible with ash sample.

W_2= Weight of empty crucible.

3.5.6 Calculation of Carbohydrate and Energy Content

Carbohydrate was calculated based on difference.

Carbohydrate = 100− {moisture +protein+ ash+ fat}.

The energy value in calorie was calculated using the following formula:

Energy = 4 × (% Protein + % carbohydrate) + 9 × % fat.

3.5.7 Determination of Mineral Content

The mineral content (Ca, Fe and P) was determined according to the method reported by Saeid Wahab (2010) with some modification.

Digestion

Biscuit (1g) was taken in 50 ml flasks. 10 ml of conc. nitric acid and 5ml of conc. perchloric acid was added to it and digested in the digestion chamber on an adjustable heater and allowed to boil until white smoke was released and solution become clear. After cooling, 20-30 ml distilled water was added to it and filtered. The volume was made up to 100 ml with distilled water. The filtrate was kept in the closed bottle.

Determination of Ca, Fe and P

i. **Measurement of Calcium:** 20 ml diluted filtrate was transferred into a 50 ml volumetric flask using a pipette. 5 ml $LaCl_3$ solution was added and made the volume with distilled water and mixed. The content of Ca was measured by atomic absorption spectrometer (AAS).

ii. **Measurement of Phosphorus:** 5 ml diluted filtrate was transferred into a 50 ml volumetric flask using a pipette. 30 ml distilled water and 10 ml ammonium molybdate-ascorbic acid solution were added, made to volume with distilled water and mixed. After 15 minutes the absorbance was measured on a spectrophotometer at 890 nm.

Calculation:

mg of Ca or P per100 gm biscuit = $(a \times 2500)/(b \times c)$

Where,

a = mg/l Ca or P measured on atomic absorption spectrophotometer,

b = ml diluted filtrate transferred,

c = g biscuit taken.

iii. **Measurement of Iron:** The iron content was measured by atomic absorption spectrophotometer (AAS) directly in the undiluted filtrate form.

Calculation:

mg of Iron per100 gm biscuit = $(d \times 100)/c$

Where,

d = mg/l Iron measured on atomic absorption spectrophotometer,

c = g biscuit taken.

3.6 Sensory Evaluation of Biscuits

A test panel evaluated the consumer's acceptability of prepared biscuits. Four biscuit samples containing various proportions of wheat flour and spinach powder were evaluated for their sensory attributes (color, flavor, texture, taste and overall acceptability). The test panels (15) were selected from students, officers and teachers of the Faculty of Engineering, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh. The coded samples were served in clean white plastic plates in individual booths with adequate florescent lights. Sample presentation to the panelists was at random and one at a time. They were provided samples to eat and check how much they liked or disliked each one and rate them as such. The panelists were given enough water to wash their mouths between each serve. For statistical analysis of sensory data, a 9-point hedonic rating test (Amerine *et al.*, 1965) was performed to assess the degree of acceptability. The taste panelists were asked to rate the sample on a 9-point hedonic scale for color, flavor, texture, taste and overall acceptability with the ratings of following: 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 and1= dislike extremely.

3.7 Statistical Analysis

All the experiments were performed in triplicate unless stated otherwise. To know the effect of different formulation methods on physical properties, physiochemical properties, and sensory quality of biscuits single factor completely randomized designed was applied. The statistical software program SAS (version 9.3) was used for the analysis of variance (ANOVA) on physical properties, physiochemical properties, and sensory quality of the biscuit. Duncan's multiple Range Test (DMRT) was also employed among the formulation methods to establish multiple comparisons of the mean values for physical properties, physiochemical properties, and sensory quality of biscuit at $p \leq 0.05$.

CHAPTER IV

RESULTS AND DISCUSSIONS

4.1. Physiochemical properties of wheat flour and spinach powder

Table 4.1 is representing the physiochemical properties of wheat flour and spinach powder used for the experiment where moisture content, protein, fiber, fat, ash, total carbohydrate and energy of wheat flour was found 12.5%, 10.17%,0.8% , 1.8%, 0.91%, 73.76% and 351.67Kcal/100g, respectively. Similar result is reported by Prodhan et al. (2015) (moisture content 12.55%, 10.5% protein, 0.6% fiber, 1.2% fat, 0.85% ash, 74.3% carbohydrate and energy 350 Kcal/100g).

Table 4.1 Chemical compositions of wheat flour and spinach powder.

Components (%)	Wheat flour	Spinach powder
Moisture	*12.5±0.30	10.2±0.10
Protein	10.17±0.32	25.73±0.50
Fiber	0.80±0.10	11.17±0.31
Fat	1.80±0.10	4.2±0.30
Ash	0.91±0.03	5.22±0.31
Total Carbohydrate	73.76±0.24	43.48±0.79
Energy (Kcal/100g)	351.67±1.07	254.67±1.32

*Values are average of triplicate with ± SE.

On the other hand, significantly higher amount of protein 25.73%, fiber 11.17% and fat 4.2% was found in spinach powder (*Spinach oleracea*) with moisture content of 10.2%, ash 20.22% and carbohydrate 28.48%. This composition of spinach powder is close to that of recorded by Kavitha *et al.* (2013) (moisture 11.17%, protein 19.10%, fiber 15.48%, fat 7.11%, ash 5.57% and carbohydrate 41.49%). Raw spinach contains 11.10%

of protein (Kavitha *et al.* 2013) and as the spinach powder is in dry and concentrated form so the protein content in the powder was found to be much higher.

4.2 Effects of spinach powder on physical quality of biscuits

The incorporation of spinach powder in wheat flour directly affected the physical quality of biscuits. Table 4.2 revealed slight increase in weight (ranging from 5.72gm to 5.81gm) where weight gain was highest for F_3.

Table 4.2 Effect of spinach powder on physical quality of biscuits

Measured	**Samples of biscuit**			
Parameters	**C**	**F_1**	**F_2**	**F_3**
Weight (gm)	5.72 ± 0.003^{d}	5.78 ± 0.003^{c}	5.79 ± 0.003^{b}	5.81 ± 0.003^{a}
Diameter (cm)	3.25 ± 0.01^{c}	3.25 ± 0.02^{a}	3.27 ± 0.01^{b}	3.27 ± 0.01^{b}
Density (gm/cc)	0.318 ± 0.003^{a}	0.301 ± 0.004^{b}	0.314 ± 0.001^{a}	0.315 ± 0.001^{a}
Thickness (cm)	0.584 ± 0.001^{d}	0.612 ± 0.001^{a}	0.593 ± 0.001^{c}	0.605 ± 0.002^{b}
Spread Ratio(D/T)	5.59 ± 0.01^{a}	5.46 ± 0.03^{b}	5.54 ± 0.01^{a}	5.43 ± 0.03^{b}

N.B. Means with different superscript within the same row differ significantly ($p<0.05$) using DMRT (Duncan's Multiple Range Test). C= Biscuit with 0% spinach powder; F_1 = Biscuit with 3% spinach powder; F_2 = Biscuit with 5% spinach powder; F_3 = Biscuit with 7% spinach powder.

An increase in the diameter was observed due to increase of spinach powder (3.25 cm, 3.27 cm and 3.27cm for incorporation of 3%, 5% and 7% spinach powder respectively). A bit change was found in density ranged from 0.301 to 0.318 which is lower in comparison to sample C. A greater biscuit thickness (ranged from 0.584 to 0.612) can be seen in Table 4.2 and the highest value was recorded for F_1 (0.612). This is because of the amount of spinach powder incorporated with wheat flour.

Spread ratio is a crucial parameter for biscuit quality which was between 5.46 and 5.54 for F_1, F_2 and F_3 (Table 4.2). The values are lower from the value of C sample (5.59).

The lowest spread ratio was obtained for F_3. Such decrease may occur due to the converse relationship between fat content and spread ratio (Srivastva *et al.* 1993).

4.3 Proximate composition of biscuits

The prepared biscuits using spinach powder at different levels (3%, 5% and 7%) were analyzed for different parameters such as moisture content, protein, ash, fiber, fat and carbohydrate. The Proximate compositions are given in the Table 4.3:

Table 4.3: Proximate composition of biscuits

Parameters (%)	C	F_1	F_2	F_3
Moisture	3.85 ± 0.03^{a}	3.26 ± 0.03^{b}	3.15 ± 0.03^{c}	3.12 ± 0.02^{c}
Protein	6.53 ± 0.23^{d}	7.41 ± 0.03^{c}	8.75 ± 0.07^{b}	10.18 ± 0.02^{a}
Fiber	1.16 ± 0.02^{d}	1.33 ± 0.01^{c}	1.63 ± 0.02^{b}	1.93 ± 0.02^{a}
Fat	20.12 ± 0.01^{a}	14.67 ± 0.01^{b}	14.57 ± 0.01^{c}	14.34 ± 0.01^{d}
Ash	1.54 ± 0.02^{d}	3.59 ± 0.01^{c}	3.63 ± 0.01^{b}	3.7 ± 0.01^{a}
Carbohydrate	66.8 ± 0.23^{c}	69.75 ± 0.06^{a}	68.26 ± 0.04^{b}	66.72 ± 0.05^{c}

N.B. Means with different superscript within the same row differ significantly ($p<0.05$) using DMRT (Duncans Multiple Range Test).

The higher portion of spinach powder resulted in lower percentage in moisture content and fat content in comparison with the control sample. The higher values for moisture content (3.26%) and fat (14.67%) were found for sample F_1 and sample F_3 gained the lowest value for moisture content (3.12%) and fat (14.34%). Ullah *et.al* (2016) found 3.49%, 3.39%, 3.31% and 3.26% moisture content for the prepared biscuits containing 5%, 10%, 15% and 20% alfalfa seed flour respectively. The values are almost similar as the formulated biscuits.

The results revealed in Table 4.3 also indicate the upward trend for the values of protein, fiber, ash and carbohydrate in comparing with the control value. The protein contents are 6.53%, 7.41%, 8.75% and 10.18% for control, F_1, F_2 and F_3 respectively. These results are in close agreement with findings of Galla *et al.* (2017) who have reported 7.54%, 8.88% and 10.79% protein content for incorporation of 5%, 10% and 15% spinach powder respectively.

The fiber and ash of prepared biscuits are ranged from 1.33% to 1.93% and 3.59% to 3.7% with the inclusion of spinach powder. The values for fiber are close to those found by Galla *et al.* (2017) who reported 1.22%, 2.31% and 2.87% for 5%, 10% and 15% incorporation of spinach powder. The variation might be due to varietal differences, different maturity and growing conditions of the crop. The values for ash are also similar to those found by Ullah *et al.* (2016) (1.54%, 1.66% and 1.78% ash content for 5%, 10% and 15% incorporation of alfalfa seed flour respectively).

From Table 4.3 the fat contents are 14.67%, 14.57% and 14.34% for $F_{1,}$ F_2 and F_3 respectively which are lower than the control sample (20.12%). The fat content is decreased with the increase of spinach powder because the crude fat content of spinach powder is lower than the fat content of wheat flour. These results are in accordance with the findings of Singh *et al.* (2006) who found that supplementation with bathua leaves decreased the fat content of wheat flour based "Paratha.

4.3.1 Energy calculation of prepared biscuits

The energy value was calculated from the proximate composition of prepared biscuits shown in Fig 4.3.1:

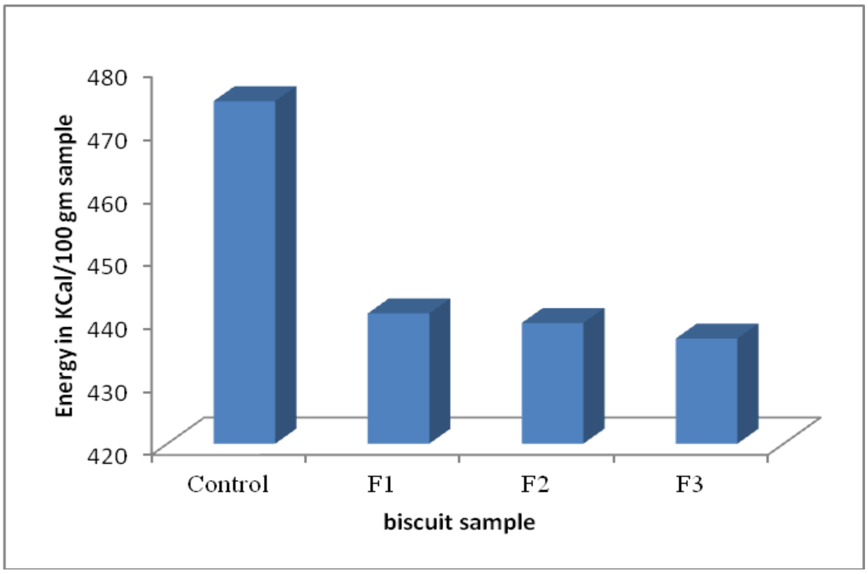


Fig 4.3.1: Graphical representation of energy calculation.

From Fig 4.3.1 it is seen that the energy level is decreased with the further incorporation of spinach powder. The energy contents are 474.38 Kcal/100g, 440.67 Kcal/100g, 439.17 Kcal/100g and 436.70 Kcal/100g for sample C, F_1, F_2 and F_3 respectively. The similar observation is reported by Galla *et.al* (2017).

4.3.2 Mineral composition of prepared biscuits

The mineral composition of prepared biscuit samples are mentioned in Table 4.3.2:

Table 4.3.2 Mineral composition of prepared biscuits

Sample(mg/100g)	**C**	**F1**	**F2**	**F3**
Calcium	123.67±1.53	170.91±1.87	211.77±1.81	261.49±0.89
Iron	1.20±0. 06	2.19±0.06	3.90±0.06	5.19±0.05
Phosphorus	61.46±0.64	72.41±1.12	91.26±1.17	112.87±1.50

The calcium contents of prepared biscuits were increased with the addition of spinach powder. It was found that 170.91 mg/100g, 211.77 mg/100g and 261.49 mg/100g calcium present in F_1, F_2 and F_3 respectively which are higher than the control sample (123.67mg/100g). This is the consequence of high amount of calcium in spinach powder. Galla *et.al* (2017) found in the biscuits supplemented with 5%, 10% and 15% spinach powder contain 203.75 mg/100g, 337.89 mg/100g and 460.18 mg/100g calcium, which is similar to this observation.

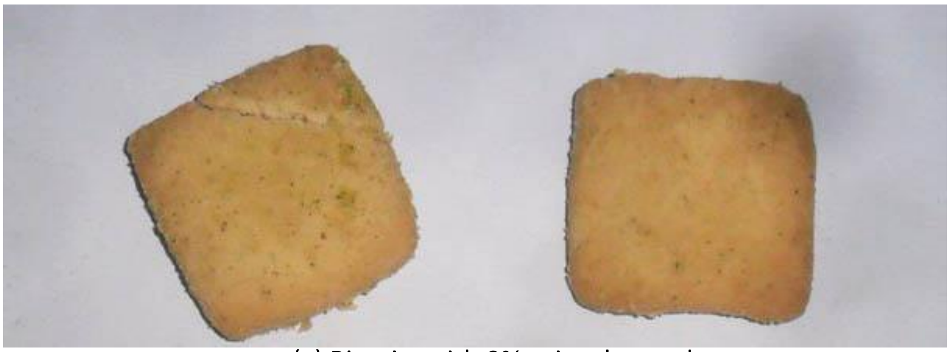
From Table 4.3.2, the inclination in value is observed for both iron and phosphorus. It was found that 2.19 mg/100g, 3.90 mg/100g and 5.19 mg/100g iron and 72.41 mg/100g, 91.26 mg/100g and 112.87 mg/100g phosphorus present in F_1, F_2 and F_3 respectively which are greater than the values of sample C. Galla *et.al* (2017) found iron content for the biscuits supplemented with 5%, 10% and 15% spinach

owder is 3.74 mg/100g, 5.64 mg/100g and 7.89 mg/100g which is similar to this experiment. The phosphorus content is also similar to the report of Galla *et.al* (2017).

4.4 Sensory evaluation of prepared biscuits

The color, flavor, texture, taste and overall acceptability of the spinach powder incorporated biscuits were evaluated by a panel consisting of 15 judges. The members scored for preferences of color, flavor, texture, taste and overall acceptability. The mean score obtained from sensory analysis is depicted on Table 4.4. The degree of differences among the samples was evaluated by Duncun's Multiple Range Test (DMRT).

BISCUITS WITH DEFERENT PERCENTAGE OF SPINACH POWDER



(a) Biscuits with 0% spinach powder

(b) Biscuits with 3% spinach powder

(c) Biscuits with 5% spinach powder

(d) Biscuits with 7% spinach powder

Fig 4.3.2 : Biscuits with deferent percentage of spinach powder.

Table 4.4 Sensory quality of spinach powder supplemented biscuits

Sensory attributes	C	F_1	F_2	F_3
Color	7.6±1.12^a	7.2±0.86^a	5.93±0.88^b	4.93±0.70^c
Flavor	7.6±1.06^a	7.13±0.99^a	5.93±0.80^b	5.07±0.80^c
Texture	7.73±1.03^a	7.0±0.76^a	7.67±0.98^b	6.13±0.74^c
Taste	7.67±0.90^a	7.2±0.86^a	6.0±0.93^b	5.13±1.13^c
Overall acceptability	7.67±0.98^a	7.73±1.03^a	7.0±0.76^b	6.13±0.74^c

All data of the samples are significantly difference from each other at 5% level of significance. Values are average of triplicate analysis with ±SD.

A two way analysis of variance (ANOVA) was carried out for all sensory attributes measurements. Sample F_1, F_2 and F_3 were found significantly different for each attribute where sample C and F_1 showed significantly same result (Table 4.4). F_1 secured the highest score for color, flavor, texture, taste and overall acceptability (7.2, 7.13, 7.0, 7.2 and 7.73 respectively) among the spinach powder incorporating samples. With the increase of spinach powder the color became dark green. The light green color of F_1 sample was mostly liked by the panelists.

Spinach-like flavor was mostly disliked by the judges. Although texture of F_2 secured higher result (7.67), F_1 scored the best value (7.2) for taste. Among the samples (F_1, F_2 and F_3) the overall acceptability value was the highest for F_1 (7.73).

So, the results outline that nutritionally F_1, F_2 and F_3 samples are significantly superior than sample C and F_1 is mostly preferred by the panelists among the spinach incorporated samples.

Finally, the result prioritized sample F_1 among F_1, F_2 and F_3 but sample F_2 and F_3 could be consumed as well.

CHAPTER V

SUMMARY AND CONCLUSION

The study was carried out to prepare value added low calorie and high fiber biscuits by supplementing wheat flour by spinach powder. The effect of spinach powder on physical and nutritional quality and consumer acceptance of the prepared biscuits were also evaluated.

After collecting the spinach sample it was dried in a multistoried universal cabinet tray dryer (55-60^{0}C) and the powder was prepared. Biscuits were prepared using wheat flour (Teer brand), spinach powder, milk powder, dalda, sugar, baking powder and egg. Changing the ratio of wheat flour and spinach powder, four formulations namely, C (40% wheat flour+ 0% spinach powder), F_1 (37% wheat flour+ 3% spinach powder), F_2 (35% wheat flour+ 5% spinach powder) andF_3 (33% wheat flour+ 7% spinach powder) were prepared. After mixing all ingredients properly, dough was prepared and cut for desired biscuit shapes and baked at 160^{0}C for 30 minutes. The physical parameters such as, weight, diameter, density, thickness and spread ratio were measured to determine the effect of spinach powder. Diameter and thickness were found to be increased of the biscuits prepared using formulation of F_1, F_2 and F_3 where the density and spread ratio decreased compared to sample C.

Quality of biscuits was analyzed in terms of moisture, protein, fiber, fat, ash and carbohydrate contents. Analysis result revealed that incorporation of spinach powder positively affected the nutrient content of prepared biscuits. The moisture content was found to be decreased with incorporation of spinach powder. The biscuit containing low moisture content usually enhance the shelf life. Biscuit supplemented with 7% spinach powder found the highest nutrient content and low energy value among samples. Sample F_1, F_2 and F_3 are also found to be rich in mineral contents (calcium, iron and phosphorus).

On the other hand, sensory evaluation of the prepared biscuits was carried out on the basis of sensory attributes including color, flavor, texture, taste and overall acceptability. Sensory analysis showed that F_1 sample (biscuit with 3% spinach powder) had highest scores for maximum sensory attributes. But the texture value was high for the F_2 sample. The sensory evaluation value was stumpy for F_3 sample. The overall acceptability was also better for F_1 sample.

From the discussion it is evident that, all biscuits prepared with spinach powder were highly nutritious than control biscuit and among those F_1 was highly accepted by the consumers. Although the level of dehydrated spinach powder incorporation was small, the increase in nutrient content is remarkable. Thus, spinach powder biscuits have potential to serve the valuable source of calcium, iron, fiber and protein in the diet of the population in Bangladesh and other developing countries. Using this study as a base, further investigations may carry out with the addition of biscuit improver to get a more acceptable quality biscuit.

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APPENDICES

Appendix-1: Statistical analysis of sensory evaluation for color.

Table 1.1 Rating scores for color of Biscuits.

Panelist No.	**Sample No.**			
	C	F1	F2	F3
1	8	8	7	6
2	7	7	6	5
3	6	7	5	4
4	9	8	6	5
5	7	6	5	4
6	8	7	6	5
7	9	8	5	6
8	6	6	5	5
9	8	7	6	5
10	9	8	7	5
11	7	7	6	4
12	8	7	5	4
13	9	9	8	6
14	6	7	6	5
15	7	6	6	5
Mean	7.6	7.2	5.933333	4.933333
SE	1.121224	0.861892	0.883715	0.703732

Appendix-2: Statistical analysis of sensory evaluation for flavor.

Table 2.1 Rating scores for flavor of Biscuits.

Panelist No	**Sample No.**			
	C	F1	F2	F3
1	8	8	7	5
2	8	7	6	6
3	6	6	5	4
4	7	6	5	5
5	9	9	7	6
6	8	7	6	5
7	9	8	7	6
8	8	7	6	6
9	7	6	5	4
10	6	6	5	4
11	8	7	6	5
12	6	8	6	5
13	7	6	5	4
14	9	8	7	6
15	8	8	6	5
Mean	7.6	7.133333	5.933333	5.066667
SE	1.055597	0.99043	0.798809	0.798809

Appendix-3: Statistical analysis of sensory evaluation for texture.

Table 3.1 Rating scores for texture of Biscuits.

Panelist No.	**Sample No.**			
	Control	F1	F2	F3
1	8	8	9	7
2	7	6	6	5
3	6	7	8	7
4	8	8	8	7
5	9	6	9	6
6	9	7	8	6
7	7	7	7	6
8	8	6	7	5
9	6	6	6	5
10	9	7	8	6
11	7	8	7	6
12	8	7	7	6
13	9	7	8	7
14	8	8	9	7
15	7	7	8	6
Mean	7.733333	7	7.666667	6.133333
SE	1.032796	0.755929	0.9759	0.743223

Appendix-4: Statistical analysis of sensory evaluation for taste.

Table 4.1 Rating scores for taste of Biscuits.

Panelist No.	**Sample No.**			
	Control	F1	F2	F3
1	8	7	7	6
2	7	8	6	7
3	7	7	6	5
4	8	6	5	4
5	9	9	8	7
6	7	6	5	5
7	9	8	7	5
8	8	7	6	6
9	7	8	6	5
10	6	7	5	3
11	8	7	7	5
12	9	8	6	6
13	7	7	5	4
14	8	7	6	5
15	7	6	5	4
Mean	7.666667	7.2	6	5.133333
SE	0.899735	0.861892	0.92582	1.125463

Appendix-5: Statistical analysis of sensory evaluation for overall acceptability.

Table 5.1 Rating scores for overall acceptability of Biscuits.

	Sample No.			
Panelist No.	Control	F1	F2	F3
1	8	7	7	6
2	9	8	8	7
3	8	9	7	7
4	7	8	7	6
5	7	7	8	6
6	8	9	7	6
7	6	6	6	5
8	7	8	6	5
9	7	7	7	6
10	8	9	7	6
11	9	9	6	6
12	8	8	8	7
13	8	6	7	7
14	6	7	6	5
15	9	8	8	7
Mean	7.666667	7.733333	7	6.133333
SE	0.9759	1.032796	0.755929	0.743223