COMPARATIVE PROXIMATE ANALYSIS OF A NEWLY DEVELOPED SOYA-MUSHROOM AND COMMERCIALLY AVAILABLE SIXTEEN BISCUITS IN DHAKA CITY

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BIOTECHNOLOGY AND GENETIC ENGINEERING DISCIPLINE
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KHULNA-9208, BANGLADESH

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DEDICATED TO MY

BELOVED PARENTS

TITLE

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DECLARATION

This thesis report is the original research work of the authors, except as otherwise stated. The material presented has not submitted either in whole or in part for a degree for this or any other university. The findings of the research has not been/will not be published anywhere without the written concurrence of the concerned supervisor. Legal action would be taken in any case of infringement regarding the declaration.

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ABSTRACT

Biscuits are high in carbohydrates, fat and calorie but low in fiber, vitamin, and mineral making them unhealthy for daily use. Because of the acceptability people of all ages, longer shelf-life, better taste and use as snacks, biscuits could be used for protein fortification and other nutritional improvement. The present study was intended to explore the possibility of fortifying the wheat flour with soya-flour and oyster mushroom (*Pleurotus ostreatus*) to formulate biscuits with improved nutritional value along with acceptable sensory properties for consumption. The proximate analyses for nutritional values in terms of (Ash, moisture, protein, sugar, carbohydrate, fiber, fat and calorific contents) of the developed soya-mushroom salt biscuits were compared to those of local biscuits. From overall acceptability rating, supplementation with 15 % soya-flour and 3% mushroom into the dough showed increased in fiber, ash and protein content without any influence on the sensory characteristics. The best values for nutritional evaluation of developed supplemented composite biscuits among the samples were obtained for moisture (1.4±0.08%), ash (2.16±0.07%), protein (9.53±0.18%), fat (19.45±1.22%), sugar (8.32±0.6%), fiber (0.46±0.04%), carbohydrate (64.35±1.6%) and calorie (485.48±5.54%) per 100g respectively. One-way ANOVA analysis ($p \leq 0.05$) showed supplementation with produced significant difference in terms of fiber content of the developed biscuits. Therefore, supplementation with soya flour and mushroom should be varied to produce biscuits with improved nutritional quality without adversely affecting the sensory parameters.

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INTRODUCTION

INTRODUCTION

1.0 Introduction

Short-dough biscuits are one of the widely consumed food products. They are appreciated for their tastes, versatility, convenience, conservation, textures and appearances. The use of natural ingredients exhibiting functional properties and providing specific health benefits, beyond traditional nutrients, is a very attractive way to design new food products. This offers an important market niche for the healthier foods and is experiencing an exponential growth.

Biscuits are a variety of quick breads popular in different forms throughout the world. They may be regarded as a form of confectionery dried to very low moisture content. According to Fayemi (1981), biscuit is defined as a small thin crisp cake made from unleavened dough. Okaka (1997) described the production of biscuits as a mixture of flour and water but may contain fat, sugar another ingredients mixed together into dough which is rested for a period and then passed between rollers to make a sheet. Biscuits may be classified either by the degree of enrichment and processing or by the method adopted in shaping them. Based on the enrichment criterion, biscuits may be produced from hand dough, soft dough or from batters (Fayemi, 1981).These products are usually cereal based and baked to keep the moisture content of less than 5%. The prime requirement of a biscuit is that it will have a smooth surface which has a slight shine or sheen and an open even texture giving a bite ranging from hard to delicate. This is achieved by a subtle balance between the requirements of recipe and processing.

Biscuits are popular as hot bread for breakfasts and other meals. They can give us nutrients important to our health and provide energy for work and play. The most important nutrients they provide are carbohydrates, fat, and B vitamins for energy; calcium for strong bones and teeth; and protein for growth and repair. They can also provide minerals, including iron (Paula M, 2002). White flour used for making biscuits is enriched. The white flour used for the production of biscuits is deficient in several nutrients including some vitamins, mineral elements as well dietary fiber (Awan et al., 1991). Wheat flour by itself lacks certain essential amino acids such as lysine, tryptophan and threonin (Kent, 1975).

The population everywhere desires to eat a healthier diet without changing their conventional dietary patterns (Becker C, et al., 1998). Eating of healthy food has received more attention in the recent years due to disliking of some having some health concerns such as fat, sugar and salt (Hilliam M, 1995). Biscuits are high in carbohydrates, fat and calorie but low in fiber, vitamin, and mineral which make it unhealthy for daily use. Among convenience foods they are very convenient and inexpensive but have only about 6 to 7% protein (Agarwal, 1990). Now days there are an ever-growing demand for high protein biscuits. This may be achieved through incorporation of protein-rich ingredients from soybean and mushroom.

Soybean is an excellent health food and it contains 40% good quality protein, 23% carbohydrates, 20% cholesterol free oil and sufficient amounts of minerals and vitamins. Amino acid profile of soy protein is excellent amongst plant proteins. Hence, it is superior to other plant proteins as it contains most of the essential amino acids except methionine, which is abundant in cereals and it is most economical source of dietary protein. It is high in vitamin, mineral and antioxidant like Isoflavones which helps in cholesterol reduction, preventing cancer and regulation of menopause. The main ingredient of any bakery products is wheat, which is having deficiency of essential amino acid lysine (Bakle, et al., 2007).Whereas soybean is richer in lysine and can be complement to wheat in bakery products. Study carried out that enrichment of defatted soya flour up to 20% improves the nutritional quality of bakery products like biscuit, bread muffins without affecting its taste, textural and overall acceptability of product (Shakuntaleb and Masum, 2008). Nutritional quality and consumer acceptability of soya cereal blends improves the nutritional status of vulnerable groups like pregnant woman, nursing mother, school going & young children (Jideani, et al., 2009). Protein energy malnutrition (PEM) is one of the most serious health problems in developing countries like India, Bangladesh (FAO, 2007).

The importance of mushroom has been recognized in recent years due to their therapeutic value, with beneficial effects upon human health. Mushroom is a unique plant food which is very low in carbohydrates. Because of their inability to photosynthesize using sugars, they are regarded ideal for diabetic patients (Hall et al., 2003). Dietary mushrooms are a good source of B vitamins, such as riboflavin, niacin and pantothenic acid and the essential minerals, selenium, copper and potassium. Fat, carbohydrate and calorie content are low, with absence of vitamin C and sodium. It also helps to regulate blood sugar levels

(Koyyalamudi et al., 2009). In this study, efforts have been made to supplement soybean flour with mushroom to develop nutritionally protein–enriched biscuits.

With the current status nutritional quality of biscuits and growing demand of nutritious foods, it seems worthwhile to take efforts in enhancing the nutritional value of biscuit. The development of a commercially viable biscuit attractive to children and adults with significant reduction in fat and sugar and which will provide fewer calories and contain nutrients designed to reduce the risk of coronary heart disease is highly desirable (Gibson and Williams, 2000). Considering these facts an attempt was made to prepare protein, fiber and mineral enriched biscuits from mushroom, soyaflour and refined wheat flour.

The present study was undertaken to fulfill the following objectives:

- To develop an enriched biscuit by modifying the recipe of traditional biscuits with the supplementation of mushroom and soya-flour.
- To compare the nutritional and quality parameters of the developed biscuits over traditional biscuits.

Chapter Two:

REVIEW OF LITERATURE

2.0 REVIEW OF LITERATURE

Increasing awareness of consumers regarding health and nutrition has led to experimentations with an aim to subsequent modifying and development of bakery products to value added health foods. This serves as the basis for further development of bakery products using low cost, nutritious ingredients. Among these bakery products cookies/biscuits are popular and well accepted as snack food. 'Cookie' is chemically leavened product, also known as 'biscuit'. Generally the term biscuit is used in the European countries and cookies in the United States of America. Biscuits and biscuit like products have been made and eaten by man for centuries (Hosney, 1986).

2.1 Development of value added biscuits

Bakery products are popular both in urban and rural area. Among these biscuits, form the most popular item because of their sensory qualities, convenience, ready to eat nature and long shelf life. These qualities make the biscuits a favorite experimental material for enhancement of nutrient composition and nutraceutical quality either by supplementation of nutrients or nutraceutical components.

2.1.1 Value addition to biscuits through substitution of flours

Refined wheat flour is the base ingredient in bakery products. Substitution wholly or partially with flours rich in nutrients and/or nutraceutical components enhance the nutritive quality of biscuits.

Ranhotra et al. (1991) developed high fiber chocolate chip and oat meal raisin cookies by substituting treated flour at 0, 50, 75 and 100 percent levels with untreated wheat flour. The treated flour was prepared by exposing the flour to repeated autoclaving and cooling cycles. Complete replacement of untreated flour with treated flour adversely affected the dimensions of the cookies and organoleptic characteristics. The total dietary fiber content in chocolate chip cookies varied from 1.50 percent in control cookies to 3.50 percent in 75.00 percent treated flour incorporated cookies. However, in oat meal raisin cookies it ranged from 2.70 percent to 3.90 percent.

Patel and Rao (1995) evaluated the effect of incorporation of untreated, roasted and germinated black gram flour (0 to 25%) on biscuit characteristics. It was indicated that the incorporation of black gram flour decreased the spread ratio with concurrent increase in thickness of biscuits. Maximum reduction was indicated in biscuits incorporated with germinated black gram. The reduction in spread was attributed to altered starch properties of the flours used. The addition of black gram flour at 25 percent levels increased the hardness values of biscuits. Hardness of control wheat flour biscuits was reported to be 2117 g, which increased to 2442 g in untreated black gram and further higher value of 2867 g in germinated black gram incorporated biscuits. Further it was inferred that black gram incorporation in any form decreased the sensory attributes of biscuits.

Singh et al., (1996 and 2000) developed defatted soy flour cookies and reported that with increased incorporation (from 0 to 50%) the cookie diameter, spread ratio and spread factors decreased (61.40 to 56.00 mm, 8.77 to 7.78 and 100 to 88.71%, respectively), where as hardness increased (26.97 N to 46.25 N). It was also proposed that soy flour incorporation up to 20 percent resulted in acceptable cookies. The level of incorporation of soy flour resulted in nutrient composition resulting in two fold increase in protein (4.91 to 10.54%) and fourfold increase in crude fiber (0.12 to 0.46 %). Soy biscuits (20%) contained higher amounts of calcium (41.46mg/100g), phosphorous (181.78mg/100g) and iron (14.53mg/100g) than control biscuits (16.53, 147.32 and 14.53mg/100g of calcium, phosphorous and iron, respectively). Soy biscuits exhibited higher non enzymatic browning (0.08 OD) than control biscuits (0.06 OD), which was attributed to higher levels of reducing sugars and protein in the formulation. These biscuits exhibited higher in vitro protein digestibility (83.82%) than control biscuits (68.46%).

Incorporation of full fat soybean flour was reported to exert no adverse effects on sensory parameters (Kulkarni, 1997). Full fat soy flour was extracted from whole soybeans containing different moisture levels (8.00 and 12.00%) and was roasted for different durations at 217 °C (10-45 seconds and 90-120 seconds, respectively for 8 and 12% moisture levels, respectively). The full fat soy flour did not affect sensory properties.

Onweluzo and Iwezu (1998) developed biscuits by blending fermented and unfermented soy flour with whole wheat and cassava flours in different proportions. It was reported that spread ratio (1.80) and biscuit weight (11.70g) were highest in control samples, which also exhibited lowest breaking strength (1.80kg). The acceptability of biscuits with whole wheat flour and fermented soy flour (1:1) was comparable to control biscuits, which contained protein (8.80%), crude fiber (1.00%) and fat (25.70%) with calorific value of 486 Kcal.

Biscuits from pearl millet and pigeon pea flour blends in the ratio of 100:0, 75:25, 65:35 and 50:50 were evaluated for sensory qualities and nutrient composition by Eneche (1999). It was indicated that all the biscuits exhibited high sensory ratings. The biscuits with blend flour of millet and pigeon pea (65:35) were highly acceptable for flavor, texture and general acceptability. Nutritional evaluation of these biscuits indicated high proportion of protein (15.20%), fat (18.10%) and digestible carbohydrate (66.50%).

Akubor (2003) developed biscuits from cowpea and plantain flour blends (60:30). The blend flour was incorporated with wheat flour at different levels ranging from 10 to 60 percent. It was indicated that at all levels of wheat flour substitution thickness and diameter of biscuits were not affected. The biscuit thickness ranged from 3.60 to 3.80 cm, weight from 7.10 to 8.20g and spread ratio from 3.10 to 3.40. The sensory scores for color, flavor and texture of the blend biscuit (85:25) were comparable with those of 100 percent wheat flour biscuits. The protein content of the cowpea plantain flour / wheat flour biscuits ranged between 15.20 to 18.96 percent, the values increased with increased levels of inclusion of cowpea flour in the blends.

Sindhuja et al., (2005) reported that increased incorporation of grain amaranth flour in biscuits (0 to 30%) significantly reduced the spread ratio from 7.82 to 6.50, breaking strength from 4.94 to 3.39kg but increased the thickness from 10.9 to 11.5 mm. Further, addition of lecithin (0.25%) and glycerol monostereate (0.25%) significantly increased the biscuits spread (8.49) and decreased the breaking strength (3.81kg). Incorporation of grain amaranth flour was reported to yield golden brown cookies which were malty and sweet flavored.

Mridula and Gupta (2008) developed high fiber biscuits by substituting bajra flour at 10 to 50 percent in wheat flour and fortified with 5 percent defatted soy flour. It was reported that with increased proportion of bajra flour, the thickness and spread ratio of biscuits decreased. This was attributed to high fiber and low dough strength of bajra flour blends. Bajra flour biscuits (50%) recorded higher crude fiber (1.08%), fat (24.16%) and ash (1.41%)

than the control biscuits (0.36, 22.10 and 1.15% of crude fiber, fat and ash, respectively). However, protein content of bajra biscuits was less (6.53%) than control biscuits (7.11%).

Masur and her associates (2009) developed high protein biscuits using bengal gram flour (10 to 25%), altering the levels of water, fat and baking powder. Results revealed that height (5.83 cm) and diameter (58.80 mm) of biscuits were unaffected up to 20 per cent level of bengal gram flour incorporation, but the spread ratio and spread factor decreased in response to 25 percent bengal gram flour incorporation. The sensory quality of biscuits prepared with 16 per cent fat and 13 ml water resulted in highest scores for flavor, texture and overall acceptability. The most acceptable biscuits (20% Bengal gram flour incorporation) exhibited higher protein (5.59%), fat (0.85%) and crude fiber (0.50%) compared to control biscuits (4.98, 0.41 and 0.14% of protein, fat and crude fiber, respectively).

2.1.2 Value addition to biscuits with unconventional ingredients

Dried and milled brewers spent grain was blended with flour (5 to 60%) in cookies preparation by Prentice et al., (1978). Addition of soy lecithin (1 and 2%) improved the spread ratio and top grain formation in cookies. The biscuits exhibited an undesirable brown color with more than 20 percent brewers spent grain addition. Acceptable physical qualities were obtained with 40 percent brewers spent grain, which resulted in 74 percent increase in nitrogen and a tenfold increase in crude fiber content from 1.50 to 15.10 percent.

Artz et al., (1990) indicated that corn fiber addition in sugar snap biscuits (15%) imparted lower shear values (144 N/g) than in no fiber control samples (207 N/g). The biscuits with lowest shear force value exhibited lowest moisture (5.77%) content, which could have contributed to increased brittleness. Addition of fiber imparted darker color to the product. Results of the triangular taste test indicated significant differences between control and corn fiber biscuits.

Awasthi and Yadav (2000) showed that liquid dairy by-products could be efficiently used to produce protein rich biscuits. Chhana whey (75%) and skim milk powder (50%) were blended separately and mixed with defatted soy flour (15%) for biscuit preparation and compared with wheat flour biscuits. It was reported that biscuits containing soy flour and skim milk powder exhibited significantly higher amount of protein (13.12 %), ash (1.50 %) and crude fiber (0.46%) than control biscuits (5.92, 0.57 and 0.14 g/100g, protein, ash and crude fiber, respectively). Biscuits with chhana whey and soy flour contributed high calcium, phosphorus, iron, reducing and non reducing sugars than control and skim milk powder biscuits. Inclusion of soy and dairy byproducts were reported to exhibit higher rates of non enzymatic browning than control biscuits.

Defatted sesame seed flour was used to increase protein content by replacing millet flour at 30, 40 and 50 percent in biscuits (Alobo, 2001). It was indicated that biscuits exhibited reduced diameter (from 6.44 to 6.22 cm), weight (from 13.52 to 13.30 g) but increased thickness (from 0.87 to 1.24 cm) and spread factors (from 41.97 to 66.58%). Incorporation of sesame significantly increased protein content ranging from 10.08 to 28.64 percent in different levels concurrently with the amount incorporated. Salama et al., (2002) reported that incorporation of dried oyster mushroom (5%) in biscuits increased minerals and improved protein quality without affecting sensory attributes. Schober and O'Brein (2003) explored the possibility of developing gluten free biscuits from commercial gluten free flour, rice bran, corn starch, potato starch, soy flour, buck wheat flour and millet flakes in different proportions and combinations. The effect of fat powders, such as high fat and low fat dairy powders, microencapsulated high fat powder and palm oil were also studied. Inclusion of buckwheat flour in the formulae resulted in dark biscuits. It was reported that the biscuits with low fat powder exhibited highest moisture content but palm oil biscuits were opined to be most dry which was attributed to reduced loss of moisture during baking because of water binding protein of fat substitutes. Use of low fat powder resulted in firmer biscuits than palm oil and other fat substitutes.

Protein enhancement through incorporation of whey protein concentrate (WPC) and sodium caseinate at different levels of 5, 10 and 15 percent in biscuit formulation were investigated by Gallagher et al., (2005). It was reported that incorporation of WPC resulted in shrinkage of biscuits. Addition of sodium caseinate resulted in thicker biscuits (8.20 mm) than control (7.98 mm). The addition of the two protein powders increased surface brownness in biscuits which was attributed to Maillard reaction. The hardness of WPC incorporated biscuits was comparable to control samples. Addition of sodium caseinate was reported to significantly increase the hardness of biscuits significantly which was attributed to the gelling ability of sodium caseinate which enabled tight matrix of the ingredients used. The incorporation of dairy products resulted in higher moisture content and water activity than control biscuits.

Raju and his associates (2007) evaluated WPC blended (0 to 30%) biscuits for sensory and nutritional characteristics. It was reported that the cutting and compression strength of biscuits increased with increased WPC incorporation, which was attributed to the possible tight matrix of protein and other ingredients. Incorporation of WPC although increased the sensory scores for color and appearance, the scores for flavour, texture and overall acceptability decreased, but were comparable to control up to 30% incorporation. With regard to nutrient composition, protein and ash content of WPC incorporated biscuits were significantly higher (14.00 and 1.50%, respectively) than the control biscuits (5.20 and 1.10% respectively). However, it was reported that the fat, carbohydrate and crude fiber contents decreased in the biscuits from 20.50 to 18.50, 70.0 to 62.60 and 0.50 to 0.40g/100g, respectively.

Chaudhary and Awasthi (2009) developed high fiber biscuits with rice bran (15 to 35%). It was indicated that there was no change in thickness, spread and sensory scores of biscuits up to 30 percent rice bran incorporation as compared to control. The biscuits recorded higher protein (7.31%), fat (20.30%), crude fiber (3.20%) total dietary fiber (11.81%) and iron (7.20 mg/100g) than control biscuits (5.20, 17.50, 1.90, 5.90 and 1.70 mg/100g of protein, fat, crude fiber, total dietary fiber and iron, respectively). Akingbala et al., (2009) compared cassava root flour biscuits with wheat flour biscuits. It was reported that the physical and sensory qualities of the biscuits were similar. The nutritional analysis indicated that the cassava biscuits exhibited lower fat (19.80%) and protein (5.60%) than wheat biscuits (23.90 and 12.70% for fat and protein, respectively).

Ajila and associates (2008) made an effort to enhance the dietary fiber content by incorporating 5 to 20 percent of mango peel powder in biscuits. The study indicated that the diameter of biscuits decreased and breaking strength increased as a result of incorporation of mango peel powder. The effect was more pronounced at higher level of incorporation. This effect was attributed to diluting effects of gluten. Biscuits with 10 percent mango peel powder were highly acceptable and exhibited 14.40 percent total dietary fiber and 145 mg/gm of carotenoid.

2.2 Shelf life of value added cookies

Good shelf life of foods is an important criteria in consumer acceptability and marketability of new products. Shelf life depends on chemical composition, packaging material, environmental factors and handling. The study of shelf life foods helps in understanding mechanism of extending storage quality for added value.

Dietetic cookies and biscuits prepared using defatted soy-flour (5 to 10%) with dietetic baking powder and packed in polyethylene pouches at ambient exhibited a poor shelf life of 7-9 days (Lakshmi, 1991). Isabgol (4, 8 and 12%) and wheat bran (20, 30 and 40%) incorporated biscuits at different proportion were evaluated for storage quality by Shubha (1994). It was reported that biscuits were acceptable only for 7 days without any significant changes in sensory qualities. The samples stored for 35 days at ambient, packed in low density polyethylene pouches exhibited lowered acceptability as a consequences of storage.

Singh et al., (2000) studied the storage quality of soy fortified biscuits. It was reported that the higher moisture uptake was recorded in biscuits packed in laminated pouches than in polypropylene material (79.19%) during 60 days of storage period. Non enzymatic browning of both control (from 0.060 to 0.110 OD) and soy biscuits (from 0.085 to 0.110 OD) increased. Soy biscuits recorded higher peroxides than control initially respectively, during storage. However, the effect was prominent in biscuits packed in laminated pouches. Similar trend was observed with free fatty acids. Shelf life of finger millet biscuits packed in two layered polypropylene was 75 days (at 90% Rh, 38°C) and 50 days in metalized polyester poly laminated packs. The shelf life was increased over 120 days (at 65% Rh, 27°C) in the two packages (Selvaraj et al., 2002). Shariff et al. (2003) investigated shelf life of rice bran oil biscuits. It was reported that the biscuits prepared by replacing normal shortenings with rice bran oil were highly acceptable even after 45 days of ambient storage. Highest thiobarbiturates were recorded in cookies with 100 per cent rice bran oil (0.05). Initial values before storage were 0.03. Thus it was concluded that, by increasing the amount of rice bran oil the thiobarbiturates number decreased and the onset of rancidity was delayed.

The storage quality of whey protein incorporated cookies (0.00 to 30.00%), stored at ambient condition for 60 days, packed in metalized polypropylene was evaluated by Raju et al., (2007). In 60 days storage period, sensory scores decreased gradually and total viable bacteria increased from 10 to 20 cfu/g in control and in whey protein concentrate biscuits from 30 to 35 cfu/g. However, the count for yeasts in the latter was (10 to 40 cfu/g) than the former (10 to 20 cfu/g). Thus, studies indicated that the cookies could be stored satisfactorily for several months at ambient condition using suitable packaging materials.

Methods for moisture, crude fat, and crude fiber are empirical, thus part of this variability can be attributed to the use of different methods of analysis. A study was organized and supported by the American Feed Industry Association, the Renewable Fuels Association, and the National Corn Grain Association to evaluate the efficacy, applicability, and the intra-laboratory variation of a number of methods for moisture, crude protein, crude fat, and crude fiber in distillers dried grains (DDG) with soluble (DDGS). The moisture methods included in the study were AOAC 930.15, AOAC 934.01, AOAC 935.29, AOAC 2003.06; the crude protein determination methods studied were AOAC 990.03 and AOAC 2001.13; the crude fat determination methods studied are AOAC 945.16, AOAC 954.02, AOAC 2003.05, and AOAC 2006.06; and the crude fiber determination methods studied were AOAC 978.10 and AOCS 6a-05.

2.3 Determination of moisture content

Knowing the moisture content of the materials used throughout the food and baking process has become one of the most critical components in the industry as many raw materials sold today are based upon weight. It is also important to know the moisture content during the mixing stage in the food manufacturing process as water influences quality and consistency of the end product. Variations and excess moisture in mixes can result in clumping and out of spec recipe formulas (Isengard, 2001). Testing food moisture during this time would allow adjustments to be made throughout the mixing process to manage moisture levels. Manufactures always strive to have the best finished product. The amount of water in a product has effects on the taste, appearance, consistency, stability as well as the shelf life (Joseph, 1994). Testing the moisture levels of the final product ensures manufactures that they have the highest quality product and consistency (Isengard, 2001). Biscuit moisture content was determined by weight loss techniques involving heating crumb from crushed or ground biscuits (Mulvaney, 1995).

Maintaining proper moisture and temperature levels in biscuits production, especially during baking ensures that the end product will be top quality and have a long shelf life. Keeping oven temperatures even and humidity low is important when it comes to reducing moisture in biscuits and achieving the desired texture, color, and taste. The principal characteristic of bakery products is the low moisture content (5% max.) (Kim and Okos, 1999). Water is added to make the dough for the biscuits nevertheless it is not among the major ingredients present in the final product. Unbound water evaporates rather easily during the baking process at high temperatures (as high as 230°C). Water bound to some functional groups of the molecules of substances present in the bakery products may also get partly released at such high temperatures, and this helps the coloring of the surface of the final products (Haghighi and Segerlind, 1988). Water activity and moisture content are always important parameters influencing stability of biscuits during storage, in particular the resistance against microbes and rheological properties of the products (Mulvaney, 1995).

A biscuit moisture model was reported on the basis of regional spectral imaging features through stepwise feature selection and linear regression towards conventional weight moisture measurements (Kim and Okos, 1999). Moisture adsorption isotherms were determined using a gravimetric static method at 25^0C and 40^0C and over a range of relative humidity from .0112 to 0.903. The equilibrium moisture content of the biscuits increased when the storage temperature at a given water activity was reduced (Mulvaney, 1995).

2.4 Determination of Ash content

Ash is the inorganic residue remaining after the water and organic matter have been removed by heating in the presence of oxidizing agents, which provides a measure of the total amount of minerals within a food (Kosata et al., 2006). The ash fraction contains all the mineral elements jumbled together. It would be more useful to know the amounts of different individual elements. It includes metal salts, which are important for processes requiring ions such as Na^+ (Sodium), K^+(Potassium), Ca^{2+} (Calcium). Ash may include any of the following: oxides e.g. Al_2O_3, CaO, Fe_2O_3, MgO, MnO, P_2O_5, K_2O, SiO_2, carbonates: Na_2CO_3 (soda ash), bicarbonates: $NaHCO_3$ (a.k.a. baking soda) (Kosata et al., 2006). Ash in biscuits analysis is conducted by pyrolysis at 550-600°C which destroys all organic matter (McClements, 2001). Analytical techniques for providing information about the total mineral content are based on the fact that the minerals can be distinguished from all the other components within a food in some measurable way. Minerals are not destroyed by heating and they have a low volatility compared to other food components (Mulvaney, 1995). The three main types of analytical procedure used to determine the ash content of foods are based on this principle: dry ashing, wet ashing and low temperature plasma dry ashing (McClements, 2001). The method chosen for a particular analysis depends on the reason for carrying out the analysis, the type of food analyzed and the equipment available. Ash contents of fresh foods rarely exceed 5%, although some processed foods can have ash contents as high as 12%, e.g., dried beef (Sivasankar, 2002).

Dry ash is the most standard method for determining the ash content of biscuit samples. The sample is commonly ignited at 550-600°C to oxidize all organic materials without flaming. The inorganic residue that does not volatilize at that temperature is called ash. The ash content is determined from the loss of weight, which occurs from complete oxidation of samples (AOAC, 1995).

2.5 Determination of Sugar content

Various chromatographic procedures including thin-layer chromatography (TLC), gas–liquid chromatography (GLC), and high-performance liquid chromatography (HPLC) have been used for the analyses of sugars. GLC is successful for determining individual sugars, but it requires a derivatization procedure with its inherent problems and the formation of anomers. In addition, the application of the GLC method is not suitable to all foods (Iverson and Bueno, 1981). HPLC has been extensively used for sugar separation and quantitation (Richmond et al., 1981). The main drawback to HPLC is that sugars do not absorb UV light at a wavelength longer than 200 nm.

A high-performance liquid chromatography (HPLC) method was developed to extract and quantities individual sugars present in dough systems throughout processing. Sugars were extracted from freeze-dried samples by boiling in 60% ethanol (v/v). No defatting of samples was required (Meyer, 2000). All sugars (fructose, glucose, sucrose, maltose and lactose) were separated in 12 min using an aminopropyl 4-μm column and a 75:25 solution of acetonitrile water mobile phase to eliminate refractive index interference from salt contained in the samples (Kishihara et al., 1992).

Reducing sugar and non-reducing sugar (after inversion) reduces the copper in Fehling's solution to the red precipitate cuprous oxide (Kishihara et al., 1992). The sugar content in a biscuit sample is estimated by determining the volume of unknown sugar solution required to reduce completely a measured volume of Fehling's solution. The Fehling's solution is an alkaline solution of copper sulphate (CuSO4. 5H2O, 6.9%) and Rochelle salt Sodium Potassium Tartrate ($KNaC_4H_4O_6.4H_2O$, 34.6%). In this method, methylene blue is used as an oxidation-reduction indicator of the end point. The methylene blue is added to the reaction mixture of sugar and Fehling's solution. Its use is based on the fact that it is reduced and completely decolorized by minute amounts of reducing sugar or invert sugar but not so long as any cupric salt is present. The reduction is carried out in a flask in which the liquid is kept boiling constantly to prevent reoxidation (Costesso et al., 1999). In acid-base indicators, the change is often in the nature of color i.e. in its position in the spectrum; but with oxidation-reduction indicators it is a change in intensity of color (Paananen and Kuisma, 2000).

2.6 Determination of Fat content

Fat determination is one of the key analyses performed in food industry and official food monitoring for controlling the production process, labeling and quality control. According to Manley (1996), fats are probably the most important ingredients used in the manufacture of biscuits. They are the third largest component after flour and sugar. A fat's functionality is determined by its fatty-acid composition. Fats have received much media attention because a number of ailments are attributed to their presence in modern diet. Research shows that certain fatty acids influence the serum levels of lipids and lipoproteins and therefore the incidence of coronary heart disease. Studies have shown that high intake of saturated fat contributes to the development of coronary heart disease. The reduction of saturated fat in the diet will eventually help in lowering blood cholesterol (Grundy, 1990; Koletzko, 1992). Fatty acids with medium chain lengths have a greater adverse effect (examples: lauric C 12:0, myristic C 14:0 and palmitic C 16:0) than those with longer chain lengths.

The fatty acid composition and trans-fatty acid (TFA) contents of biscuits products were determined by gas chromatography, using a highly polar 100m capillary column (HP-88) and flame ionization detection. Trans-fatty acid is defined as unsaturated fatty acids containing one or more isolated double bonds in a trans-configuration (Food and Drug Administration, 2003). Trans-fatty acids in human diet can comes from natural sources like meat and milk of ruminant animals in small quantities through biological hydrogenation process of unsaturated fatty acids (Huang et al., 2006). However, the major source of trans fatty acids in human diet come from industrial food processing by a process called hydrogenation (Koletzko and Decsi, 1997). In the food industry, vegetable oils containing high unsaturated fatty acids are hydrogenated in the presence of metal catalyst like nickel and hydrogen to produce solid or semisolid fat. Total hydrogenation of fat rarely occurs during food processing and most often is partial hydrogenation.

Total lipids were extracted using Soxhlet Method (AOAC Methods 920.39 C for cereal fat). Petroleum spirit 40 - 60°C (AOAC, 1995) was the solvent used for the determination of fat content. Ten-fourteen grams of ground crackers or biscuits were weighed into a pre-dried extraction thimble and the oil was extracted using the solvents. The time of each extraction was 16 hours. The oil was recovered after stripping off the solvent in a rotary evaporator. Total fat contents of the biscuit samples ranged between 18.5% and 32.0%. The highest fat content was determined in salt cheese biscuits and the lowest in soya-mushroom biscuits. Total fat contents varied even for the same type of biscuit as a result using of different recipes by each company. The major fatty acids in the samples were C16:0, C18:0, trans C 18:1, C 18:1, trans C 18:2 and C 18:2. Depending on the biscuit type, total unsaturated fatty acid contents were between 52.1% and 72.8% (Manley, 1991).

2.7 Determination of Protein content

Bakery products are being made since long, where bread and biscuits are the main constituents. Majority of the biscuits are manufactured from wheat flour. Addition of soya flour and mushroom would increase the protein contents substantially. Protein rich biscuits can thus serve the purpose of providing additional nutrition at a reasonable price.

The population everywhere desires to eat a healthier diet without changing their conventional dietary patterns (Becker et al., 1998). Good eating quality makes biscuits attractive for protein fortification and other nutritional improvements. Present dietary scenario necessitates exploring the possibility of incorporating novel ingredients in commonly consumed foods rather than developing new food product. With the current status of nutritional quality of biscuits and growing demand of nutritious foods, it seems worthwhile to take efforts in enhancing the nutritional value of biscuit (Aleem et al., 2012).

Protein is used by our body for repairing and building muscles and other tissues. Protein is also used to produce hormones and enzymes and regulate other bodily processes. For example, water balance in the body -- the amount of water that is excreted or kept within the blood system must be regulated. Protein also helps to transport nutrients and provide a source of energy to improve stamina and keep us from getting easily fatigued. Protein is found in muscle, bone, hemoglobin and antibodies, and it makes up approximately 45 percent of human body. The quality of the protein is determined by the amino acids that can be used for bodily functions (Malabika, 1999). Eight essential amino acids must be consumed in sufficient amounts from food. The foods that contain these eight essential amino acids have a higher quality of protein for the body. These eight essential amino acids are tryptophan, lysine, methionine, valine, leucine, isoleucine, threonine and phenylalanine (Peterson, 1983).

The monitoring of protein amount, through the estimation of nitrogen, must be accurate in order to determine the nutritional quality of biscuits. If the amount of nitrogen is multiplied by a factor depending on the kinds of protein expected to be present in the food, then the total protein content can be determined. This is a method for the quantitative determination of nitrogen in chemical substances (Peterson, 1983). For many years, the Kjeldahl method has been the internationally-recognized method for estimating protein concentration in food, due to its universality, precision and reproducibility. Currently, it is the standard method against which all other methods are judged. Nevertheless, the Kjeldahl method is associated with many major downfalls. It is not capable of providing measurements of true protein content and different correction factors are required for different proteins to account for different amino acid sequences.

The Kjeldahl method is a multi-stepped process, including sample digestion in boiling sulphuric acid, neutralization with sodium hydroxide solution, distillation of the resulting ammonia gas into a trapping solution, titration with an acid solution and determination of the amount of nitrogen and protein by calculation. Kjeldahl analyses also generate toxic waste since they involve the use of mercury or selenium catalysts during the digestion step. All these disadvantages have ended the dominance of the Kjedahl method as the preferred technique for nitrogen/protein determination in biscuits (AOAC, 1999). On the contrary, the capabilities of the combustion method have been greatly improved to make it faster, safer and more reliable than the traditional Kjeldahl method.

2.8 Determination of Fiber content

Due to a great variety of positive health effects, fibers are gaining growing interest in modern nutrition, especially in the production of functional food products. In that respect, due to frequent consumption of various confectionery products in the modern lifestyle, the application of fibers in the production of various confectionery products is desirable (Martin, 1999). Published reports indicate numerous health benefits are associated with an increased intake of dietary fiber, including reduced risk of coronary heart disease, diabetes, obesity, and some forms of cancer (Miller, 2004). Not only the dietary fiber helps to evade hydrolysis, digestion and absorption in the human small intestine, but it achieves at least one of these functions: increases the fecal bulk, stimulates colonic fermentation, and reduces postprandial blood glucose (reduces insulin responses) and also pre-prandial cholesterol levels (Mann and Cummings, 2009).

According to recommendations (Food and Nutrition Board, Institute of Medicine 2001) the average daily requirement of dietary fiber is 25 gm per day for women younger than 50, 21 gm per day for women older than 50; 38 gm per day for men younger than 50, and 30 gm per day for men older than 50. Most nutritionists and diet experts suggest that ca 20–30% of our daily fiber intake should come from soluble fiber.

Dietary fiber (DF) can also impart some functional properties to foods, e.g., increase water holding capacity, oil holding capacity, emulsification and/or gel formation. Dietary fiber incorporated into food products (bakery products, dairy, jams, meats, soups) can modify textural properties, avoid synaeresis (the separation of liquid from a gel caused by contraction), stabilize high fat food and emulsions, and improve shelf-life. Among foods enriched in fiber, the most known and consumed are breakfast cereal and bakery products such as integral breads and biscuits (Nelson, 2001) as well as milk and meat derived products. The addition of DF to bakery products also improves their nutritional quality since it makes possible to decrease the fat content, by using DF as a substitute of fat without loss of quality (Byrne 1997; Martin 1999).

The chemical nature of fibers is complex; dietary fibers are constituted of a mixture of chemical entities. The choice of analytical method to investigate fibers depends on the composition of each particular fiber. The measurement of dietary fibers in foods is a complex issue, associated with the definition of fiber in the analytical method chosen. Methods for the determination of dietary fiber may be divided into three categories: non-enzymatic-gravimetric, enzymatic-gravimetric, and enzymatic-chemical methods. The later includes enzymatic-colorimetric and enzymatic-chromatographic (GLC/ HPLC) methods. Nowadays, the most commonly used methods for dietary fiber measurement are the enzymatic-gravimetric Association of Official Analytical Chemists (AOAC) method (Prosky et al., 1988) and enzymatic-chemical method (Englyst et al., 1994).

2.9 Determination of carbohydrate content

Health professionals argue that a healthy diet is one which provides us with at least 50% of our daily energy intake in the form of carbohydrates, 35% or less from fats and the remainder from proteins. The consensus view on the diet for athletes and active people is that it should include more carbohydrate-containing foods than recommended by the health professionals (Martin, 1999). Their diets should be such that about 60% of their daily energy intake is obtained from carbohydrates, 30% or less from fat and 10 to 15% from proteins (Miller, 2004).

Carbohydrates are one of the most important components in many foods. Carbohydrates may be present as isolated molecules or they may be physically associated or chemically bound to other molecules. Individual molecules can be classified according to the number of monomers that they contain as monosaccharides, oligosaccharides or polysaccharides (Dyke, 1960). Molecules in which the carbohydrates are covalently attached to proteins are known as glycoproteins, whereas those in which the carbohydrates are covalently attached to lipids are known as glycolipids (Olson et al., 2000). Some carbohydrates are digestible by humans and therefore provide an important source of energy, whereas others are indigestible and therefore do not provide energy. Indigestible carbohydrates form part of a group of substances known as dietary fiber, which also includes lignin (Meyer et al., 2001). Consumption of significant quantities of dietary fiber has been shown to be beneficial to human nutrition, helping reduce the risk of certain types of cancer, coronary heart disease, diabetes and constipation. As well as being an important source of energy and dietary fiber, carbohydrates also contribute to the sweetness, appearance and textural characteristics of many foods (Takematsu et al., 2008). It is important to determine the type and concentration of carbohydrates in biscuits for a number of reasons.

A large number of analytical techniques have been developed to measure the total concentration and type of carbohydrates present in foods. The carbohydrate content of a food can be determined by calculating the percent remaining after all the other components have been measured: % carbohydrates = 100 - % moisture - % protein - % lipid - % mineral (Southgate, 1976). Nevertheless, this method can lead to erroneous results due to experimental errors in any of the other methods, and so it is usually better to measure directly the carbohydrate content for accurate measurements. Due to the widespread use and importance of carbohydrates, their determination is of considerable interest in biological, environmental, clinical and medical research. Additionally, quality control of foodstuffs ensures consumers top-quality carbohydrate supply.

Chapter Three:

MATERIALS & METHODS

3.0 MATERIALS AND METHODS

3.0 Preparation of soya-mushroom biscuits

3.1 Source of materials

This research was carried out at Bangladesh Council of Scientific and Industrial Research (BCSIR). The materials used such as wheat flour, baking fat, granulated sugar, eggs, baking powder and salt were bought from Meena bazaar, Dhanmondi, Dhaka. Biscuits samples of different brands were purchased from the local market with adequate number of replicates. Soybean and mushroom (Oyster) were collected from Bangladesh Agricultural Research Institute (BARI) and National Mushroom Development and Extension Centre, Savar respectively. The chemicals were of analytical grade.

3.1.1 Preparation of soybean flour

The soybean seeds were processed into flour using the method of IITA (1990). It ensures effective removal of most anti-nutritional factors. Soybean was soaked for 12 hours. The roasted seeds were boiled for 30-40 min. Thereafter, seeds were drained and dried at 60-70°C for overnight. The dried seeds were milled into flour. The flours were screened through a 0.25mm sieve and stored at 4°C in a refrigerator to prevent spoilage particularly rancidity until usage. Wheat and soy flour were mixed for biscuits preparation.

Soybean seeds
↓
Washing
↓
Soaking in water (for 12h)
↓
Dehulling
↓
Draining and Removal of hulls
↓
Boiling (100^0Cfor 30 min)
↓
Drying (65^0C for 6 hr)
↓
Milling (Attrition mill)
↓
Sieving (Fine sieve, 0.25mm)
↓
Stored at 4°C in a refrigerator
↓
Packaging

Fig 3.1: Flow chart for the production of soyabean flour

3.1.2 Preparation of mushroom

Fresh mushrooms were collected and kept in a big tray. The fresh mushroom samples were sorted and graded according to size by hand trimmed using a stainless steel knife and cleaned with a soft brush. They were separated into caps and stalk and cut into uniform sizes of about 5cm diameter and 4cm long for caps and stalk respectively. The caps and stalk were dried separately as the caps dries faster than the stalk. These samples were then washed with softbush in 0.1% sodium meta-bisulphite solutions to prevent browning. It was then dried in a oven (Gallenkamp, model OV–160) at 55°C for 2hrs to obtain 5% moisture content. The dried mushroom was milled using a milling machine (PC 4021/Britain). The flour obtain was sieved using 0.25mm sieve (Model BS 410). The Flow chart is down in figure.

Fresh mushroom samples
↓
Mushroom Sorting
↓
Cleaning (0.1% sodium metabisulplute)
↓
Drying (55°Cfor 2 hrs)
↓
Dry milling (Screening (0.25mm aperture)
↓
Mushroom flour.
↓
Drying (65°C for 6 hr)

Figure 3.2: Flow chart for the production of Mushroom flour.

3.2 Recipe for biscuits

The recipe for biscuits used in this study are shown in table 3.1

Table 3.1: Types and amount of ingredient for biscuit manufacturing

Ingredients	Quantity
Wheat flour	35 gm
Soya flour	15 gm
Sugar	8 gm
Mushroom	3 gm
Baking powder	.6gm
Soda (sodium bicarbonate)	.5gm

Salt	1.25gm
Oil	8 ml
Butter	12gm
Ghee	2ml
Egg	15ml
Flavor	4 /5 drops
Lecithin	.2gm
Cumin seed	.25gm

3.3 Process of biscuit manufacturing

- First take butter (at room temperature) add sugar to it and mix well until it becomes smooth
- Then add oil into a work, add lecithin, ghee, egg and mixed properly for few minutes
- Add the baking powder, soda and salt
- Dry mush rooms that were soaked into 10ml water for about 15 to 20 minutes. Then add into the mixture
- Mix well and add vanilla essence
- Now add the white flour and soya flour to the mixture and also add cumin seed and mix well together until it becomes a soft dough
- Preheat the oven to $200^{0}C$
- Now take a baking sheet and sprinkle some flour on it
- Take small dough (small ball size) and make it like round and place it on the sheet. Continue the same procedure with the remaining dough
- Place the baking sheet in the oven and bake it for 45 to 50 minutes
- Remove the baking sheet and wait for it cool and remove the salt biscuits

The whole process of biscuit manufacturing is outlined in figure 3.3

Egg, sugar, butter, oil, ghee and lecithin
↓
Mixed
↓
Baking powder, soda, salt
↓
Then mushroom and flavor
↓
Mixed properly

↓
Wheat and soya flour
↓
Dough prepared and cut
↓
Heated 170-180 °C for 45- 50 minutes in oven

Fig 3.3: Flow chart for the preparation of soya-mushroom biscuits

3.4 Physical analysis

The weight of the biscuits was measured by weighing on a weighing balance (Model Mettler PE1600, Mettler Instruments Corporation, Greifensee, Zurich, Switzerland) with an accuracy of 0.1 mg. Width (W): Six biscuits were placed horizontally (edge to edge) in a row and taken their average diameter using digital vernier caliper with 0.01 mm accuracy. Thickness: Six biscuit were placed one another and taking their average thickness using digital Vernier Caliper with 0.01 mm accuracy.

3.5 Spread ratio determination

Three rows of five well formed biscuits were made and the height measured. The biscuits were arranged horizontally edge to edge and the sum of the diameter was measured with the height. The spread ratio (Gomeaz et al., 1997) was calculated as diameter/height.

3.6 Sensory Evaluation

The biscuits were evaluated by a panel of fifteen untrained judges drawn from BCSIR for attributes of color, texture, flavor and general acceptability.

3.7 Proximate analysis

The proximate composition (i.e. moisture, ash, protein, fat, fiber, carbohydrate) and total energy of soya-mushroom biscuits and different local biscuits were determined according to the standard analytical methods (AOAC).

3.8 Equipments

The equipments used for experiments are given below:

- Weighting Balance
- Porcelein Dish
- Conical Flask
- Volumetric Flask
- Pasture pipette
- Pasture pipette Holder
- Glass Rod
- Funnel
- Beaker
- Muffle Furnace
- Electric Oven
- pH meter
- Water Bath
- Burner
- Hot Plate
- Filter Paper
- Test-tube
- Aluminum Foil
- Thermometer
- Desiccators
- Soxhlet
- Burette

3.9 Chemicals and Reagents

The chemicals used for experiments are below

- Ethanol 95%
- Fehlings' Solution-A
- Fehlings' Solution-B
- Clearifing Solution-I (Zinc-Acetate)
- Distilled Water
- Clarifying Solution-II, Potassium Ferrocyanide
- 6.35 N Hydrocloric Acid
- 0.1N Hydrocloric Acid
- 40% Sodium Hydroxide
- 0.095 N Sodium Hydroxide
- Methyl Blue
- Methyl Red
- Petroleum Ether
- Sulfuric Acid
- Digestion Mixture

3.10 Determination of Moisture

3.10.1 Procedure

At first, weight of a porcelain dish was taken. Approximate 4-5 gm sample was taken into dish. Then weight of dish with sample was taken. The dish was placed in a previously dried electric oven for 10-12 hours at 105^{0}C. After 5 hours the dish was cooled in a desiccator for 30 minutes. Finally, the weight of dish was taken.

3.10.2 Calculation

$$\text{Moisture\% by mass} = \frac{100 \times (W_1 - W_2)}{W_1 - W} \quad \text{......................} (3.1)$$

Where,

W_1 = Weight of dish + Sample ⟶ Before drying

W_2 = Weight of dish + Sample ⟶ After drying

W = Weight of the empty dish

3.11 Determination of Ash

3.11.1 Procedure

At first, weight of a porcelain dish was taken. Approximate 1-2 gm sample was taken into dish. Weight of dish with sample was taken. The sample was burned completely with a burner. After burning the dish was kept in a muffle furnace at 700^0c until grey ash results. The dish was cooled in desiccators. Finally, the weight of dish was taken.

3.11.2 Calculation

$$\text{Ash\% by mass} = \frac{100 \times (W2 - W)}{W_1 - W} \quad \text{........................} (3.2)$$

Where,

W_1 = Weight of dish + Sample

W_2 = Weight of dish + Ash

W = Weight of the empty dish

3.12 Determination of Sugar

3.12.1 Procedure

At first, approximate 15-20 gm sample was taken in a 250 ml volumetric flask. 125 ml of 50% ethanol was mixed with it and was boiled 1 hour on a steam bath. After 1 hour it was cooled and kept for overnight. The volume of sample was made 250 ml with 95% ethanol, mixed thoroughly and was kept for settle. It was filtered into a conical flask and the volume of supernatant was measured. The ethanol was evaporated from supernatant upto 20 ml on a steam bath and was transferred to a 100 ml volumetric flask. 10 ml Clarifying solution-I was added and was mixed thoroughly. 10 ml Clarifying solution-II was added and was mixed thoroughly.

Then 100 ml volume was made with distilled water and was stand for about 45 minutes. It was filtered and the supernatant was used for fresh sugar analysis. 20 ml supernatant was taken into a 100ml volumetric flask. 5 ml of 6.35 N HCl was added into it, mixed well and hydrolysis was done on a boiling water bath for 30 minutes. It was cooled and few drop of phenolphthalein was added and was neutralized with 40% NaOH. It was filtered and the volume was made 100ml with distilled water. 5 ml of Fehling solution-B and 5 ml of Fehling solution-A was taken in a 250ml conical flask. 50ml distilled water was added into it, mixed thoroughly and was heated on a hot plate. At the starting of the boiling point 2-3 drops of methyl blue was added. Sugar solution was taken into a burette. Finally, Fehling solution was titrated with sugar solution, one for total sugar another for reducing sugar.

3.12.2 Calculation

$$\text{Reducing Sugar} = \frac{.063 \times 100 \text{ (Final volume)}}{\text{Titration Reading}} \quad \text{......................} \quad (3.3)$$

$$= \frac{\text{Result from eqn } 3.3 \times 250 \text{ (Initial volume)}}{\text{Total volume}} \quad \text{......................} \quad (3.4)$$

$$= \frac{\text{Result from eqn } 3.4 \times 100}{\text{Weight of sample}}$$

$$\text{Total sugar} = \frac{.063 \times 100 \text{ (Volume after hydrolysis)}}{\text{Titration reading}} \quad \text{......................} \quad (3.5)$$

$$= \frac{\text{Result from eqn } 3.5 \times 100 \text{ (Volume after clarification)}}{20 \text{ (Volume used for hydrolysis)}} \quad \text{..........} \quad (3.6)$$

$$= \frac{\text{Result from eqn } 3.6 \times 250 \text{ (Initial volume)}}{\text{Total volume}} \quad \text{......................} \quad (3.7)$$

$$= \frac{\text{Result from eqn } 3.7 \times 100}{\text{Weight of sample}}$$

$$\% \text{ of Sugar} = (\text{Total sugar} - \text{Reducing sugar}) \times 0.95$$

3.13 Determination of Protein

3.13.1 Procedure

At first, weight of approximate 0.4-0.5 gm sample was taken. Digestion mixture and 10ml H_2SO_4 was added. The mixture was heated in low temperature for 2 days until color became white. After digestion the sample was cooled and was poured into 100 ml volumetric flask. The volume of sample was made 100 ml with distilled water. 10 ml 0.1N HCl was taken into a conical flask and 1-2 drop of methyl red was added. The sample was distillated with 0.1N HCl which was taken into conical flask. Finally, the sample was titrated with 0.095 N NaOH from burette.

3.13.1 Calculation

$$\%\text{ of protein} = \frac{\begin{array}{c}\text{Difference between blank and}\\ \text{sample titration result}\end{array} \times 1.4 \times 6.25 \times \text{strength of NaOH}}{\text{Original weight of sample}} \quad \text{................ (3.8)}$$

3.14 Determination of Fat

3.14.1 Procedure

At first, weight of approximate 10-15 gm sample was taken. The sample was refluxed in a Soxhlet with petroleum ether for 2 days. Then sample was distillated. Weight of a small conical flask was taken. The sample was poured into conical flask and Petroleum ether was evaporated from Sample with a burner. It was placed into an electric oven until the smell of petroleum ether was completely removed. Finnaly, the sample was cooled in a desiccators and then weight of sample was taken

3.14.2 Calculation

$$\%\text{ of fat} = \frac{W_2 - W_1 \times 100}{W} \quad \text{...................... (3.9)}$$

Where,

W_1 = Weight of the empty container

W_2 = Weight of container with Fat

W = Original weight of sample

3.15 Determination of crude fiber

3.15.1 Procedure

Moisture and fat free sample was boiled with 0.255N H_2SO_4 and 0.313N NaOH consecutively for 30 minutes under a reflux condenser and each time the sample was washed with boiling water properly to remove acid and alkali residue. The sample was then transferred in a crucible, dried overnight at 110^0C and weighed (W_1) in an analytical balance. The crucible was heated in a muffle furnace at 600°C for 20 minutes, cooled and weighed again (W_2). The difference in the weights (W_1- W_2) represents the weight of crude fiber.

3.15.1 Calculation

$$\text{Crude fiber (g/100g)} = \frac{(W_1\text{-}W_2) \times 100}{W} \quad \text{...................... (3.10)}$$

Where,

W_1 = Weight after burning at 110^0C

W_2 = Weight after burning at 600^0C

W = Weight of the sample taken for fat extract

3.16 Determination of Carbohydrate

3.16.1 Calculation

The content of the available carbohydrate was determined by the following equation.

Total carbohydrate (g/100g of sample) = [100-(moisture + ash + protein + fat + crude fiber)]

3.17 Determination of energy content:

Metabolizable energy was calculated as the following formula (Joshi, S. A. 2002):

Energy (kcal/100g)= [(4xcarbohydrate)+ (4x protein) + (9x fat)] ....(3.11)

3.18 Software packages used for analysis

Samples were prepared in three replicates and analyzed using the Statistical Packages for the Social Sciences (SPSS) database version 16.0. Simple one factor analysis of variance (ANOVA) was performed and the mean separation was done at ($p < 0.05$).

Chapter Four:

RESULTS

4.0 RESULTS

4.1 Sensory Evaluation of Soya-mushroom Biscuits

A number of compositions as outlined in Table 4.1 were carried out to find out the best combination for development of soya-mushroom biscuits. A standard recipe for the preparation of the biscuits with wheat flour was considered as control for this purpose.

Table 4.1 Composition of prepared biscuits and their qualitative attributes

Serial No.	Composition	Temp. & Time	Color	Texture	Flavor	Taste
01	Wheat flour 30gm, Soya flour 20gm, Sugar 12.5gm, Mushroom 5gm, Baking powder 0.6gm, Soda (sodium bicarbonate) 0.5gm Salt 1gm, Oil 22.5ml, Egg 10ml, water 10ml, Cumin seed 0.5gm (B1)	200^0C 25min	Blackish	Not crispy	Off flavor	Not good
02	Wheat flour 30gm, Soya flour 20gm, Sugar 12.5gm, Mushroom 5gm, Baking powder 0.6gm, Soda (sodium bicarbonate) 0.5gm Salt 1gm, Oil 25ml, butter 5gm, Egg 10ml, water 10ml, Cumin seed 0.5gm (B2)	200^0C 15min	Deep brown	Not crispy	Off flavor	Not good
03	Wheat flour 30gm, Soya flour 20gm, Sugar 12.5gm, Mushroom 4gm, Baking powder 0.6gm, Soda (sodium bicarbonate) 0.5gm Salt 1gm, Oil 25ml, butter 5gm, Egg 15ml, water 10ml, Cumin seed 0.5gm (B3)	180^0C 25-30min	Deep Brown	Crispy	Off flavor	Salty but not good
04	Wheat flour 35gm, Soya flour 15gm, Sugar 12.5gm, Mushroom 4gm, Baking powder 0.6gm, Soda (sodium bicarbonate) 0.5gm Salt 1.25gm, Oil 12ml, butter 5gm, ghee 3ml, Egg 15ml, water 10ml, Cumin seed 0.25gm (B4)	140^0C 40-50min	Brown	Crispy	Off flavor	Salty
05	Wheat flour 35gm, Soya flour 15gm, Sugar 10gm, Mushroom 3gm, Baking powder 0.6gm, Soda (sodium bicarbonate) 0.5gm, Salt 1.25gm, Oil 10ml, butter 5gm, ghee 3ml, Egg 15ml, water 10ml, Cumin seed 0.25gm (B5)	200^0C 20min	Brown	Crispy	Flavor good	Salty and good
06	Wheat flour 35gm, Soya flour 15gm, Sugar 8gm, Mushroom 3gm, Baking powder 0.6gm, Soda (sodium bicarbonate) 0.5gm, Salt 1.25gm, Oil 8ml, butter 10gm, ghee 2ml, Egg 15ml, water 10ml, Cumin seed 0.25gm, Lecithin .2gm, Vanilla Flavor 4/5 drops. (B6)	170 -180 ^{0}C 40-50min	Brown	Crispy	Vanilla essence	Salty and good

It has been observed that the qualitative parameters for the biscuits differed significantly. Table 4.2 gives the percentage score of panelists' mean ratings for the biscuit samples and the comparative sensory evaluation of their properties in regard to taste, color, flavor, texture and overall acceptability. The method of Larmond (1977) was used for sensory evaluation. The samples of biscuits were subjected to sensory evaluation with 100% white wheat flour biscuit as control. A twenty five member experienced panelists Bangladesh Council for Scientific and Industrial Research (BCSIR) were used to evaluate the samples using a modified 5-point hedonic scale, where 5 was extremely acceptable and 1 was extremely unacceptable. Room temperature water and unsalted crackers were given to the panelists for mouth rinsing between samples after to eliminate carry over effects.

Table 4.2 Mean panelist ratings for soya-mushroom biscuit samples.

Parameter	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**Control**
Taste	1.15 ± 0.75^{a}	1.47 ± 0.66^{b}	2.74 ± 0.63^{c}	3.32 ± 0.59^{d}	3.9 ± 0.47^{e}	4.2 ± 0.50^{f}	2.55 ± 0.27^{g}
Color	0.85 ± 0.12^{a}	1.55 ± 0.28^{b}	2.99 ± 0.25^{c}	3.4 ± 0.56^{d}	3.88 ± 0.69^{e}	4.8 ± 0.20^{f}	2.75 ± 1.23^{g}
Flavor	0.98 ± 0.19^{a}	1.35 ± 0.21^{b}	2.5 ± 0.35^{c}	3.12 ± 1.26^{d}	3.75 ± 0.37^{e}	4.3 ± 0.30^{f}	2.58 ± 0.26^{g}
Texture	1.05 ± 0.07^{a}	1.35 ± 0.22^{b}	3.8 ± 0.37^{c}	3.5 ± 0.92^{c}	3.95 ± 0.24^{c}	4.1 ± 0.70^{c}	3.71 ± 0.15^{c}
Overall acceptability	1.04 ± 0.28^{a}	1.45 ± 0.45^{b}	2.4 ± 0.35^{c}	3.16 ± 0.26^{d}	3.97 ± 0.33^{e}	4.5 ± 0.97^{f}	2.46 ± 0.33^{g}

Different superscript (a, b, c, d. e, f & g) within each row in the same group indicate significant ($P<0.05$) difference. Values are means of triplicate determination.

The taste of the biscuits refers to the salt sensation caused in the mouth by contact with the biscuit due to the salting agent. The panelists preferred the taste of the soya-mushroom flour supplemented biscuit type B6 over other types of biscuits. In terms of taste, the least preferred was sample B1. The mean of different samples were found to be significantly different from the control biscuits.

The crust as applied here was the outside layer of the biscuits. It should be smooth and golden brown which is more or less related to the color of the biscuits. The color of the biscuits provided information about the appearance of the biscuit, how it should look, if it was appealing to the eyes, inviting and bright. The panelists prefer the crust and color of sample B6 while B1 was least preferred. It can be seen from Table 4.2 that the soya-mushroom biscuits were rated significantly ($p < 0.05$) different from the biscuits from wheat flour. The means of the soya-mushroom biscuits were significantly different from the other sample types at varying composition levels at 5% significant level.

Texture is the quality of the biscuits that can be decided by touch, the degree to which it is crispy or not crispy, rough or smooth, hard or soft. The panelists slightly preferred the texture of sample B6 to that of the biscuits made from other biscuits with varying composition levels (B5, B4 and B3). The least preferred sample was found to be B1. The mean indicates that there was insignificant difference between the textures of samples B6, B5, B4 and B3 at 5% significant level.

The overall acceptance expressed how the consumers or panelists accepted the product in general. It was observed that the panelists accepted the soya-mushroom supplemented biscuit sample B6 over other sample types. The analysis of variance indicated that there were significant differences among the samples with whole wheat flour and those with soya flour and mushroom supplementation at 5% significance level.

4.2 Proximate Analysis

Comparative analyses of different biscuits from different companies (Table 4.3) were carried out in the laboratory of BCSIR to determine moisture, ash, protein, fat, sugar, fiber, carbohydrate and calorific content.

Table 4.3 Biscuit varieties used

Serial no	Name of the brands	Serial name
1	Bakery	S1
2	Capital	S2
3	Crack jack	S3
4	Diasalt	S4
5	Digestive	S5
6	Good time	S6
7	Grand Choice	S7
8	Kasuan Sugar free	S8
9	Lavender	S9
10	Protein biscuit	S10
11	Salt cheese	S11
12	Salt puff	S12
13	Sugar free biscuit	S13
14	Tip	S14
15	Top	S15
16	Zero sugar	S16
17	**Soya-mushroom**	**S17**

4.2.1 Moisture content

The mean value of total moisture was highest in the sample S1 (3.83±0.03 %) and lowest in SM (1.4±0.08 %) (Appendices: Table 01). The moisture content of all the local biscuits was higher than that of the newly developed biscuit. The lower the moisture content of the biscuits, the greater is its quality. Therefore, the samples were ranked as; SM > S3 > S10 > S4 > S14 > S12 > S5 > S9 > S6 >

S13 > S2 > S11 > S16 > S7 > S8 > S15 > S1. Significant differences (p<0.05) among the mean values were found non-significant through one way ANOVA.

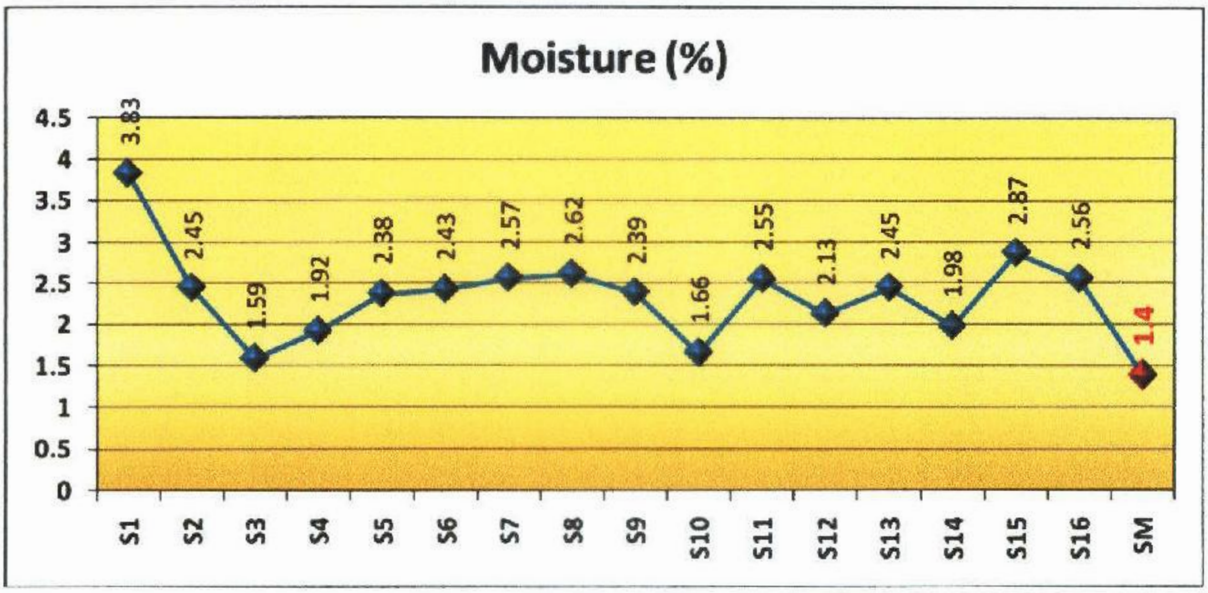

Figure 4.1: Moisture percentages of biscuit varieties tested

4.2.2 Ash content

The mean value of total ash was highest in SM (2.16±0.07 %) and lowest in S15 (0.99±0.05 %) (Appendices: Table 02). The ash content of all the local biscuits was lower than that of the newly developed biscuit. The higher the ash content of the biscuits, the greater is its quality. So, soya-mushroom biscuits contain greater quality in comparison to the local varieties tested and the samples were ranked as; SM > S3 > S11 > S4 > S12 > S1 > S14 > S8 > S13 > S10 > S7 > S2 > S5 > S16 > S9 > S6 > S15.

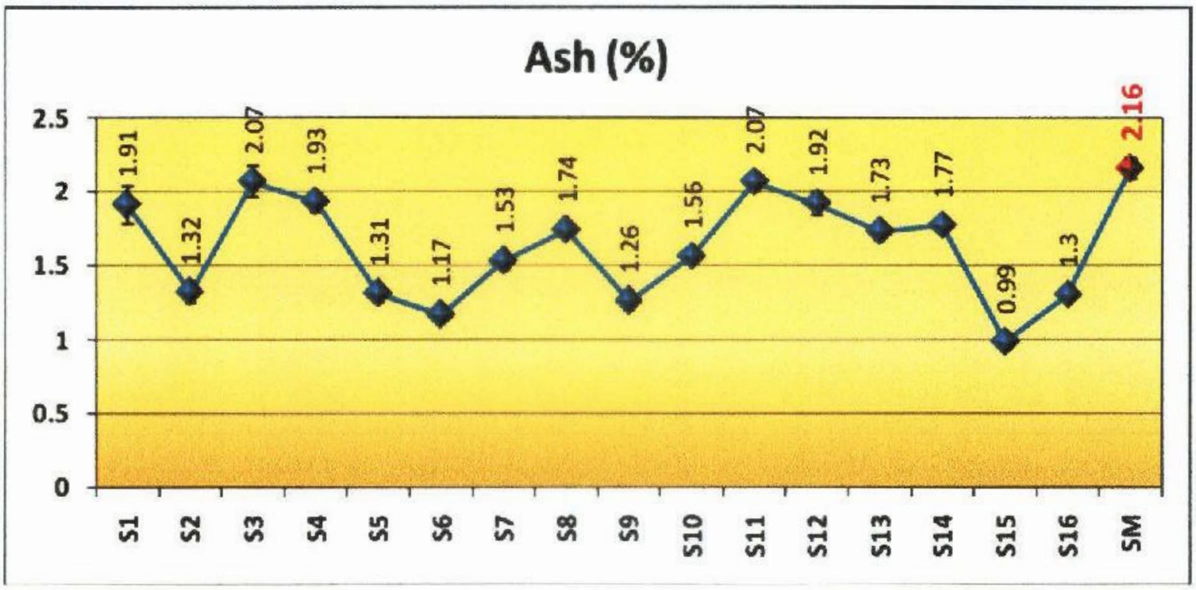

Figure 4.2: Ash percentages of biscuit varieties tested

4.2.3 Fat content

Fat percentage for the composite soya-mushroom (SM) biscuit were maintained at a lower range (19.45±1.22 %), but among the varieties salt cheese showed the highest percentage (32.91±0.45 %) (Appendices: Table 03) so SM is a biscuit of choice for adult and diabetic patients. Other biscuits contain moderate percentage of fat which is acceptable for all classes of people. Hence, the rank is noted as; SM > S6 > S4 > S7 > S3 > S13 > S15 > S14 > S10 > S5 > S1 > S16 > S8 > S12 > S9 > S2 > S11.

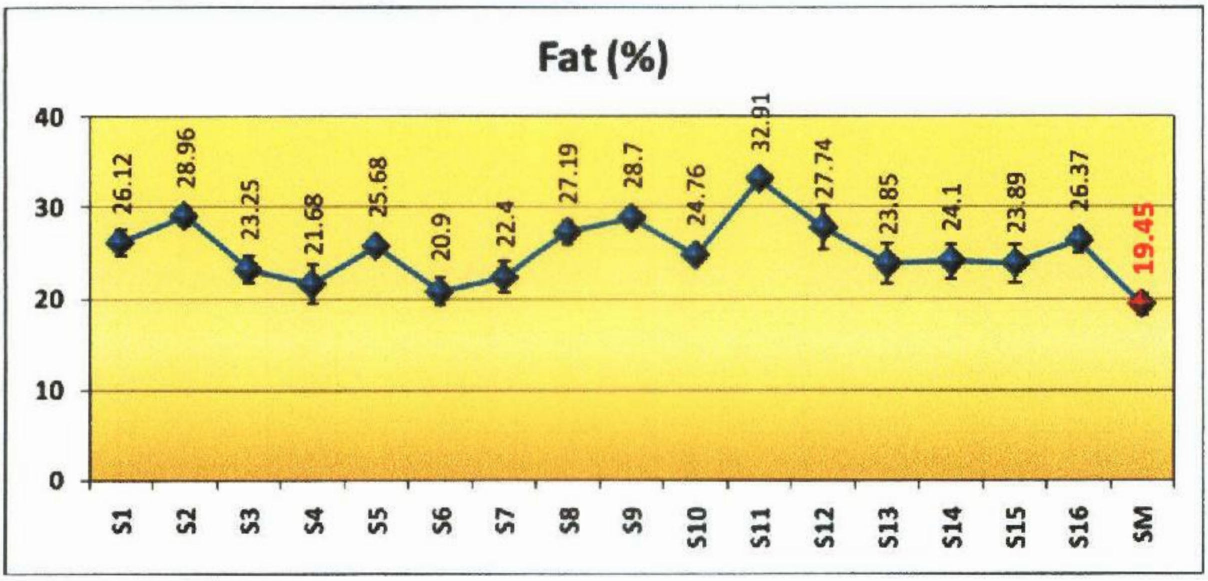

Figure 4.3: Fat percentages of biscuit varieties tested

4.2.4 Protein content

Soya-mushroom biscuit contains highest percentage of protein; because it contains soya flour and mushroom which are rich in protein. The mean value of total protein was highest in SM (9.53±0.18 %) and lowest in S1 (3.5±0.54 %) (Appendices: Table 04). The protein content of all the local biscuits was lower than that of the newly developed biscuit. Thus, the order regarding protein content is given here in descending way; SM > S11 > S10 > S8 > S16 > S4 > S5 > S12 > S7 > S6 > S13 > S3 > S9 > S14 > S15 > S2 > S1.

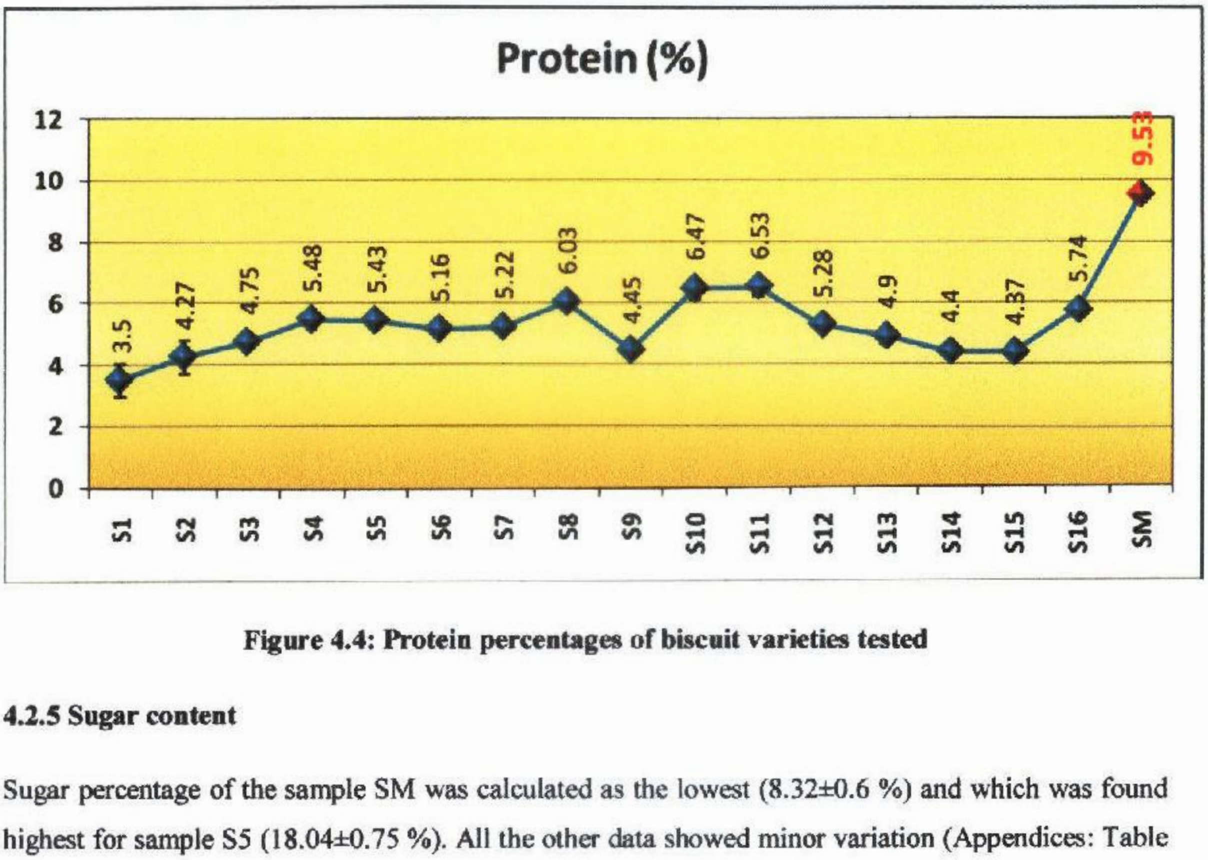

Figure 4.4: Protein percentages of biscuit varieties tested

4.2.5 Sugar content

Sugar percentage of the sample SM was calculated as the lowest (8.32±0.6 %) and which was found highest for sample S5 (18.04±0.75 %). All the other data showed minor variation (Appendices: Table 05). Therefore, samples are arranged in lower to higher value; SM > S8 > S3 > S13 > S6 > S16 > S14 > S9 > S12 > S1 > S15 > S7 > S2 > S11 > S10 > S4 > S5.

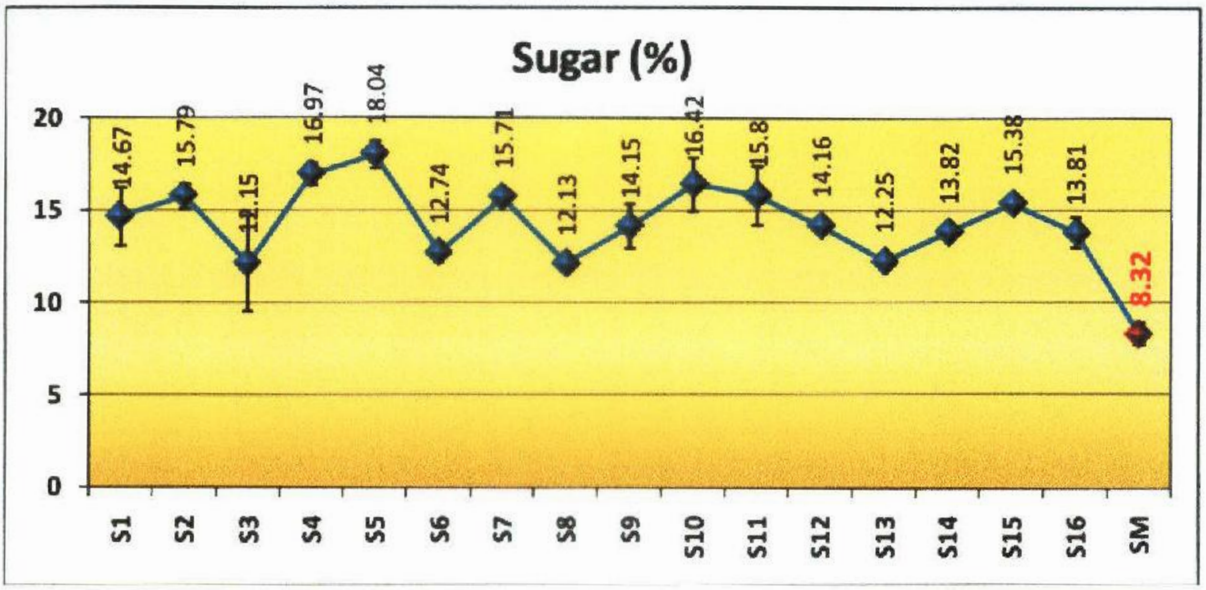

Figure 4.5: Sugar percentages of biscuit varieties tested

4.2.6 Fiber content

Both soya-flour and mushroom are rich in fiber content thus soya-mushroom supplemented biscuits (SM) showed the highest value (0.46±0.04 %), whereas, S9 represented the lowest value (0.1±0.01 %) among the tested samples (Appendices: Table 06). This result indicates that the composite biscuit will be helpful for digestion and which was supported by the one way analysis of variance (ANOVA) test (Appendix 02), where at $p<0.05\%$ these data were found highly significant. So, they were categorized as; SM > S11 > S16 > S13 > S6 > S15 > S5 > S10 > S3 > S8 > S7 > S14 > S4 > S2 > S12 > S1 > S9.

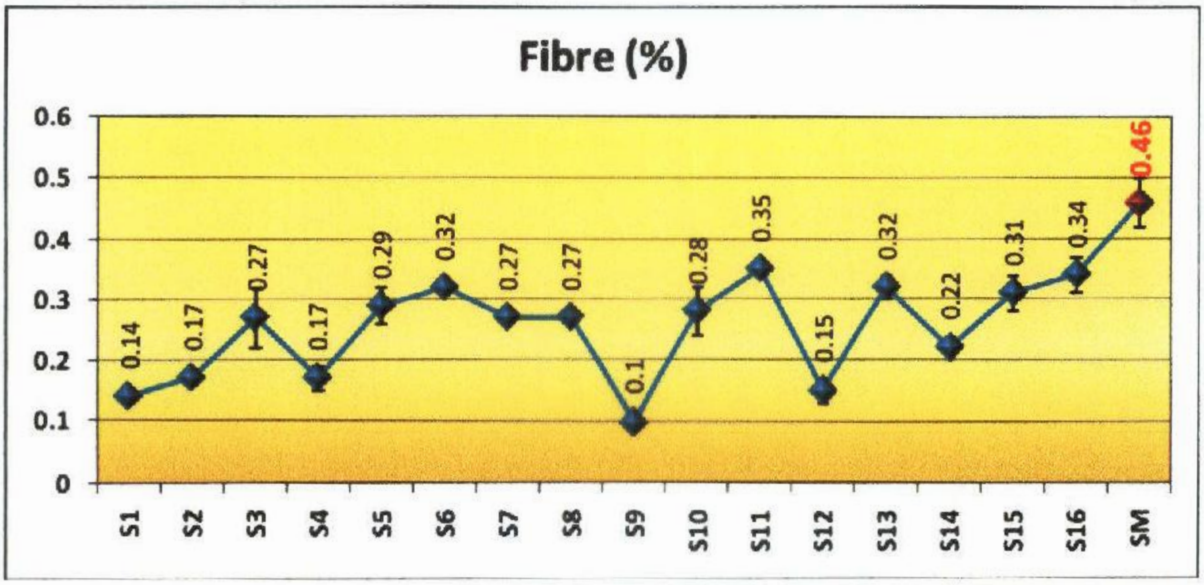

Figure 4.6: Fiber percentages of biscuit varieties tested

4.2.7 Carbohydrate content

Biscuits are generally rich in carbohydrate because cereals are mainly rich in carbohydrate. Biscuits prepared for middle age and adult people contain lower amounts of carbohydrate, because higher amounts of carbohydrate are harmful to their health. Soya-mushroom biscuits (SM) contain acceptable range of carbohydrate (64.35±1.6 %), where approximately similar values were recorded for the local varieties rather than sample S6 which showed the highest value (71.04±0.21 %) among all (Appendices: Table 07). Therefore, the order of biscuit samples regarding this property is; S11 > S1 > S8 > S2 > S9 > S13 > S12 > S16 > SM > S5 > S10 > S7 > S4 > S14 > S15 > S3 > S6.

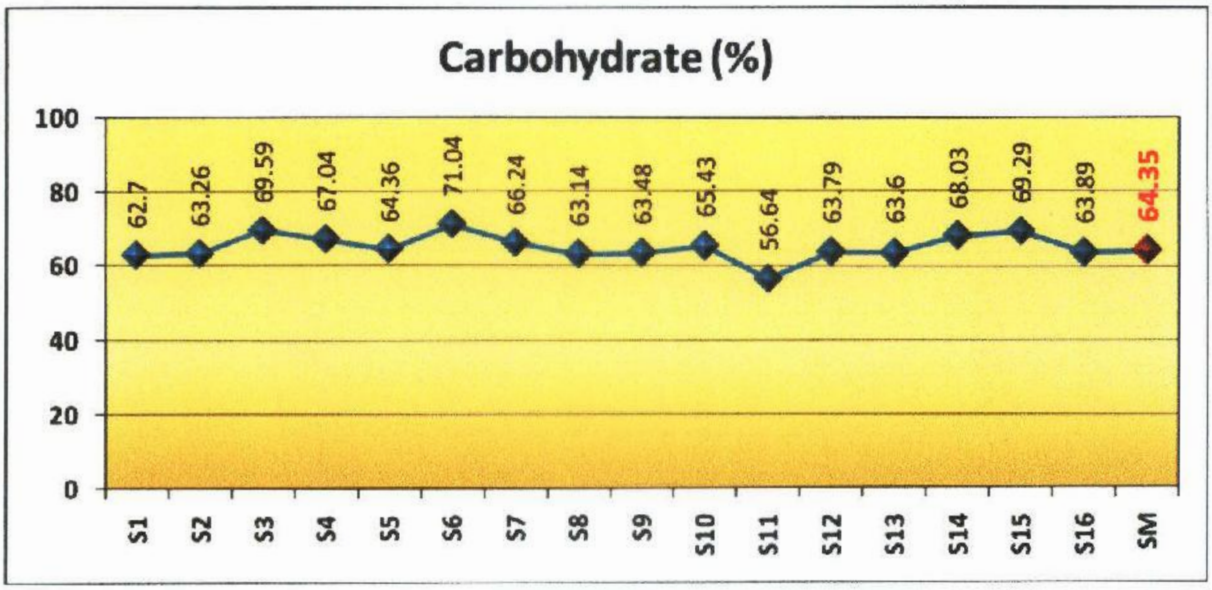

Figure 4.7: Carbohydrate percentages of biscuit varieties tested

4.2.8 Calorie content

Low calorie content is essential factor to be maintained while supplemented biscuit preparation. Among the tested varieties sample SM showed the least calorie content (485.48±5.54 %) (Appendices: Table 08). So, supplementation with mushroom has beneficial effect on the product. Therefore, the samples were sorted in a ascending way concerning their values; SM > S7 > S6 > S15 > S3 > S4 > S14 > S12 > S13 > S16 > S8 > S1 > S10 > S5 > S9 > S2 > S11.

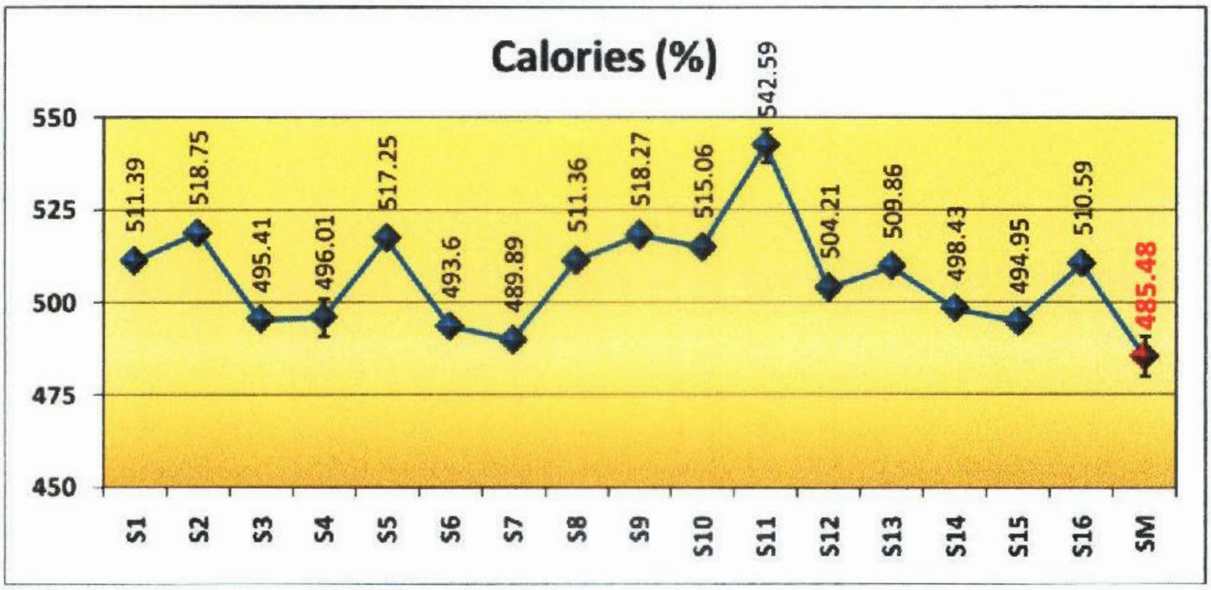

Figure 4.8: Calorie percentages of biscuit varieties tested

From all graphs and the data table (Appendices: Table 09), it is clear that the quantity of different ingredients is generally different in different types of biscuits. The higher content of protein, fiber and ash in the soya- mushroom biscuit might be attributed to the addition of soya-flour, mushroom. As the prepared biscuits contain low sugar it may be suitable for diabetes patients. Significant amount of protein may be an alternative protein source for the vegetarians and the people suffering from protein energy malnutrition. The higher amount of dietary fiber present in the soya-mushroom biscuit may aid in diabetics, constipation and peptic ulcers. It might be provide nutritional support towards malnourished different age group of peoples.

Table 4.4: Overall comparison among the tested characteristics of samples and the soya-mushroom composite biscuit

Name of Biscuits	Moisture (%)	Ash (%)	Fat (%)	Sugar (%)	Protein (%)	Fiber (%)	Carbohydrate (%)	Calories (%)
Bakery (S1)	3.83±0.03	1.91±0.13	26.12±1.40	14.67±1.57	3.5±0.54	0.14±0.01	62.7±0.78	511.39±1.59
Capital (S2)	2.45±0.08	1.32±0.07	28.96±1.03	15.79±0.66	4.27±0.54	0.17±0.01	63.26±0.04	518.75±2.08
Crack jack (S3)	1.59±0.06	2.07±0.11	23.25±1.43	12.15±2.58	4.75±0.07	0.27±0.05	69.52±0.37	495.41±0.75
Diasalt (S4)	1.92±0.02	1.93±0.07	21.68±2.11	16.97±0.59	5.48±0.3	0.17±0.02	67.04±0.64	496.01±5.03
Digestive (S5)	2.38±0.05	1.31±0.06	25.68±0.7	18.04±0.75	5.43±0.17	0.29±0.03	64.36±0.69	517.25±1.52
Good time (S6)	2.43±0.02	1.17±0.04	20.9±1.44	12.74±0.54	5.16±0.23	0.32±0.01	71.04±0.21	493.6±0.69
Grand Choice (S7)	2.57±0.08	1.53±0.06	22.4±1.68	15.71±0.26	5.22±0.13	0.27±0.01	66.24±0.83	489.89±1.90
Kasuan Sugar free (S8)	2.62±0.02	1.74±0.02	27.19±1.31	12.13±0.34	6.03±0.1	0.27±0.01	63.14±0.16	511.36±2.09
Lavender (S9)	2.39±0.05	1.26±0.06	28.7±0.93	14.15±1.22	4.45±0.18	0.1±0.01	63.48±0.13	518.27±0.16
Protein biscuit (S10)	1.66± 0.06	1.56±0.02	24.76±0.6	16.42±1.46	6.47±0.35	0.28±0.04	65.43±0.16	515.06±0.16
Salt cheese (S11)	2.55±0.05	2.07±0.06	32.91±0.45	15.8±1.57	6.53±0.3	0.35±0.01	56.64±0.35	542.59±4.53
Salt puff (S12)	2.13±0.03	1.92±0.08	27.74±2.34	14.16±0.4	5.28±0.07	0.15±0.02	63.79±0.78	504.21±1.39
Sugar free biscuit (S13)	2.45±0.02	1.68±0.06	23.85±2.16	12.25±0.33	4.9±0.28	0.32±0.02	63.6±1.90	509.86±1.93
Tip (S14)	1.98±0.05	1.77±0.03	24.1±1.81	13.82±0.24	4.4±0.21	0.22±0.01	68.03±1.30	498.43±0.35
Top (S15)	2.87±0.05	0.99±0.05	23.89±2.07	15.38±0.44	4.37±0.22	0.31±0.03	69.29±0.18	494.95±0.09
Zero sugar (S16)	2.56±0.01	1.3±0.04	26.37±1.36	13.31±0.81	5.74±0.11	0.34±0.03	63.89±0.93	510.59±0.72
Soya-mushroom (SM)	**1.4±0.08**	**2.16±0.07**	**19.45±1.22**	**8.32±0.6**	**9.53±0.18**	**0.46±0.04**	**64.35±1.6**	**485.48±5.54**

Note: - S1: Bakery, S2: Capital, S3: Crack Jack, S4: Diasalt, S5: Digestive, S6: Good time, S7: Grand choice, S8: Kasuan sugar free, S9: Lavender, S10: Protein biscuit, S11: Salt cheese, S12: Salt puff, S13: Sugar free biscuit, S14: Tip, S15: Top, S16: Zero sugar, SM: Soya-mushroom

Table 4.5: Various types and amount of ingredient for biscuit manufacturing

Serial No.	Composition	Temp. & Time	Color	Texture	Flavor	Taste	Acceptability
01	Wheat flour 30gm, Soya flour 20gm, Sugar 12.5gm, Mushroom 5gm, Baking powder 0.6gm, Soda (sodium bicarbonate) 0.5gm Salt 1gm, Oil 22.5ml, Egg 10ml, water 10ml, Cumin seed 0.5gm	200^0C 25min	Blackish	Not crispy	Off flavor	Not good	**1**
02	Wheat flour 30gm, Soya flour 20gm, Sugar 12.5gm, Mushroom 5gm, Baking powder 0.6gm, Soda (sodium bicarbonate) 0.5gm Salt 1gm, Oil 25ml, butter 5gm, Egg 10ml, water 10ml, Cumin seed 0.5gm	200^0C 15min	Deep brown	Not crispy	Off flavor	Not good	**1**
03	Wheat flour 30gm, Soya flour 20gm, Sugar 12.5gm, Mushroom 4gm, Baking powder 0.6gm, Soda (sodium bicarbonate) 0.5gm Salt 1gm, Oil 25ml, butter 5gm, Egg 15ml, water 10ml, Cumin seed 0.5gm	180^0C 25-30min	Deep Brown	Crispy	Off flavor	Salty but not good	**2**
04	Wheat flour 35gm, Soya flour 15gm, Sugar 12.5gm, Mushroom 4gm, Baking powder 0.6gm, Soda (sodium bicarbonate) 0.5gm Salt 1.25gm, Oil 12ml, butter 5gm, ghee 3ml, Egg 15ml, water 10ml, Cumin seed 0.25gm	140^0C 40-50min	Brown	Crispy	Off flavor	Salty	**3**
05	Wheat flour 35gm, Soya flour 15gm, Sugar 10gm, Mushroom 3gm, Baking powder 0.6gm, Soda (sodium bicarbonate) 0.5gm, Salt 1.25gm, Oil 10ml, butter 5gm, ghee 3ml, Egg 15ml, water 10ml, Cumin seed 0.25gm	200^0C 20min	Brown	Crispy	Flavor good	Salty and good	**4**
06	Wheat flour 35gm, Soya flour 15gm, Sugar 8gm, Mushroom 3gm, Baking powder 0.6gm, Soda (sodium bicarbonate) 0.5gm, Salt 1.25gm, Oil 8ml, butter 10gm, ghee 2ml, Egg 15ml, water 10ml, Cumin seed 0.25gm, Lecithin .2gm, Vanilla Flavor 4/5 drops,.	190^0C 20-25min	Brown	Crispy	Vanilla essence	Salty and good	**5**

Note: Acceptability is expressed with numbers where the highest acceptance is noted as 5 and the lowest as 1. All the other numbers in between 1 and 5 denotes partial approval. But the composition and sensory properties may vary due to the quality of raw materials and differences in treatments.

Chapter Five:

DISCUSSION

5.0 DISSCUSSION

Biscuits are predominantly consumed by people of different ages, more particularly by children in both rural and urban areas. The Biscuit industry is ever changing its products to cater the diverse demands of consumers including those belonging to low income groups as well as health conscious people (Hussein et al., 2006). It is one of the few universal staples, which is complete in it and requires no additional preparation. Thus, for many, biscuits have become an important source of carbohydrates, vegetable proteins, vitamins and minerals. But it is important to know that, as compared to animal proteins, refined wheat flour is deficient in certain essential amino acids, thus has a low nutritional value. The nutritional value of biscuit can be enhanced by fortification and supplementation with a wide variety of proteins, vitamin and mineral sources (Awasthi et al., 2012).

Normally biscuits are made with refined wheat flour, hydrogenated fat and sugar as the major ingredients. Refined flour loses much of its dietary fiber and micronutrients during its manufacturing process. Hydrogenated fats are prepared from conventional oils to suit its application in bakery products by the process of hydrogenation during which the natural cis fatty acids are converted to trans-isomers. The trans-fats are implicated in several metabolic disorders such as diabetes, hyperlipedima and cancer among the populations of developed countries whose diet is predominantly based on processed foods. Hence, in the present study experiments were conducted to develop high protein and low sugar contained biscuits and enhance its value using different ingredients to provide nutrients and/or nutraceuticals components. Soy flour, mushroom, wheat flour and other ingredients were used to enrich the nutritional value (Hui, 1992; Elkhalifa and El-Tinay, 2002; Ghattas et al., 2008).

The proximate composition showed that soya flour and mushroom produced to some extent significant nutritional influence on biscuits. They were will be used as a substitute to wheat in varying ratios for biscuits production as a local alternative. They are reported to cater good source of non-starchy carbohydrates, dietary fiber, protein, mineral and vitamins (Bano, 1988).

Soyabean is a wonder bean having higher proportion of protein (40 to 45%), fat (18 to 20%) but low in carbohydrate (20 to 30%). The amino acid pattern is well balanced which is qualitatively almost equivalent to that of animal proteins (Gopalan et al., 2004). Experiments have proved that soy components exert number of health benefits such as cancer prevention, cholesterol lowering, combating osteoporosis and menopause problems (Messina, 1999). Soybean protein constitutes about 40% of the total solids and plays a very important role in the enrichment of cereal-based baked goods (Fukushima, 1999). It is also a rich source of vitamin, minerals and is relatively low in crude fiber (Oyenuga, 1968). Soybean is one such protein sources, which when used partially to replace or complement wheat flour in the production of bakery products such as biscuits, bread and other confectionery could go a long way in improving the nutritional status of such products (Fukushima, 1999). The objective of the study was to conduct the proximate analysis and acceptability of biscuits prepared from wheat flour supplemented with soyabean flour at varying ratios. Hence, experiments showed some promise in terms of nutritional enhancement of biscuits.

Edible mushrooms are widely consumed as an important food item for their significant roles in human health, nutrition and disease. Historically, mushrooms have been used for both medicinal and culinary properties in Asia and many parts of the world. Mushrooms have also been associated with many pharmacological properties by both eastern and western medicine. The functions include reducing cholesterol (Bobek et al., 1997), lowering blood pressure, strengthening the immune system against diseases (Reguła and Siwulski 2007), combating tumors (Mau et al., 2004) and improving liver function (Wang et al., 2000). Oyster mushroom (*Pleurotus sajor-caju*) inhibits hypertensive effects through its active ingredients, which affect the renin-angiotensin system (Chang, 1996). It possesses a very pleasant flavor and taste. The saccharidic complex of oyster mushroom is characterized by low content of digestible carbohydrates and by a relatively high content of polysaccharides which take part in formation of edible fiber (Strmiskova et al., 1992). Freshly harvested oyster mushrooms were reported to contain low fat content in average ranged from 0.38% to 2.28%, indicated low calorific value (kcal) contribution of mushrooms on total daily energy intake (Chye et al., 2008). On the other hand, the cultivated edible mushroom normally had high moisture content at more than 80%. It contains up to 90% water, but owing to its protein and amino acids content, low fat and contain 9-group vitamins and a wide spectrum of mineral substances it represents a good source of biologically valuable substances for human nutrition (Strmiskova et al., 1992). Dry matters of mushrooms contain more than 25% protein, less than 3% crude fat and almost 50% of total carbohydrate. It also contains appreciable amount of dietary fiber and β-glucan, vitamin B groups, D and other useful nutrients. (Kotwaliwale et al., 2007). Therefore, mushrooms form an important constituent of a diet for a population suffering from atherosclerosis (Dunkwal et al., 2007).

Bakery based products including biscuit are popularly consumed by consumers in the Asia-Pacific regions. Extensive studies have been done using various types of legumes, cereal and plant dietary fiber in bakery based products in attempting to enhance nutritional qualities and dietary fiber. It is expected that by partially replacing wheat flour with oyster mushroom powder into biscuit formulation, an improvement of nutritional composition especially dietary fiber without affecting sensorial properties can be achieved (Singh et al., 1996).

Protein content is one of the most essential properties to formulate any kind of food items. The protein content of the biscuit increased with the increase in supplementation. The increase in protein content could be due to the soya fraction of the blended flour as the soya flour has higher protein (40.2%) in comparison to wheat flour (12.1%). Soyabean is an excellent source of protein and complement to lysine-limited cereal protein. Addition of mushroom improve the quantity and quality of protein content of the food product, thereby has the great potential in combating with protein energy malnutrition (Wadsworth, 1992). Oyster mushrooms are high in B complex vitamins and protein (Chang, 1999). This indicates that supplementing soya flour with mushroom flour would greatly improve the protein content and nutritional quality of biscuits. This could obviously be due to the significant quantity of protein in mushroom (Kadir and Fasidi, 1992). The high protein content in the soya-mushroom supplemented food would be of nutritional importance in most developing countries where many people can hardly afford high proteinous foods because of their high cost. The protein content of the soya-mushroom composite biscuit has higher protein content (9.53%) than the others. The combination has been found more nutritious than wheat flour alone because they are richer in essential amino acids than wheat flour. This similar observation was erlier reported (Fayemi, 1999) in which it was showed that an increase in the protein content with corresponding increase in the proportion of breadfruit flour supplementation in biscuit production from wheat- breadfruit flour blends. Therefore, the use of mushroom raise the protein content up to 12% as compared to cookies made from only from wheat flour.

For biscuit like food stuff fiber is needed to maintain a definite structure as well as to enhance the nutritional value. Dehulled soya flour and mushroom has higher fiber content than wheat flour. Dry matters of mushrooms contain more than 25% protein, less than 3% crude fat, 14% crude fiber and almost 50% of total carbohydrate (Kotwaliwale et al., 2007). Total dietary fiber (TDF) intake for adult men and women should be 38 and 25g/d respectively (IOM, 2002). Soluble fibers are effective in reducing total blood cholesterol and promoting satiety, and insoluble fibers help in treating constipation and reduce the risk of colon cancer and cardiovascular disease (Best, 1987). Soy fiber, of course, is effective in lowering serum lipids and cholesterol. By mechanically interfering with reabsorption of cholesterol, as well as by decreasing transit time in the gut, fiber contributes to decreased blood serum cholesterol levels. Besides the isolated types of soy fiber, defatted soy flour itself contains about 19% dietary fiber and can be a useful source of this healthy food component (Cole, 1990). The fiber enriched biscuit may be helpful incurring the constipation and other aliments related to fast food habits (Hegstad, 2008). The fiber content of the composite biscuit has higher fiber content than the local varieties tested. An increase in fiber content could be due to the oyster mushroom fraction of the blended flour. Anderson, (1987) showed in a very similar study the usefulness of dietary fiber in the management of diabetes. Soluble fiber is thought to interfere with the active transport of sugars across the luminal wall, perhaps by forming a thin layer of hydrated gums on the wall. The end result is increased glucose tolerance and reduced fasting glucose levels, both desirable outcomes for diabetics.

The moisture content of the composite biscuit was found to be has slightly lower moisture content (1.4%) than that of local varieties. This is due to the fact that soya flour contained greater amount of total dry solid with high emulsifying properties compared to wheat flour. The moisture content of the biscuit decreased with the increasing amount of soya flour in the blend due to low moisture content of the beans. This was in agreement with the findings of Sutharshan et al., (2001) in which they reported that increase in proportion of soy flour reduced the moisture content of the soya bean flour supplemented biscuits. Low level of moisture content might serve as an indicator of long self-life of composite biscuit as they might minimize microbial growth leading to spoilage.

Soyabean is a protein rich oil seed, which is presently number one edible oil source globally. Soybean is rich in polyunsaturated fats, including the two essential fatty acids, linoleic and linolenic, that are not produced in the body. Linoleic and linolenic acids aid the body's absorption of vital nutrients and are required for human health (Hegstad, 2008). Soyabean oil is 61% polyunsaturated fat and 24% monounsaturated fat which is comparable to the total unsaturated fat content of other vegetable oils (85%). The fat content of the biscuits increased from 14.6 to 24.0% with increase in soybean flour from 0 to 25%. The increase in the fat content could be due to the increase in the proportion of soyabean in the flour blend. This could be due to the fact that soya flour contained higher percentage of fat than wheat flour. The fat content of the composite biscuit showed lower (19.5%) value than that of local varieties. Though the additions of soy-flour help to increase the protein content along with a boosted fat percentage, so to minimize the fat content, the supplementation with soy-flour was maintained 15%. In addition, mushroom fraction was put in to decrease the fat level due to its very low oil content (< 3%). Our results are in agreement with the finding of Akubor and Ukwuru (2005).

Sugar percentage of biscuit is important factor to be measured and maintain low, because that would help the diabetes patients to consume those products. Sugar content of the soya- mushroom composite biscuit was maintained as the lowest (8.32±0.6 %) among all the samples tested. Interestingly, kasuan sugar free (12.13±0.34 %), crack jack (12.15±2.58 %), sugar free biscuit (12.25±0.33 %) and good time (12.74±0.54 %) showed up with quite similar values.

Ash content is indicated an estimate of the total mineral content in a given quantity of food substance. The mineral content of the prepared composite biscuit was higher (2.16%) as compared to the local variety. The increase in mineral content could be due to the fraction of the mushroom powder as it had higher mineral content compared to soya flour and wheat flour. This result was in akin to the findings of Neha and Ramesh, (2012).

The carbohydrate content were highest for the biscuits in terms of all proximate composition parameters (that is, crude protein, crude fiber, ether extract, ash, moisture and carbohydrate) determined in this study. This was expected as the ingredients composed of mainly carbohydrate rich materials, which were wheat and soyaflour.

Food energy is the amount of calorie available from food available through oxidation. Nutritionists usually regard amount of calorie in a gram of a nutrient, but this implies that the food actually contains energy. Fats have the greatest amount of food energy which is 9 kcal/gm while proteins and most carbohydrates have about 4 kcal/gm. The calorific content of the biscuits has been measured 485.48 kcal with the addition of soya flour (15%). These results were in concordance with Rani et al., (2008) and Awasthi et al., (2012).

Taste is the primary factor which determines the acceptability of any product, which has the highest impact as far as market success of product, is concerned. The taste had been decreased with the increase in the level of substitution by mushroom. Biscuit containing 5-6% of mushroom as well as 25% soyabean flour was rated poorest in taste. The color of the biscuit changed from creamy to pale brown. The brown color might be due to Maillard reaction between reducing sugar and protein (Dhingra and Jood, 2000). The texture of the crust was related to the external appearance of the biscuit top which implies smoothness or roughness of the crust.

Flavor of biscuit decreased with increasing in the substitution of soybean flour and mushroom. This could be due to the beany flavor of soy flour (Akubor and Ukwuru, 2005). Overall acceptability includes many implications, which is the important parameter in organoleptic estimation. At 15% level of soy flour and 2.5-3% mushroom incorporated biscuits had higher scores for all the sensory attributes evaluated. Above this level, biscuits received lower sensory scores.

Biscuits packed in polythene bags and stored for four weeks were placed for sensory evaluation at weekly intervals for judging color, taste, flavor, texture and overall acceptability. The judges observed no quality deterioration during storage at room temperature (20 to 25°C). Similar observations were reported by Sirokman and Soboleva (1977).

CONCLUSION

6.0 CONCLUSION

The proximate analyses of nutrient in biscuits are generally done for the quantitative and qualitative analysis of different nutritional factors like ash, moisture, protein, sugar, carbohydrate, fiber, fat, calorie etc. To ensure proper quality of biscuits, the quantities of these nutrients should be properly maintained. This study has confirmed that traditional high-fat and high-sugar biscuits which are not associated with healthy diets by most consumers can be modified. This modification procedure was performed by the addition of soya flour and mushroom (Oyster mushroom, *Pleurotus ostreatus*), thereby converting a traditional food product into a functional one. This resulted in a healthy alternative, not only acceptable to consumers, but also could be manufactured under strict commercial conditions. The study had an opportunity to look into different brands of salted biscuits produced in Bangladesh by different manufacturers and compared them with soya-mushroom composite biscuits.

Biscuits are typically high in both fat and sugar and have been identified as a food contributing to negative health (Department of Health and Social Services). Reducing the materials that were thought to be essential in the standard product was difficult. The complex interaction between these raw materials necessitated a large number of formulation modifications, resulting in complicated manufacturing issues. For example, reduction in fat resulted in several undesirable effects such as toughening of base dough, shrinkage of final product, loss of color and eating quality. Addressing one problem often created another.

In this study, a healthy biscuit was successfully produced under strict commercial manufacturing conditions, and furthermore the biscuit was initially acceptable to the consumer. The quality of biscuits could be improved with supplementation of soya flour and/or mushroom, in terms of increased level of crude fiber, ash and protein. Fiber, ash and protein content of the experimental biscuit samples increased with the level of incorporation of soya flour and mushroom while fat content of biscuit sample decreased slightly. The study showed that soya-mushroom biscuits may be a suitable source of proteins. It could be used as a vehicle for protein fortification and other nutritional improvement in biscuits. Addition of soy flour in wheat products increases the cost but if the functionality of soy protein is considered, addition of soy flour is economical with nutrition improvement as bonus. Soy protein is also a cost effective way to reduce fat, increase protein, and improve the overall characteristics of various bakery products.

At a time when obesity is a major concern and foods high in energy and low in nutrients are linked to the causes of obesity and negative health, the manufacturers of these products are under increasing pressure to produce healthy alternatives. Such developments across a range of products will have a profound effect on the food industry as children and adults alike are becoming increasingly aware of the need to consume a health promoting diet. Hence, development and utilization of such functional foods will not only improve the nutritional status of the population but also helps those suffering from degenerative diseases. Further work is necessary in terms of the microbiological examinations of the products. In dept analyses into the microbiological qualities and shelf life will help in improvement in the product and also research on the level of blood sugar in rats with low sugar contented biscuits for the management of diabetes.

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APPENDICES

Appendix 01: Table 01: Moisture content of biscuits

Name of Biscuits	Moisture		
	Data 01	Data 02	Result (Av±SD)
Bakery	3.81	3.85	3.83±0.03 %
Capital	2.39	2.5	2.45±0.08%
Crack jack	1.54	1.63	1.59±0.06%
Diasalt	1.91	1.93	1.92±0.02%
Digestive	2.34	2.41	2.38±0.05%
Good time	2.44	2.42	2.43±0.02%
Grand Choice	2.51	2.63	2.57±0.08%
Kasuan Sugar free	2.63	2.6	2.62±0.02%
Lavender	2.42	2.35	2.39±0.05%
Protein biscuit	1.62	1.7	1.66± 0.06%
Salt cheese	2.51	2.58	2.55±0.05%
Salt puff	2.15	2.11	2.13±0.03%
Sugar free biscuit	2.46	2.44	2.45±0.02%
Tip	2.01	1.94	1.98±0.05%
Top	2.83	2.9	2.87±0.05%
Zero sugar	2.56	2.55	2.56±0.01%
Soya-mushroom biscuit	1.34	1.45	1.4±0.08%

Table 02: Ash content

Name of Biscuits	Ash		
	Data 01	Data 02	Result (Av±SD)
Bakery	1.82	2	1.91±0.13%
Capital	1.27	1.37	1.32±0.07%
Crack jack	1.99	2.15	2.07±0.11%
Diasalt	1.98	1.88	1.93±0.07%
Digestive	1.26	1.35	1.31±0.06%
Good time	1.14	1.19	1.17±0.04%
Grand Choice	1.49	1.57	1.53±0.06%
Kasuan Sugar free	1.72	1.75	1.74±0.02%
Lavender	1.3	1.21	1.26±0.06%
Protein biscuit	1.57	1.54	1.56±0.02%
Salt cheese	2.03	2.11	2.07±0.06%
Salt puff	1.97	1.86	1.92±0.08%
Sugar free biscuit	1.77	1.68	1.68±0.06%
Tip	1.75	1.79	1.77±0.03%
Top	0.96	1.03	0.99±0.05%
Zero sugar	1.27	1.33	1.3±0.04%
Soya-mushroom biscuit	2.21	2.11	2.16±0.07%

Table 03: Fat content

Name of Biscuits	Fat		
	Data 01	Data 02	Result (Av±SD)
Bakery	27.11	25.12	26.12±1.40%
Capital	28.23	29.68	28.96±1.03%
Crack jack	22.24	24.26	23.25±1.43%
Diasalt	23.17	20.19	21.68±2.11%
Digestive	26.17	25.18	25.68±0.7%
Good time	21.91	19.88	20.9±1.44%
Grand Choice	23.59	21.21	22.4±1.68%
Kasuan Sugar free	26.26	28.11	27.19±1.31%
Lavender	28.04	29.35	28.7±0.93%
Protein biscuit	24.34	25.18	24.76±0.6%
Salt cheese	32.59	33.22	32.91±0.45%
Salt puff	26.08	29.39	27.74±2.34%
Sugar free biscuit	25.37	22.32	23.85±2.16%
Tip	22.82	25.38	24.1±1.81%
Top	22.42	25.35	23.89±2.07%
Zero sugar	25.41	27.33	26.37±1.36%
Soya-mushroom biscuit	20.11	18.38	19.45±1.22%

Table 04: Protein content

Name of Biscuits	Protein		
	Data 01	Data 02	Result (Av±SD)
Bakery	3.88	3.11	3.5±0.54%
Capital	4.65	3.89	4.27±0.54%
Crack jack	4.7	4.8	4.75±0.07%
Diasalt	5.27	5.69	5.48±0.3%
Digestive	5.31	5.55	5.43±0.17%
Good time	4.99	5.32	5.16±0.23%
Grand Choice	5.31	5.12	5.22±0.13%
Kasuan Sugar free	6.1	5.96	6.03±0.1%
Lavender	4.58	4.32	4.45±0.18%
Protein biscuit	6.71	6.22	6.47±0.35%
Salt cheese	6.73	6.32	6.53±0.3%
Salt puff	5.33	5.23	5.28±0.07%
Sugar free biscuit	5.1	4.69	4.9±0.28%
Tip	4.25	4.55	4.4±0.21%
Top	4.52	4.21	4.37±0.22%
Zero sugar	5.81	5.66	5.74±0.11%
Soya-mushroom biscuit	9.4	9.65	9.53±0.18%

Table 05: Sugar content

Name of Biscuits	Sugar		
	Data 01	Data 02	Result (Av±SD)
Bakery	15.78	13.56	14.67±1.57%
Capital	16.25	15.32	15.79±0.66%
Crack jack	13.97	10.32	12.15±2.58%
Diasalt	17.38	16.55	16.97±0.59%
Digestive	17.51	18.57	18.04±0.75%
Good time	13.12	12.36	12.74±0.54%
Grand Choice	15.52	15.89	15.71±0.26%
Kasuan Sugar free	12.37	11.89	12.13±0.34%
Lavender	15.01	13.29	14.15±1.22%
Protein biscuit	17.45	15.39	16.42±1.46%
Salt cheese	16.91	14.69	15.8±1.57%
Salt puff	14.44	13.88	14.16±0.4%
Sugar free biscuit	12.48	12.01	12.25±0.33%
Tip	13.99	13.65	13.82±0.24%
Top	15.07	15.69	15.38±0.44%
Zero sugar	12.74	13.88	13.31±0.81%
Soya-mushroom biscuit	8.74	7.89	8.32±0.6%

Table 06: Fiber content

Name of Biscuits	Fiber		
	Data 01	Data 02	Result (Av±SD)
Bakery	0.15	0.13	0.14±0.01%
Capital	0.17	0.16	0.17±0.01%
Crack jack	0.29	0.22	0.27±0.05%
Diasalt	0.18	0.15	0.17±0.02%
Digestive	0.27	0.31	0.29±0.03%
Good time	0.31	0.33	0.32±0.01%
Grand Choice	0.27	0.26	0.27±0.01%
Kasuan Sugar free	0.27	0.26	0.27±0.01%
Lavender	0.09	0.1	0.1±0.01%
Protein biscuit	0.25	0.3	0.28±0.04%
Salt cheese	0.34	0.35	0.35±0.01%
Salt puff	0.13	0.16	0.15±0.02%
Sugar free biscuit	0.3	0.33	0.32±0.02%
Tip	0.21	0.23	0.22±0.01%
Top	0.29	0.33	0.31±0.03%
Zero sugar	0.32	0.36	0.34±0.03%
Soya-mushroom biscuit	0.49	0.43	0.46±0.04%

Table 07: Carbohydrate content

Name of Biscuits	Carbohydrate		
	Data 01	**Data 02**	**Result (Av±SD)**
Bakery	63.25	62.15	62.7±0.78%
Capital	63.29	63.23	63.26±0.04%
Crack jack	69.25	69.78	69.52±0.37%
Diasalt	67.49	66.59	67.04±0.64%
Digestive	64.85	63.87	64.36±0.69%
Good time	71.19	70.89	71.04±0.21%
Grand Choice	66.83	65.65	66.24±0.83%
Kasuan Sugar free	63.02	63.25	63.14±0.16%
Lavender	63.57	63.38	63.48±0.13%
Protein biscuit	65.54	65.32	65.43±0.16%
Salt cheese	56.39	56.88	56.64±0.35%
Salt puff	64.34	63.23	63.79±0.78%
Sugar free biscuit	64.94	62.25	63.6±1.90%
Tip	68.95	67.11	68.03±1.30%
Top	69.16	69.42	69.29±0.18%
Zero sugar	64.54	63.23	63.89±0.93%
Soya-mushroom biscuit	65.48	63.22	64.35±1.6%

Table 08: Calorie content

Name of Biscuits	Calories		
	Data 01	**Data 02**	**Result (Av±SD)**
Bakery	512.51	510.26	511.39±1.59%
Capital	520.22	517.28	518.75±2.08%
Crack jack	495.94	494.88	495.41±0.75%
Diasalt	499.57	492.45	496.01±5.03%
Digestive	516.17	518.32	517.25±1.52%
Good time	494.08	493.11	493.6±0.69%
Grand Choice	491.23	488.55	489.89±1.90%
Kasuan Sugar free	512.84	509.88	511.36±2.09%
Lavender	518.15	518.38	518.27±0.16%
Protein biscuit	515.17	514.95	515.06±0.16%
Salt cheese	545.79	539.39	542.59±4.53%
Salt puff	505.19	503.23	504.21±1.39%
Sugar free biscuit	508.49	511.22	509.86±1.93%
Tip	498.18	498.68	498.43±0.35%
Top	494.88	495.01	494.95±0.09%
Zero sugar	510.08	511.1	510.59±0.72%
Soya-mushroom biscuit	489.4	481.56	485.48±5.54%

Table 09: Nutritional value of local available biscuits compared with newly developed biscuit

Name of Biscuits	Moisture* (%)	Ash (%)*	Fat (%)^	Sugar (%)^	Protein (%)^	Fiber (%)#	Carbohydrate (%)^	Calories (%)^
Bakery	3.83±0.03	1.91±0.13	26.12±1.40	14.67±1.57	3.5±0.54	0.14±0.01	62.7±0.78	511.39±1.59
Capital	2.45±0.08	1.32±0.07	28.96±1.03	15.79±0.66	4.27±0.54	0.17±0.01	63.26±0.04	518.75±2.08
Crack jack	1.59±0.06	2.07±0.11	23.25±1.43	12.15±2.58	4.75±0.07	0.27±0.05	69.52±0.37	495.41±0.75
Diasalt	1.92±0.02	1.93±0.07	21.68±2.11	16.97±0.59	5.48±0.3	0.17±0.02	67.04±0.64	496.01±5.03
Digestive	2.38±0.05	1.31±0.06	25.68±0.7	18.04±0.75	5.43±0.17	0.29±0.03	64.36±0.69	517.25±1.52
Good time	2.43±0.02	1.17±0.04	20.9±1.44	12.74±0.54	5.16±0.23	0.32±0.01	71.04±0.21	493.6±0.69
Grand Choice	2.57±0.08	1.53±0.06	22.4±1.68	15.71±0.26	5.22±0.13	0.27±0.01	66.24±0.83	489.89±1.90
Kasuan Sugar free	2.62±0.02	1.74±0.02	27.19±1.31	12.13±0.34	6.03±0.1	0.27±0.01	63.14±0.16	511.36±2.09
Lavender	2.39±0.05	1.26±0.06	28.7±0.93	14.15±1.22	4.45±0.18	0.1±0.01	63.48±0.13	518.27±0.16
Protein biscuit	1.66± 0.06	1.56±0.02	24.76±0.6	16.42±1.46	6.47±0.35	0.28±0.04	65.43±0.16	515.06±0.16
Salt cheese	2.55±0.05	2.07±0.06	32.91±0.45	15.8±1.57	6.53±0.3	0.35±0.01	56.64±0.35	542.59±4.53
Salt puff	2.13±0.03	1.92±0.08	27.74±2.34	14.16±0.4	5.28±0.07	0.15±0.02	63.79±0.78	504.21±1.39
Sugar free biscuit	2.45±0.02	1.68±0.06	23.85±2.16	12.25±0.33	4.9±0.28	0.32±0.02	63.6±1.90	509.86±1.93
Tip	1.98±0.05	1.77±0.03	24.1±1.81	13.82±0.24	4.4±0.21	0.22±0.01	68.03±1.30	498.43±0.35
Top	2.87±0.05	0.99±0.05	23.89±2.07	15.38±0.44	4.37±0.22	0.31±0.03	69.29±0.18	494.95±0.09
Zero sugar	2.56±0.01	1.3±0.04	26.37±1.36	13.31±0.81	5.74±0.11	0.34±0.03	63.89±0.93	510.59±0.72
Soya-mushroom	**1.4±0.08**	**2.16±0.07**	**19.45±1.22**	**8.32±0.6**	**9.53±0.18**	**0.46±0.04**	**64.35±1.6**	**485.48±5.54**

*Non-significant
Significant
^ Post hoc tests are not performed for Biscuits because at least one group has fewer than two cases

Appendix 02: One way Analysis of Variance (ANOVA)

ANOVA data (by missing analysis)

Ash

ANOVA

Biscuits

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	376.000	15	25.067	.783	.724
Within Groups	32.000	1	32.000		
Total	408.000	16			