PROXIMATE AND MINERAL ANALYSIS OF MOST WIDELY USED MUSHROOM CULTIVATED IN CHATTOGRAM AND DEVELOPED MUSHROOM BISCUITS

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Roll No.: 0118/03

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

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June, 2020

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

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DEDICATED TO MY

RESPECTED AND BELOVED

PARENTS AND TEACHERS

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The Author

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Tables

Abbreviations

AD	Anno Domini
AIDS	Acquired Immunodeficiency Syndrome
AOAC	Association of Official Analytical Chemists
CHO	Carbohydrate
CAD	Coronary Artery Disease
CMC	Carboxymethyl cellulose
DPPH	2,2-diphenyl-hydrazyl-hydrate
dl	Deci-liter
^{0}C	Degree Celsius
%	Percent
&	And
FAO	Food and Agriculture Organization
GABA	Gamma aminobutyric acid
GAE	Gallic Acid Equivalent
G	Gram
KJ	Kilo joule
Hr	Hour
IARI	Indian Agriculture Research Institute
SDA	Sabouraud dextrose agar
TVC	Total viable count
TCC	Total Coliform Count
MPN	Most probable number
ML	Milliliter
mmol	Millimole
N	Normal

Abstract

Mushroom is a rich source of nutrients and widely consumed in Asian countries. Biscuits can be easily fortified with Mushroom rich flour to provide convenient foods, in order to supplement protein in the diet. The research study was conducted to evaluate proximate and mineral analysis of most widely used mushroom cultivated in Chattogram and developed mushroom biscuits. The mushroom varieties (*Pleurotus ostreatus, Pleurotus sajor-caju, Agaricus bitorquis, Ganoderma lucidum*) were collected and dried, then taken to the laboratory for proximate and mineral analysis by following standard assay methods. Results demonstrated that the moisture, total solids, protein, fat, carbohydrate, fiber, ash and energy content of different types of mushrooms were ranged from 53.10to 90.10 %, 9.90 to 47.00 %, 3.23 to 19.70 %, 0.47 to 4.20 %, 4.21 to13.02 %, 0.51 to 3.90 %, 1.28 to 6.18 and 34.78 to 172.37 (Kcal/100g) respectively. The highest value of sodium, phosphorus and copper content was found in *Agaricus bitorquis*. Magnesium content was found the highest in *Pleurotus ostreatus.* Potassium, calcium, chloride, iron and zinc content was found the highest in *Ganoderma lucidum*. In mushroom biscuits, the moisture, ash, protein, fat, fiber, carbohydrate and energy value were ranged from 4.03 to 3.89 %, 3.47 to 4.11 %, 12.15 to 13.43 %, 5.43 to 5.83 %, 4.71 to 5.09 %, 67.03 to 70.35 % and 374.15 to 378.87 (Kcal/100g) respectively. From the sensory and microbiological point of view, mushrooms biscuits exhibited a significant acceptability and stability. Mushroom and mushroom based products are valuable food source of some human needs, able to meet day-to-day nutritional requirements as a food and help the economy of the country.

Keywords: Mushroom, Proximate, Minerals, Mushroom biscuits, Sensory attributes, Microbiological.

Chapter I: Introduction

Mushroom is soft delicate white fruit body of fungi, those are Agaricaceae family. The real plant is the microscopic fine thread-like body called mycelium, grows on the substratum or under the surface of soil. The commercial cultivation first started in Europe with the beginning of this century. Edible mushrooms are one of the most valuable and least expensive sources of protein that is of great importance in the world (Nasiri et al., 2013). More than 2000 species of mushrooms exist in the nature; however, less than 25 species are widely accepted as foods. *Agaricus bisporus*" is the most common and is cultivated worldwide and totally accounts for 38% of the world edible mushrooms. The greatest producer of mushroom is China that produces more than one million tons a year (Kapahi, 2018). Mushrooms are categorized as healthy and valuable foodstuffs that contain combinations of protein, carbohydrates, minerals and vitamins. Edible mushrooms contain fibers and some nutrients that have medicinal aspects; also, they are poor source of carbohydrates and for this reason they can be included in the diet of people with diabetic disorder. The edible mushrooms are considered as a healthy food for human (Valverde et al., 2015).

During researches about the different species of edible mushrooms, it is found that the protein content in mushrooms is higher than fruits and vegetables. Mushroom also contains some unsaturated fatty acids; provide several types of the B vitamins, and vitamin D. They are a good source of B vitamins, especially niacin and riboflavin, and rank the highest among vegetables for protein content. Among the minerals, potassium has the highest concentration in all species of edible mushrooms. Major mineral constituents in mushrooms are potassium, sodium, phosphorus, calcium, magnesium, and elements like copper, zinc, iron, molybdenum, cadmium form minor constituents (Gucia et al., 2012). Mushrooms have been used in health care for treating simple and age-old common diseases like skin diseases to present day complex and pandemic disease like AIDS (Wani et al., 2010). Mushroom contains various bioactive compounds including terpenoids, steroids, phenols, alkaloids, lectins and nucleotides, which have been isolated and identified from the fruit body, mycelium and culture broth of mushrooms are shown to have promising biological effects. Mushrooms are considered as a functional food, which can provide health benefits beyond the traditional nutrients they contain (Rathee et al., 2012).

Using mushroom in production of products such as biscuit could improve the nutritional quality of mushroom food products (Van Toan and Thu, 2018). In addition, enriching Mushroom flour with flour could provide a product suitable for diabetes and choleric people. This could encourage local farmers to produce mushroom food products commercially also; the great potential of mushroom could be fully exploited in enriching other food products. Bakery based products including biscuits are among top five items popularly consumed by consumers in Bangladesh (Islam and Ullah, 2010). Biscuits are baked, edible and commonly flour-based products. Biscuits may be regarded as a form of confectionery dried to very low moisture content. Biscuits are ready to eat, convenient and inexpensive food products containing dietary and digestive principles of vital importance. These are becoming popular among both rural and urban populations in Bangladesh. Mushroom which is rich in protein and fiber content also available but underutilize could be used to increase it nutritional value of baked food products (Friedman, 1996). Mushrooms have a great potential due its high and good quality protein (20–40% on dry weight basis), Vitamins (Vitamin B-complex), and minerals (Farzana and Mohajan, 2015). So, mushroom can be dried and converted into powdered form which then used for fortification in baked products like breads, biscuits etc.

Objective of the study

1. To determine the proximate composition and mineral content of the edible mushroom available in Chattogram region.
2. To develop a mushroom biscuit and evaluate its proximate composition, sensory attributes and microbiological quality of developed mushroom biscuits.

Chapter II: Review of Literature

2.1 History of mushroom production in Bangladesh

Bangladesh is one of the most adequate countries in the world for mushroom production for its favorable climate condition. It has low production cost and high market value. Commercial mushroom cultivation was initiated by Bangladesh Agricultural Research Council and Mushroom Culture Centre at Savar (Alom and Bari, 2010). A number of projects were initiated by the Government of Bangladesh under Department of Agricultural Extension to promote commercial production. Oyster, Shitake, Reishi, Milky White, Straw, Wood ear and White Button mushroom are the varieties which have so far been produced or attempted for production in Bangladesh. Oyster mushroom produced huge amount in all over the country. Private company namely Panbo Bangla Mushroom Ltd. (PBML) a member company of ORION GROUP has been producing white button mushroom utilizing best European raw materials and technology since 2012. The production capacity of fresh mushroom is 1500-2200 MT/year and 6-7 million pieces of 285 g canned mushroom per year. The government introduced a Mushroom Development Project under Agriculture Extension department. Different research and training works are being conducted under the project (Rahman, 2015). The project activities done in various district in Bangladesh like Savar, Dinajpur, Jessore, the aim of the project is motivating people to cultivate mushroom (Sarker, 2014). Currently 13 species of mushroom are cultivated in Bangladesh of which oyster Mushroom is produced commercially to a large extent. Mushroom production is a very easy job. It is giving good profit by investing a little amount of capital and labor. One can earn Tk 3-5 thousand a month by investing only Tk 10- 12 thousand (Oseni et al., 2012).

Fig. 1: Mushroom

2.2 Present status of mushroom in Bangladesh

A survey was conducted to assess the present status of mushroom-scenario in 8 meteorological regions of Bangladesh (Kamal et al., 2009a). More than 620-675 tons of edible mushrooms production in Bangladesh per annum (Kamal et al., 2009b). It was

also estimated that 66% of mushrooms were consumed as fresh, 23% in dried form, 10% in powdered form and 1% in other forms (pickling, frying etc) as processed or preserved ones. From the results of the survey, it was also shown that among the cultivators, 34% cultivated mushrooms on account of its nutritional and medicinal values, 27% to reduce unemployment, 14% for growing mushrooms as more profitable vegetable.

2.3 Mushroom production in Chattogram area

The Chinese have cultivated the mushroom for centuries (Chang, 1999). More than 300 spices are edible. Only about 8 species can be commercially grown in Chattogram area (Begum, 2013). Mushroom sub-center Hatazari introduced the mushroom production in this area. This institution gave various training facilities among the farmer. Those 8 species are

1. Button mushroom (*Agaricus bitorquis*)

2. Shiitake (*Lentinus edodes*)

3. Common oyster mushrooms (*Pleurotus ostreatus, Pleurotus sajor-caju, Pleurotus florida*)

4. Reishi mushroom (*Ganoderma lucidium)*

5. Shimaji mushroom (*Hypsizygus tessulatus*)

6. Enoki (*Flammjlina Flutes*)

7. Straw mushroom (*Volvariella volvacea*)

8. Milky white mushroom (*Calocybe indica*)

2.4 Mushrooms as a source of food

Mushrooms are considered as a very nutritional food source because of its tastiness and exclusive flavor for chemical compositions (Yamaguchi and Ninomiya, 2000), that's why had been hunting since the ancient times indeed. In recent decades, mushrooms are also used as a special diet for combat disease due to their chemical components as well. There are lots of scientific studies which revealed nutritional requirements for human and its different compositions in different species of mushrooms. In addition, the research by (Thatoi and Singdevsachan, 2014) also recorded the nutritive value of

Pleurotus flabellatus in which 2.75% (dry weight) protein, 0.974% (dry weight) ash, 0.105% (dry weight) fat, 1.084% (dry weight) crude fiber, 90.95% (dry weight) moisture and 0.14% (dry weight) of non-protein nitrogen have found, respectively. Moreover, (Cheung, 2008) reported that mushrooms contain 90% water and 10% dry matter in general, protein (% dry weight) content varies between 27 and 48% dry weight, carbohydrates vary less than 60% and lipids between 2% to 8% dry weight, respectively. Therefore, the edible mushroom is highly nutritious compared to meat, milk, eggs and value deceits between meat and vegetables as well (Okoro and Achuba, 2012) reported only around 2000 species of mushrooms considered as edible among thousands of species, and about only 20 species (with only 4 to 5 under industrial production) cultivated commercially, respectively.

2.5 Health benefit of mushroom consumption

Mushrooms have a great nutritional value since they are quite rich in protein, with an important content of essential amino acids and fiber, and poor in fat (Reis et al., 2012). Edible mushrooms also provide a nutritionally significant content of vitamins (B1, B2, B12, C, D and Edible mushrooms could be a source of many different nutrients such as unsaturated fatty acids, phenolic compounds, tocopherols, ascorbic acid and carotenoids (Alzand et al., 2019). Thus, they might be used directly in diet and promote health, taking advantage of the additive and synergistic effects of all the bioactive compounds present. Mushrooms are important constituents of minor forest produce, that grow on the most abundant biomolecule of this biosphere, that is, cellulose (Pusa, 2012). Presently mushrooms are regarded as a macro-fungus with a distinctive fruiting body which can be either epigeous or hypogenous and large enough to be seen with the naked eyes and to be picked by hand (Wani et al., 2010). Only fruiting body of the mushroom can be seen whereas the rest of the mushroom remains underground as mycelium. Geologically, mushrooms existed on the earth even before man appeared on it, as evidenced from the fossil records of the lower cretaceous period. Mushrooms offer tremendous applications as they can be used as food and medicines besides their key ecological roles. They represent as one of the world's greatest untapped resources of nutrition and palatable food of the future. Mushrooms have been found effective against cancer, cholesterol reduction, stress, insomnia, asthma, allergies and diabetes (Vishwakarma et al., 2011). Due to high number of proteins, they can be used to bridge the protein malnutrition gap. Mushrooms as functional foods are used as nutrient

supplements to enhance immunity in the form of tablets. Due to low starch content and low cholesterol, they suit diabetic and heart patients. One third of the iron in the mushrooms is in available form. Their polysaccharide content is used as anticancer drug. Even, they have been used to combat HIV effectively. Biologically active compounds from the mushrooms possess antifungal, antibacterial, antioxidant and antiviral properties, and have been used as insecticides and nematicides as well. Thus, keeping in view, the tremendous applications of mushrooms, the present study reviews different aspects of mushrooms towards human health benefits such as food, medicine, minerals, drugs etc (Wasser, 2017).

2.6 Medicinal benefits

In the medicinal point of view, mushrooms are very important and have been used since the Neolithic and Paleolithic eras (Das et al., 2020) since 100 A.D. in China, but the active principles of mushroom as health encouraging had explored in 1960 as well. Mushrooms have been used for 'consumption' treatment (tuberculosis disease), skin diseases, pandemic disease like AIDS and also have valuable status for anti-allergic, anti-cholesterol, anti-tumor and anti-cancer properties as well. The first successful research by (Wasser, 2002) showed that the anti-tumor activities of several mushrooms and also proved the main components of polysaccharides especially β–D- glucans. Another research by (Hamzah et al., 2013) also reported that mushrooms cure epilepsy, lesions, skin diseases, heart ailments, rheumatoid arthritis, cholera besides intermittent fevers, diaphoretic, diarrhea, dysentery, cold, anesthesia, liver disease, gall bladder diseases and used as vermicides, respectively.

2.7 Mushroom based products

Technologies for production of some other products like mushroom-based biscuits, nuggets, preserves, noodles, papad, candies and readymade mushroom curry in retort pouches have been developed but are yet to be popularized (Priyadarsini and Mishra, 2020). Attractive packaging of the value-added products is yet another area, which may be called the secondary value-addition (Rai and Arumuganathan, 2007). While small growers may add value by grading and packaging, industry may go for the processed products for better returns as well as improvement in the demand, which shall have cascading positive effect on the production.

A. Mushroom Soup Powder

Soups are commonly used as appetizers but also as main course by the diet conscious. Experiments were conducted at DMR, to prepare good quality ready-to-make mushroom soup powder using quality mushroom powder produced from the button and oyster mushroom, dried in the dehumidifying air cabinet-drier (Priyadarsini., et al 2020).

B. Mushroom Biscuit

Delicious and crunchy mushroom biscuits were prepared by using the button/ oyster mushroom powder and various ingredients viz., maida, sugar, ghee (bakery fats), mushroom powder, coconut powder, backing soda, ammonium bichromate and milk powder (Nagulwar et al., 2020). Mushroom biscuits combine two of my very favorite things – mushrooms and buttery dough.

C. Mushroom Nuggets

'Nuggets' are generally prepared out of 'pulse' powder namely, black gram powder, soybean powder, urad dhal powder, etc., and used in the preparation of vegetable curry in North India (Wakchaure, 2011).

D. Mushroom Ketch-Up

Ketch-up is made by concentrating the juice/ pulp of the fruits/ vegetables without seeds and pieces of skin. It does not flow freely and is highly viscous in nature. They also contain more of sugar and less of acid (Priyadarsini., et al 2020).

E. Mushroom Candy

A fruit or vegetable impregnated and coated with sugar, subsequently taken out and dried is called a candied fruit or vegetable. The total sugar content of the impregnated

produce is kept at about 75% to prevent fermentation (Wakchaure, 2011).

F. Mushroom Preserve (Murabba)

Murabba (preserve) is made from matured fruits or vegetables, by cooking it whole or in the form of pieces in heavy sugar syrup, till it becomes tender and transparent. (Wakchaure, 2011). In murabba preparation, around 45 kg of fruit or vegetable is used

for every 55 kg of sugar and cooking is continued till a concentration of at least 68% of soluble solid is reached.

G. Mushroom Chips

The freshly harvested button mushrooms are washed, sliced (2 mm) and blanched in 2% brine solution. The mushrooms are dipped overnight in a solution of 0.1% of citric acid + 1.5% of NaCl + 0.3% of red chili powder. After draining off the solution, the mushrooms are subjected to drying in a cabinet dryer at 600C for 8 h. Then it is fried in the refined oil and good quality chips are prepared. Garam masala and other spices can be spread over the chips to enhance the taste. After mixing the spices, the chips are packed in polypropylene packets and sealed after proper labelling (Wakchaure, 2011).

2.8 Current demand of Mushroom biscuits

Edible mushrooms formerly called the "food of the gods." Edible mushrooms still treated as a garnish or delicacy can be taken regularly as part of the human diet or as functional food (Prodhan et al., 2015). Mushrooms have been consumed and appreciated for their flavor, economic and ecological values and medical properties for many years. Although mushroom contains approximately 90% water, but its protein and amino acids content, low fat and 9-group vitamins and a wide spectrum of mineral substances it represents a high-quality source of biological substances for human nutrition. Mushrooms contain (dry basis) more than 25% protein, less than 3% crude fat and almost 50% of total carbohydrate (Mattila et al., 2002). Mushrooms are considered to be a healthy diet because it is low in calorie, sodium, fat and cholesterol (Valverde et al., 2015).

Chapter III: Materials and Methods

The research work was conducted at the Department of Food Processing and Engineering, Department of Applied Food Science and Nutrition of Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh, 1st Jan 2020 to 30th Dec 2020 for the proximate and mineral analysis of most widely used mushroom cultivated in Chattogram region and development of mushroom biscuits.

3.1 Collection of Mushrooms

The mushrooms (*Pleurotus ostreatus, Pleurotus sajor-caju, Agaricus bitorquis, Ganoderma lucidum*) were bought from super market in Chattogram, Bangladesh.

3.2 Proximate analysis of mushrooms and mushroom biscuits

Moisture, protein, fat and ash contents of mushrooms samples were measured in triplicate according to AOAC (Association of Official Analytical Chemists) methods. The moisture was measured by oven drying at 105^0C to constant weight (AOAC, 2000). The crude protein content was measured by the Kjeldahl procedure (6.25×N). Total lipid was extracted by the AOAC (2000) method using the Soxhlet apparatus. Ash was measured gravimetrically in a muffle furnace by heating at 550^0C to constant weight (AOAC, 2000).

3.2.1 Moisture/Water

At first weight of empty crucibles were dried and 5g of sample was placed on it. Then the crucible was placed in an air oven (thermostatically controlled) and dried at temperature of 105^0C for 24 hrs. After drying, the crucible was removed from the oven and cooled in desiccator. It was then weighed with cover glass. The crucible was again placed in the oven, dried for 30 minutes, took out of the dryer, cooled in desiccator and weighed. Drying, cooling and weighing were repeated until the two consecutive weights were same. From these weights, the percentage of moisture in food samples was calculated as follows:

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

3.2.2 Protein

Reagents used: Concentrated H_2SO_4, Digestion mixture (Potassium sulphate 100g + Copper sulphate 10g + Selenium dioxide 2.5g), Boric acid solution, Alkali solution, Mixed indicator solution, Standard HCl (0.1N)

For estimation of protein, the steps were followed:

Digestion: 2g sample, 3g digestion mixture and 25 ml H_2SO_4 was taken in a kjeldahl digestion flask. It was heated for 4 hours in a kjeldahl digestion and distillation apparatus. The digestion was completed when the color of the substance was pale yellow.

Distillation: After digestion 100ml water, 100 ml 40% NaOH and glass blitz were added to kjeldahl flask which containing about 10 ml 2% boric acid and 2-3 drops mixed indicator. About 100 ml distillate was collected just before the distillation was stopped. The receiving flask was moved so that the tip of the distilling tube was out the distillate. Some distillate was collected in this way to make sure the condenser tube was free from traces of ammonia.

Titration: The ammonia collected was titrated with 0.1N HCl solution and titer value was recorded.

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

$$\% \text{ Nitrogen} = \frac{(T_s - T_b) \times \text{Normality of acid} \times \text{meq. } N_2}{\text{Weight of sample (g)}} \times 100$$

Where, T_s = Titer value of sample (ml), T_B = Titer value of Blank (ml), meq. of N_2 = 0.014, % Protein = % Nitrogen × 6.25

3.2.3 Fat

The fat content of the samples was determined by the standard AOAC method (AOAC, 2003). The dried sample remaining after moisture determination was transferred to a thimble and plugged the top of the thimble with a wad of fat free cotton. The thimble was dropped into the fat extraction tube attached to a Soxhlet flask. Approximately 75ml or more of anhydrous ether was poured into a flask. The top of the fat extraction tube was attached to the condenser. The sample was extracted for 16hrs or longer on a water bath at 80^0C. At the end of the extraction period, the thimble was removed from

the apparatus and distilled off most of the ether by allowing it or collected in Soxhlet tube. The ether was poured off when the tube was nearly full. When the ether reached a small volume, it was poured into a small, dry beaker through a small funnel containing a plug of cotton. The flask was rinsed and filtered thoroughly, using ether. The ether was evaporated on a steam bath at low heat; it was then dried at 100^0C for 1hr, cooled and weighed. The difference in the weights gave the ether soluble material present in the sample.

The presence of fat was expressed as follows:

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

3.2.4 Ash

The ash content of the samples was determined by the standard AOAC method (AOAC, 2003). This method performs oxidization of all organic matter by incineration and determines the weight of remaining ash. Briefly, five grams (5 g) of sample was burned and put into muffle furnace with crucible at 550ºC for 8 hrs It was calculated using the following formula:

$$\%\ \text{Ash content} = \frac{\text{Weight of ash}}{\text{Initial weight of sample}} \times 100$$

3.2.5 Crude fiber determination

Crude fiber was determined according to AOAC method (2005). Principle: Crude fiber is the water insoluble fraction of carbohydrate consists mainly of cellulose, hemicellulose and lignin. It is estimated through digestion of fat free known amount of food sample by boiling it in a weak solution of acid (1.25% H_2SO_4) for 30 minutes followed by boiling in weak solution of alkali (1.25% NaOH) for 30 minutes at constant volume and then deducting ash from the residue obtained.

Apparatus: Liebig condenser, Reflux condenser, Gooch crucible

Reagent required:

1. 0.255N Sulphuric acid solution

2. 10.0% Potassium sulphate solution;
3. Asbestos- Gooch grade.

Calculation: The loss in weight represents crude fiber

$$\text{Crude fiber \%} = \frac{\text{Weight of residue with crucible - weight of ash with crucible}}{\text{Weight of sample (moisture and fat free)}} \times 100$$

3.2.6 Determination of total carbohydrates

It was given as the difference between 100 and a sum total of the other proximate components. Hence it was calculated using the formula below:

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

3.2.7 Determination of energy value

The energy value of the samples was determined by multiplying the protein content by 4, carbohydrate content by 4 and fat content by 9 according to standard James formula (Matin et al., 2019).

Energy Value = (Crude protein × 4) + (Total carbohydrate × 4) + (Crude fat × 9)

3.3 Mineral analysis

3.3.1 Sample preparation

10 ml Nitric and 5 ml $HClO_4$ acid were added to a sample of 1 g in a digestion flask. The mixture was digested for 1 hr. The digested mixture was filtered. The filtrate was made up to 100 ml with distilled water. Mineral contents were determined by using a biochemical analyzer (Humalyzer 3000). Commercially available biochemical kit (Randox®) was used for biochemical assay (Akther et al., 2020).

3.3.2 Determination of Sodium (Na^+)

Principle: Sodium is precipitated as a triple salt with magnesium and uranyl acetate. The excess of uranyl ions is reacted with ferrocyanide in an acidic medium to develop a brownish color. The intensity of the color produced is inversely proportional to the concentration of sodium in the sample

Assay:

Wavelength / filter:	530 nm (Hg 546) /Green
Temperature:	Room Temperature
Light path:	1 cm

Calculations

$$\text{Sodium in}\frac{\text{mmol}}{\text{L}} = \frac{\text{(A) sample}}{\text{(A) Standard}} \times \text{Standard conc. } (\frac{\text{mg}}{\text{dl}})$$

3.3.3 Determination of Calcium (Ca)

Principle: Calcium ions form a violet complex with O-Cresol phthalein complex one in an alkaline medium.

Colorimetric method: O-Cresol phthalein complex one, without deproteinization

Assay:

Wavelength / filter:	Hg 578nm (550-590)
Spectrophotometer:	570nm
Temperature:	20-25^{O}C / 37^{O}C
Light path:	1 cm

Calculations

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

3.3.4 Determination of Potassium (K^+)

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

Assay:

Wavelength / filter:	630 nm (Hg 623) /Green
Temperature:	Room Temperature
Light path:	1 cm

Calculations

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

3.3.5 Determination of Magnesium

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

Assay

Wavelength / filter:	520 nm, Hg 546 nm 500-550nm (Increase of absorbance)
	628 nm, Hg 623 nm, 570-650 nm (Decrease of absorbance)
Temperature:	20-25^{O}C / 37^{O}C
Light path:	1 cm
Measurement:	Against reagent blank

Calculation

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

3.3.6 Determination of Phosphorous

Assay:

Wavelength / filter:	340 nm, Hg 334 nm, Hg 365 nm
Temperature:	20-25^{O}C / 37^{O}C
Light path:	1 cm
Measurement:	Against reagent blank

Calculation

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

3.3.7 Determination of Chloride ion (Cl^-)

Principle: Chloride ions combine with free mercuric ions and release thiocyanate from mercuric thiocyanate. The thiocyanate released combines with the ferric ions to form a red brown ferric thiocyanate complex. Intensity of the color formed is directly proportional to the amount of chloride present in the sample.

Assay:

Wavelength / filter:	505 nm (Hg 546) /Green
Temperature:	Room Temperature
Light path:	1 cm

Calculation

$$\text{Chloride in } \frac{\text{mmol}}{\text{L}} = \frac{\text{(A) sample}}{\text{(A) standard}} \times \text{Standard conc. } (\frac{\text{mg}}{\text{dl}})$$

3.3.8 Determination of Iron (Fe)

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

Assay:

Wavelength / filter:	562nm
Temperature:	37^{O}C / 15-25^{O}C
Light path:	1 cm

Calculations:

$$\text{Iron in } \frac{\mu g}{dl} = \frac{\text{(A) sample - (A) sample blank}}{\text{(A) standard}} \times \text{Standard conc. } (\frac{\text{mg}}{\text{dl}})$$

3.3.9 Determination of Copper (Cu)

Principle: Chloride ions combine with free mercuric ions and release thiocyanate from mercuric thiocyanate. The thiocyanate released combines with the ferric ions to form a red brown ferric thiocyanate complex. Intensity of the color formed is directly proportional to the amount of chloride present in the sample.

Assay:

Wavelength / filter:	505 nm (Hg 546) /Green
Temperature:	Room Temperature
Light path:	1 cm

Calculation

$$\text{Copper in } \frac{\text{mmol}}{\text{L}} = \frac{\text{(A) sample}}{\text{(A) standard}} \times \text{Standard conc. } (\frac{\text{mg}}{\text{dl}})$$

3.3.10 Determination of Zinc (Zn)

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

Assay:

Wavelength / filter:	562nm
Temperature:	37^{O}C / 15-25^{O}C
Light path:	1 cm

Calculations:

$$\text{Zn in } \frac{\mu g}{dl} = \frac{\text{(A) sample - (A) sample blank}}{\text{(A) standard}} \times \text{Standard conc. } (\frac{\text{mg}}{\text{dl}})$$

3.4 Development of mushroom biscuits

3.4.1 Raw materials for mushroom biscuits

Ganoderma lucidum mushroom powder, wheat flour, soy flour, milk powder, egg, sugar, sodium bi carbonate, salt and oil were purchased from local market and super shop of Chattogram.

3.4.2. Preparation of mushroom powder

For the preparation of mushrooms powder, at first fresh mushrooms was washed by clean water. The mushrooms was cut into small pieces. Then the mushrooms was placed in-tray and kept in the cabinet dryer (Genlab 1000-L, UK) at 60^0C for 24 hours. Finally, the dried mushrooms was grinded by using mixer grinder (Panasonic MX-AC300). The mushrooms powder was packed in the poly bag and storage in the refrigerator for further use.

Fig-2: Mushroom Powder

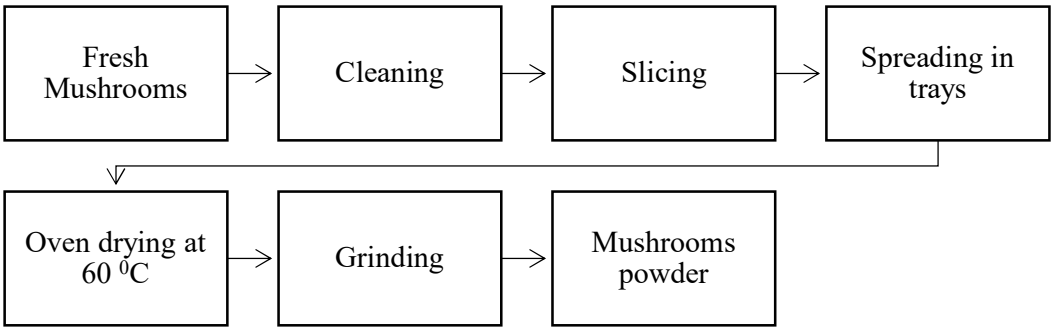

Fig. 3: Flow chart of the preparation of mushroom powder

3.4.3 Formulation of mushroom biscuits

Various trails were carried out to formulate the mushroom biscuits. From table 1; Sample A, B, C, and D represented Formulation one (control), Formulation two (5g mushroom powder), Formulation three (10g mushroom powder) and Formulation three (15g mushroom powder) respectively.

Table 3.2 Formulation of mushroom biscuits

Ingredients	Formulation 1	Formulation 2	Formulation 3	Formulation 4
Mushroom powder (g)	0	5	10	15
Wheat flour (g)	75	70	65	60
Soy flour (g)	10	10	10	10
Milk powder (g)	8	8	8	8
Egg (ml)	20	20	20	20
Sugar (g)	35	35	35	35
Sodium bi carbonate (g)	1.0	1.0	1.0	1.0
Salt (g)	0.5	0.5	0.5	0.5
Oil (ml)	20	20	20	20

3.4.4 Processing of mushroom biscuits

The oil and sugar were initially mixed until fluffy in a Kenwood mixer (model HM 430). Egg white and milk powder were added while mixing continues for about 40 min. Appropriate flour and salt were slowly introduced into the mixture. Thereafter, consistent dough was formed after thoroughly mixing with water. The dough obtained was kneaded on a smooth clean board for about 5 min, thinly rolled on a wooden board with rolling pin to uniform thickness of 5 mm and cut out to desired shapes of uniform sizes. The cut-out dough pieces were placed on greased baking tray and baking was carried out at 160°C for 15 min. The biscuits were cooled hygienically and packaged in airtight polythene, kept at room temperature until needed for sensory attributes and other analyses (Wakchaure, 2011). Biscuits samples from wheat flour (white) served as control. The flow chart for the biscuit production is as shown Fig. 2.

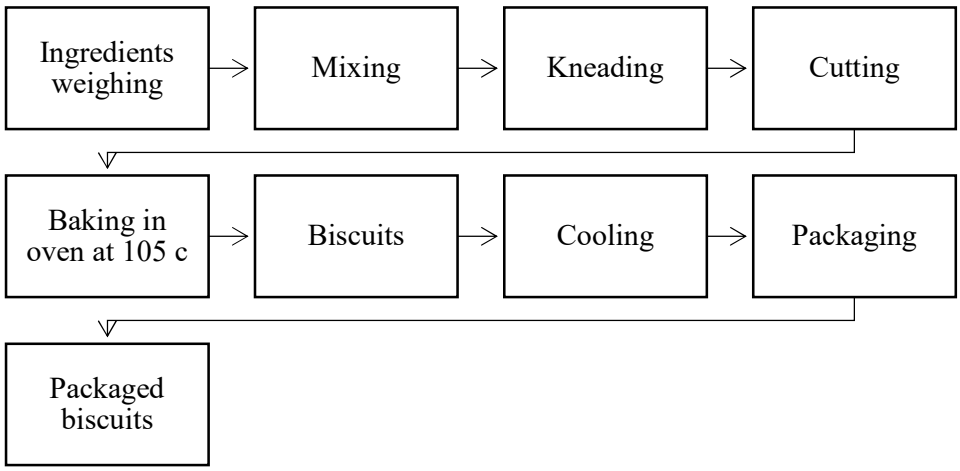

Fig. 4: Flow chart of the processing of mushroom biscuits

3.4.5 Sensory attributes of mushroom biscuits

The evaluation of mushroom biscuits was carried out using a score card developed for the purpose. Score card was prepared considering the quality characteristics of the products. Descriptive terms were given to various quality attributes like appearance/ color, flavor, taste and general acceptance. Each attribute of the samples was scored by the panelists on a visual analogue scale. The scale was arranged such that: Like extremely = 9, Like very much = 8, Like moderately = 7, Like slightly = 6, Neither like nor dislike = 5, Dislike slight = 4, Dislike moderately = 3, Dislike very much = 2, Dislike Extremely = 1. While scoring, highest score (9) was assigned to most preferred characteristic and least score (1) to the least desired characteristic. While this evaluation does not reflect actual consumer perception, it gives an idea of what attributes a good quality product should possess (Mamun et al., 2020).

3.4.6 Microbiological quality of the mushroom biscuits

Total viable count was carried out by using plate count agar medium according to published standard procedure (Matin et al., 2019). Colony count was done using digital colony counter and total viable count was expressed as colony forming units per ml (CFU/ml). Total coliform and *E. coli* count was done according to Standard methods (Matin et al., 2018). Yeast and mold count were carried out according to previously published methods (Matin et al., 2018). The result was expressed as yeast and mold growth was present or not.

3.5 Statistical analyses

Data for statistical analysis were determined and recorded in Microsoft Excel 2013 spread sheet. All samples were in three replicates. Data were then sorted, coded and recorded in IBM SPSS Statistics 23. These data were further analyzed using one-way ANOVA tests to assess significant levels of variation at 95% confidence interval. The statistical analysis was conducted at 5% level of significance ($P < 0.05$) (Matin et al., 2019).

Chapter IV: Results

4.1 Proximate analysis of mushroom

The result of proximate analysis of mushrooms are presented in Table 4.1. The results of moisture, total solids, protein, fat, carbohydrate, fiber, ash and energy were ranged from 53.10 to 90.10 %, 9.90 to 47.00 %, 3.23 to 19.70 %, 0.47to 4.20 %, 4.21 to13.02 %, 0.51 to 3.90 %, 1.28 to 6.18 and 34.78 to 172.37 (Kcal/100g) for different types of mushrooms respectively.

Table 4.1 Proximate composition of different types of mushrooms

Parameter	*Pleurotus ostreatus*	*Pleurotus sajor-caju*	*Agaricus bitorquis*	*Ganoderma lucidum*	Level of sign.
Moisture (%)	89.80 ± 0.05^{b}	89.20 ± 0.02^{c}	90.10 ± 0.10^{a}	53.10 ± 0.07^{d}	*
Total Solids	10.20 ± 0.07^{c}	10.80 ± 0.05^{b}	9.90 ± 0.10^{d}	47.00 ± 0.15^{a}	*
Protein (%)	3.49 ± 0.03^{c}	3.69 ± 0.05^{b}	3.23 ± 0.10^{d}	19.70 ± 0.03^{a}	*
Fat (%)	0.54 ± 0.02^{b}	0.58 ± 0.07^{b}	0.47 ± 0.02^{b}	4.20 ± 0.05^{a}	*
Carbohydrate (%)	4.31 ± 0.09^{b}	4.37 ± 0.02^{b}	4.21 ± 0.11^{b}	13.02 ± 0.15^{a}	*
Fiber (%)	0.58 ± 0.04^{bc}	0.69 ± 0.03^{b}	0.51 ± 0.03^{c}	3.90 ± 0.10^{a}	*
Ash (%)	1.28 ± 0.04^{c}	1.47 ± 0.03^{b}	1.46 ± 0.11^{bc}	6.18 ± 0.08^{a}	*
Energy (Kcal/100g)	36.89 ± 0.14^{c}	38.32 ± 0.02^{b}	34.78 ± 0.06^{d}	172.37 ± 0.02^{a}	*

* Significant at P <0.05; Values followed by different superscript letters denote a significant difference

4.2 Mineral contents different types of mushrooms

The result of Mineral contents different types of mushrooms is presented in Table 4.2. The results of sodium, potassium, calcium, magnesium, phosphorus, chloride, iron, copper, zinc was ranged from 463.21 to 413.12 mg/dl, 32.14 to 15.25 mg/dl, 1.81 to 0.90 mg/dl, 1.90 to1.10 mg/dl, 13.20 to 7.40 mg/dl, 13.57 to 7.20 mg/dl, 0.17 to 0.11 mg/dl, 0.07 to 0.02 mg/dl and 0.50 to 0.30 mg/dl for different types of mushrooms respectively.

Table 4.2 Mineral contents of different types of mushrooms

Parameter	***Pleurotus ostreatus***	***Pleurotus sajor-caju***	***Agaricus bitorquis***	***Ganoderma lucidum***	Level of sign.
Sodium	437.69±0.03^{c}	442.29±0.08^{b}	463.21±0.04^{a}	413.12±0.02^{d}	*
Potassium	21.51±0.10^{c}	15.25±0.05^{d}	27.13±0.03^{b}	32.14±0.04^{a}	*
Calcium	1.20±0.05^{b}	0.90±0.10^{c}	1.78±0.04^{a}	1.81±0.10^{a}	*
Magnesium	1.90±0.05^{a}	1.70±0.10^{b}	1.10±0.05^{c}	1.80±0.02^{ab}	*
Phosphorus	11.70±0.05^{b}	8.30±0.03^{c}	13.20±0.05^{a}	7.40±0.10^{d}	*
Chloride	9.23±0.03^{b}	9.24±0.04^{b}	7.20±0.01^{c}	13.57±0.06^{a}	*
Iron	0.15±0.02^{a}	0.13±0.01^{a}	0.11±0.03^{a}	0.17±0.04^{a}	NS
Copper	0.02±0.01^{a}	0.03±0.01^{a}	0.07±0.03^{a}	0.05±0.03^{a}	NS
Zinc	0.31±0.03^{b}	0.30±0.03^{b}	0.43±0.05^{a}	0.50±0.02^{a}	*

** Significant at P <0.05; NS = Non significant; Values followed by different superscript letters denote a significant difference.

4.3 Proximate composition of developed of mushroom biscuits

The result of proximate analysis of four formulated mushroom biscuit are presented in Table 4.3. The results of moisture, ash, protein, fat, fiber, carbohydrate and energy value were ranged from (4.03 to 3.89) %, (3.47 to 4.11) %, (12.15 to 13.43) %, (5.43 to 5.83) %, (4.71 to 5.09) %, (67.03 to 70.35) % and (374.15 to 378.87) Kcal/100g for four formulated of mushrooms biscuits respectively.

Table 4.3 Proximate analysis of developed of mushroom biscuits

Parameter	A	B	C	D	Sign.
Moisture (%)	3.89 ± 0.05^{c}	4.03 ± 0.03^{b}	4.43 ± 0.06^{a}	4.51 ± 0.05^{a}	*
Ash (%)	3.47 ± 0.03^{d}	3.71 ± 0.01^{c}	3.85 ± 0.02^{b}	4.11 ± 0.02^{a}	*
Protein (%)	12.15 ± 0.04^{d}	12.68 ± 0.02^{c}	13.27 ± 0.01^{b}	13.43 ± 0.03^{a}	*
Fat (%)	5.43 ± 0.01^{c}	5.62 ± 0.05^{b}	5.79 ± 0.02^{a}	5.83 ± 0.04^{a}	*
Fiber (%)	4.71 ± 0.02^{c}	4.91 ± 0.02^{b}	4.94 ± 0.03^{b}	5.09 ± 0.06^{a}	*
CHO (%)	70.35 ± 0.15^{a}	69.05 ± 0.13^{b}	67.72 ± 0.14^{c}	67.03 ± 0.20^{d}	*
Energy (Kcal/100g)	378.87 ± 0.35^{a}	377.50 ± 0.01^{b}	376.07 ± 0.34^{c}	374.15 ± 0.22^{d}	*

** Significant at P <0.05; Values followed by different superscript letters denote a significant difference; comparison done across formulation. Where, A = Formulation 1, B = Formulation 2, C = Formulation 3, and D = Formulation 4.

4.4 Sensory attributes of developed of mushroom biscuits

Sensory attributes of four formulated mushroom biscuit are presented in Table 4.4. The results of appearance & color, flavor & aroma, taste, crispness & texture, Overall acceptability score were ranged from 6.6 to7.7, 7.3 to 7.9, 7.1 to 7.7, 6.9 to 8.4 and 7.3 to 8.0 for four formulated of mushrooms biscuits respectively.

Table 4.4 Sensory attributes of developed of mushroom biscuits

Sensory attributes	A	B	C	D	Sign.
Appearance & Color	6.7 ± 1.25^{a}	6.6 ± 0.51^{a}	7.2 ± 1.13^{a}	7.7 ± 0.94^{a}	NS
Flavor & Aroma	7.4 ± 0.84^{a}	7.9 ± 0.87^{a}	7.3 ± 0.82^{a}	7.5 ± 0.70^{a}	NS
Taste	7.4 ± 0.51^{a}	7.7 ± 1.15^{a}	7.1 ± 1.28^{a}	7.1 ± 0.73^{a}	NS
Crispness & Texture	7.3 ± 0.67^{bc}	8.4 ± 0.51^{a}	6.9 ± 0.73^{d}	7.9 ± 0.73^{ab}	*
Overall acceptability	7.6 ± 1.34^{a}	7.3 ± 0.67^{a}	8.0 ± 0.81^{a}	7.8 ± 0.60^{a}	NS

** Significant at P <0.05; NS = Non significant; Values followed by different superscript letters denote a significant difference; comparison done across formulation. Where, A = Formulation 1, B = Formulation 2, C = Formulation 3, and D = Formulation 4.

4.5 Microbiological quality of developed of mushroom biscuits

Microbiological characteristics are indicators of safety, quality and shelf life of prepared mushroom biscuits. Total viable count, Coliform, yeast and mold count of the mushroom biscuits were determined. The result of microbiological quality of four formulated mushroom biscuits are presented in Table 4.5.

Table 4.5. Microbiological quality of developed of mushroom biscuits

Samples	TVC (/ml CFU)	Yeast & mold
A	ND	ND
B	ND	ND
C	ND	ND
D	ND	ND

Where, A = Formulation 1, B = Formulation 2, C = Formulation 3, and D = Formulation 4.

Chapter V: Discussions

5.1 Proximate analysis of mushroom

The results of proximate analysis of four types of mushrooms are presented in Table 4.1. The highest value of moisture content (90.10±0.10) % was found in *Agaricus bitorquis*, and the lowest value (53.10±0.07) % was for *Ganoderma lucidum* types of mushrooms. The moisture content of mushroom samples was similar reported by Nasiri et al., (2013). The moisture content of five species of edible mushrooms were ranged from 85.95-90.07 % reported by Zahid et al. (2010). Roy et al. (2015) observed the moisture content of two edible mushroom varieties species include oyster (*Pleurotus ostreatus*) 84 % and Reshii (*Ganoderma lucidum*) 47%.

The highest value of total solids 47.00±0.15 % was found in *Ganoderma lucidum*, and the lowest value 9.90±0.10 % was for *Agaricus bitorquis* types of mushrooms. Total solids of five species of edible mushrooms were ranged from 9.93-14.05 % reported by Zahid et al. (2010).

The highest value of protein content 19.70±0.03 % was found in *Ganoderma lucidum*, and the lowest value 3.23±0.10 % was for *Agaricus bitorquis* types of mushrooms. The protein content of mushroom samples was lower than reported by Nasiri et al., 2013 and Nasiri et al., 2012**.** The protein content of five species of edible mushrooms were ranged from 3.22-4.83 % reported by Zahid et al. (2010).

The highest value of fat content 4.20±0.05 % was found in *Ganoderma lucidum*, and the lowest value 0.47±0.02 % was for *Agaricus bitorquis* types of mushrooms. The fat content of mushroom samples was similar reported by Nasiri et al., (2013) and lower than Nasiri et al., 2012. The fat content of five species of edible mushrooms were ranged from 0.43-1.05 % reported by Zahid et al. (2010). Roy et al. (2015) observed the fat content of two edible mushroom varieties species include oyster (*Pleurotus ostreatus*) 1.05 % and Reshii (*Ganoderma lucidum*) 3%.

The highest value of carbohydrate content 13.02±0.15 % was found in *Ganoderma lucidum*, and the lowest value 4.21±0.11 % was for *Agaricus bitorquis* types of mushrooms. The Carbohydrate content of mushroom samples was lower than reported by Nasiri et al, 2013; Nasiri et al., 2012 and higher than reported by Zahid et al. (2010).

The highest value of fiber content 3.90±0.10 % was found in *Ganoderma lucidum*, and the lowest value 0.51±0.03 % was for *Agaricus bitorquis* types of mushrooms. The fiber content of mushroom samples was lower than reported by Nasiri et al, 2013; Nasiri et al., 2012 and higher than reported by Zahid et al. (2010). Roy et al. (2015) observed the fiber content of two edible mushroom varieties species include oyster (*Pleurotus ostreatus*) 2.4 % and Reshii (*Ganoderma lucidum*) 3.5%.

The highest value of ash content 6.18±0.08 % was found in *Ganoderma lucidum*, and the lowest value 1.28±0.04 % was for *Pleurotus ostreatus* types of mushrooms. The ash content of mushroom samples was lower than reported by Nasiri et al, 2013; Nasiri et al., 2012. The ash content of five species of edible mushrooms were ranged from 0.98-2.30 % reported by Zahid et al. (2010). Roy et al. (2015) observed the ash content of two edible mushroom varieties species include oyster (*Pleurotus ostreatus*) 5.5 % and Reshii (*Ganoderma lucidum*) 6.3%.

The highest value of energy value 172.37±0.02 % was found in *Ganoderma lucidum*, and the lowest value 34.78±0.06 % was for *Agaricus bitorquis* types of mushrooms. The results of energy value ash of five species of edible mushrooms were ranged from 35.51-50.03 (Kcal/100g) reported by Zahid et al. (2010).

5.2 Mineral contents different types of mushrooms

The highest value of sodium, phosphorus and copper content was found in *Agaricus bitorquis*. Magnesium content was found highest in *Pleurotus ostreatus.* Potassium, calcium, chloride, iron and zinc content was found highest in *Ganoderma lucidum.* The lowest value of sodium, phosphorus content was found in *Ganoderma lucidum.* Copper content was found lowest in *Pleurotus ostreatus.* Chloride, iron, magnesium content was found lowest in *Agaricus bitorquis*. Zinc, calcium, potassium content was found lowest in *Pleurotus sajor-caju.* The results of sodium, potassium, calcium, magnesium, phosphorus, iron, copper, zinc of five species of edible mushrooms were ranged from 3.18 to 37.23 mg/100g, 19.83 to 197.24 mg/100g, 0.12 to 0.58 mg/100g, 27.26 to 51.21 mg/100g, 22.20 to 62.10 mg/100g, 1.81 to 0.81 mg/100g, 0.14 to 0.91 mg/100g and 0.68 to 1.24 mg/100g reported by Zahid et al. (2010). Roy et al. (2015) observed sodium, potassium, calcium, magnesium, phosphorus, iron, copper, zinc content of two edible mushroom varieties species includes oyster (*Pleurotus ostreatus*) 2.9 mg/100g, 425 mg/100g, 1.78 mg/100g, 7.74 mg/100g, 212 mg/100g, 2.19 mg/100g, 28 mg/100g,

0.5 mg/100g and Reshii (*Ganoderma lucidum*) 2.82 mg/100g, 432 mg/100g, 1.88 mg/100g, 7.95 mg/100g, 225 mg/100g, 2.22 mg/100g, 26 mg/100g, 0.7 mg/100g.

5.3 Proximate Composition of developed of mushroom biscuits

The results of moisture content ranged from 3.89 to 4.03 % for a different type of formulated mushroom biscuit. The highest value of moisture content 3.89±0.05 % was found in Formulation A, and the lowest value 4.03±0.03 % was for Formulation B. The moisture content of different type of formulated mushroom biscuit samples were higher than reported by Farzana and Mohajan, (2015) and lower than reported by Prodhan et al., (2015); Bello et al., (2017) and Ayo et al., (2018).

The results of ash content ranged from 3.47 to 4.11 % for a different type of different type of formulated mushroom biscuit. The highest value of ash content 4.11±0.02 % was found in Formulation D, and the lowest value (3.47±0.03) % was for Formulation A. The ash content of different type of formulated mushroom biscuit samples were higher than reported by Farzana and Mohajan, (2015). The ash content of mushroom (*pleurotussajor-caju*) enriched biscuits, Acha-Mushroom Flour Blend Biscuits was ranged from 3.61-4.13 % and 2.56-3,06 % reported by Prodhan et al. (2015) and Ayo et al. (2018). Bello et al. (2017) observed the ash content was 7.35 % of Mushroom flour biscuit (% dwb.).

The results of protein content ranged from 13.43 to 12.15 % for a different type of formulated mushroom biscuit. The highest value of protein content 13.43±0.03 % was found in Formulation D, and the lowest value12.15±0.04 % was for Formulation A. The protein content of different type of formulated mushroom biscuit samples were lower than reported by Farzana and Mohajan, 2015 and similar than reported by Prodhan et al. (2015) and higher than reported by Ayo et al. (2018). Bello et al. (2017) observed the protein content was 24.23 % of Mushroom flour biscuit (% dwb.).

The results of fat content ranged from 5.43 to 5.83 % for a different type of formulated mushroom biscuit. The highest value of fat content 5.83±0.04 % was found in Formulation D, and the lowest value 5.43±0.01 % was for Formulation A. The fat content of different type of formulated mushroom biscuit samples were lower than reported by Farzana and Mohajan, 2015; Ayo et al. (2018) and similar than reported by Prodhan et al. (2015). Bello et al. (2017) observed the fat content was 2.59 % of Mushroom flour biscuit (% dwb.).

The results of fiber content ranged from 4.71 to 5.09 % for a different type of formulated mushroom biscuit. The highest value of fiber content 5.09±0.06 % was found in Formulation D, and the lowest value 0.51±0.03 % was for Formulation A. The fiber content of different type of formulated mushroom biscuit samples were higher than reported by Farzana and Mohajan, 2015; Ayo et al. (2018) and similar than reported by Prodhan et al. (2015). Bello et al. (2017) observed the fiber content was 30.12 % of Mushroom flour biscuit (% dwb.).

The results of carbohydrate content ranged from 67.03 to 70.35 % for a different type of formulated mushroom biscuit. The highest value of carbohydrate content 70.35±0.15 % was found in Formulation A, and the lowest value (67.03±0.20 % was for Formulation D. The carbohydrate content of different type of formulated mushroom biscuit samples were higher than reported by Farzana and Mohajan, 2015; Ayo et al. (2018) and similar than reported by Prodhan et al. (2015). Bello et al. (2017) observed the carbohydrate content was 35.71 % of Mushroom flour biscuit (% dwb.).

The results energy value ranged from 374.15 to 378.87 (Kcal/100g) for a different formulated mushroom biscuit. The highest value of energy value 378.87±0.35 (Kcal/100g) was found in Formulation A, and the lowest value 374.15±0.22 (Kcal/100g) was for Formulation D. The energy value of different type of formulated mushroom biscuit samples were lower than reported by Farzana and Mohajan, 2015. Bello et al. (2017) observed the energy value was 263.07 (KJ/100g) of Mushroom flour biscuit (% dwb.).

5.4 Sensory attributes of developed of mushroom biscuits

Organoleptic tests of the biscuits depend on its first color, texture, taste, aroma, flavor and overall acceptability of the sample. Table 4.4 shows the summary of Sensory attributes of developed of mushroom biscuits A, B, C, and D respectively. Sample D scored highest 7.7±0.94 for appearance & Color, while Sample B received highest scores for crispness & texture 8.4±0.51, flavor & aroma 7.9±0.87 and taste 7.7±1.15. Sample C scored highest 8.0±0.81 for overall acceptability. Conversely, lowest score 6.6±0.51 for appearance & color, 7.3±0.67 overall acceptability was recorded for sample B. Sample C received lowest scores for flavor & aroma 7.3±0.82, crispness & texture 6.9±0.73 and Sample D was worst 7.1±0.73 in terms of taste. This is why mushroom biscuits, with its great taste and nutritional value, are highly preferred during the whole season. The results of mushroom biscuits revealed that Sample C scored high in the hedonic scale for most attributes, indicating its overall acceptability. The results of sensory attributes (Taste, color, texture, flavor and overall acceptability) of soy-mushroom biscuits incorporated with different levels of soy flour were ranged from 7.2 to 8.2, 7.6 to 8.6, 6.9 to 7.6, 7.2 to 7.7 and 7.2 to 8.4 respectively reported by Farzana and Mohajan, 2015. The sensory attributes are the primary factor which determines the acceptability of any product, which has the highest impact as far as market success of product, is concerned.

5.5 Microbiological quality of developed of mushroom biscuits

Microbiological attributes are pointers of security, quality and timeframe of realistic usability of the developed products. Safety, quality, and shelf life of a developed of mushroom biscuits are reflected by its microbiological characteristics. Total viable count, yeast and mold count of all developed of mushroom biscuits were determined. The results obtained are shown in Table 4.5. In all samples total, the viable count and yeast and mold were not detected. During the time of the processing mushroom biscuits, maintaining proper hygienic condition, it increased security, quality and timeframe of realistic usability of the developed products.

Chapter VI: Conclusion

The present study reveals an inter and intra genus variation in nutrient content of mushrooms. Despite the differences in their nutrient content, the overall nutritive picture of these mushrooms appears to be quite sound. They hold out a promise to contribute significantly to the intake of micro-nutrients amongst our people. So, the mushrooms should surely be incorporated into our diets more frequently in order to improve the quality of our habitual diets. This endeavor will certainly improve our micronutrient situation, improving the overall health and ensuring the general well-being of the people. The findings from this study revealed that mushrooms are highly nutritious foods. They are rich in macro nutrients and minerals. Their protein content is high, offering higher than the protein content of most vegetables. Mushrooms are foods that can be eaten by anybody, both the old and young. They are also good food for hypertensive patients as its high potassium content can help to control blood pressure. The proximate composition of the biscuit composites revealed that the sample contained low moisture contents, appreciable amount of other nutritional component and moderate energy value that is required in the body for normal physiological and metabolic processes. The results of mineral composition indicate that potassium, calcium, sodium, and phosphorous are the predominant mineral elements present in the mushroom biscuits.

Chapter VII: Recommendations & future perspectives

The worldwide eating of mushroom and mushroom based products has importantly increased during recent periods, due to a number of distinct factors. Foremost among these factors is the growing knowledge that mushroom and mushroom constitutes an important and healthy part of the human diet, mainly owing to the presence of minerals, vitamins and energy value, which play an essential role in human health. Present study is conducted to analysis the proximate composition and minerals of most widely used mushroom cultivated in Chattogram and developed mushroom biscuits. Establishment of proper processing, it will be helpful to get this product during all season, ultimately increased the utilization of mushroom in human diet. The following recommendation are:

- Bioactive compound and vitamins of Mushroom should be evaluated.
- Prepared the product maintaining hygienic condition.
- There should be create awareness about the importance of mushroom and mushroom based product being a scope for food as human diet.

REFERENCES

Akther, S., Alim M. A., Badsha, M. R., Matin, A., Ahmad, M., and Hoque, S. M. Z. 2020. Formulation and quality evaluation of instant mango drinks powder. Food Research, 4(4): 1287 – 1296.

Alom, M.M. and Bari, M.W. 2010. Investment in mushroom cultivation at Savar Upazilla: A prospective sector for Bangladesh. ASA University Review, 4: 57-63.

Alzand, K.I., Bofaris, M.S.M., Ugis, A. 2019. Chemical Composition and Nutritional Value of Edible Wild Growing Mushrooms: A Review. World J. Pharm. Res 8: 31-46.

AOAC. (2000). Official methods of analysis. 17th ed. Maryland, USA: Association of Official Analytical Chemists.

Ayo JA, Ojo MO, Omelagu CA, Kaaer RU. 2018. Quality Characterization of Acha-Mushroom Blend Flour and Biscuit. Nutrition &. Food Science International Journal, 7(3):1-9.

Begum, P. 2013. Nutrient composition and medicinal properties of plant foods consumed by ethnic groups in Chattogram Hill Tracts. University of Dhaka.

Bello M, Oluwamukomi M.O., Enujiugha V.N. 2017. Nutrient composition and sensory properties of biscuit from mushroom-wheat composite flours. Archives of Current Research International, 9(3) :1-11.

Chang, S.-T. 1999. Global impact of edible and medicinal mushrooms on human welfare in the 21st century: non-green revolution. International journal of medicinal mushrooms 1.

Cheung, P.C. 2008. Nutritional value and health benefits of mushrooms. Mushrooms as functional foods, 71-109.

Das, B., De, B., Chetree, R., Mandal, S.C. 2020. Medicinal Aspect of Mushrooms: A View Point. Herbal Medicine in India. Springer, 509-532.

Farzana, T., Mohajan, S. 2015. Effect of incorporation of soy flour to wheat flour on nutritional and sensory quality of biscuits fortified with mushroom. Food science & nutrition, 3: 363-369.

Friedman, M. 1996. Nutritional value of proteins from different food sources. A review. Journal of agricultural and food chemistry, 44: 6-29.

Gucia, M., Jarzyńska, G., Rafał, E., Roszak, M., Kojta, A.K., Osiej, I., Falandysz, J. 2012. Multivariate analysis of mineral constituents of edible Parasol Mushroom (Macrolepiota procera) and soils beneath fruiting bodies collected from Northern Poland. Environmental Science and Pollution Research, 19: 416-431.

Hamzah, R., Jigam, A., Makun, H., Egwim, E., 2013. Antioxidant properties of selected African vegetables, fruits and mushrooms: A review. Mycotoxin and food safety in developing countries.

Islam, N., Ullah, G. 2010. Factors affecting consumers' preferences on fast food items in Bangladesh. The Journal of Applied Business Research, 26: 131.

Kamal, A., Fauzia, B., Abul, K., 2009. Mushroom production in Bangladesh: Present scenario and potentialities. SAARC J. Agric, 7: 91-105.

Kamal, A.S.M., Khair, A., Dawlatana, M., Hassan, M.T., Begum, F., Rahim, M., 2009b. Evaluation of aflatoxins and pesticide residues in fresh and different processed mushrooms. Bangladesh Journal of Scientific and Industrial Research, 44: 193-198.

Kapahi, M. 2018. Recent advances in cultivation of edible mushrooms. Biology of Macrofungi. Springer, 275-286.

Matin, A., Akhter, S., Badsha, M.R., 2019. Microbiological Quality Assessment of Powder Milk at Dhaka Metropolitan Area. Journal of Advances in Food Science & Technology, 6: 88-94.

Matin, A., Banik, T., Badsha, M.R, Hossain, A., Haque, M.M., Ahmad, M. 2018. Microbiological quality analysis of yoghurt in some selected areas of Bangladesh. International Journal of Natural and Social Sciences, 5: 82-86.

Matin A, Ahmed S, Badsha M. 2019. Proximate Analysis of Dry Cake Available in Local Market of Bangladesh. Journal of Advances in Food Science & Technology, 27:130-134.

Mattila, P., Salo-Väänänen, P., Könkö, K., Aro, H., Jalava, T. 2002. Basic composition and amino acid contents of mushrooms cultivated in Finland. Journal of Agricultural and Food Chemistry, 50: 6419-6422.

Nagulwar M.M., More D.R., Mandhare L.L. 2020. Nutritional properties and value addition of mushroom: A review. The Pharma Innovation Journal, 9(10): 395-398.

Nasiri, F., Tarzi, B.G., Bassiri, A., Hoseini, S., Aminafshar, M. 2013. Comparative study on the main chemical composition of button mushrooms (Agaricus bisporus) cap and stipe. Journal Food Biosciences Technology, 3, 41-48.

Nasiri F, Tarzi BG, Bassiri A, Hoseini SE. 2012. Comparative study on some chemical compounds of button mushrooms (Agaricus bisporus) cap and stipe during the first to third flushes. Ann Biol Res, 3(12):5677-80.

Okoro, I., Achuba, F. 2012. Proximate and mineral analysis of some wild edible mushrooms. African Journal of Biotechnology, 11: 7720-7724.

Oseni, T.O., Dlamini, S.O., Earnshaw, D.M., T MasarirambI, M. 2012. Effect of substrate pre-treatment methods on oyster mushroom (Pleurotus ostreatus) production. International journal of agriculture and biology 14.

Priyadarsini, P., Mishra, A. 2020. A storage study of value-added oyster mushroom products. IJCS, 8: 266-270.

Prodhan, U.K., Linkon, K.M.M.R., Al-Amin, M.F., Alam, M.J., 2015. Development and quality evaluation of mushroom (Pleurotus sajor-caju) enriched biscuits. Emirates Journal of Food and Agriculture, 27(7): 542-547.

Pusa, R., 2012. Mushrooms: Nutritional and Medicinal Properties. Modern Trends in Microbial Biodiversity of Natural Ecosystem, 255.

Rahman, M.S., 2015. Effectiveness of Training Programme on Mushroom Cultivattion. Dept. Of Agricultural Extension & Information System.

Rai, R., Arumuganathan, T. 2007. Value-addition in mushrooms. Mushroom Biology and Biotechnology 213, 265.

Rathee, S., Rathee, D., Rathee, D., Kumar, V., Rathee, P. 2012. Mushrooms as therapeutic agents. Revista Brasileira de Farmacognosia, 22: 459-474.

Reis, F.S., Barros, L., Martins, A., Ferreira, I.C., 2012. Chemical composition and nutritional value of the most widely appreciated cultivated mushrooms: an inter-species comparative study. Food and Chemical Toxicology, 50: 191-197.

Roy DN, Azad AK, Sultana F, Anisuzzaman AS, Khondkar P. 2015. Nutritional profile and mineral composition of two edible mushroom varieties consumed and cultivated in Bangladesh. The Journal of Phytopharmacology, 4(4):217-20.

Sarker, M.R. 2014. Economics of Value-Added Mushroom Production and Marketing: A Study at The Farmers and Consumers Level of Dhaka District. Dept. Of Agribusiness and Marketing.

Thatoi, H., Singdevsachan, S.K. 2014. Diversity, nutritional composition and medicinal potential of Indian mushrooms: A review. African Journal of Biotechnology 13.

Valverde, M.E., Hernández-Pérez, T., Paredes-López, O. 2015. Edible mushrooms: improving human health and promoting quality life. International journal of microbiology.

Van Toan, N., Thu, L.N.M. 2018. Preparation and improved quality production of flour and the made biscuits from shitake mushroom (Lentinus edodes). Clinical Journal of Nutrition and Dietetics 1, 1-9.

Vishwakarma, M., Bhatt, R., Gairola, S. 2011. Some medicinal mushrooms of Garhwal Himalaya, Uttarakhand, India. Int. J. Med. Arom. Plants, 1: 33-40.

Wakchaure G.C. 2011. Mushrooms- Value Added Products Mushrooms. Mushrooms: Cultivation, Marketing and Consumption, Solan, India: Directorate of Mushroom Research, Indian Council of Agricultural Research (ICAR). 2011:233-238.

Wani, B.A., Bodha, R., Wani, A. 2010. Nutritional and medicinal importance of mushrooms. Journal of Medicinal Plants Research, 4: 2598-2604.

Wasser, S. 2002. Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. Applied microbiology and biotechnology 60, 258-274.

Wasser, S.P. 2017. Medicinal mushrooms in human clinical studies. Part I. Anticancer, oncoimmunological, and immunomodulatory activities: a review. International Journal of Medicinal Mushrooms 19.

Yamaguchi, S., Ninomiya, K. 2000. Umami and food palatability. The Journal of nutrition 130, 921S-926S.

Zahid, M., Barua, S. and Huq, S.M. 2010. Proximate composition and mineral content of selected edible mushroom varieties of Bangladesh.

Appendix A: Photo Gallery

Pictorial presentation of processing and laboratory activities

Mushroom

Drying

Weight

Grinding

Mushroom Powder

Others Ingredients

Dough

Baking

Mushroom Biscuits

Proximate

Mineral analysis

Appendix B: Hedonic Rating Test (Mushroom Biscuits)

Name of Tester……. Date……

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

HEDONIC	**Appearance & Color**				**Flavor & Aroma**				**Crispness & Texture**					**Taste**				**Overall acceptability**			
	Sample				**Sample**				**Sample**					**Sample**				**Sample**			
	1	2	3	4	1	2	3	4						1	2	3	4	1	2	3	4
Like extremely																					
Like very much																					
Like moderately																					
Like Slightly																					
Neither like nor dislike																					
Dislike slightly																					
Dislike moderately																					
Dislike very much																					
Dislike extremely																					

Extra comments on each sample if any:

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

……..

Signature of Judge

Brief Biography

Md. Zahid Hasan passed the Secondary School Certificate Examination in 2009 and then Higher Secondary Certificate Examination in 2011. He obtained his B.Sc. (Hon's) in Food Science and Technology from the Faculty of Food Science and Technology of Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh. Now, he is a candidate for the degree of Master of Science in Applied Human Nutrition and Dietetics under the Department of Applied Food Science and Nutrition, Faculty of Food Science and Technology, Chattogram Veterinary and Animal Sciences University (CVASU). His research interests are functional food product development and nutritional value analysis.