EFFECT OF BLEND RATIO ON THE QUALITY AND SENSORY PROPERTIES OF WHEAT BREADS MADE FROM OYSTER MUSHROOM (*Pleurotus ostreatus*) POWDER

By

KALEAB HAILU

Advisor: MELESE ABDISSA (PhD)

ACKNOWLEDGEMENTS

My deepest gratitude goes to my family, the greatest human beings I have known so far, who are willing to risk everything to ensure my success and happiness. I feel incomplete if I do not extend my heartfelt thanks to my precious friend, Sofanit Nigussie who continues to lighten my life every single day, just by her presence. I promise to continue to make you feel proud in the years ahead of us.

My sincere gratitude is to all my friends who always helped me to complete this work. It has also been pleasurable to work with you all; always helping, friendly, empathetic and encouraging. It is hard to mention all the names here, yet I would like to mention few out of bunch: Aduigna, Abiy, Woinshet and of course Delnesaw. May my love and respect remain encrypted in your hearts. I hope the team spirit and inspiration we showed each other during our path continue, to come up with what is best for our blessed mother land- Ethiopia.

I am also indebted to thank all the staff at the department of Food Science and Nutrition in Addis Ababa University, my advisor, Melese Abdissa (PhD), who has tolerated my short comings throughout this work. It is worth mentioning here the constant support and priceless advices of Ato. Dawde and Ato. Ashagre, which has been a stepping stone to me to overcome every obstacle and challenge I faced in this work.

Last but not least, I want all praises and thanks to go to Almighty God, the Merciful, the only creator of the universe and source of all knowledge and wisdom, who blessed me with health, thoughts, talented teachers, helping friends and opportunity to complete this study. May glory be to his name. Amen!

Kaleab Hailu

ABSTRACT

Breads were prepared by substituting wheat flour with oyster mushroom (*Pleurotus ostreatus*) powder (OMP). The objectives of the study was to study the possibility of bread making by using OMP and wheat flour (WF) at levels of 2%, 4%, 6%, 8% and 10% and evaluate the effects of substitution on flour functional properties, bread qualities, nutritional composition and sensory attributes. Wet gluten content of composite flours was lower as compared to control flour, indicating a destruction of gluten matrix of wheat flour. Substitution of OMP at all levels except 2% had significant effect ($p < 0.05$) on mean wet gluten content of flour, values ranging from 0.153g/g - 0.301 g/g for control flour and 10% substituted flour respectively. Blending proportion generally significantly ($p < 0.05$) affected the mean water absorption capacities. The values varied between 0.689 ml/g and 0.821 ml/g for control and 10% substituted flour respectively. Blending proportion also had an effect in bread characteristics, resulting a significant ($p < 0.05$) increase on loaf weight of breads and a significant decrease ($p < 0.05$) in loaf volume and height of breads as blending proportion increased. The mean loaf weight of breads varied from 127.14g- 137.41g for control and 10% substituted flour respectively. Control breads had mean loaf volume 686.67cm^3 while breads substituted with 10% OMP had mean loaf volume of 236.67cm^3. The proximate composition of breads resulted an increase in moisture content of breads at the crumb as blending proportion increased. Substitution also caused an increase in protein content of breads up to 16.5%. Lysine content of experimental breads varied from 0.23 g/100g – 0.31 g/100g resulting a significant ($p < 0.05$) increase. The total ash values had a significant increase ($p < 0.05$) as substitution levels increased, means ranging from 1.51 g/100g – 2.48 g/100g for control breads and breads with 10% OMP respectively. The sensory evaluation using a nine point hedonic scale indicated that control breads were more preferred by panalists than OMP substituted breads. Breads substituted with 6% OMP had no significance difference ($p > 0.05$) in terms of appearance and color but recorded significant differences in terms of taste, flavor and mouth feel. Sensory evaluation revealed that breads with appreciable nutritional value and acceptable scores can be prepared with inclusion of 6% OMP, since their overall acceptability scores lies above the “like slightly” score.

Key Words: *Oyster Mushroom, OMP supplemented bread, proximate composition, acceptability*

List of Tables

List of Figures

List of Abbreviations

AACC	American Association of cereal chemists
AOAC	Association of Official Analytical Chemists
BP	Blend Proportion
EHNRI	Ethiopian Health Nutrition Research Institute
FAO	Food and Agricultural Organization
OMP	Oyster Mushroom Powder
SE	Standard Error
SPSS	Statistical Package for Social Science
WF	Wheat Flour
WAC	Water Absorption Capacity
WHO	World Health Organization

1. Introduction

1.1. Background

Bread has been regarded for centuries as one of the most popular and staple food products. Basic ingredients are cereal flour, water, yeast or another leavening agent, and salt. Wheat (*Triticum aestivum*) is by far the most important crop for bread making because of its supreme baking performance in comparison with all other cereals. Wheat flour is preferred because of its mild, nutty flavor and formation of gluten when mixed with water (DiMuzio, 2010) However, there may also be very good reasons to use other cereals to improve the nutritive value of bread.

In Ethiopia, bread is mainly prepared from wheat flour (*Triticum aestivum*) and is gaining increasing popularity. Apart from being a good source of calories and other nutrients, bread is considered to be nutritionally poor, as the cereal proteins are deficient in essential amino acids such as lysine, tryptophan, and threonine. The deficiencies of essential amino acids lead to poor utilization of proteins and thus contribute to malnutrition. More than 10% of lysine is reported to be further destroyed during baking, (Rasool, 2004) which calls for appropriate enrichment and optimization process, to make breads with acceptable quality.

The fact that breads are more amenable to variation in the formulation makes them excellent vehicles to meet a wide spectrum of consumer demands with respect to taste and nutritional requirements. (Islam *et al*, 2007) In Ethiopia, bread usage is not limited by the income of the consumer. The addition of bread from composite flour to the daily diets may reduce the malnutrition problems of most Ethiopians, particularly those living around the cities. Thus, the desire of increasing the protein consumption, Food security and health conditions of Ethiopians can be effectively realized by enhancing the nutritional quality of wheat flour for bread, without significantly affecting the sensory properties of the final product.

Mushrooms are found out to be nutritious foods that are rich in protein, minerals, and vitamins, and they contain an abundance of essential amino acids. On the other hand, they provide little calories, fats, and sugars and are commonly recommended in healthy diets (Chang *et al*, 2004). With their great variety of species, they have huge potential of providing a cost- effective means of supplementing the nutrition of the majority of the population. However, in Ethiopia, ignorance has led many to become skeptical whether they hold any great nutritional promise. The "mushrooming" mushroom industry, in the capital, is challenged by lack of market, leading to spoilage of the produce. Processing is hardly effective due to lack of knowledge, expertise and finance, which makes the call for academic research for value added products from mushrooms vital. If such researches are encouraged and commercialized, they will reduce wastage associated with mushrooms by maximizing their utilization, which in turn encourages people to cultivate mushrooms.

The most common value added products from mushrooms are powdered mushrooms and mushroom extracts, alcoholic and aqueous, in liquid or dried form. Mushroom powder has been proposed to be used as a direct food additive to increase content of dietary fibers in various foods or as a partial substitute for wheat flour in bakery products and meat patties (Zivanovic, 2006). This study will thus be conducted with the objective of determining the appropriate substitution level of oyster mushroom powder for wheat flour in the formulation of bread. The physicochemical and sensory properties, nutritive value and consumer acceptance of the final products will be investigated as well.

1.2. Statement of the problem

The malnutrition due to protein deficiency is a serious problem, particularly in populations whose diet mainly consists of cereals. Good quality proteins have a significant impact to meet the nutritional requirements of the fast growing population in developing countries like Ethiopia, but consumption of animal proteins is low in Ethiopia, and thus protein is derived mainly from cereals. Mushrooms, on the other hand, are very nutritious products that can be generated from lingo cellulosic waste materials; and are rich in protein and crude fiber. In Ethiopia, fresh mushroom market is largely a contribution of small entrepreneurs who face challenges in marketing their produce, mainly due to lack of awareness by the society as a food item. To add to the challenge, quality of cultivated mushrooms starts declining soon after harvesting because of their high moisture content, metabolism and susceptibility to enzymatic browning. Knowledge of effective mushroom preservation methods (apart from sun drying) being limited leads to loss of produce. To add to the challenge, dried mushrooms are hygroscopic and absorb moisture from the air unless packaged effectively. Moreover, there is little know how on how to utilize dried mushrooms as food item in the Ethiopian society. These factors make preservation of mushrooms by processing vital and thus, call for the exploration of utilizing dried oyster mushrooms in the production of nutritious foods without sacrificing the functional and sensory properties of the final product.

1.3. Significance of the study

- Contribute in overcoming malnutrition, as daily consumption of such blended products will fill the gap of protein deficiency
- Reduce mushroom losses thereby enhancing the income of growers by value-addition since off-grade mushrooms can be utilized in composite flour making
- Provides information on how and to what extent the benefits of both mushroom production and mushroom products can be maximized in Ethiopia
- Creates job opportunities, especially for the unemployed educated people because both the cultivation and drying of mushrooms is an indoor task
- Encourages further research and development on value added products by utilizing other mushroom species

1.4. Objectives

General objective

- To study the possibility of bread making by using oyster mushroom powder and wheat flour blends which will result in acceptable bread quality

Specific objectives

- To determine the functional properties of wheat flours enriched with oyster mushroom powder
- To evaluate the effect of blend proportion of oyster mushroom powder and wheat flour on the proximate composition of bread
- To assess the sensory properties of breads made by blending Oyster mushroom powder with wheat flour

2. Literature review

Nutritional supplementation of staple food products such as bread which is widely used as a major part of most people's diet has become an effective means to alleviate malnutrition problems. (Salehifar *et al*, 2007) Different research works have used various protein rich food materials for enriching wheat flour. Several studies about the influence of fortification of wheat flour by protein sources from either animal or plant on the physico-chemical properties of bread dough and its final products quality have been reported by various researchers. Milk and its products, fish protein concentrate and egg protein, grain legumes and oil seed proteins, soybean, cottonseed, sunflower, peanut, lupin and rice bran are reported to be protein supplements for bread. Some roots, including cassava (*Manihot esculenta Crantz*) and sweet potato (*Ipomoea batatas*), some tubers including potato (*Solanum tuberosum*) and yam (*Dioscorea spp.*) and some edible aroids, including taro (*Colocasia esculenta*) and cocoyam (*Xanthosoma sagittifolium*) were used as important calorie sources and wheat flour substitutes (Ammar *et al*, 2009) The studies include the chemical, technological, biological and sensory characteristics of enriched wheat flour and optimization of the ingredients and operating conditions for the bakery products. However, the information pertaining to mushroom protein- enriched wheat flour for bread making is scarce.

The Department of Food Science and Technology of University of Tennessee cited research works that showed wheat flour substitution with freeze dried Portablella powder up to 20% produced dough without compromising dough quality. The same project report included successful studies conducted in India and China that proposed use of oyster mushroom powder in breads and noodles. (Rai *et al*, 2008). The informations reported however are scarce and hardly reproducible, as nutritional composition of mushrooms itself depends on type of mushroom species, type of substrate used for cultivation, environmental conditions during harvest and post harvest handling of cultivated mushrooms.

2.1. Composite flour

Composite flour is a binary or ternary mixture of wheat flour with flour from other crops and is considered advantageous in developing countries as it reduces the importation of wheat flour and encourage the use of locally grown crops as flour (Aziah *et al* 2009, Hugo *et al*, 2000). Cereal based products made from composite flours are widely accepted in many parts of the world these days. The mandate passed by the Federal Government of Nigeria to use of composite cassava-wheat flour for baking by adding minimum of 10% cassava flour to wheat flour can be cited. This mandate aimed in cutting the nation's expense on wheat importation and find wider utilization for the increasingly produced cassava roots (Shittu *et al*, 2007)

A variety of wheat flour substitutes have been tried in bakery formulations with varying success. Ammar *et al* (2009) studied the effect of partial substitution of wheat flour using taro flour on the rheological properties, organoleptic and chemical characteristics in Egyptian bread. The Farinograph results showed that the increase of substitution level increase the water absorption (56% for the control up to 59.5%) and dough weakening but decrease the mixing time and dough stability, while the organoleptic evaluation showed that the substitution of wheat flour with taro flour up to 10 % produce bread similar to the control (wheat bread) in all the organoleptic properties, increased substitution resulting decrease in Organoleptic quality of bread samples. The increase of the taro flour level resulted decrease in the crude protein and ether extract while, ash, total carbohydrates and fiber contents increased from 0.95 – 2.15%, 74.60- 79.63% and 1.15 -2.20% respectively. They concluded that the substitution of wheat flour with taro flour in bread making with substitution level up to 10 % produces bread with rheological and organoleptic properties similar to the wheat flour bread.

The performance of 10, 20 and 30% cassava composite bread was carried out by evaluating the colour, aroma, texture, acceptability and buying preference. (Eddy *et al* 2007). The result showed that bread baked with 10 and 20% composite flour were not significantly different in all sensory attributes, acceptability and readiness to buy from the control. There was a tendency for bread baked with 10 and 20% composite flour to be rated higher than the control especially in flavour, acceptability and desire to buy. Values obtained for proximate composition of cassava composite bread samples were comparable to those obtained for whole wheat bread, protein contents dropping with increasing amounts of cassava flour. Both wheat and cassava are poor sources of protein, but the corresponding researchers advocated for 10-20% wheat/cassava flour for bread making as an alternative to 100% wheat to ensure economic goals by cutting wheat expenses.

In another research, the use of plantain flour substitution in wheat flour from 0 to 15% for the production of bread was investigated by Olaoye *et al* (2006). The crude protein of the plantain supplemented breads and the whole wheat bread ranged between 6.88 and 7.01%, with the whole wheat bread recording the highest value. The ash content increased with progressive increase in the proportion of the plantain flour, the highest value (0.95%) was recorded for the 15% plantain supplemented breads. There were no significant differences between the whole wheat bread and the plantain supplemented breads up to 10% plantain flour substitution in all the sensory attributes tested; crust, taste, aroma, shape, internal texture, appearance and general acceptability. Based on these findings, they concluded that the plantain supplemented breads had comparable sensory and nutritional qualities to the whole wheat breads

The effect of Oat flour on dough rheology, texture and Organoleptic properties of bread was studied by Salehifar *et al (2007)* by substituting wheat flour with oat flour by 0, 10, 20, 30 and 40%. Oat flour was observed to have pronounced effects on dough properties yielding a higher water absorption and dough development time and lower dough stability and extensibility compared with the control flour. Dough softening increased with an increased level of oat flour while the addition of oat flour improved bread shelf life and nutritional values. Panelists preferred breads with no Oat flours, followed by breads produced with up to 20% oat flour. Breads produced with 30% and 40% oat flour had a bitter after taste, and were unacceptable by the sensory panelists.

Adawy (1995) studied the effect of sesame seed protein supplementation on the nutritional, physical, chemical and sensory properties of wheat flour bread by adding sesame products (sesame meal, roasted and autoclaved sesame meals, sesame protein isolate and concentrate) to wheat flour at levels of 14, 16, 18 and 20%. His results suggested the addition of sesame products to wheat flour up to 18% level (sesame protein isolate) and up to 16% level (for other sesame products) without any observed detrimental effects on bread sensory properties and no significant differences in loaf volume between control and sesame supplemented breads. Also, the addition of sesame products to bread increased the concentration of total essential amino acids, valine, lysine, leucine (except sesame meal) and total sulphur amino acid up to 80-125% compared to control. (Adawy, 1997)

In an attempt to introduce nutritious products by utilizing indigenous foods, a study was conducted by Gayle *et al* (1986) to determine the physical, sensory and nutritional characteristics of all- purpose wheat flour breads supplemented with pigeon pea flour. An increase in pigeon pea flour from 0 to 25% increased protein content from 9.19 to 13%, (lysine increased from 0.3 to171 mg/ 16g N), ash increased from 0.89 to 1.1% and moisture increased from 29 to 31%. Conversely, fat and carbohydrate- fiber percentages declined as pigeon pea flour was increased. However, there were no differences in the sensory scores of the breads as the amount of pigeon pea flour was increased in the formulation, even though the breads were slightly more squat and compact.

Another study which involved soy flour substitution in wheat flour from 0 to 15%, for the production of bread was investigated conducted by Olaoye. *et al* (2006), showed that the crude protein, crude fiber, ether extract and ash contents of the soy supplemented breads increased with progressive increase in the proportion of soy flour, with the 15% soy supplemented breads having highest values of 8.39, 0.14, 2.46 and 1.17%, respectively, while lowest values were recorded for the whole wheat bread. The carbohydrate content was observed to decrease with corresponding increase in the percentage of soy flour in soy supplemented breads.

The sensory evaluation resulted highest Hedonic mean scores for whole wheat breads, while no significant differences were observed between the whole wheat bread and the 5% soy supplemented breads in the sensory attributes of aroma, internal texture, taste and general acceptability. But differences were significant in crust, shape and appearance. In their conclusion, breads of good nutritional and sensory qualities could be produced from up to 10% soy flour substitution in wheat flour, despite the fact that the high amount soy supplemented breads had higher proteins contents.

These results parallel similar studies undertaken by Tariqul *et al (2007)*, which showed that the protein content and other nutrients of bread were increased by addition of soy flour, with the exception of carbohydrates, which significantly decreased upon addition of soy flour. The analysis of bread containing 10% soy flour showed moisture 34.90%, protein 10.75%, fat 2.91%, ash 1.58%, crude fibre 0.61%, sugar 1.59% and total carbohydrate 45.59% respectively. Statistical analysis of sensory attributes through a taste-testing panelists proved that wheat flour could be substituted for soy flour in the formulation of soy flour breads up to 10% level to achieve bread with acceptable quality attributes, since it resulted the highest score of three quality attributes (colour, flavour, and overall acceptability) and the lowest score for one quality attribute (texture).

Functional properties (solubility, foamability, gelation and emulsification properties) are the intrinsic physicochemical characteristics which may affect the behaviour of food systems during processing and storage (Oshodi *et al*, 1989) Flour protein content is not only an indicator of direct nutritional value, but is also an important influence on dough rheological properties. It is often related to bread-making quality. Good bread flour has strong gluten that is indicated by high protein quantity. In addition, wheat of high protein content usually commands a premium price because it is in demand for blending with low protein wheat for the production of bread flour. Water absorption is the amount of water absorbed by the flour to produce dough of workable consistency. It is determined by the protein content of the flour, the amount of starch damaged during milling and the presence of non-starch carbohydrates. It is desirable that flours for bread-making possess a high water absorption capacity at normal working consistencies so that the yield of dough, and hence bread, will be relatively high. (Wujun *et al*, 2007)

2.2. Nutritional composition of Oyster mushrooms

Mushrooms, which are members of the class Basidiomycetes, have for long been known as "nature's trash burners", a descriptive but unromantic name they share with bacteria because of their role in decomposition. (Chang et al, 2004) Mushrooms have long been consumed as a food high in nutritive value and as a contribution to a healthy diet. Today, mushrooms are produced not only for their good nutritional and high digestibility values, but also for their metabolic products, many of which are said to possess antitumor, immunoregulatory and hypertensive functions without adverse side effects. Current studies estimate that 1.5 million species of fungi may actually exist and that there may be 140,000 species that produce fruiting bodies of sufficient size and structure to be considered macro fungi, thus fulfilling the definition of mushroom. (Chang *et al*, 2004) According to FAO, China is the leader in mushroom production, followed by the United States of America, Netherlands, Poland and France (FAO, 2007)

Available data in the chemical composition reveal that they are nutritious foods rich in non-starchy carbohydrates, dietary fibre, minerals which are essential for human health, vitamins B1, B2, B6, B12, C, D and are quite low in fat value (P. Mattila *et al*, 2002, Atikpo *et al*, 2007). However, the results of analyses vary widely for all constituents. This variation can be caused by differences in strain, substrate, and the developmental stage of the mushroom. Furthermore, cultivation, watering, fruiting, and storage conditions affect the contents of biologically active compounds. (Mattila *et al*, 2002)

Much of the interest in Pleurotus spp. as a food source has centred on its high protein content, and the high nutritional value of the protein. Published values for the protein content of four popular edible mushrooms, *Agaricus bisporus, Lentinula edodes, Pleurotus* spp., and *Volvariella volvacea*, which are commercially cultivated in various countries, range from 1.75 to 3.63% of their fresh weight. On a dry weight basis, mushrooms normally contain 19 to 35% protein, as compared to 7.3% in rice, 13.2% in wheat, 39.1% in soybean, and 25.2% in milk. (Braaksma *et al*, 1996)

The protein question also should be examined in reference to quality. Because proteins are made up from over 20 amino acids in varying amounts, they are quantitatively different. The human body can convert some of these amino acids into others, but there are nine essential amino acids (lysine, methionine, tryptophane, threonine, valine, leucine, isoleucine, histidine, and phenylalanine) which must be present simultaneously and in correct relative amounts for protein synthesis to occur. If one or more should be in inadequate supply, the utilization of all others in the cellular pool will be reduced in the same proportion. (Chang *et al*, 2004)

Amino acid	White button	Shitake	Oyster	Egg
Leucine	7.5	7.9	6.8	8.8
Iso leucine	4.5	4.9	4.2	6.6
Valine	2.5	3.7	5.1	7.3
Trytophan	2.0	ND	1.3	1.6
Lysine	9.1	3.9	4.5	6.4
Theronine	5.5	5.9	4.6	5.1
Phenyaline	4.2	5.9	3.7	5.8
Methionine	0.9	1.9	1.5	3.1
Histidine	2.7	1.9	1.7	2.4
Total Ess. AA	38.9	36.0	33.4	47.1

Table 1: Amino acid composition (in grams per 100 grams of corrected protein) of some edible mushrooms as compared to egg: ND – Not determined
(Source: *Mushroom: Cultivation, nutritional value, medicinal effect and environmental impact*)

The proteins of commonly cultivated mushrooms contain all nine amino acids essential for humans (Table 1). For all mushrooms listed in the table, the most abundant essential amino acid is leucine, and the lowest levels among the essential amino acids are those of tryptophan and methionine. Considering the essential amino acid index, biological value, in vitro digestibility, nutritional index, and protein score, Pleurotus species fall between high grade vegetables and low grade meats. There are reports that various species of mushrooms contain, in addition to the common amino acids and amides, the less common amino acids and related nitrogenous compounds such as methionine sulfoxides, β-alanine, cystic acid, hydroxyprolines, aminoadipic acid, phosphoserine, cystathione, canavanine, creatinine, citrulline, ornithine, glucosamine, and ethanolamine. (Chang *et al*, 2004)

The use of mushrooms may contribute significantly in overcoming protein deficiency in the developing countries where there has been a decline of about 6% in the protein intake in developing countries since World War II, following the shift towards protein derived from grains from that of animal sources. Food products of animal origin always provide a better-balanced and higher-quality protein than do plant foods, which often lack some of the important amino acids; e.g., cereal grains have too little of the essential amino acid lysine, and legumes usually lack the essential amino acids methionine and tryptophan (Abbott, 1966)

2.3. Need for mushroom processing

Mushroom being highly perishable and cannot be kept for more than 4 to 6 hours under normal atmospheric condition and hence need immediate disposal after harvest. (Das *et al*, 2006) Even after harvesting, mushrooms continue to grow, respire, mature and senesce resulting in weight loss, veil-opening, browning, wilting and finally in spoilage. Since mushrooms contain sugars, amino acids and proteins, non-enzymatic browning is inevitable when stored above 5°C. Most damaging post harvest changes in mushrooms vary with species—it is blackening in the button mushroom while mucilage in the oyster mushroom, which affect their marketability significantly. Weight loss is very serious problem in all the mushrooms as these contain very high moisture (85-90 %). Due to very high moisture and rich nutritive value, microbial spoilage in mushrooms is also a problem. (Rai *et al*, 2008)

The protein content of mushrooms will also decrease sharply following harvest, as they develop into fully opened stage. Rai and Arumuganathan cited findings that reveled storage of mushrooms at higher temperature (18 to 20°C) resulted in reduction in protein with accumulation of free amino acids due to activated protease enzyme activity and decrease in free amino acids and cell wall glucan. At higher temperature urea accumulation and breakdown of nucleic acids and other nitrogenous substances may occur (Braaksma *et al*, 1996, Rai *et al*, 2008)

Proper packaging and storage for the fresh mushrooms and processing for long-term storage are some of the most common post harvest practices of mushrooms to increase the shelf- life of mushrooms, even though the market continuity for fresh mushrooms is likely to dominate (Rai *et al*, 2008) Processing techniques such as canning, freezing, pickling, steeping Preservation, radiation preservation and drying are the most suitable techniques, but not all conservation methods are suitable for all type of mushrooms. Canning of oyster mushrooms may result in products having horrible taste. (Marshall *et al*, 2009) Drying of mushrooms is an excellent method and has several advantages: it is easy, quick and safe and well-dried mushrooms can be stored for a long time, making it a more convenient ingredient to be used in recipes (Combs, 2004, Marshall *et al*, 2009)

Drying can be achieved by various dryers, air-cabinet drier, freeze drier, fluidized bed drier, dehumidified-air drier and solar drier. Sun-drying is the cheapest and oldest method among various drying methods. It is a very simple operation, where no fuel or mechanical energy is required. However, it is completely dependent on weather and it is not possible round-the-clock and round the- year. Quality of sun-dried mushrooms is generally less than that of artificially dried ones as the moisture content of sun-dried mushrooms is higher, thus adding to the disadvantages of sun -drying. (Rai *et al*, 2008) Artificial during methods using instruments is effective and efficient means of dehydration as it provides temperature control and drying is not restricted by time or weather conditions.

Studies which deal with the changes in color and texture and safety of dried mushrooms have been conducted by different researchers (Kotwaliwale *et al*, 2007, Walde *et a*l, 2006, Bala *et al*, 2009, Kausar, 1988, Dunkwal *et al*, Czapski *et al*, 2000). The color changes during drying of oyster mushrooms are mostly in the form of browning caused by enzymatic or non-enzymatic reactions between carbohydrate and amino acids at elevated temperature. (Kotwaliwale *et al*, 2007) Non-enzymatic browning is due mainly to Maillard reaction, which involves interaction between reducing sugars, amino acids and proteins or may be caused by reaction of oxidation products of ascorbic acid with proteins or amino acids. (Rai *et al,* 2008)

Results obtained suggest longer drying at low temperatures to be safer than faster drying at high temperatures, as the mushrooms could become toasted at high temperatures. Furthermore, if the fresh mushrooms are very wet, the starting temperature should not be low as they might start to rot. (Marshall *et al*, 2009) Dried products (from whole mushrooms to powders) are also found out to be microbiologically safe as long as they are kept at low humidity (Zivanovic, 2006). Dehydrated mushrooms obtained in either ways are used as an important ingredient in several food formulations, including instant soups, pasta, snack seasonings, casseroles, and meat and rice dishes. (Rai *et al*, 2008)

2.4. Value added products from mushrooms

Studies have shown that dehydrated mushrooms have shelf life of about than six months (Das *et al*, 2006). This short shelf life is further challenged by appropriate packaging materials and appropriate storage conditions, because dehydrated products are hygroscopic and can be attacked by moisture from the air. Besides canning, drying, steeping and pickling currently resorted to for the long-term storage and trade, the production and consumption of the readymade or ready-to-make value-added mushroom products are receiving the attention the mushroom research and industry lately. (Rai *et al*, 2008) The gourmet appeal of mushroom and the consumers demand for variety has led to the development of ready made or value added processed foods from mushrooms.

Mushroom powder enhances the protein, vitamin and mineral content of the product. Fast foods like spring rolls, mushroom burger, mushroom chow, mushroom cutlets, pizzas are results of dried mushrooms. Mushroom-based soup powder, noodles and biscuits are already on the shelves of Indian supermarkets. Preparation of highly nutritious and energetic soup is reported to be prepared from dried mushroom powder by blending with mixed vegetable, chicken and tomato. Dried mushroom powder can also be utilized in preparing weaning food. (Das *et al*, 2006) Dry mushrooms can be powdered and used in infant food preparations for increased nutritional value and to combat protein energy malnutrition problems of the vulnerable group (Atikpo *et al*, 2007)

Dried mushrooms powder made either from *Pleurotus sp, Agaricus bisporus, calocybe indica* can be incorporated to various conventional recipes to increase their nutritional quality(Das *et al*, 2006) As a result, technologies for ready-to make mushroom pizza, mushroom curry in retortable pouches, nuggets, ketchup, preserve in sugar syrup (murabba) are reported to be developed in Asian countries, particularly in China and India.(Rai *et al*, 2008) According to the same report, four types of foods have been produced from low quality mushrooms in China:

i. Ingredients such as seasoning and mushroom-soy sauce
ii. Snacks such as extruded mushroom products, candied mushrooms, fried mushrooms, and jerky-like snacks
iii. Beverages, such as reishi (Ganoderma lucidum) wine, reishi tea, white jelly mushroom (Tremella fuciformis) concentrate, and shiitake soda ,and
iv. Mushroom based cakes and candies such as cakes, reishi candy, and wood ear (Auricularia auricular) candy

Tasnim Kausar studied the effect of incorporating Oyster mushroom powder on the nutritional and Organoleptic properties of various traditional Pakistani dishes- “Pea”, “Pakoray”, “Roti”*, “Liver” “Omelette”, “Allu kabab” and “Samosa”**. Increase in protein content of “Allu kabab” and “Samosa” supplemented with oyster mushroom was 93.33% and 62.24 % respectively while it ranged from 4.25 to 25.73% in the case of Pea, Pakoray, Liver and Omelette dishes, maximum being in pea and minimum being in Omelete. (Kausar, 1988)

---

* *an unleavened flatbread made from stone ground whole meal flour, traditionally known as atta flour. Its defining characteristic is that it is unleavened.(Source: Wikipedia, the free encyclopedia)*

** *stuffed pastry and a popular snack in Asia, Arabian Peninsula, Mediterranean (Turkey), North and South Africa. It generally consists of a fried or baked triangular pastry shell, which may include spiced potatoes, onions, peas, coriander, and lentils, or ground beef or chicken.(Source: Wikipedia, the free encyclopedia)*

Sensory evaluation of these Pakistani dishes resulted an overall acceptability with 85% (rated excellent) to mushrooms supplemented with Omelette and Pakoray where as Samosa, Pea and Allu kabab had an overall acceptability with 70-84% and were rated very good. (Kausar, 1988) While determining the Quality and safety of halawa modified with Pleurotus sajor-caju mushroom, Eissa et al (2006) supplemented halawa with 10–120 g mushroom.

Test results showed that halawa samples containing 10 and 20 g mushroom had similar characteristics to those of the control halawa sample and that these samples had good keeping quality and were closest to controls in terms of consistency and color. From their results, they concluded that supplementation of halawa with mushroom can help to overcome lysine deficiency or essential amino acids deficiency and mushrooms can be used instead of food additives to maintain the quality and safety of halawa (Eissa *et al*, 2006)

The Project report prepared by the University of Tennessee in India in 2006 (Zivanovic, 2006) included in its report research works that indicated the possibility of substitution of wheat flour with freeze dried Portablella powder up to 20% to produce dough without compromising dough quality. The same report cited studies that proposed use of oyster mushroom powder as an effective supplement in Indian breads, and the preparation of bread and noodles using mushrooms of inferior quality in China.

Another report proposed that mushroom powder can be incorporated with wheat flour, maize and millet flour to make rotis, bread for daily consumption, following acceptability studies which showed rotis and bread made from combination of wheat flour and mushroom powder were well accepted than those from millet and maize. (Das *et al*, 2006)

The effects of mushroom (Lentinus Tuber-Regium) powder on the physicochemical characteristics of dough and bread-making properties were studied by Lee *et al* (2004). In mixogram test, 2-10% mushroom powder increased the water absorption from 67-79%. Highest loaf volume was obtained when 2 and 4% mushrooms were added, whereas decreased above 6%. Bread weight, chewiness and gumminess increased with increasing mushroom powder. Although sensory values decreased with increasing mushroom powder, use of mushroom powder to replace up to 4% wheat flour is recommended in bread making.

In a study to see the effect of oyster mushroom powder on bread quality and dough rheology, Hong et al prepared breads from wheat flour supplemented with oyster mushroom powder. (Hong *et al*, 2005). Farinograph results indicated gradual increase in water absorption, dough development time and decrease in dough stability with increased amount of oyster mushroom powder. The supplementation of oyster mushroom powder had effect on bread quality also, resulting in an increase in loaf weight and a decrease in loaf volume. (Hong *et al*, 2005). These changes in dough rheological properties were also reported by Bothina Abd-Elateef (Abd-Elateef, 2008). Very delicious and crunchy biscuits were also successfully prepared in India from oyster and button mushrooms and other necessary ingredients (Rai *et al*, 2008)

2.5. Bread baking ingredients and their functions

Bread has remained vital to the human diet despite the competition of today's increasing diversity of food products. The evolution of bread is tied to the evolution of human life and dates back to the Neolithic era (Liu *et al*, 2003). The discovery of bread for the first time could only be speculated; however it is believed it resulted from people accidentally spilling bits of porridge onto the hot stones of a hearth. (Dimuzio, 2010) Thus, the first bread man ate was an unleavened product, which was cooked from a paste of crushed wild cereal grains (Liu *et al*, 2003). They accidently introduced air borne wild yeasts to the paste, and baked the first leavened bread. Around 4000 BC, Egyptian slaves made bread from wheat that acted differently than barley or oats. The critical difference between the flour made from wheat and that made from grains like barley or oats was that wheat contained some unique proteins called *gluten*, which are present either as monomers or, linked by interchain disulphide bonds, as oligo- and polymers. (Dimuzio, 2010, Wieser, 2007) Gluten is the rubbery mass that remains when wheat dough is washed to remove starch granules and water-soluble constituents. Depending on the thoroughness of washing, the dry solid contain 75–85% protein and 5–10% lipids; most of the remainder is starch and non starch carbohydrates (Wieser, 2007)

The bread making procedure differs from place to place, from person to person even though the principles of baking bread have been established for hundreds of years. The basic ingredients are flour, yeast, salt and water. Bread Improvers (flour treatment agents), usually Vitamin C (ascorbic acid), are used in commercial production. (Federation of Bakers, 2002) The flour used for bread making can be from a single grain or a combination of grains, such as wheat, rye, corn, or barley, but when flour is referred in a recipe with no other descriptors, it is to mean that the flour is made from wheat only. (Dimuzio, 2010) Quality is a term that encompasses a large number of parameters with respect to the product delivered from the flourmill to the baker and it means satisfying the baker's requirement for consistent performance from the flour (Webb *et al,* 2003)

2.5.1. Flour function

Flour can be defined as the powder or particles that result from the crushing or milling of starchy seeds, grains, tubers, or legumes (Dimuzio, 2010). Wheat flour is the principal component in bread making because of its characteristic ability to capture gases from fermentation and thereby leaven bread (Dimuzio, 2010). The quality of the flour is particularly important in bread making as it will have a significant impact on the finished product attributes, such as texture and appearance (Federation of Bakers, 2002).Gluten and starch are responsible for much of the structure-building properties of flour. Gluten is formed from two proteins in flour, glutenin and gliadin, when flour is mixed with water. While not as important as gluten and starch, pentosan gums also contribute to flour structure. Gums appear either to form their own structure or to interact with gluten (Figoni, 2003)

To the extent the gluten-forming proteins present in flour, there will be dough elasticity and structure due to formation of a gluten matrix. (Vaclavik *et al*, 2008) Protein content is thus a key specification for wheat and flour purchasers since it is related to many processing properties, such as water absorption and gluten strength (*Wheat and Flour Testing Methods*) Hard wheat is the term applied to varieties that are high in protein (10–18%) while soft wheat is the term for varieties that are lower in protein (8–11%). (Dimuzio, 2010) The later are desired for crisp or tender products, such as snacks or cakes while the wheat high in protein content are desired for products with chewy texture, such as pan bread and hearth bread (*Wheat and Flour Testing Methods*)

Starch on the other hand contributes structure to dough as it gelatinizes. Starch gelatinization is the swelling of starch granules when heated with water, often resulting in thickening. By gelatinization, starch becomes flexible and takes water from gluten, a process which helps gluten to set and makes the crumb more rigid. (Vaclavik *et al*, 2008)

Gluten and starch thus share the role of structure building in yeast leavened products, gluten being most important for developing structure in unbaked dough, while starch is arguably more important to the structure of the final baked product. Moreover, flour is a source of fermentable sugar that is acted upon by yeast in producing CO_2 for leavening (Figoni, 2003) Although wheat flours are the most common types of flour used in baked products, non wheat flours such as rye, barley, corn, rice, and soy also are popular in bread and the formulation combines the flour with wheat flour for more desirable baking results. (Dimuzio, 2010, Vaclavik *et al*, 2008)

Another function of flour is that it absorbs liquids, water or oil. The absorption value of flour is an important quality factor in Dimuzio, 2010. It is defined as the amount of liquid absorbed by flour when forming bread dough. High absorption values are desirable in Dimuzio, 2010 because the added moisture slows staling. Higher water absorption also means that less flour is needed to make a loaf of bread, which accounts for cost effective production of bread. (Figoni, 2003) Starches, proteins, and gums are the three main components in flour that absorb moisture (water) and oil, helping to bind ingredients together. These are the same components that form structure, the difference being that all proteins in flour (not just glutenin and gliadin) absorb moisture, while only glutenin and gliadin form structure (Figoni, 2003)

Wheat flours also contribute to color and flavor of finished products. Wheat flours have a relatively mild, slightly nutty flavor that is generally considered desirable. Essentially all flours and grain products contribute complex carbohydrates (starch), vitamins, minerals, and protein. However, the protein in wheat is low in *lysine*, an essential amino acid. White flour is also a poor source of fiber, but whole wheat flour and whole white wheat flour, being whole grain products, are good sources of insoluble dietary fiber from the bran, important in the diet (Figoni, 2003)

2.5.2. Water function

Water has critical effects on dough making as it hydrates both proteins required for gluten formation and starch that undergoes gelatinization (Vaclavik *et al*, 2008) The major proteins of bread, gliadin and glutenin, can be viewed as coiled or folded chains, with the structure stabilized by bonds between sulfur atoms (disulfide bonds) on adjacent areas of the molecules (intramolecular bonds). Mixing stretches the molecules and breaks the relatively weak bonds. During resting, the disulfide bonds can re−form either within (as before) or between molecules (intermolecular). When bonds form between molecules, the resulting structure (gluten) is much stronger than the individual proteins. (Tenbergen, 1999)

Additionally, water is the solvent for dissolving many recipe ingredients such as the leavening agents, baking powder and baking soda, as well as salt and sugar. Yeast and bacteria, cannot thrive without sufficient water, amylase which is an enzyme that is critical to successful fermentation cannot degrade starch into sugars without sufficient water (Dimuzio, 2010, Vaclavik *et al*, 2008). It is important that the water is the correct temperature and quantity when making bread, because it affects the dispersal of the other ingredients. (Federation of Bakers, 2002)

2.5.3. Yeast functions

Yeast is one of the essential ingredients in bread production. It is a one cell plant which multiplies by a process known as budding. Under the right conditions of water, sugars, temperature, and dissolved minerals, yeast causes fermentation. There are thousands of known species, but the one that is the concern of bread bakers is *saccharomyces cerevisiae*, which prefers to feed on sugars such as glucose, fructose, sucrose, and maltose. Yeast cells produce their own enzyme, *zymase*, to help them obtain energy from those sugars, leaving behind the by-products *carbon dioxide* (thus enabling the dough to rise) and *alcohol*. (Dimuzio, 2010) In bread making, the leavening step is fundamental to creating a bread loaf that conforms to the textural requirements of its class. Leavening brings about changes in the cellular structure of the dough, and hence the textural properties of the bread by expanding gas cell sizes in the dough with carbon dioxide. (Bellido *et al*, 2009)

Though yeast is the typical source of leavening gas in bread making, it is by no means the only source. Chemical leavening systems (also known as dough conditioners or dough improvers) are off-white dry, granular products that look like flour. Because they contain mix of ingredients, they are added to dough to raise or leaven bread dough faster, more consistently and often times more conveniently than yeast because they do not require lengthy activation periods or stringent environmental conditions for evolving gas. One major disadvantage of chemical leaveners, though, is that they are unable to duplicate the distinctive flavor imparted to bread by yeast. This undesirable effect can be mitigated by reformulation with other flavored bakery ingredients or chemical leavening systems may be used in conjunction with yeast (Bellido *et al*, 2009)

2.5.4. Salt functions

Salt is another essential ingredient in quality bread production. It has several functions. It enhances the flavor of other dough ingredients and regulates fermentation. Too little salt causes the dough to ferment too fast and wild, while too much salt slows down the fermentation process. In the proper amount, salt produces good grain and texture; because it tightens the gluten structure and adds strength to it, by allowing the gluten to fully mature during fermentation. This improves the ability of the dough to capture and hold the carbon dioxide gas from fermentation, and it ensures the loaf will have good volume. It also prevents the growth of wild yeast and bacteria. A whiter crumb is produced and last but not least, it brings out the taste and flavor of bread. (Federation of Bakers, 2002, Dimuzio, 2010)

2.5.5. Sugar functions

Sugar is not considered an essential ingredient according to Dimuzio (2010). This is because flour contains a small amount of natural sugars and some starch is converted to sugar during the fermentation process. It does have many important functions. Some of the added sugar is converted to carbon dioxide and alcohol by the yeast enzymes. It provides necessary sweetness, helps to produce a golden brown color of the crust, improves the texture of the crumb, helps to retain moisture in the crumb and adds to the nutritional value of the bread (DiMuzio, 2010)

However, the secondary effects on fermentation and dough strength can make the process of adding any form of sugar (table sugar, honey, molasses, and others) undesirable. While it is true that sugar acts as food for yeast, its hygroscopic nature draws a lot of moisture from the dough, and, at levels of 12% of flour weight or higher, it ties up so much water that the yeast don’t have enough moisture available to maintain their typical rate of fermentation. So, at levels below 12%, sugar can help increase the rate of fermentation, but at higher levels, it actually begins to slow it. (DiMuzio, 2010)

3. Materials and methods

3.1. Location of the study

The experiment was conducted in Addis Ababa, Ethiopia, which lies at an altitude of 2,300 meters and is located at 9.03°N 38.74°E. Bread was baked at the department of Hotel Management in Entoto Vocational and Technique College while all chemical analysis was done at the laboratory of Food Science and Nutrition Programme, Food Technology laboratory, in Addis Ababa University and Ethiopian Health and Nutrition Research Institute.

3.2. Material collection and sample preparation

White wheat flour on 74% extraction rate was purchased from Misrak Flour and Bread factory, while fresh Oyster Mushrooms that were harvested on cotton seed were purchased from Absynia Mushroom, Addis Ababa, Ethiopia. Mushrooms were then sliced using stainless steel knife and dried in oven at $55 \pm 2^{0}C$ for 18 hours. Dried mushrooms were then milled until they pass 0.184 mesh size, packed in air tight polyethylene containers, and stored in refrigerator until used. Compressed yeast (Saf Instant), bread improver (Ethio Improver) and other dough ingredients were purchased at a local supplier in Addis Ababa.

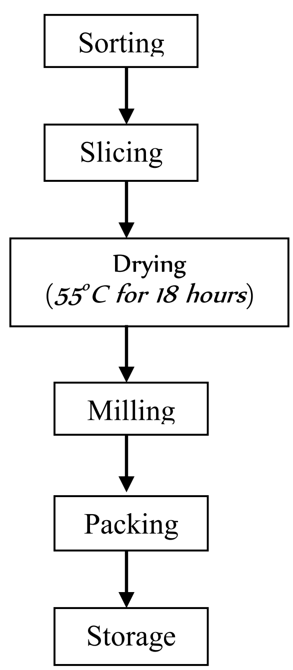

Fig1: Preparation of Oyster Mushroom Powder

Five blends were prepared by substituting wheat flours with oyster mushroom powder at maximum levels of 10% of wheat flour. Composite flours were mixed using blending machine for 3 minutes. The different levels of wheat flour blended with oyster mushroom powder are given in Table 2.

BP	Wheat Flour (%)	Oyster Mushroom Powder (%)
Control	100	0
BP1	98	2
BP2	96	4
BP3	94	6
BP4	92	8
BP5	90	10

Table 2: Preparation of Oyster mushroom enriched wheat flour
BP – Blend proportion

3.3. Analysis methods

3.3.1. Flour Functional properties

3.3.1.1. Gluten content

The gluten quality was evaluated by the standard methods of AACC test procedure (AACC 2000). Ten grams of flour samples were weighed and dough was prepared by mixing it with water. The dough was then washed with 2% salt (NaCl) solution for 5 minutes. Then the elastic material that remains behind the washing was taken as wet gluten. Gluten yield was calculated as the ratio of the weight of wet gluten to the weight of wheat flour or mushroom/wheat flour blend used (10g).

3.3.1.2. Water Absorption Capacity

WAC was determined by using the method of Sosulski (1992) with some modification as cited by Emanuel et al, 2010. Sample of 3g composite flour was mixed with 25 ml of water and stirred six times for 1 min at 10 min intervals. The mixtures were centrifuged at 3000 rpm for 25 min and supernatants removed. Pellets were dried at 50 °C for 25 min and weighed. Water absorption capacity was expressed as the milliliteres of water retained by 100 g of material.

3.3.2. Bread Processing and Characterization

3.3.2.1. Bread making procedure

The bread making process consisted of three major stages: mixing, fermentation and baking. Breads were made by using the straight dough method (AACC, 1984) with slight modifications. All the ingredients (Table 2) were mixed manually for 10 minutes and kneading was done until consistent dough was obtained. The dough was divided into 150 g divisions and molded and panned on aluminum baking pans (10.5cm X 7.5cm X 4 cm) and allowed to stand for 45 minutes at room temperature to complete fermentation. Baking was done in triplicates for each formulation using an electric oven at 200 ± 5^{o}C for 20 min. Following removal from the oven, breads were cooled for 20 minutes in racks and their weight was recorded. Breads were then dried at 50^{o}C for 6 hours and dried samples were milled using a blending machine. Powdered bread samples were then packed in polyethylene bags, labeled and stored in dry place until used for analysis.

Ingredient	Composition (%)
Flour	100
Yeast	2
Salt	0.75
Sugar	2
Improver	2.5
Water	Variable

Table 2: Recipe used in dough formulation per loaf (*the federation of bakers*)

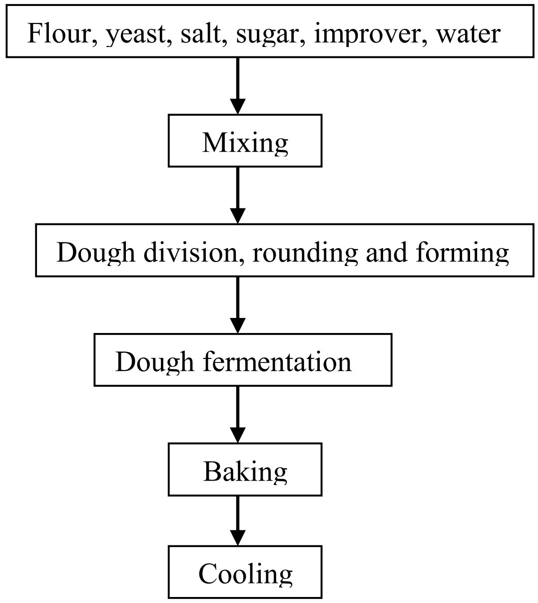

Fig 2: Flow chart for bread making procedure

3.3.2.2. Determination of Loaf Weight, Height and Volume

Breads were baked in triplicates and loaf weight was determined immediately after cooling the breads using digital balance. Height of breads was recorded using scale in centimeters. The volume of respective breads was determined by seed displacement method (AACC, 2000), using chickpea (*Cicer arietinum*). A rectangular box made from plastic was used. The box was fixed with cleaned chickpea, leveled and poured out. The bread was then placed in the same box and filled with the measured chickpea grain and leveled. The volume of the remaining grains from the same measured grains was taken as the volume of the loaf.

3.3.3. Sensory analysis

The sensory evaluation of the samples were carried out for consumer acceptance using nine panelists, comprising of students from the Food Science and Nutrition programme of Addis Ababa University and staff of Hotel Management department in Entoto Technique and Vocational Training Institute. Nine point Hedonic Scale was used to rate the attributes namely appearance, color, taste, flavor, mouth feel and over all acceptance .Samples were sliced using knife, coded with arbitrary three digit numbers and served randomly to panelists along with unsliced loaf so that they evaluated appearance. Panelists were provided with water to rinse their mouth after evaluating each sample.

3.4. Proximate Analysis

Nutritional composition of the mushroom was analyzed using AOAC (2000) standard methods at Addis Ababa University, Food Science and Nutritional Programme laboratory and Ethiopian Health and Nutritional Research Institute laboratory.

- **Determination of moisture content**

Moisture of the white wheat flour and mushroom was determined according to AOAC (2000) using the official method 925.09. A clean dried and covered, flat dish containing 5g of the sample was transferred to an oven at 100-105 ^{o}C for 4-6 hours, cooled in a desiccators for 30 minute and weighed. Moisture content was determined as follows:

$$\text{Moisture content (\%)} = \frac{\text{Weight of fresh sample} - \text{Weight of dry sample}}{\text{Weight of fresh sample}} \times 100$$

- **Determination of crude protein**

Protein content was determined according to AOAC (2000) using the official method 979.09. A digestion flask containing approximately 0.5g sample, to which some amount of concentrated sulphuric acid and and catalyst mixture (K_2SO_4 and Selenium) were added was left overnight. The digest was then exposed to a temperature of about 370^{o}C in order to allow digestion. Then distillation was performed by adding 40% NaOH to the compartment and using 4% boric acid solution and 3-4 drops of methyl red solution as indicator. Finally the distillate was titrated with 0.1N Hydrochloric acid to a pink color. The protein content was estimated using the formula given below.

$$\text{Total Nitrogen Content (\% / Weight)} = \frac{(T- B) \times N \times 14.007 \times 100}{W}$$

Where:

T= Volume in ml of the standard Hydrochloric acid used in titration for the test material

B= Volume in ml of the standard Hydrochloric acid used in titration of the blank

N= Normality of standard Hydrochloric acid

W= Weight in g of the test material

Crude Protein Content (% / Weight) = % Total Nitrogen Content × 4.38 (for Mushroom)

Crude Protein Content (% / Weight) = % Total Nitrogen Content × 5.25 (for breads)

- **Determination crude fat**

A clean, dried thimble containing about 5 g of dried sample and covered with fat free cotton at the bottom and top was placed in the extraction chamber. The extraction was take place for at least 4h AOAC (2000) official method 4.5.01. Then, the crude fat content was determined as follows:

$$\text{Crude Fat} = 100\,\frac{(W_2 - W_1)}{W}$$

Where: W = Weight in g of the dried test material

W_1 = Weight of extraction flask

W_2 = Weight in g of the extraction flask and dried crude fat

- **Total ash determination**

The porcelain dish used for the analysis was cleaned by drying at 105^0C for 30 minutes and cooled in desiccators for 30 minutes. The mass of the dish was measured by analytical balance (M_1). About 3gram of the sample was weighed in to porcelain dish (M_2). The sample was dried at 120^0C for one hour in drying oven. When gas evolution ceased from the drying oven, the sample was placed in furnace at about 550^0C until free from carbon and the residues appear grayish white (about 6 hours).The sample was then removed from the furnace and if the ashing was incomplete it was moistened by the few drops of water and placed in an oven at 120^0C for one hour and re-ash at 550^0C until white ash color was obtained. It was removed from the furnace and placed in the desiccator. Finally the mass was weighed (M_3).

$$Ash\ (\%) = \frac{M_3 - M_1}{M_2 - M_1} x\ 100\%$$

Where:

M_1 = *Weight of the dish*

M_2 = *Weight of fresh sample and dish*

M_3 = *Weight of ash and dish*

- **Determination of crude fiber**

Crude fiber analysis was conducted using the method of AOAC (2000) official method 962.09. About 1.5g weighed sample was transferred into a 600 ml beaker and about 200 ml 1.25% sulfuric acid was added and boiled for 30 minutes. Recording was taken place by placing a watch glass over the mouth of the beaker. After 30 minutes heating by gently keeping the level constant with distilled water, 20 ml 28% KOH was added and again boiled gently for further 30 minutes. Subsequently, washing was conducted with 1% sulfuric acid and NaOH solution. Then, it was filtered and dried in the electric oven at 130 ^{o}C for 2h. Furthermore, it was cooled at room temperature for 30 minutes in a desiccator and weighed, and then it was ashed in a muffle furnace at 550 ^{o}C. Finally, it was cooled again in desiccators and re-weighed. The crude fiber content was determined by using the formula:-

$$\text{Crude fiber content} = \frac{\lfloor (w_{1} - w_2)(100 - m) \rfloor}{w_3}$$

where, w_1 = Crucible weight after drying, g

w_2 = Crucible weight after ashing, g

w_3 = Dry weight, g

m = % moisture of the sample

- **Crude carbohydrate determination**

Total carbohydrate was calculated by difference by subtracting the sum percentage of crude protein, crude fat, total ash and crude fiber from 100.

3.5. Determination of Lysine

To determine lysine, the first step was to determine the tryptophan content. Accordingly, tryptophan percentage was determined using Glyooxilic Acid following the method developed by (Nurit *et al*., 2008) as it is cited by (Galicia *et al.*, 2008). The sample was grinded finely and defatted for 6 hours in a soxhelt apparatus after that the defatted sample was air dried to make sure that all the solvent was evaporated. From each of the defatted sample about 80 mg were weighed in a 15ml falcon tubes then 3ml of papain solution (papain dissolved in 0.165 M sodium acetate solution) was added in each of them followed by closing of the tubes to ensure that no evaporation will take place during incubation. Then the mixture was Vortexed thoroughly and incubated in an oven at 64 ºC for 16 h. Then the tube was taken out of the oven and was cooled to room temperature after that the sample was vortexed followed by centrifuging at 600rpm for 5min. After that 1ml of supernatant was taken from each sample and transferred to a new glass tube. Then 3ml of calorimetric reagent (a mixture of ferric chloride dissolved in 0.1M Glyoxilic Acid and 30N sulfuric acid) was slowly poured down to the inner wall of each of tube. After that they were closed and vortexes followed by incubation in the oven at 640C for 30min for color development. Then the sample was removed from the oven and cooled to room temperature. Finally the absorbance of each sample was read at 560nm in a spectrophotometer.

Lysine was then determined by multiplying the tryptophan results obtained above by 4 as follows:

$$\%\text{Lysine} = 4 \text{ X } (\% \text{ tryptophan})$$

3.6. Mineral Analysis

Calcium, Phosphorous and Iron content of breads were determined by Spectrophotometric technique from the ash obtained through dry ashing.

- **Calcium and Iron analysis**

About 1g of ash was treated with 5 ml of 6N HCl to wet it completely and carefully dried on a low temperature hot plate to dryness. Seven point five ml of 3N HCl was added on each crucible and placed on the hot plate until the solution just boiled. The solution was cooled to room temperature and filtered through a filter paper (whatman 42,125mm) into 50ml volumetric flask. Again 5ml of 3N HCl was added to the crucible to dissolve the residue and it was heated until the solution just boiled. Then the solution was cooled and filtered into the previous 50ml volumetric flask. The crucible was washed with de ionized water three times and filtered and combined the washings into the 50ml volumetric flask. Then 2.5ml of lanthanum chloride solution was added in 50ml volumetric flask which contained the filtrate and the flask was filled with de ionized water up to the mark. Blank was prepared in parallel with the sample dissolving process using the same amount of reagents which were used for the sample. The sample solutions were transferred to polyethylene bottle.

Standard solutions: Five series of working standard for Fe and Ca were prepared from 1000ppm metals stock solutions with de ionized water containing 2.4 ml 3N HCl and 0.5ml lanthanum chloride in 10 ml volumetric flask used to make the sample and standard matrix similar. Iron, Zinc and Calcium Hallo cathode lamps were used as a radiation source for each elements and Air acetylene gas mixture was used as source of flame. After Flame Atomic Absorption Spectrophotometer (Varian SpectrAA-20 Plus, Varian Australia Pty., Ltd., Australia) turned on and optimized for each elements one after the other, maximum absorbance was obtained by adjusting the hallo Cathode lamps at specific slit width and wave lengths. Before the analysis of sample calibration graph (concentration versus absorbance) for each element using the standard solutions was prepared, then first Sample blank solution was run followed by the sample solutions. After each solution of standard or sample run the system was rinsed by aspirating de ionized water to avoid contamination. The measured samples blank concentration was deducted from that of the sample concentration. Duplicates of each sample were measured, and the concentrations were calculated using the average of each value.

Calculation:

Metal content (mg/100g) = [(A-B) x V]/ (10 *W)

Where,

W= Weight of sample in gram

V=Extract volume (50ml)

A = Concentration (μg/ml) of sample solution;

B = Concentration (μg/ml) of blank solution

- **Phosphorus determination**

The sample solutions prepared for mineral determination was used for phosphorous determination. One ml of the clear extract (sample solution prepared for mineral determination) was diluted into 100 ml with deionized water. Five ml of the sample solution was added into test tubes. Exactly 0.5 ml of molybdate and 0.2 ml aminonaphtholsulphonic acid were added into the test tubes (sample solution) and mixed thoroughly step by step. The solution was allowed to stand for 10 minutes. Six series of working standard phosphorous solutions (0- 1µg/ml) were prepared by appropriate dilution of the phosphorous stock solution (1000 µg P/ml of KH2PO4) with deionized water using 10 ml volumetric flask. After manipulating the instrument operation procedure, the absorbance (A) the sample solution was measured at 660 nm against distilled water using UV-VIS spectrophotometer. The standard and sample blank solution was run with the sample. Calibration graph (concentration versus absorbance) prepared and used to quantify the amount of phosphorous in the sample.

$$\textbf{Phosphorus in mg/100gm} = \frac{(A_s - A_B) * \text{dilution factor} * \text{extracted volume} * 100}{\text{Slope} * \text{weight of sample} * 1000}$$

Where,

A_s= absorbance of sample

A_B= absorbance of blank

Slope= obtained from the calibration curve

3.7. Experimental Design and Statistical Analysis

In the experiments conducted, the effect of addition level of Oyster mushroom powder to wheat flour on the functional properties of flours, proximate composition and sensory characteristics of bread were examined using a complete randomized design. All data were analyzed using SPSS version 16 software. Significant differences between means were identified by Duncan's multiple range tests at $p < 0.05$ significance level. All the values reported are means of two replicates unless specified.

4. Result and Discussion

4.1 Flour functional properties

4.1.1 Wet gluten content

The wet gluten test provides information on the quantity and estimates the quality of gluten in wheat or flour samples. Gluten is noted for its strong, three-dimensional, visco-elastic structure and is created by the hydrophobic, insoluble gliadin proteins (that contribute sticky, fluid properties to the dough) and the insoluble glutenins (that contribute elastic properties to the dough). Upon hydration and manipulation, the two proteins aggregate and form disulfide bridges producing a gluten protein matrix that subsequently is coagulated upon baking (Vaclavik *et al*, 2008).

Blending proportion generally significantly ($p < 0.05$) affected mean gluten content of flours. The values varied between 15.27 g/g and 30.16 g/g for 10% and 0% blending proportion, respectively. The mean gluten generally significantly ($p < 0.05$) decreased as blending proportion of OMP increased. Manal *et al* (2000) also reported that the weights of wet and baked gluten in experimental doughs were less than those in the control doughs and the dough added with powdered oyster mushroom at 10% level had significantly less amount of gluten.

Table 4: Mean scores of Wet gluten content and water absorption capacity of OMP- WF composite flours

Substitution level	Wet Gluten Content (g/g)	Water Absorption (ml/g)
0%	$0.301^{a} \pm 1.70$	$0.689^{d} \pm 0.47$
2%	$0.271^{a} \pm 0.39$	$0.774^{c} \pm 0.72$
4%	$0.219^{b} \pm 1.43$	$0.786^{bc} \pm 1.33$
6%	$0.193^{bc} \pm 1.03$	$0.801^{abc} \pm 0.75$
8%	$0.159^{cd} \pm 0.44$	$0.806^{ab} \pm 0.88$
10%	$0.153^{d} \pm 1.1$	$0.821^{a} \pm 0.46$

Reported values are mean ± SE, (n = 3) Means followed by different letters within a column are different at $p < 0.05$

The high water absorption capacity of OMP supplemented doughs must have resulted in the domination of the viscous component of dough, with a decreased resistance to extension (elasticity), increased extensibility (viscosity) and the development of sticky dough. (Mirsaeedghazi *et al*, 2008) In the current study, dough stickiness has been experienced while making breads from flour supplemented at higher ratios (6%-10%), which is in complete agreement with the findings of Manal *et al* (2000) and Mirsaeedghazi *et al* (2008), who cited (Launay, 1990) and stated that hydrophobic reactions have key role in elastic behavior of dough (gluten development).

Another explanation as to why the presence of OMP in WF has interfered with the gluten development might be because of high fiber content of OMP. Fiber dilutes wheat gluten functionality for two reasons; due to its high water holding capacity as well as lack of viscoelasticity (Mohamed *et al*, 2010)

4.1.2. Water Absorption Capacity

The absorption value of flour is an important quality factor. It is defined as the amount of water absorbed by flour when forming bread dough. The mean water absorption capacities of wheat flour and OMP were 0.69 ml/g and 3.17 ml/g respectively. Blending proportion significantly ($p < 0.05$) affected mean water absorption values, composite flours generally having significantly ($p < 0.05$) higher mean water absorption values than control flours. These experimental findings were also observed while dough preparation; composite flours required additional amount of water than control flour. Composite flour prepared from 6% OMP had no significance difference ($p > 0.05$) from composite flours prepared from 4% and 8% OMP. The results are within agreement with the Farinograph results reported by Hong *et al* (2005). Dhingra *et al* (2003) also reported increase in water absorption values of composite soy-wheat flours upon increased substitution.

The increase in water absorption values is due to the increase in protein and fiber contents in supplemented flours (Hong *et al*, 2005). Proteins absorb twice their weight of water (Vaclavik *et al*, 2008), which makes water absorption a function of the protein content of the flour. As of increased dietary fiber contents in composite WF-OMP flours, they contribute to increased water holding capacity, resulting an increase in water absorption. (Hong *et al*, 2005, Mohamed *et al*, 2010)

4.2. Evaluation of Bread Quality

4.2.1. Loaf Weight

Loaf weight is determined by the quantity of dough baked, the amount of moisture present in loaf and the amount of moisture and carbon dioxide diffused out of the loaf during baking. (Shittu *et al*, 2007) Doughs used to prepare breads in this study were carefully weighed (150 ± 2 grams) and transferred into baking pans and thus weight loss from doughs was all caused by water evaporation. Table 5 gives the mean percent moisture loss of breads and mean weights of experimental breads.

Blending proportion resulted significant difference ($p < 0.05$) in mean weight of loaves. Breads supplemented with 10 % OMP had highest mean weight (137.41g) while control breads had lowest mean weight (127.14g). The increase in weight of breads upon increased supplementation of OMP indicates that an extra amount of water was retained in breads supplemented with mushroom powder following baking. From Table 5 it can be seen that control breads have highest moisture loss (15.24 %) whereas breads that contained 10% OMP had the lowest (8.39%), which explains the reason for high loaf weight of the latter breads (Fan *et al*, 2010) The significant increase in weight of supplemented breads may also be attributed to higher water absorption of oyster mushroom powder than that of wheat flour (Ga- Hyung *et al*, 2005). Moreover, the less retention of gas in the blended doughs contributes to the gain in loaf weight, since less gas retention provides denser bread texture (Dhingra *et al*, 2004).

In the study which deals with the effects of Mushroom (*Lentinus Tuber-Regium*) powder on bread baking properties of wheat flour, Lee et al (2004) reported that loaf weight increased only up to 4% substitution and then decreased from 6% substitution onwards. The results in this study were comparable to those reported by earlier works done on yeast leavened breads by substituting wheat flour with non- wheat flour (Hyung *et al*, 2005, Ribotta *et al*, 2010, Tariqul Islam *et al*, 2007).

4.2.2. Loaf Volume

Blending proportion resulted significant ($p < 0.05$) decrease in loaf volume (up to 65.5%), with loaf volume decreasing significantly ($p < 0.05$) as blending proportion of OMP to WF increased (Table 5). Breads supplemented with 2% OMP having the highest mean bread volume (700 cm^3) and breads supplemented with 10% OMP the lowest mean bread volume (236.67 cm^3). The decrease in loaf volume was also reported by Hyung (2005), who supplemented WF with OMP up to 4%, but in his study, the decrease in loaf volume was as high as 27% at this respective blend proportion. Decrease in loaf volumes of breads was also obtained by incorporating high levels of soy proteins (Ribotta *et al*, 2010, Dhingra *et al*, 2004). It is widely accepted that large bread volume is a quality criteria for breads.

Bread physical qualities are influenced by many factors, such as wheat flour compositions, additives and dough fermentation conditions (Fan *et al*, 2010). Since the bread samples studied here have been produced from same dough size (250 g), and the same fermentation time (45 minutes), the variation in loaf volume could be attributed mainly to different rate of gas evolution of flours (which is a function of type of flour used).

To produce bread with good volume quality, two factors should be considered: (a) Dough should have a high viscosity to prevent gas cells rising and (b) dough should remain extensible at high level to prevent sudden breakage in gas cell membranes (Mirsaeedghazi *et al*, 2008) The protein content (quantity) and the gliadin/glutenin ratio (protein quality or gluten content) have primary roles in determining loaf volume of bread (Shittu *et al*, 2007**,** Uthayakumaran *et al*, 1999**).** Gliadins generally contribute to dough viscosity where as the glutenins contribute to dough elasticity, and it is the unique combinations of the dough viscosity and dough elasticity that comprises the functional properties of dough (Uthayakumaran *et al*, 1999**).** Substitution of OMP to WF must have caused an increase in gliadin/glutenin ratio. Mirsaeedghazi et al (2008) cited (Angioloni & Rosa, 2005) and stated that dough extension stability decreases and extensibility increases with increase in gliadin/glutenin ratio.

Table 5: Mean scores of the of blend proportion on loaf weight, volume and height of OMP substituted wheat breads after baking

Substitution level	**Loaf Weight (g)**		**Loaf Volume (cm^3)**		**Specific Volume(cm^3/g)**	**Loaf Height (cm)**	
	Weight	**Decrease * (%)**	**Volume**	**Decrease ** (%)**		**Height**	**Decrease (%)**
0%	$127.14^{d} \pm 0.84$	15.24	$686.67^{e} \pm 8.82$	**-**	$5.4^{a} \pm .04$	$6.43^{a} \pm 0.19$	**-**
2%	$129.65^{c} \pm 0.65$	13.56	$700.00^{e} \pm 5.77$	**ND**	$5.4^{a} \pm 0.06$	$6.1^{a} \pm 0.17$	5.13
4%	$133.49^{b} \pm 0.82$	11.00	$586.67^{d} \pm 17.64$	14.6	$4.39^{b} \pm 0.12$	$5.3^{b} \pm 0.21$	17.57
6%	$130.83^{c} \pm 0.71$	12.78	$450.00^{c} \pm 17.32$	34.47	$3.44^{c} \pm 0.15$	$4.8^{bc} \pm 0.20$	25.35
8%	$136.89^{a} \pm 0.40$	8.74	$396.67^{b} \pm 8.82$	42.23	$2.90^{d} \pm 0.06$	$4.67^{c} \pm 0.12$	27.37
10%	$137.41^{a} \pm 0.40$	8.39	$236.67^{a} \pm 20.28$	65.53	$1.72^{e} \pm 0.15$	$3.7^{d} \pm 0.15$	42.46

Reported values are the mean ± SE (n = 3). Means with different superscripts in the same column are significantly different ($p < 0.05$).
* Weight loss calculation based on the total weight of dough, 150 g used for each loaf of bread.
** Compared with the bread without OMP flour.
ND. Not determined

Decrease in dough extension capacity is an unfortunate event for the baker, and refers to the escape of CO_2 from the dough system, thus becoming unavailable for leavening. (Vaclavik *et al*, 2008). Loss of gas retention properties in OMP supplemented doughs may also be due to presence of small pores in the gluten fibrils, which must have allowed loss of gas during fermentation and baking (Ribotta *et al*, 2010, Dhingra *et al*, 2004)

4.2.3. Specific Volume

The specific volume, which is the ratio of the loaf volume to loaf weight, has been generally adopted in the literature as a more reliable measure of loaf size. In this study the specific volume of breads decreased as blending proportion of OMP to WF increased. Control breads exhibited highest mean specific volume (5.4 cm^3/g) while breads supplemented with 10% OMP had the least mean specific volume (1.72 cm^3/g). Apart from the specific volumes of control breads and breads supplemented with 2% OMP, there was a significant difference ($p < 0.05$) among all breads. The decrease in wet gluten content of WF upon increased supplementation of OMP (Table 5), explains that the gluten structure is not sufficiently developed to allow extension with the $CO_{2,}$ which accounts for the decreased gas retention capacity and volume of OMP supplemented WF doughs and breads. (*Vaclavik et al, 2008*, Dhingra *et al*, 2004)

Similar findings were reported by Dhingra et al (2004) in breads prepared from WF supplemented with barley and soy at different levels of inclusions. Hyung *et al* (2005) also concluded that low specific volumes could be from the dilution of gluten protein in dough system with the increase in addition level of OMP to WF. In addition, OMP could cleave gluten strands during mixing and change crumb texture. (Hyung *et al* , 2005).

4.2.4. Loaf Height

The effects of blend ratio of OMP on loaf height were similar to the effects observed on loaf volume; blending ratio resulted a significant decrease ($P < 0.05$) in mean loaf height of breads as substitution level increased. Mean scores of loaf height of breads made with 2% OMP did not show a significant difference ($p < 0.05$) in the loaf height when compared with the control breads. Control breads had the highest mean loaf height (6.43 cm) while breads baked by substituting 10% OMP had the least mean height (3.7 cm) that accounts a reduction of 42.26% as compared to the control breads. Moreover, irregularity in the shape of breads was observed in the breads baked from flours with 8% and 10% substitution level of OMP.

Higher loaf size (loaf weight, loaf volume and loaf height) have positive economic effect on bread at the retail end, as consumers often get attracted to bread loaf with higher size, believing that it has more substance for the same price. (Shittu *et al*, 2007**)** Once again, the reduction in loaf height as substitution of WF with OMP increased can be associated with the presence of increased dietary fibers, which leads to the presence of less wet gluten in the dough (Table 4). Fibers not only compete with proteins for water in the dough system, but also greatly interfere with protein associations by occupying the space of the proteins in the gluten network (Rosell *et al*). Fan *et al* (2006) obtained comparable results in breads blended with Auricularia auricular Polysaccharide flour and related the reduction in loaf height to the presence of less gluten in the dough formulations at increased substitution level, which caused less retention of fermentation gas.

4.3. Sensory Analysis

The sensory characteristics of breads from WF substituted with different ratios of dried oyster mushrooms were evaluated. The choice of food is dependent up on consumer's beliefs and attitudes, and thus sensory analysis is an important criterion for assessment of quality while developing new product. Table 6 gives the mean scores of evaluated sensory attributes for control and OMP substituted breads.

Table 6: Mean scores of the effect of blend ratio on the sensory qualities of OMP substituted WF breads

Breads	Appearance	Taste	Flavor	Color	Mouth Feel	Overall Acceptability
Control	$7.89^{a} \pm 0.20$	$7.75^{a} \pm 0.16$	$7.88^{a} \pm 0.23$	$7.67^{a} \pm 0.29$	$7.62^{a} \pm 0.46$	$8.00^{a} \pm 0.00$
BP1	$7.11^{a} \pm 0.26$	$6.50^{ab} \pm 0.68$	$6.62^{ab} \pm 0.32$	$6.78^{a} \pm 0.52$	$6.75^{ab} \pm 0.56$	$6.89^{ab} \pm 0.48$
BP2	$7.44^{a} \pm 0.24$	$6.75^{ab} \pm 0.31$	$5.88^{bc} \pm 0.23$	$7.33^{a} \pm 0.17$	$6.62^{ab} \pm 0.26$	$6.78^{ab} \pm 0.22$
BP3	$7.00^{a} \pm 0.33$	$5.88^{bc} \pm 0.55$	$5.88^{bc} \pm 0.40$	$7.11^{a} \pm 0.31$	$6.38^{ab} \pm 0.38$	$6.56^{bc} \pm 0.38$
BP4	$6.00^{b} \pm 0.37$	$4.62^{cd} \pm 0.63$	$5.00^{cd} \pm 0.71$	$6.56^{a} \pm 0.48$	$4.62^{c} \pm 0.63$	$5.44^{cd} \pm 0.63$
BP5	$5.78^{b} \pm 0.36$	$4.12^{d} \pm 0.55$	$4.12^{d} \pm 0.55$	$6.44^{a} \pm 0.41$	$5.25^{bc} \pm 0.68$	$4.78^{d} \pm 0.55$

Reported values are the means ± SE (n = 9). Means followed by different letters within a column are significantly different ($p < 0.05$) BP1-98%WFP, 2%OMP BP2-96%WFP, 4%OMP BP3-94%WFP, 6%OMP BP4-92%WFP, 8%OMP BP5-90%WFP, 10%OMP

4.3.1. Appearance

The highest mean score for appearance of breads was recorded for control breads, followed by breads supplemented with 4% OMP and 2% supplemented ones. Blending ratio did not cause significant difference ($p < 0.05$) in appearance of breads until 8% substitution of WF with OMP. Moreover, there was non- significant difference ($P < 0.05$) in mean appearance scores between breads with 8% and 10% OMP.

The appearance of a food includes its size, shape, color, structure, transparency or turbidity, and degree of wholeness or damage (Vaclavik *et al*, 2008). The decreased loaf size of 8% and 10% substituted breads might have contributed to their decreased appearance score. The odor of these breads might also have contributed for their appearance to be liked least, as input from other senses, especially smell, may contribute while evaluating appearance (ISO, 1992)

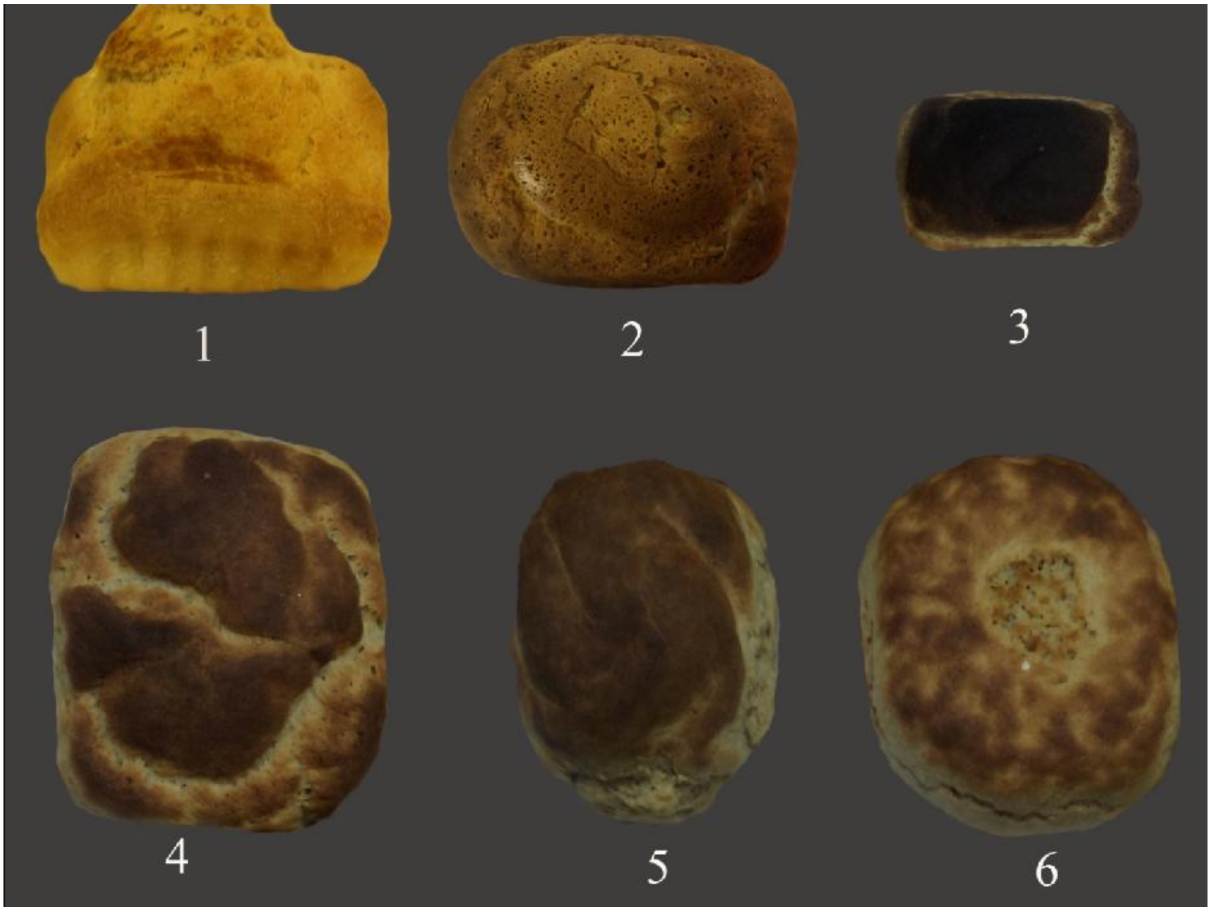

Fig 3: Pictures showing breads made from wheat flour and OMP composite flours
1. Control **2.** 98% WF, 2% OMP **3.** 96% WF, 4% OMP
4. 94% WF, 6% OMP **5.** 92% WF, 8% OMP **6.** 90% WF, 10% OMP

4.3.2. Taste

The trend for taste generally indicated significant decrease ($p < 0.05$) as blending proportion increased. Breads supplemented with 4% OMP had higher mean taste scores than those with 2% OMP. Mean bread taste scores varied significantly ($p < 0.05$) among the breads except for breads supplemented with 2% OMP and control breads. Supplemented breads were reported to have salty taste by panalists (data unavailable). Salty taste might be due increased lysine contents in breads (Rosenberg *et al*, 1951). Previous studies also reported oyster mushroom contained free bitter amino acids, such as arginine, histamine, isoleucine, leucine, methionine, phenylalanine, trypsine and valine, which cause bitterness (Mau *et al*, 2001)

4.3.3. Flavor

The mean scores for flavor were significantly different ($p < 0.05$) as a result of blending proportion. Control breads had the highest mean flavor score (7.88) while breads supplemented with 10% had the least mean flavor score (4.12). Panalists described breads having OMP to have an aftertaste and a meaty flavor (data unavailable), which implies the need to seek preliminary processing methods (e.g. heat treatment) to improve the performance of WF-OMP composite flours. Heat treatment (steaming for 30 min at 100^0C) was found to be effective for improving the flavor of cowpea flour used in cookie-preparation (Arshad *et al*, 2007)

Flavor, which is the combination of taste and aroma, could be correlated to the changes in generated volatiles during the baking process. (Vaclavik *et al*, 2005, Mohsen *et al*, 2009) The characteristic flavour of mushrooms, mainly dried, can be classified as derivatives of octane and octenes, lower terpenes, derivatives of benzaldehyde, sulphur compounds and others. The derivatives of octane, 1-octene and 2-octene, alcohols and their esters with volatile fatty acids, and ketones, form the very characteristic group of mushroom aroma. Free linoleic acid which is oxidised under catalysis of lipoxygenase and hydroperoxide lyase is characteristic for mushrooms and it is very intensive during drying (Kalac, 2009**)**

4.3.4. Color

Crust color did not show significant difference ($p > 0.05$) within all breads, which shows that the exterior of the product was browned to the preferences of the panelists. Mean scores for color of breads varied between 6.44 to 7.62 for 10% substituted and control breads respectively. Browning in breads is the result of Maillard browning, (which is the browning that results from proteins reacting with sugars) and Caramelization (which is the browning that results from sugars breaking down under heat) (Vaclavik *et al*, 2008)

4.3.5. Mouth Feel

Mouth feel evaluation refers to the textural properties of breads in the mouth, their ease of chewability and viscosity in the mouth. The mouth feel of supplemented breads were explained to be dry by Panelists (data not available) Once again, control breads had the highest mean mouth feel scores (7.62), but had no significant difference ($P > 0.05$) with breads prepared by substituting 2%, 4% and 6% OMP. Increase in blend proportion of OMP to WF generally resulted mean score values for mouth feel to decrease, indicating that control breads were easily chewable than substituted breads.

In this study, the visual texture of bread crumbs revealed that control breads had a light, smooth crumb texture while OMP substituted breads had a dense, rough and coarse crumb structure. The crumb of bread made with 10% OMP substitution was very dense, almost not developed. This could be explained by the fact that 10% OMP gave tough and sticky dough, with poor viscoelastic properties, even though extra water had been added.

Starch gelatinization is reported to be the main factor in structuring the bread crumb as starch becomes flexible and takes water from gluten, a process which helps gluten to set and become rigid by gelatinization **(**Hugo *et al*, 2000). During this process the starch goes from an ordered, crystalline state to a disordered, amorphous state. Upon cooling the disordered starch state begins to re-order (or retrograde), returning the starch back to its more rigid crystalline state, resulting in the firming of crumb texture in baked goods (Vaclavik *et al*, 2008). Hong et al (2005) included viscoamylograph data in his study, which showed a rapid increase in pasting temperature of wheat flour (from 63^{o}C to 74.5^{o}C) by supplementation of OMP, indicating that starch gelatinization within wheat flour was retarded by OMP. This factor might be responsible for the dry mouthfeel felt by panalists, as starches that gelatinize at temperatures higher than wheat starch (68^{o}C-78^{o}C) will have poor baking characteristics, with greater hardness and chewiness values. **(**Hugo *et al*, 2000**,** Ribotta *et al*, 2010)

4.3.6. Overall Acceptability

The overall acceptability rating in this study was done by panalists themselves. The mean overall acceptability of breads by panalists was in close range with the mean score of all the organoleptic characteristics evaluated in the present study. Control breads had highest mean overall acceptability (8.00) and had significant difference ($p < 0.05$) with 6%, 8% and 10% OMP substituted breads but not with those with 2% and 4% OMP substituted breads. Breads with 2% OMP were accepted better (6.89) than those with 4% OMP (6.78) and 6% OMP (6.56) although there was no significance difference ($p > 0.05$) between their acceptability means.

Breads with 10% OMP were least liked by panalists (4.78), which might be related to their taste flavor and mouth feel as well. These three organoleptic parameters are the ones that varied significantly among breads, which makes the relation between them and overall acceptability vital. Moreover, the decreased loaf volume and height as well have effects on overall acceptability. Scanlon *et al*, (2001) stated that bread crumb visual texture accounts for approximately 20% of the weighting used in judging bread quality.

4.4. Proximate Analysis

The mean values for moisture, crude protein, crude fat, total ash, total fiber and total carbohydrate are tabulated in Table 7. The effect of blend ratio on the nutritional quality of breads is described below.

4.4.1. Moisture

The mean moisture of breads at the crust and crumb was determined. Statistical analysis for mean moisture content of breads at the crust were not significantly different ($p > 0.05$) though there was an irregular decrease as substitution level increased. The decrease might be because OMP contained more total solids than wheat flour (Tariqul Islam *et al*, 2007). The crumb moisture on the other hand showed an increase in moisture content. Breads substituted with 10 % OMP had the highest crumb mean moisture (41.03 g/100g) while the control bread had the least (28.88 g/100g). The mean crumb moisture content of control breads were significantly different ($p < 0.05$) than substituted breads, but there was no significant difference ($p > 0.05$) among the crumb mean moisture content of substituted breads at all levels. Results regarding moisture in this study are different from moisture contents of breads from composite breads; Tariqul Islam (2007) reported moisture content of soy-substituted breads ranged from 33.95 - 35.65g/100g while Olaoye et al (2006) reported the moisture content of soy substituted whole wheat breads in the range 30.98 - 35.59g/100g. Given the high water absorption capacities of OMP- WF composite flours, the moisture results obtained in the current study are convincing (Zhang, 2004).

4.4.2. Crude Protein

The mean protein content of breads ranges from 10.44 – 12.16 g/100g. Control breads had the lowest mean protein content (10.44 g/100g) while bread substituted with 10% OMP had the highest (12.16 g/100g), resulting a 16.5% increase in the protein content of control breads. OMP used in the current study had a mean protein content of 27.13 g/100g (on dry weight basis), whereas, the protein content of wheat flour on 70% extraction rate is expected to be 12.7 g/100g (Dewettinck *et al*, 2008). The protein results for the later are higher because they used 6.25 as a conversion factor. The increase in protein content of breads can thus be related to the high protein present in OMP as compared to WF (Tariqul Islam *et al*, 2007). The statistical results despite the increase however show that control breads did not significantly vary ($P > 0.05$) in their protein contents with breads substituted up to 6% OMP level. But there were variation in protein contents ($p < 0.05$) among control breads and those substituted with 8 and 10% OMP. Consumption of 100g of 6% OMP substituted bread is close to meet half the nutritional minimum daily requirement set by FAO/WHO (25-30g per day) as recommended by FAO/WHO (2002) for children aged between 5 and 19 years.

4.4.3. Crude Fat

The crude fat values among breads showed a slight increase as supplementation with OMP increased, but the increase was not significant ($p > 0.05$). It can be concluded that no substantial enrichment in fat content of WF resulted by substituting it with OMP up to 10% level. This can be justified by the fact that oyster mushrooms are poor sources of fat and thus substitution of WF with OMP did not affect the fat content of breads. Rasool (2004) noted the fat content of chapattis substituted with mustard seed up to 20 g/100g ranged from 1.50 - 1.90g/100g. He also cited works of Gandhi et al (2000) who reported non-significant increase in fat content of breads made by substituting defatted oilseeds, irrespective of the level of substitution. The results of this finding are in line with the findings of Rasool (2004).   .

Table 7: Mean scores of blend proportion on proximate composition (g/100g) of OMP substituted wheat breads (dry weight basis)

Blend Ratio	Moisture*		Protein**		Crude Fat	Total Ash	Total Fiber	Total Carbohydrate***
	Crust	Crumb	Crude Protein	Protein increase (%)				
0%	$26.06^{a} \pm 0.81$	$28.88^{b} \pm 0.91$	$10.44^{c} \pm 0.21$		$1.68^{a} \pm 0.27$	$1.51^{e} \pm 0.20$	$1.87^{d} \pm 0.10$	55.62
2%	$25.43^{a} \pm 0.25$	$37.74^{a} \pm 2.06$	$10.70^{bc} \pm 0.05$	2.5	$1.78^{a} \pm 0.37$	$1.88^{d} \pm 0.20$	$1.93^{d} \pm 0.76$	45.97
4%	$25.04^{a} \pm 0.25$	$38.96^{a} \pm 1.17$	$11.19^{abc} \pm 0.64$	7.2	$1.79^{a} \pm 0.30$	$2.05^{c} \pm 0.70$	$2.40^{c} \pm 0.17$	43.61
6%	$26.32^{a} \pm 1.3$	$40.19^{a} \pm 0.84$	$11.45^{abc} \pm 0.45$	9.7	$1.80^{a} \pm 0.01$	$2.18^{bc} \pm 0.60$	$2.61^{bc} \pm 0.14$	41.77
8%	$24.85^{a} \pm 0.21$	$40.54^{a} \pm 0.10$	$11.77^{ab} \pm 0.30$	12.7	$1.81^{a} \pm 0.15$	$2.31^{b} \pm 0.60$	$3.06^{ab} \pm 0.19$	40.51
10%	$23.88^{a} \pm 1.17$	$41.03^{a} \pm 1.69$	$12.16^{a} \pm 0.15$	16.5	$1.82^{a} \pm 0.11$	$2.48^{a} \pm 0.30$	$3.33^{a} \pm 0.09$	39.18

Reported values are the mean ± SE (n = 2). Means with different letters in the same column are significantly different ($p < 0.05$).
*Wet basis **N X 5.25 *** Calculated by difference

Table 9: Mean Mineral Content of breads (mg/100g)

	Blend Ratio					
Mineral	0%	2%	4%	6%	8%	10%
Phosphorus	$16.01^{d} \pm 0.45$	$16.67^{cd} \pm 1.11$	$17.28^{cd} \pm 0.84$	$18.54^{bc} \pm 0.66$	$19.65^{ab} \pm 0.82$	$20.89^{a} \pm 0.62$
Iron	$14.62^{c} \pm 1.06$	$16.18^{bc} \pm 1.51$	$17.06^{bc} \pm 1.4$	$21.06^{ab} \pm 1.5$	$23.06^{a} \pm 0.60$	$25.60^{a} \pm 2.27$
Calcium	$1.33^{d} \pm 0.02$	$1.44^{dc} \pm 0.1$	$1.35^{d} \pm 0.09$	$1.61^{bc} \pm 0.06$	$1.70^{b} \pm 0.02$	$1.93^{a} \pm 0.05$

Reported values are the mean ± SE (n = 2). Means with different letters in the same row are significantly different ($p < 0.05$).

4.4.4. Total Ash

Mineral elements must be supplied from food because the body cannot synthesise them.The mean ash values of breads ranged from 1.53 - 2.50g/100g, resulting an increase following increased substitution of WF with OMP. All breads showed significant differences ($p < 0.05$) in their mean ash values, which resulted significant enrichment of WF with minerals at all levels of substitution with OMP in the current study. The mean ash values of WF and OMP were 0.84 g/100g and 7.55 g/100g respectively. Thus the accounted increase in substituted breads can be related with the presence of high ash content in OMP relative to WF.

4.4.5. Total Fiber

The mean fiber content of breads ranged from 1.87 - 3.3g/100g. Control breads had the lowest mean fiber content, while breads substituted with 10% OMP exhibited the highest mean fiber content. The statistical analysis regarding analysis of variance indicated that crude fiber content varied significantly ($p < 0.05$) as substitution level of WF with OMP increased. However, the means for crude fiber of control breads and bread substituted with 2% OMP did not show any significant variation ($p > 0.05$). The mean fiber content of OMP was 8.4 g/100g. Consequently increase in mean crude fibers of substituted breads is expected as the fiber content of WF is within the range 1.5g/100g - 2.2g/100g (Dewettinck *et al*, 2008). Foods with high fiber can reduce calorie uptake and provide health benefits related to chronic ailments like obesity, diabetes, cancer and cardiovascular disease (Gajula, 2007).

Mushrooms offer both soluble and insoluble fiber. The soluble fiber is mainly beta-glucans, a polysaccharide which may have anti-cancer properties and helps to lower total cholesterol levels and regulate blood sugar levels. Insoluble fiber of mushrooms constitutes of chitin and promotes regularity and good bowel health. It also helps slow digestion and adds satiety or staying power to foods (Mushrooms.ca, Kurtzman, 2005).

4.4.6. Total Carbohydrate

The carbohydrate content (calculated by difference) of different bread samples ranged from 39.18 - 55.62 g/100g. It may be noted that the total carbohydrate content of OMP free (Control sample) bread was higher (55.62 g/100g) than that of OMP substituted breads. The variations in carbohydrate content among these bread samples may result from the difference in the level of protein, fat, ash and moisture content of WF and OMP (Tariqul Islam *et al*).

4.5. Lysine Analysis

The lysine content of breads is given in Table 8. There was a significant ($p < 0.05$) increase in lysine content of breads as blending ratio of OMP to WF increased. Mean lysine content of breads made from 8% and 10% OMP however did not show any difference. The similarity in the mean lysine content of breads substituted with 8% and 10% OMP might be associated with the loaf size decrease in 10% OMP breads. A decrease in loaf size brings the crumb of breads closer to the crust, which might have destroyed the lysine due to Milliard reactions. However, lysine destruction can only be confirmed by quantifying the lysine in dough systems of each of these breads. The low levels of lysine in control breads can be associated with the low levels of lysine present in proteins from grains, such as wheat (Tariqul Islam *et al*, 2007, Elango *et al* 2007, Fleming *et al*, 1977). Although lysine is the most limiting essential amino acid in cereal proteins, its concentration is still high enough to meet the requirements of adults, but not those of children (Dewettinck *et al*, 2008). High lysine content of OMP substituted breads indicated that these breads can meet about a quarter of the daily lysine requirement for children (30 mg/Kg of body weight) as recommended by FAO/WHO (2002).

Table 8: Mean scores of the effect of blend proportion on Lysine Content of breads (g/100g)

Blend Ratio	0 %	2%	4%	6%	8%	10%
Lysine	$0.23^{d} \pm 0.01$	$0.25^{cd} \pm 0.01$	$0.26^{bc} \pm 0.01$	$0.28^{b} \pm 0.11$	$0.31^{a} \pm 0.01$	$0.31^{a} \pm 0.01$

Reported values are mean ± SE ($n = 2$). Means with different letters are significantly different ($p < 0.05$).

4.6. Mineral Analysis

Phosphorus

The effect of blend proportion on the phosphorus content of breads is shown in Table 9. Phosphorus content of breads generally increased significantly ($p < 0.05$) as blend proportion increased. Increase in phosphorus content of substituted breads might be associated with the presence of high amounts of phosphorus in OMP. Oyster mushrooms are reported to be rich sources of minerals, with 56-70% of total ash content to be constituted of potassium, phosphorus, calcium and magnesium (Chang *et al*, 2004, Feeney, 2006)

Iron

The mean iron content of control and substituted breads is given in Table 9. Control breads had the lowest mean iron content (14.62 mg/100g) while 10% OMP substituted breads had the highest (25.60 mg/100g). Blending ratio significantly ($p < 0.05$) increased the mean iron content of breads. It is reported that over 60 % of the world population is iron deficient (Skrbic *et al*, 2009), and thus utilization of OMP can be a means of overcoming iron deficiencies. Consumption of 100 grams of 6% OMP substituted bread will significantly enhance to meet the daily nutritional requirement (29.2 mg/day for males and 28 mg/day for females on 5% bioavailability) of children aged between 11 and 14 (FAO/WHO, 2002).

Calcium

The mean Calcium content of breads is given in Table 9, and ranged from 1.33 mg/100g (control bread) to 1.93 mg/100g (10% OMP substituted bread). Generally the calcium content of breads can be considered low. It is reported that higher levels of calcium in food products will interfere with the absorption of iron (FAO/WHO, 2002). Blend ratio significantly ($p < 0.05$) increased the Calcium content of breads. It can be conclude that OMP can be used to increase the calcium content of breads without interfering the absorption of iron.

5. Conclusion and Recommendations

5.1 Conclusions

Oyster Mushroom powder drastically decreased dough strength and negatively affected loaf size (volume, weight and height) due to the destruction of gluten matrix in wheat flour. The wet gluten content of composite flours decreased significantly from the control flour following increased supplementation with OMP. Breads prepared from OMP substituted flours had lower crust moisture but the crumb moisture increased. This increase in moisture accounts the fact that bread doughs needed more water than control flours as compared to control doughs. There was an increase in protein level of breads prepared by substituting WF with OMP as well. OMP substitution was also found to significantly increase the level of lysine in breads, indicating their supplementation with a cereal diet may help to overcome lysine deficiency. Moreover, the ash content and crude fiber content of substituted breads were also higher in substituted breads than control breads, leading to be conclusion that OMP can be a good supplement to breads in terms of nutritional improvement.

The results from the study showed that substitution of WF with OMP will greatly improve the nutritional values of white wheat breads, particularly in terms of lysine content, fiber and minerals that are important for health to consumers. However, the desirability of a food product is not necessarily correlated with its nutritional value. Appearance, taste, and aroma are often important in stimulating the appetite and determining preference. The sensory evaluation using a nine point hedonic scale indicated that control breads were more preferred by panalists than OMP substituted breads. Composite breads prepared by utilizing 6% OMP had an acceptability score which was above the "like slightly" range, which leads to the conclusion that acceptable breads can be prepared by utilizing OMP up to 6% in WF. In addition to nutritional enhancement, such breads had higher loaf weight since the water absorption of composite flour is high. Higher water absorption means that less flour is needed to make a loaf of bread, so if cost is a factor, this is an important point. Finally the output of this work will pave the way for researchers to work on the utilization of composite flour from OMP in Ethiopian bakery industry.

5.2 Recommendations

- Production of value added products from mushroom is a promising enterprise especially for the unemployed educated person because it is basically an indoor activity and can be effectively managed. Value added products, such as WF-OMP breads to the daily diets of Ethiopians, will not only cater to the protein requirement within the country but at the same time will encourage mushroom cultivation in rural as well as urban sectors, adding to the economy. As a result, stake holders like mushroom cultivators, governmental and non-governmental organizations, and research institutes need to promote the utilization of OMP via trainings and workshops.
- Breads with OMP above 6% level were least acceptable since they did not have appreciable loaf volume. But unleavened breads with greater nutritional value could be produced by inclusion of OMP more than 6% level. At the same time, the possibility of utilizing OMP to produce cookies need to be studied, as the outcome can be used to tackle the protein needs of consumers. Protein enriched cookies are attractive for target areas, such as child-feeding programmes, low-income groups and disaster relief operations.
- The key factor in producing acceptable breads is gluten structure of the flour, and this is the reason why the use of OMP was limited to 6% in the current study. Further investigations to improve the quality of WF-OMP composite breads can be done by utilizing other protein sources such as dairy proteins and egg proteins since they have functional properties similar to gluten and as a result may enhance strong cohesive viscoelastic structure within the dough system.
- The utilization of OMP in other cereals (which do not possess gluten) and the acceptability of the final products from these flours need to be studied to make maximum use of OMP in daily foods of Ethiopians, which is expected to result in value added products with enhanced nutritional qualities.
- The control of parameters like temperature, fermentation time and baking time combination during baking has to be understood and studied if acceptable breads with appreciable lysine values are to be produced on large scale by utilizing OMP composite flour.

References

AACC. 1984. American Association of Cereal Chemists. Approved method of the AACC). St. Paul, MN: AACC.

AACC. 2000. American Association of Cereal Chemists. Approved method of the AACC). St. Paul, MN: AACC.

Abbott, J. C. 1966. Protein Supplies of the World. Advances in Chemistry; American Chemical Society

Al-Adawy, T.A.1997. Effect of sesame seed protein supplementation on the nutritional, physical, chemical and sensory properties of wheat flour bread. *Food Chemistry* **59**:7-1

AOAC. 2000. Association of Official Analytical Chemists. Official methods of analysis Association of Official Analytical Chemists, Washington, DC

Ammar, M.S., Hegazy, A.E. and Bedeir, S.H.2009. Using of Taro Flour as Partial Substitute of Wheat Flour in Bread Making. *World Journal of Dairy & Food Sciences* **4 (2)**: 94-99

Atikpo,M., Onokpise, O., Abazinge, M., Louime, C., Dzomeku, M., Boateng, L. and Awumbilla, B. 2007. *Sustainable mushroom production in Africa: A case study in Ghana African Journal of Biotechnology* .**7 (3)**: 249-253

Aziah, N.A.A. and Komathi, C.A. 2009. Acceptabi;ity attributes of crackers made from different types of Composite flour. *International Food Research Journal.* **16**:479-482

Bala, B. K. , Morshed, M. A. and Rahman, M. F. 2009. Solar Drying of Mushroom using Solar Tunnel Dryer. *International Solar Food Processing Conference*

Bellido, G.G., Scanlon, M.G. and Page, J.H. 2009. Measurement of dough specific volume in chemically leavened dough systems. *Journal of Cereal Science* **49**:212–218

Bothina, A.-F. Evaluation of Mushroom Fortified Bread Made Using Wheat Flour at Different Rates of Extraction.2008. Master's Thesis

Braaksma, A., Schaap, D.J. 1996.Protein Analysis of the Common Mushroom Agaricus biosporous. *Postharvest Biology and Technology* **7**:119-127

Bryan, C.2004. Mushroom Drying and the Benefit to the Industry

Chang, S.–T. and. Miles, P.G.2004.Mushroom: Cultivation, Nutritional value, Medicinal effect and environmental impact, CRC Press 2nd ed, PP 27-35

Combs, B. 2004. Mushroom during and benefit to the Industry. CASNR Honors and Creative Achievements project

Czapski, J. and Szudyga, K.2000. Frozen Mushrooms Quality as Affected by Strain, Flush, Treatment Before Freezing and Time of Storage. *Journal of Food Science* **65**: 4

Das, M. and Kalita, M.C.2006. Value addition of Mushrooms. *Science Tech Entrepreneur*

DiMuzio, D. T. 2010. Bread Baking: An Artesian Perspective. John Wiley & Sons PP 3-5, 13-23

Dewettinck, K., Van Bockstaele, F., Kuhne, B., Van de Walle, D.,Courtens, T.M. and Gellynck, X. 2008. Nutritional value of bread: Influence of processing, food interaction and consumer perception. *Journal of Cereal Science* **48**:243-257

Dhingra, S. and Jood, S. 2004. Effect of flour blending on functional, baking and organoleptic characteristics of bread. *International Journal of Food Science and Technology*. **39**:213–222

Dunkwal, V., Jood, S., and Singh, S. Physico- Chemical Properties and Sensory Evaluation of Pleurotus sajor caju Powder as influenced by Pre-treatments and Drying Method. *British Food Journal*. **109(9):**749 - 759

Eddy, N. O., Udofia, P. G. and Eyo, D. (2007).Sensory Evaluation of Wheat/Cassava Composite Bread and Effect of Label Information on Acceptance and Preference. *African Journal of Biotechnology* **6 (20)**: 2415-2418

Eissa, H.A. and Zohair, A.2006. Quality and Safety of Halawa Modified with Mushroom. *Journal of the Science of Food and Agriculture* **86**: 2551-2559

El-Adawy, T. A. 1997. Effect of sesame seed protein supplementation on the nutritional, physical, chemical and sensory properties of wheat flour bread. *Food Chemistry*.**59**:7–14

Elango, R. ,Humayun,M.A.,Ball, R.O. and Pencharz, P.B.2007. Lysine requirement of healthy school-age children determined by the indicator amino acid oxidation method.*Am J Clin Nutr*. **86**:360 –365

Emmanuel, C. A. I., Osuchukwu, N. C. and Oshiele, L. 2010**.** Functional and sensory properties of wheat (*Aestium triticium*) and taro flour (*Colocasia esculenta*)composite bread. *African Journal of Food Science.* **4(5)**:248-253

Fan. L, Zhang.S, Yu. L.,Ma.L. 2010. Evaluation of antioxidant property and quality of breads containing Auricularia auricula polysaccharide flour. *Food Chemistry.***101**:158–1163

FAO. 2007**.** Mushroom production training for disabled people, Thailand- FAO

FAO/WHO. 2002. Protein and Amino acid requirements in human nutrition. Report of joint FAO/WHO/UNU expert consultation Geneva, Switzerland. PP 78-80

Feeney, M.J.2006. Mushrooms- Intake, Composition, and Research. *Nutrition Today*.**41**:5

Gajula, H. 2007. Fiber-Enriched Wheat Flour Precooked using Extrusion Processing: Rheological, Nutritional and Sensory Properties. Msc Thesis Kansas State University.

Gayle, P.E., Knight, E.M., Adkins, J.S. and Harland, B.F.1986. Nutritional and Organoleptic Evaluation of Wheat Breads Supplemented with Pigeon pea (Cajanus cajan) Flour. *Cereal Chem*. **63**(2): 136-138

Hong, G.-H., Kim,Y.-S. and Song, G.-S. 2005. Effect of oyster Mushroom (Pleurotus ostreatus) Powder on Bread Quality. *Journal of Food Science and Nutrition.* **10**: 214-218

Hugo, L. F., Rooney, W. and Taylor, J. R. N. 2000. Malted Sorghum as a Functional Ingredient in Composite Bread. *Cereal Chem*. **77(4)**:428–432

Kalac, P. 2009.Chemical composition and nutritional value of European species of wild Growing mushrooms: A review. *FoodChemistry*.**113**:9–16

Kausar, T.1988. Cultivation of mushrooms using crop residues as substrates. University Of Punjab

Kotwaliwale, N. , Bakane, P., Verma, A. 2007. Changes in textural and optical properties of oyster mushroom during hot air drying. *Journal of Food Engineering.***78**:1207-1211

Kurtzman, R. H. 2005. Mushrooms: Sources for Modern Western Medicine. *Micologia Aplicada International*.**17(2)**:21-33

Lee, M.-J.,Kyung, K.-H and Chang, H.-G.2004. Effect of Mushroom (Lentinus tuber- regium) Powder on the Bread Making Properties of Wheat Flour. *Korean Journal of Food Science and Technology* **36 (1)**: 32-37

Liu, Z. and Scanlon, M.G. 2003. Predicting Mechanical Properties of Bread Crumb. *Trans IChemE*. **81**

Mane, A., Bhoge, N., Kale, M.and Roodkar, P. 2004. Effect of incorporation of mushroom powder on gluten formation in wheat dough used for bread making. *Recent trends in biotechnology*.**35**:102-105

Marshall, E. and Nair, N.G .T. 2009. Make Money by Growing Mushrooms. *Food and Agricultural Organization of the United Nations* PP 33-34

Mattila, P., Vaananen, P. S., Konko, K., Aro, H. and Jalava, T.2002. Basic Composition and Amino Acid Contents of Mushroms Cultivated in Finland. *Journal of Agriculture and Food Chemistry* **50**:6419-6422

Mau, J.-L., Lin, H.-C ., Ma, J.-T. and Song, S.-F. 2001. Non-Volatile taste components of several specialty Mushrooms. *Food Chemistry*.**73**:461-466

Mirsaeedghazi, H., Z. Emam-Djomeh and S.M.A. Mousavi. 2008. Rheometric measurement of dough rheological characteristics and factors affecting It. *Int. J. Agri. Biol*. **10**:112–119

Mohamed. Abdellatif, Xu.J, Singh.S. 2010. Yeast leavened banana-bread: Formulation, processing, colour and texture analysis. *Food Chemistry* **118**:620–626

Mohsen, S.M., Fadel, H.M., Bekhit, M. A., Edris, AmrE. and Ahmed, M.Y. S.2009. Effect of substitution of soy protein isolate on aroma volatiles, Chemical composition and sensory quality of wheat cookies. *International Journal of Food Science and Technology*.**44**:1705–1712

Noor Aziah, A.A. and Komathi, C.A. 2009. Acceptability attributes of crackers made from different types of composite flour. *International Food Research Journal **16**:479-482*

Olaoye, O. A. , Onilude, A. A. and Idowu, O. A. 2006. Quality characteristics of bread produced from composite flours of wheat, plantain and soybeans. *African Journal of Biotechnology*.**5 (11)**:1102-1106

Oshodi, A. A. and Ekperigin, M. M. 1989. Functional properties of pigeon pea (*Cajanus cajan*) flour. *Food Chemistry.* **34(3)**: 187-191

Rai, R.D. and Arumuganathan, T. 2008. Post Harvest Technology of Mushrooms. *Indian Council of Agricultural Research*

Rasool, G.2004. Characterization and Evaluation of Wheat Flour Enriched with Oilseed Proteins for Chapati Making. Institute of Food Science and Technology University of Agriculture, Faisalabad

Ribotta, P. D., Pérez, G.T., Añón, M.C. and León, A.E.2010. Optimization of Additive Combination for Improved Soy–Wheat Bread Quality. *Food Bioprocess Technol* **3**:395–405

Rosell, C. M., Santos, E.and Collar, C. Physical Characterization of fiber-enriched bread doughs by dual mixing and temperature constraint using the mixolab. Cereal Group, Department of Food Science. Instituto de Agroquímica y Tecnología de Alimentos (CSIC)

Rosenberg, H.R. and Rohdenburg, L 1951. The Fortification of Bread with Lysine. I. The loss of Lysine during Baking. *Journal of nutrition*

Salehifar, M. and Shahedi, M.2007. Effects of Oat Flour on Dough Rheology, Texture and Organoleptic Properties of Taftoon Bread. *Journal of Agriculture Science and Technology*.**9**: 227-234

Scanlon, M.G., and Zghal, M.C.2001. Bread properties and crumb structure. *Food Research International.* **34**:841–864

Shittu, T. A., Raji, A. O., and Sanni, L. O. 2007. Bread from composite cassava- wheat flour: I. Effect of baking time and temperature on some physical properties of bread loaf. *Food Research International*. **40**:280-290

Skrbic, B., Milovac, S., Dodig, D. and Filipcev, B.2009. Effects of hull-less barley flour and flakes on bread nutritional composition and sensory properties. *Food Chemistry.***115**: 982–988

Tariqul Islam, A.F.M., Chowdhury, M. G. F., Islam, M. N. and Islam, M. S. 2007. Standardization of Bread Preparation from SoyFlour. *Int. J. Sustain. Crop Prod*. **2(6)**:15-20

Uthayakumaran, S, Gras, P. W., Stoddard, F. L. and. Bekes, F. 1999. Effect of Varying Protein Content and Glutenin-to-Gliadin Ratio on the Functional Properties of Wheat Dough. *Cereal Chem*. **76(3)**:389–394

Vaclavic, V., and Christian, E. W. *2008*. Essentials of Food Science. Springer Science. 3rd ed. PP 32-35, 46-52

Walde, S.G.,Velu, V., Jyothirmayi, T., Math, R. G.2006. Effects of Pretreatments and Drying Methods on Dehydration of Mushrooms. *Journal of Food Engineering* **74**:108- 115

Webb, C., and Owens, G. W. 2003. Milling and flour quality. 200-219 in: Bread Making—Improving Quality. S. P. Cauvain, ed. CRC Press: Boca Raton, FL.

Wieser H. Chemistry of gluten proteins. *Food Microbiol* 2007. **24**:115–119

Wujun, Ma and Sutherland, Mark, W. and Kammholz, Stephen and Banks, Phillip and Brennan, Paul and Bovill, William D. and Daggard, Grant. 2007. Wheat flour protein content and water absorption analysis in a doubled haploid population. *Journal of Cereal Science*. **45(3)**:302-308

Zhang, Y.C. Physicochemical Properties of and Isoflavone content of Bread made with Soy. 2004. Phd Dissertation. Ohio State University

Zivanovic, S.2006. Identifcation of oppourtnities for Production of ingredients based on Further processed Fresh Mushrooms, off- grade Mushrooms, bi-products and Waste Materials. Department of Food Science and Technology. *Mushroom Council*

Diatery Fiber. Mushrooms Canada at www.mushrooms.ca. Retrived on May 2010

Federation of bakers. 2002. www. Federationof bakers.com. Reterived on April 2010.

Figoni.2003.http://media.wiley.com/product_data/excerpt/69/04712685/0471. Retrieved on Feb.2011.

Tenbergen, K. 1999. Dough and Bread. http://sci.tech−archive.net/Archive/sci.med.nutrition/2005−06/msg00879.html. Retrieved on sepetember 2011

Wheat and Flour Testing Methods: A Guide to Understanding Wheat and Flour Quality.*www. Wheatflourbook.org*. (retrieved on November 2010)

Wikipedia, the free encyclopedia. www.wkipedia.org. Retrieved on February 2011

Appendices

Appendix-1: Score sheet for sensory evaluation of breads

Sample code: ______________ **Date: _________________**

Instruction

You are kindly requested to taste each of the six samples you are provided and choose the descriptor which, in your option, is the most applicable to the characteristics being evaluated. Eat the entire portion which is served to you before you make up your decision unless you decide immediately that the food is definitely unpleasant. Rinse your mouth with water provided after you have finished evaluating each sample and wait for approximately three minutes before evaluating the next sample.

Degree of Like/dislike	Appearance	Taste	Flavor	Color	Mouth Feel	Overall Acceptability
Like Extremely						
Like Very much						
Like Moderately						
Like Slightly						
Neither Like nor Dislike						
Dislike Slightly						
Dislike Moderately						
Dislike Very Much						
Dislike Extremely						

Comments: ....

....

....

Thank You!