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SCHOOL OF CHEMICAL AND BIO-ENGINEERING

**Development and Characterization of Bread from Mushroom and
Wheat Composite Flour**

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Abstract

This study was focused on substituting a part of whole wheat flour (WWF) with mushroom flour (MF) to develop mushroom bread for children, as an attempt to solve the problem in protein energy malnutrition of children from 1-5 years. The effect of mushroom supplementation on some physico-chemical and sensory properties of wheat bread was determined, as well as the dough rheological properties (farinograph) of the whole wheat flour with various proportions of the protein- rich mushroom flour were investigated. Examination of the functional properties of two different flours and breads were carried out. Analyses were made for different formulated bread and compared with the recommended daily allowance (RDA). The crude protein, crude fat, crude fibre, ash and energy of the composite bread loaves increased significantly ($p < 0.05$) from 10.21% to 23.92%, 1.72% to 1.92%, 1.59% to 2.57%, 0.88% to 2.69% and 275.4kcal to 276.45kcal, respectively; while the moisture content and carbohydrate decreased with increased level of supplementation from 30.84% to 28.02%, and 54.76% to 40.89% respectively. There was also a decrease in bulk density and bread volume by 22.22% and 44.86% respectively, with progressive inclusion of the mushroom flour. The sensory analysis showed that there was no significant difference ($p < 0.05$) in texture, taste, flavour and overall acceptability up to 12.5% substitution with mushroom flour. In this study, it was attempted to optimize the ingredient formulation and processing parameters of MF incorporated bread such as nutrient and sensory score responses. D – Optimal design was used which consists of WWF and MF, with 13 formulations for the nutrient and sensory score of the MF incorporated bread. The result showed that the optimized acceptability of the MF incorporated bread containing WWF 80.86%, MF 19.14%, protein 34g, energy 455.78kcal and overall acceptability of 6.2 was suitable for children between the age groups of 1-5 years being not lower than the recommended dietary allowance for this group of children. It was concluded that a substitution of 12.5% mushroom flour into wheat flour gave the bread with the best overall quality indices. Therefore Mixture Design was used to investigate the effects of Whole Wheat Flour (WWF) and Oyster Mushroom flour (MF) on the bread.

Keywords: Bread, characterization, mixture design, mushroom, optimization, protein, wheat.

List of Abbreviations

AACC – Americans Associations of Cereal Chemist

ANOVA - Analysis of Variance

AOAC - Association of Analytical Chemist

CSA - Central statics agency

EDHS -Ethiopia Demographic and Health Surveys

FAO - Food and agricultural organization of the united nation

FQN - Farinograph Quality Number

FU - Farinograph Unit

ICMBMP - International Conference on Mushroom Biology and Mushroom Products

Kcal - Kilo calorie

MDG -Millennium Development Goal

MF- Mushroom Flour

PEM - protein energy malnutrition

RDA - Recommended Dietary Allowance

UNICEF –United nation international children’s emergency fund

WWF – Whole wheat flour

WHO- World Health Organization

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1. Introduction

1.1 Background

Protein energy malnutrition is the major nutritional problems of the third world countries. Its prevalence ranges from 20-40% in Africa and Southeast Asia. In Ethiopia, according to CSA

rural nutrition survey in 1992, the highest prevalence of stunting was recorded in South Gondar (74.5%) and the lowest prevalence in South Omo (49.2). Whereas the highest prevalence of wasting was recorded in Tigray (14.2%), and the lowest in Bale (4.4%). Concerning the prevalence of underweight, the highest (59.9%) was recorded in Tigray and the lowest in Bale (29.2%). Generally, the prevalence of moderate and severe forms of stunting and underweight in Ethiopia showed an increasing trend over a decade according to the report on rural nutrition survey in 1992. However, PEM is mostly common in children under five years of age. Marasmus is common in children less than 12 months of age and kwashiorkor is prevalent in children less than 5 years, commonly in the age groups of 2-3 years. Children under 5 years of age are the most visible victims of PEM and most susceptible to PEM's characteristic growth impairment because of their high energy and protein need and their vulnerability to infection (WHO, 2010).

There is a very high incidence of malnutrition, especially of protein deficiency in developing countries. The problem of protein shortage in developing countries including Ethiopia is an existing reality and will continue for the foreseeable future. Protein malnutrition will become even more acute since the supply of protein for the diet has not kept pace with population growth (DHS, 2005).

Ethiopia is the 6th highest infant mortality rate in the world. Available data shows that, 504,000 children under 5 years old die per year in Ethiopia, Apart from the large number of child deaths, 52% of Ethiopian children suffer from stunting and 11% wasting; resulting from the long term effects of malnutrition. UNICEF (2004).

Many studies show that this problem is associated with different factors like improper weaning practice (early abrupt weaning with dilute and dirty formula), infections (diarrhea, measles, tuberculosis, pertusis, etc.), harmful traditional practices (age bias in feeding, sex bias, in feeding, food prejudices- omission from family diet), and child neglect. These factors do operate in the Ethiopian context. In Ethiopia, there is a cyclic occurrence of malnutrition in most rural agrarian communities following the turn of the seasons. The winter (rainy) season is therefore called the hunger (lean) season and that of the summer (dry) season is the harvest season. This seasonality of energy and protein intake is reflected in the variations in the prevalence of PEM in those two seasons.

Food fortification according to (Henkel, 2000) has been investigated that the addition of one or more nutrients to a food to improve its quality for the people who consume it, usually with the goal of reducing or controlling nutrient deficiency. Normal bread does not contain enough protein for human nutrition. Addition of mushroom powder is a cost-effective way to increase protein and other nutrient content in wheat bread. Taste and flavour of mushroom can also increase sensory quality of breads. Mushroom cultivation is comparatively an easy process and maybe cultivated in small piece of land with minimal maintenance and could be cultivated even indoor places. Industrial production might decrease the cost drastically.

However, ICMBMP (2012) reported that food supply should be both an economic and ecological subject, although health and nutrition involve balanced and sufficient functional food components. The amount of edible oyster mushrooms produced in modern plants for public nutrition that need balanced foods has increased. The production plant can bring economical benefits to unemployed people in these countries. Therefore, with the emphasis on utilization of Oyster Mushroom as a source of protein to enrich human diet especially in developing countries (Rabiee , 1990), appropriate processing technique was developed to preserve oyster mushroom which was utilized in bread enrichment.

Worldwide bread consumption accounts for one of the largest consumed foodstuffs, with over 20 billion pounds (9 billion kg) of bread being produced annually (Heenan et al., 2008). Water and flour are the most significant ingredients in a bread recipe, as they affect texture and crumb the most. Whole wheat or whole meal flour is made from the complete wheat kernel with nothing removed. This flour makes a fuller flavoured, nutritious but denser loaf than plain flour. (Trowell,1948).

1.2 Statement of the problem

Protein inadequacies are a problem facing risk population groups of the world, particularly populations in developing countries where diets consist mainly of cereals. Even in Ethiopia where governmental programs supply food and/ or food stamps, there are populations of low-income expectant mothers and children with inadequate diets. Increased utilization of high

protein source in basic food, such as bread, is a method of dealing with food shortages on a world scale.

The problems of malnutrition in Ethiopia, although different in magnitude and severity among different groups, are due to protein, vitamins, and mineral deficiencies since the diet of an average Ethiopia consists of foods that are mostly carbohydrate based, there is therefore, need for strategic use of inexpensive high protein and micronutrient food sources that will increase the protein content of the staple diet in order to enhance their nutritive value.

Currently, little information is available on nutritional characters of mushroom species that are native to Ethiopia. A balanced and sufficient diet is a problem for low-income people which results in protein malnutrition marasmus and kwashiorkor in the country. Supplementation of wheat flour with mushroom species will provide vital information to characterize them. Sufficient daily calorie intake is the main problem of our countries, hence the people cannot supply an adequate intake of essential food compounds such as proteins containing essential amino acids, vitamins, minerals and essential fatty acids. The protein content of Wheat flour is low due to separation of germ and bran during milling so that normal wheat bread naturally low in protein and nutritionally not a balanced diet due to lack of essential amino acid, lysine which is deficient in most cereal like wheat but edible oyster mushroom have these essential amino acid and can contribute formulation of balanced diet. Therefore, this study is dedicated to alleviate the above mentioned problems through blending wheat with oyster mushroom flour to improve the protein content and to enrich overall quality of the bread.

The multi-sectoral nature of nutrition contributes to this state and the integration of nutrition programs with health, agriculture, education and poverty reduction programs have been challenging. Protein energy malnutrition is mostly common in children under five years of age. Marasmus is common in children less than 12 months of age and kwashiorkor is prevalent in children less than 5 years, commonly in the age groups of 2-3 years. Protein malnutrition will become even more acute since the supply of protein for the diet has not kept pace with population growth. In order to meet the deficit most developing countries tend to import essential protein sources of food from abroad, spending large sums of their meager foreign exchange earnings. Such a situation has forced planners and nutritionists to think about unconventional alternative sources of protein such as mushrooms

1. 3 Objective of the study

1. 3.1 General objective

The main objective of this study was to develop and characterize bread from the blends of wheat and mushroom flours using mixture design.

1.3. 2 Specific objectives

- To determine the physico- chemical and functional properties wheat and mushroom flours
- To evaluate the effect of blend proportions on the nutritional content of the bread
- To study the effect of mushroom substitution with whole wheat flour on rheological properties of dough
- To determine optimal levels for the responses of mushroom flour incorporated bread.
- To suggest a manufacturing flow sheet for bread production from wheat and mushroom

1.4 Significance of the Study

- Using the research findings as reference for other projects.
- Encourages further research and development on value added products
- Findings of this study have potential to promote the production and diversification of mushroom consumption in Ethiopia and other African countries.
- Provide basic technical knowledge to develop appropriate processing technique in order to improve productivity and market success for bread maker
- Can bring economical benefits to unemployed people in these countries.
- To enhance the nutritive value of bread to fulfill the nutritional demand.

2. Literature review

2.1 The food habit in Ethiopia

In Ethiopia, foods are rarely modified to increase nutrient density to meet the needs of calorie for normal person. Traditionally prepared foods made of cereals or tubers may be low in several nutrients including protein, zinc and iron; these nutrients are of special importance due to their impact on physical and cognitive development. Furthermore, the bulkiness of traditional

foods and low concentrations of fiber and high concentration of inhibitors are major factors reducing their nutritional benefits.

Children at the age of five to twelve have the incomplete diet. This means most of the children do not have a balanced diet during feeding and results in low weight and slow growth. Moreover, when the malnourished children are grown up they become low resistant to diseases and, most of them will be of lower IQ (i.e. the ability to understand things and making decisions). When we see all these production of nutritious food is a critical importance in Ethiopia (Yewelsew, 2006).

2.1.1 Nutritional requirements and sources of balanced diet

The basic nutrients that a human being needs to have to be a healthy person are carbohydrate, protein, fat, vitamins, minerals and water. Depending on the sex, age, work, and basal metabolic rate the energy required for an individual can be grouped in to different categories but when we see the energy required depending upon age a man of weight of 60 kg who is heavy worker requires 3800 Kcal and above, but for office worker only requires 2425 Kcal. For a woman who is a sedentary worker requires 1875 Kcal, for moderate worker reaches to 2225 Kcal, and for heavy worker goes up to 2900 Kcal. Keeping all these, for pregnant women requires additional 300 Kcal more and for lactating women requires additional 500 Kcal more (Swaminathan, 1989).

Table-2.1. Nutrients for different population groups

Nutrients	Adults(sedentary)		Adolescent (16 – 18 years)		Children	
	Male	Female	Boy	Girl	7 –9 years	1 – 3 years
Protein (g)	67	53	76	61	51	39
Fat (g)	46	41	49	48	46	39
Energy(kcal)	2402	1890	2660	2085	1943	1384
Calcium(mg)	1242	1175	1490	1421	1208	1054
Iron (mg)	31	25	30	32	20	12

Vit.A(μ mg)	821	1025	1048	1470	835	626
Thiamine(mg)	1.88	1.5	2.19	1.63	1.35	0.91
Riboflavin(mg)	1.23	1.11	1.41	1.29	1.04	0.82
Nyasine(mg)	1.7	12	18	14	11	7
Ascorbic acid(mg)	93	99	106	148	82	55
Pyridoxine (mg)	1.9	1.30	1.9	1.3	1.1	0.7

Source: Swaminathan, 1989

The energy requirement of an individual, in a state of desirable equilibrium, is equal to the energy expenditure. In some clinical situations, where an improvement in nutritional status may be advisable, the energy requirement may be set at a higher level than the energy expenditure in order to produce, temporarily, a positive energy balance. In certain physiological states, such as during growth in children, or in pregnancy and lactation, the energy requirement may also be higher than the energy expenditure.

The energy requirement of an individual is the level of energy intake from food that will balance energy expenditure when the individual has body size and body composition, and level of physical activity, consistent with long-term good health; and that will allow for the maintenance of economically necessary and socially desirable physical activity. In children, the energy requirement includes the energy associated with the deposition of tissues at rates consistent with good health.

Table-2.2. Energy requirements of infants from birth to one year

Age (months)	Total requirement		
	(Kcal/kg/day)	Boys (Kcal/day)	Girls (Kcal/day)
0-5	124	470	445
1-2	116	550	505
2-3	109	610	545
3-4	103	655	590
4-5	99	695	630
5-6	96.5	730	670
6-7	95	765	720
7-8	94.5	810	750
8-9	95	855	800
9-10	99	925	865
10-11	100	970	905
11-12	104.5	1050	975

Source: European Journal of Clinical Nutrition, 1996

For children of age between zeros to six month it is required about 118 Kcal/ body weight of energy. If the child is at the age of 6 to 12 month, the energy required is about 108 Kcal/body weight for detailed data see Table-2.2 and Table-2.3. Children are growing fast and need body building nutrients, which are mainly carbohydrate, protein and fat. Children require a protein content of 1.25 g/kg body weight to 2 g/ Kg body weight depending on the health and body composition. The conversion factor for of energy from carbohydrate is four Kcal per one gram of carbohydrate, for proteins it is also the same, but for fat, the conversion factor is nine Kcal per one gram of fat.

The need for balanced diet is essential for any human being and, to fulfill this, people have to eat different kinds of foods, each food has its own nutritional value and Table-2.4 shows the nutritional content of each food and the required nutrition for a specific group of age and gender. The nutritional requirement of different groups is also shown in Table 2.5 and this shows the daily requirement of a person. In this Table it shows that children require high amount of protein and as they grow to adult stage the need for protein increases but this is due to the continued movement and work of that group.

Table-2.3 Food items for different population groups

Food	Adults (sedentary)		Adolescent (16-18 year)		Children (years)	
	Male (g/d)	Female(g/d)	Boys (g/d)	Girls (g/d)	7-9 (g/d)	1-3 (g/d)
Cereals	400	300	450	300	250	150
Pulses	50	40	60	50	40	35
Green leafy veg.	75	100	100	150	75	50
Other vegetable	75	50	50	100	50	20
Roots and tubers	75	50	75	50	50	10
Fruits	100	100	100	100	100	100
Milk	200	200	200	200	250	300
Sugar	20	20	20	20	20	15

Source: Swaminathan, 1989.

2.2 Malnutrition

The term malnutrition generally refers both to under nutrition and over nutrition, but in this guide we use the term to refer solely to a deficiency of nutrition. Many factors can cause malnutrition, most of which relate to poor diet or severe and repeated infections, particularly in underprivileged populations. Inadequate diet and disease, in turn, are closely linked to the general standard of living, the environmental conditions, and whether a population is able to meet its basic needs such as food, housing and health care. Malnutrition is thus a health outcome as well as a risk factor for disease and exacerbated malnutrition and it can increase the risk both of morbidity and mortality. Although it is rarely the direct cause of death (except in extreme situations, such as famine), child malnutrition was associated with 54% of child deaths (10.8 million children) in developing countries in 2001 (WHO, 2004).

Malnutrition continues to be a major public health problem in developing countries. It is the most important risk factor for the burden of disease causing about 300, 000 deaths per year directly and indirectly responsible for more than half of all deaths in children (Olaf , 2005, WHO, 2000). Malnutrition at the early stages of life can lower child resistance to infections, increase child morbidity and mortality, and decrease mental development and cognitive achievement and nutritional status is the best global indicator of well being in children (Leonor R, 2011). Adequate nutrition is the keystone of survival, health and development not

only of current generations but also of the ones to come. Malnutrition is the largest single underlying cause of death worldwide and is associated with over 1/3 of all childhood deaths(Ingunn ,2008 ,WHO, 2010)

Malnutrition is an underlying cause of the death of 2.6 million children each year, and one-third of the global total of children's (7.6 million child) deaths each year before their fifth birthday through weakening the body's resistance to illness. Malnutrition is a silent killer that are under reported, under addressed and, as a result, under prioritized. Every hour and minute of every day, 300 and 5 children die because of malnutrition respectively. In the world today, one child in four is stunted due to malnutrition, and in developing countries this figure is as high as one in three and specifically in Africa two out of five children's will suffered with malnutrition (Ibekwe,1994).

Globally, PEM continues to be a major health burden in developing countries and the most important risk factor for illnesses and death especially among young children (Muller, 2011). The World Health Organization estimates that about 60% of all deaths, occurring among children aged less than five years in developing countries, could be attributed to malnutrition (Faruque ASG 2008). The improvement of nutrition therefore, is the main prerequisite for the reduction of high infant and under five mortality rates, the assurance of physical growth, social and mental development of children as well as academic achievement. (Anwar, 2011).

Sub-saharan Africa bears the brunt of PEM in the world. On the average, the PEM associated mortality in subSaharan Africa is between 25 and 35% (Gernaat, 2011).

PEM is also associated with a number of co morbidities such as lower respiratory tract infections including tuberculosis, diarrhea diseases, malaria and anaemia (le Roux1, 2010). These co-morbidities may prolong the duration of hospital stay and death among affected children.

Several strategies have been used to improve the nutritive value of weaning foods. The traditional East - African weaning foods could be improved upon by combining locally available foods that complement each other in such a way that new pattern of amino - acids created by this combination is similar to that recommended for infants WHO (1999).

2.2.1 Malnutrition and child growth

Malnutrition commonly affects all groups in a community, but infants and young children are the most vulnerable because of their high nutritional requirements for growth and development. Another group of concern is pregnant women, given that a malnourished mother is at high risk of giving birth to a LBW baby who will be prone to growth failure during infancy and early childhood, and be at increased risk of morbidity and early death. Malnourished girls, in particular, risk becoming yet another malnourished mother, thus contributing to the intergenerational cycle of malnutrition. In developing countries, poor perinatal conditions are responsible for approximately 23% of all deaths among children younger than five years old. (Mahraj, 2003)

There is ample evidence that the growth (height and weight) of well-fed, healthy children from different ethnic backgrounds and different continents is remarkably similar, at least up to six years of age (Leonor et al., 2011). Based on this finding, WHO has been recommending that a single international reference population be used worldwide, with common indicators and cut-offs, and that standard methods be used to analyze child growth data (WHO, 1995). Moreover, growth assessment is universally applicable: it does not pose any cultural problems; measuring equipment is easy to transport; the tools are simple and robust, can be set up in any environment; users require little training; and the procedure is inexpensive and non-invasive (WHO, 1995).

Even though it has long been recognized that malnutrition is associated with mortality among children (Trowell, 1948; Gomez et al., 1956), a formal assessment of the impact of malnutrition as a risk factor was only recently carried out. In the early 1990s, results of the first epidemiological study on malnutrition showed that malnutrition potentiated the effects of infectious diseases on child mortality at population level (Pelletier et al., 1993).

Table 3 shows the global estimates for the prevalence's of underweight, stunting, and wasting in developing countries, as well as the estimated absolute number of children affected by growth retardation. Available data for these prevalence's are based on surveys covering 94% of the total population of under-5-year-olds in Latin America, 90% in Asia, 77% in Africa, and 66% in Oceania (see Table 1).

Table 2.4: Global estimates for the prevalence and number of under weight, stunted, and wasted children in developing countries

	% underweight	% stunted	% Wasted
Africa	27.4(31.6)	38.6 (44.9)	7.2 (8.3)
Asia	42.0(154.1)	47.1 (47.1)	10.8 (39.6)
Latin America	11.9(6.5)	22.2 (12.1)	2.7 (1.5)
Oceania	29.1(0.3)	41.9 (0.4)	5.6 (0.1)
All developing countries	35.8(192.5)	42.7 (229.9)	9.2 (49.5)

Figures in parentheses are millions of children

2.2.2 Child malnutrition in Ethiopia

Ethiopia is the second-most populous country in Africa, at nearly 84 million. Approximately 14% are children under five years of age (CSA 2007). These children and their mothers suffer disproportionately from the poor health and nutrition situation in the country. In fact, malnutrition is the underlying cause of 57% of child deaths in Ethiopia (SCUK 2009), with some of the highest rates of stunting and underweight in the world. As for women in the country, over a quarter had a low body mass index (BMI) (<18.5) in 2005; nearly one third among women 15-19 years of age. Contributing factors to under nutrition include widespread poverty, limited employment opportunities, poor infrastructure, high population pressure, low education levels, inadequate access to clean water and sanitation, high rates of migration and poor access to health services (Getahun ,2001;Bhutta, 2008). Without increased efforts to improve the nutrition status of vulnerable groups such as mothers and children under two years old, Ethiopia risks falling short of reaching the Millennium Development Goals (MDGs) of halving underweight and reducing child mortality by two-thirds by 2015.

Malnutrition is one of the leading causes of morbidity and mortality in children under five years of age in Ethiopia. The country has the second highest rate of malnutrition in Sub-Saharan Africa (FMOH, 2008). Malnutrition in children is one of the most serious public health problem in Ethiopia and the highest in the world (Gugsu, 2000). According to the 2011 DHS of Ethiopia, the prevalence of underweight, stunting, and wasting was very high; 30.7%, 33% and 45.3%, respectively for Somali region (CSA,2012) . Somali region is one of the most underserved regions in terms of access to essential services and characterized by a high level of child malnutrition, food insecurity, and vulnerable livelihoods.

While there has historically been an understandable focus on recurring humanitarian crises in Ethiopia, which have at times manifested as nutrition emergencies, there has also been a lack of policy and implementation around the problem of long-term, chronic malnutrition. This analysis focuses mainly on chronic malnutrition among young children and the current program and policy environment in place to prevent it, rather than emergencies and acute malnutrition, which tend to have different actors engaged in responsive action rather than preventive work (Taylor, 2011).

Nutrition outcome data (Table 2.4) are taken from the three most recent Ethiopia Demographic and Health Surveys (EDHS) (Measure DHS 2000, 2005, 2011). As of 2011 the under-five mortality rate was 88 per 1000, stunting prevalence was 44.4%, and underweight prevalence was 28.7%. These rates have decreased quite a bit in the past decade, most notably with mortality almost halving. Additionally, at the current rate of 1.22 percentage points per year, Ethiopia is finally on track to meet the first (MDG1) target of halving the number of underweight children under five years of age. However, Ethiopia still needs a concerted effort to accelerate reductions in under nutrition.

Table 2.5 EDHS data on prevalence of key child (<5 years) under nutrition outcomes

	2000	2005	2011	2015 Estimate ¹	2015MDG ²
U5 mortality rate	166	133	88	59	61
Stunting	57.8	51.5	44.4	39.5	N/A
Wasting	11	12	9.7	-	N/A
Underweight	42.1	34.9	28.7	23.8	23.8
Anemia (6-59)	N/A	53.5	44.2	-	N/A

¹Based on the current rate from 2000-2011

²Based on 1992 estimates from www.mdgss.un.org and www.mdgmonitor.org

*Not re-calculated using 2006 WHO growth standards; re-calculated figure will be higher

2.2.3 Protein malnutrition

Protein-energy malnutrition (PEM) is a diagnosis that includes several overlapping syndromes. The scientific basis for PEM was questioned in the early 20th century and different terms were introduced to describe it and there were different views as to its etiology. Controversies raged since 1930 and in 1935 Cicely Williams introduced the Ghanaian diagnosis Kwashiorkor (a disease of child displaced from breast by birth of the next one).

The term kwashiorkor -remained constant in spite of the criticisms because it doesn't describe the cause. Over the next 20 years around 50 different alternative names have been given to the same syndrome. In 1959, Jelliffe, proposed the term protein calorie malnutrition (PCM) to include all syndromes relating to inadequate feeding. This has been largely replaced by protein energy malnutrition (PEM) or malnutrition.

Protein energy malnutrition (PEM) develops in children whose consumption of protein and energy is insufficient to satisfy the body's nutritional needs. While pure protein deficiency can occur when a person's diet provides enough energy but lacks the protein, in most cases the deficiency will be dual. PEM may also occur in persons who are unable to absorb vital nutrients or convert them to energy essential for healthy tissue formation and organ function. Malnutrition is a major factor in causing infant mortality in the tropics and sub-tropics. Current treatment for children involves the use of special formulated foods which are either labelled as F-100 or F-75 which is expensive and not sustainable in the long term. (Srikanth, 1990).

2.2.4 Protein malnutrition in Ethiopia

According to Ethiopia public health training initiative in 2001 reported that ,causes of protein energy malnutrition are multi-factorial having a number of interwoven factors operating simultaneously. The causes could be categorized as immediate, underlying and basic.

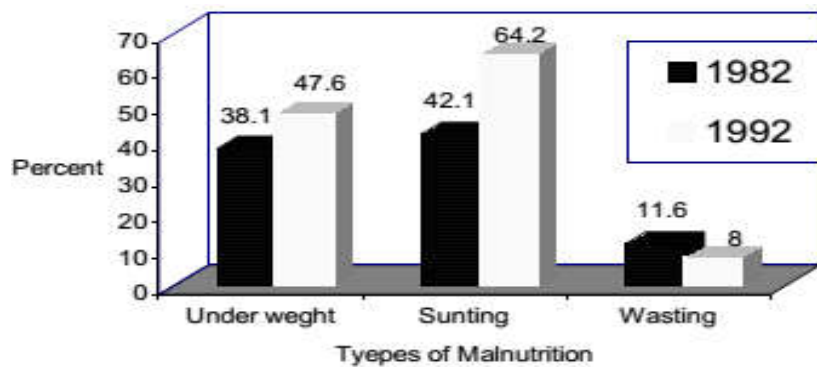


Figure 2.1. Trend of protein Energy malnutrition in children 5-59 months in Ethiopia over 10 years (1982-1992)

Source: CSA report on rural nutrition surveyor module, 1992

2.3 Wheat

In Ethiopia, the total yield of bread grains does not satisfy the needs of the country. The total production of bread wheat grains cover only about 60% of the total needs. The way to overcome this problem is to search for the native cereal sources or others which could be used with wheat flour bread making. To be able to make our particular bread type we must have an understanding of the complex interactions between the raw materials and the methods used in the conversion processes from ingredients to baked product. The raw materials will change and our processes are time and temperature sensitive. Given the intricate nature of the process it is a wonder that we manage to make bread at all. So that because of accumulated knowledge (craft) augmented these days by scientific and technological understanding. Most of bakery products are used as a source for incorporation of different nutritionally rich ingredients for their diversification (Gandhi et al., 2001). The enrichment of protein may be achieved through the incorporation of protein-rich non-wheat flours

Although wheat flour is not the only ingredient employed in bread production, it plays a pivotal role in determining the overall quality of bread (World Grain Statistics, 2007). As a matter of fact, it is common to consider bread quality in terms of loaf dimensions, crumb texture and crust visual aspect and consistency, which are in a whole a set of properties deeply related to the characteristics of the flour employed in production, either directly, in terms of strength and technological behaviour of the dough (Samaan, 2006 ; El-Khayat,2006) or indirectly, by

influencing the different bread-making phase conditions (MacRitchie,1987 and Graybosch,1993).

The baking potential of wheat flours is influenced by many factors, most notably protein content. Protein content is in turn influenced mainly by nitrogen fertilization, while the protein quality is determined primarily by the wheat genotype (Samaan,2006 : El-Khayat,2006). On the other hand, both the quality and the content of the wheat protein are affected by the climatic conditions during wheat maturation (Abboud , 2012).

When considering all these aspects, and moreover when moving from the hand-crafted to the industrial baking process, it is clear that a precise knowledge of flour chemical and rheological properties is of paramount importance for the optimization of the process itself. In an industrial baking context, the modifications of the recipe and of the production parameters are a direct consequence of flour features, such as its strength and extensibility, which for example regulate the amount of yeast, water and other ingredients to be added, the mixing time and the temperature and humidity in the leavening step. The main target is to be able to evaluate the effect of flour properties on production, in terms of dough workability, which has to be such that no huge and sudden changes in the recipe and process parameters are required, and of bread quality, which should remain as constant as possible in order to satisfy the consumer's needs

2.4 Mushroom (*Pleurotus ostreatus*)

The oyster mushroom, (*Pleurotus ostreatus*), and white button mushroom, (*Agaricus bisporus*), are the most widely cultivated edible mushrooms worldwide (Kues and Liu, 2000; Sánchez 2010). Oyster mushrooms are a diverse group of saprotrophic fungi belonging to the genus *Pleurotus*(Kong, 2004). According to Croan (2004), these mushrooms are a good source of non-starchy carbohydrates, with high content of dietary fiber and moderate quantity of proteins, including most amino acids, minerals, and vitamins. Moreover, Randive (2012) reported that oyster mushrooms are rich in Vitamin C, B complex, and mineral salts required by the human body. Therefore, mushrooms can be a good supplement to cereals (Chang and , 1996)

World production of cultivated edible mushrooms is estimated to be almost 5 million tonnes, valued at about \$9.8 billion per year, to which Africa contributes a very small proportion. Total global production of mushrooms has increased more than tenfold in the past 25

years and the market for mushrooms is growing. The production of mushrooms, therefore, has the potential to generate a significant cash income.

However, many people are apprehensive about mushrooms as a food source. Ignorance has led many to become skeptical about whether food of fungal origin can hold any great nutritional promise. It seems much education is needed before full advantage can be taken of this readily available, nutritionally rich food source (Crisan and Sands, 1978; Chang and Mshigeni, 2001). There is a very high incidence of malnutrition, especially of protein deficiency in developing countries. The problem of protein shortage in developing countries including Ethiopia is an existing reality and will continue for the foreseeable future.

A detailed account of the compositional analyses of cultivated and wild species of edible mushrooms has been reported elsewhere (Crisan and Sands, 1978). Also mushrooms have been used as human food for centuries, being valued particularly for the variety of flavours and textures they can provide (Sadler, 2003). However, they have nutritional value and can be useful food supplements, although species vary in their nutritional value (Crisan and Sands, 1978). Protein tends to be present in an easily digested form and on a dry weight basis. Mushroom normally ranges between 20 and 40% protein which is better than many legume sources like soybeans and peanuts, and protein-yielding vegetable foods (Chang and Buswell, 1996; Chang and Mshigeni, 2001). Moreover, mushroom proteins contain all the essential amino acids needed in the human diet and are especially rich in lysine and leucine which are lacking in most staple cereal foods (Chang and Buswell, 1996; Sadler, 2003). Mushrooms are low in total fat content and have a high proportion of polyunsaturated fatty acids (72 to 85%) relative to total fat content, mainly due to linoleic acid. The high content of linoleic acids is one of the reasons why mushrooms are considered a health food (Chang and Mshigeni, 2001; Sadler, 2003). Furthermore, they contain significant amounts of carbo-hydrates and fibres (Crisan and Sands, 1978; Chang and Buswell, 1996). In Turkey, there have been important studies and development strategies done on mushroom productions at the universities and in the developed private companies. Not only the quantity and the numbers of cultivated mushroom species, but also scientific researches about cultivation techniques of edible wild mushrooms which have nutritional and medicinal aspects are increasing). Increasing consumption of

mushroom is good for preventing malnutrition, although mushrooms cannot be an alternative protein source for of meat, fish, and egg (Çalarirmak et al., 2002).

Edible oyster mushrooms have been traditionally appreciated due to their excellent sensory characteristics, including their unique aroma and taste. These type of Mushrooms contain a wide range of molecules responsible for the fungal flavor comprising of volatile compounds (Chiron and Michelot, 2005) and unsaturated fatty acids, as well as amino acids (Maga,1981). Besides their excellent flavor, mushrooms have attracted much attention due to their proven healthy properties. Oyster Mushrooms contain low fat levels and low energy values, though they contain high amounts of proteins and essential amino acid

Addition of this type of mushroom powder is a cost-effective way to increase protein and other nutrient content in wheat bread. Taste and flavor of oyster mushroom can also increase sensory quality of breads. Mushroom cultivation is comparatively an easy process and may be cultivated in small piece of land with minimal maintenance and could be cultivated even indoor places. Industrial production might decrease the cost drastically. Some of the most well known food fortification examples are Iodine in salt, Niacin in bread, Calcium in beverages etc. Wheat (*Triticum aestivum*) is a grass, originally from the Fertile Crescent region of the Near East, but now cultivated worldwide. Globally, wheat is the leading source of vegetable protein in human food, having a higher protein content than either maize (corn) or rice, the other major cereals (Belderok et al., 2000).

2.5 Health benefit of mushroom

During the past 50 years, several major advancements in medicine came from lower organisms such as molds, yeast, and mushrooms. Of approximately 140,000 known species of mushroom, 2000 are safe for people's health, and about 300 of them possess medicinal properties. Of about 300 mushroom species with known medicinal properties, only about 20 species are in use at the present. Most of traditional knowledge about medicinal properties of mushrooms came from the Far East (China, Japan, Korea, Siberia), where such mushrooms as Reishi, Shiitake and others were collected, cultivated and used for thousands of years (Wasser, 2002) .

Of all cultures, mushrooms were least valued in the West, especially in regard to their use as medicine (Halpern, 2007). But now the western countries have just started to

ponder into diversity and great potential of mushrooms. Many pharmaceutical substances with potent and unique properties were recently extracted and made their way all around the world (Wasser, 2002). They are known to contain pharmacologically active components which cause no harm nor place additional stress to the body (Oyetayo, 2002). They are also probiotic, help our body strengthen itself and fight off illness by maintaining physiological homeostasis, restoring our bodies balance and natural resistance to disease. Mushrooms have a beneficiary effect on prebiotics in the gastrointestinal tract, helping promote healthy bacteria. They are also adaptogens, substances that help the body cope during times of stress. The compounds they contain have been classified as host defense potentiators (HDP) which can have immune system enhancement properties. That is the reason why currently used as adjuncts to cancer treatments in Japan and China (Halpern, 2007).

2.6 Bread

Bread is a staple food prepared by cooking a dough of flour and water and often, additional ingredients. Doughs are usually baked, but in some cuisines breads are steamed, fried, or baked on an uncoiled skillet. It may be leavened or unleavened. Salt, fat and leavening agents such as yeast and baking soda are common ingredients, though bread may contain other ingredients, such as milk, egg, sugar, spice, fruit (raisins), vegetables (onion), nuts or seeds (poppy). Referred to colloquially as the "staff of life", bread has been prepared for at least 30,000 years. The development of leavened bread can probably also be traced to prehistoric times

The simplest breads are made from grains such as wheat, oats, barley, rye, millet and corn mixed with water or milk. These ingredients are mixed into dough, shaped and cooked usually by baking. Salt, eggs, sugar and other ingredients may be added to give the bread flavor, change its texture or increase its nutritional value. A special ingredient is added often called leavening agent to make the dough rise by enlarging the air spaces in the dough, giving it a lighter texture and more volume (Adow et al., 1991).

Since bread is an important food that is generally accepted, they could be an excellent and convenient food item for protein fortification to improve the nutritional well being health of the people and in nutritional programs which will enhance reduction in protein malnutrition that is prevalent in Ethiopia and other developing countries. Fortification of wheat flour with high protein materials from plant sources to increase the protein and improve the essential amino acid

balance of the resultant baked product such as bread has been recognized (Misra et al., 1991). Fortification of wheat flour with high protein materials from plant sources to increase the protein and improve the essential amino acid balance of the resultant baked product such as bread has been recognized (Misra et al., 1991). The enrichment of protein may be achieved through the incorporation of protein-rich non-wheat flours (Gandhi et al., 2001; Sharma & Chauhan, 2002). Among them chick peas, kidney peas and Oyster mushroom has a great potential, due to their high and good quality-protein, whereas legumes and mushrooms have better nutritional qualities (higher lysine and soluble dietary fiber and lower methionine than wheat) and better baking qualities than rye, but oyster mushrooms have soluble and insoluble dietary fibre especially β -glucan. Therefore, fortification with high protein legume flours could provide a good opportunity to improve the nutritional quality of protein consumed by many people. Also, fortification of wheat flour with non-wheat proteins increases protein quality by improving its amino acid profiles.

3. Materials and methods

3.1 Materials

The study was conducted at different laboratories of Chemical and Food Engineering, Bahir Dar University and Kality Food Share Company. Wheat grain variety L-20” Digilu” variety was obtained from Holetta agricultural research center. Fresh Oyster Mushroom (*Pleurotus ostreatus*) was purchased from commercial mushroom producer in Addis and kept at 3–4°C for 2 days. Sugar, yeast, shortening (fat), salt, and other ingredients were collect from the local market. High density polyethylene bags were used for packaging and storage of samples. Major equipment used in the study was roller miller, oven dryer, mixer, and farinograph.

3.2 Methods

3.2.1 Preparation of composite flours

The whole wheat seeds and oyster mushroom were cleaned from dirt by sorting out contaminants such as sands, sticks and leaves, and were later washed and oven dried. . Both the dried whole wheat and oyster mushroom were later milled using roller mill and sieved into fine flour of uniform particle size, by passing them through a 2 mm mesh sieve as shown in Figure 3.1.

Table 3.1: Baking formula* and conditions of WF-MP bread

Wheat flour** (%)	100
Dry yeast (%)	1
Salt (%)	2
Sugar (%)	6
Fat (%)	1
Water	Variable
Fermentation	1½ h at 30°-32°C
Proving	1 h at 30-35°C

Baking	25-30 min at 220-250°C
RH	85-90%

Source : Okafor (2012)

*Ingredients listed as percent of flour.

**The wheat flour (WF) was replaced by different ratio of mushroom flour (MP)

3.2.2 Dough Preparation

The whole wheat flour was mixed with different proportion of the mushroom-flour. The composite flours were blended with other baking ingredients (Table 3.1) in a mixer, kneaded for 7 min into consistent dough and the resulting dough was mold and placed in a pre-oiled baking bowl (Hesham et al). The dough was proved for 45 to 60 min at 35°C and 85% relative humidity and baked in a reel oven for 35 min at 217°C.

3.2.3 Baking Process

Bread was baked from the flour samples using the Straight Dough Method of Chauhan et al.,1992. All ingredients were thoroughly mixed in a dough mixer to form dough, which was put into a baking pan greased with plasticized fat and covered with greased bread wrapper. The doughs were fermented for 90 minutes at room temperature (28°C - 30°C), proved at 35°C - 40°C for 90 minutes, and baked at 250°C for 30 minutes. The bread loaves were packaged in low density polyethylene bags for consumption and stored at room temperature for further analysis.

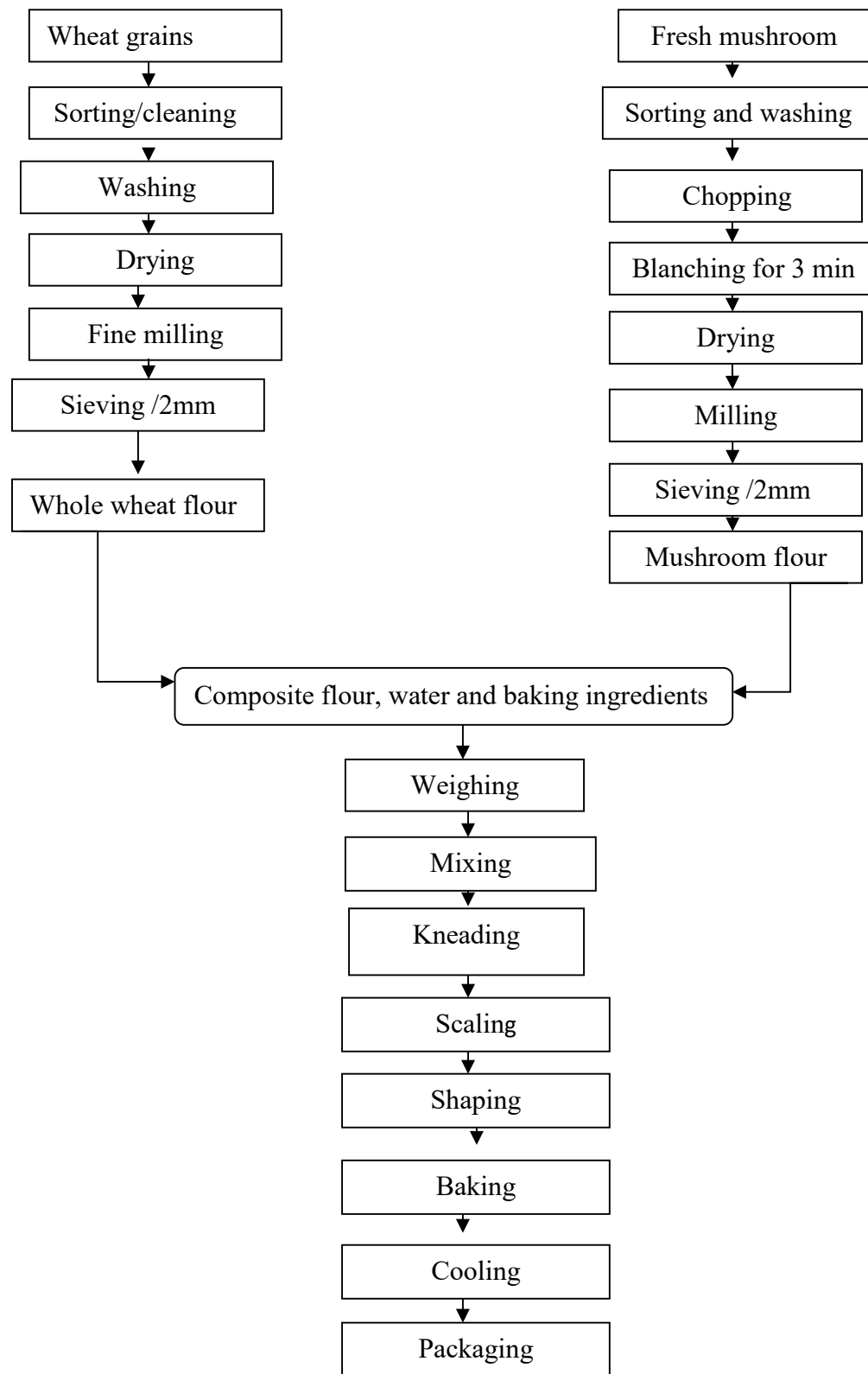


Figure 3.1. Flow chart for the production of functional composite bread

3.2.4 Analysis method

3.2.4.1 Proximate analysis flour and bread sample

The Association of Official Analytical Chemist (AOAC, 2000) procedure was used to determine the proximate compositions of the wheat flour, mushroom flour and the bread samples made from the various blends of the above flours.

Determination of Moisture Content

Three (3) grams of each of the samples was weighed out with the aid of an analytical balance into dried, cooled and weighed dish in each case. The samples in the dishes were then put into a Genlab moisture extraction oven set at 105⁰c and allowed to dry for 3 hours. When this time elapsed, the samples were then transferred into a dessicator with the aid of a laboratory tong and then allowed to cool for 30 minutes. After cooling in the dessicator, they were weighed again and their respective weights recorded accordingly. The above processes were repeated for each sample until a constant weight was obtained in each case. The difference in weight was calculated as a percentage of the original sample.

$$\text{Percentage moisture content} = \frac{W_2 - W_3}{W_2 - W_1} \times 100 \quad \dots\dots\dots(3.1)$$

Where W_1 = Initial weight of the empty dish, W_2 = Weight of the dish + undried sample and W_3 = Weight of the dish + dried sample.

Determination of Ash Content

Three (3) grams of each of the samples was weighed out with the aid of an analytical balance into a dried cooled and weighed crucible in each case. The samples were then charred by placing them on a Bunsen flame inside a fume cupboard to drive off most of the smoke for 30 minutes. The samples were thereafter transferred into a pre-heated muffle furnace already at 550⁰C with the aid of a laboratory long. They were allowed to stay in the furnace for 3 hours until a white or light grey ash resulted. Samples that remained black or dark in colour after this time had elapsed were moistened with small amount of water to dissolve salts, dried in an oven and then the ashing processes repeated again. After ashing, the crucibles were then transferred into a dessicator with a laboratory long. When they cooled, they were each weighed again and recorded accordingly.

$$Ash(\%) = \frac{W_3 - W_1}{W_2 - W_1} \times 100 \quad \dots\dots\dots (3.2)$$

where W_1 = Initial weight of the empty dish, W_2 = Weight of the dish + undried sample and W_3 = Weight of the dish + dried sample

Determination of Crude Fibre Content

Weigh about 3-20 g sample according to the content of crud fiber. The sample were exactly weighted and transfer to the round bottom flask and shake first with 60 ml acid mixture and then washed the inside wall of the flask with the rest of 20 ml acid mixture. Then the flask were heated for about 30 min long under condenser. After disassembling the condenser and the flask, the flask were cooled first in air and then with running water from the tape.

Ashe free filter paper was heated 1h long by $103 \pm 2^\circ\text{C}$ and cooled in desiccators and exactly weighted. The treated analysis sample was filtered with the treated filter paper, which is already exactly weighted. (Use suction flask). The flask was rinsed with hot water many times and washed the sample until it is free from the acid (check with PH-paper). Then after wash the sample three times with 10 ml ethanol and two times with each 10 ml diethyl ether.

The filter paper with the sample was placed on heat resistant porcelain crucible, which was heated, cooled and weighted. The crucible with the sample was heated 1 h long by $103 \pm 2^\circ\text{C}$ and then cooled in desiccators and exactly weighted. Filter paper with the sample were heated in muffle oven 1h long by 700°C and then cooled and exactly weighted.

The percentage of the crude fiber content (R) is calculated as follows.

$$R(\%) = \{(m_1 - m_f) - m_2/E\} * 100 \quad \dots\dots\dots (3.3)$$

Where m_1 = weight of the residual + weight of Filter paper

m_f = weight Blank probe

m_2 = Weight of the ash

E = Sample weight in g

Determination of Crude Protein Content

(0.1 -0.5g) of each of the samples was mixed with 10ml of concentrated H_2SO_4 in a kjeldahl digestion flask. A tablet of selenium catalyst was added to each of samples which were then digested (heated) inside a fume cupboard until a clear solution was obtained in a separate flask in each case. Also, a blank was made by digesting the above reagents without any sample in it. Then, all the digests were carefully transferred into a 100ml volumetric flask in each case and were made up with distilled water. A 100ml portion of each digest was mixed with equal volume of 45% NaOH solution in a kjeldahl distilling unit. The resulted mixtures were each distilled and the distillates collected in each case into 10ml of 4% boric acid solution containing three drops of mixed indicators (bromocresol green and methyl red). A total of 50ml of each distillate was obtained and titrated with 0.02 molar H_2SO_4 solution. Titration was done from the initial green colour to a deep red end-point.

The nitrogen contents of each sample were calculated thus;

$$\text{Nitrogen\%} = \frac{V_{HCl} \text{ in } L \times N_{HCL} \times (ca.0.1) \times 14.00 \times 100}{\text{sample weight on dry basis}} \dots\dots\dots(3.4)$$

Where V is volume of HCl in L consumed to the end point of the titration, N is the normality of HCl used often is about 0.1N and 14.00 is the molecular weight of nitrogen. Percentage of nitrogen is converted to percentage of protein by using appropriate conversion factors as follows:

$$\% \text{ Protein} = F \times \% N \dots\dots\dots(3.5)$$

Where, F is the conversion factor in most cases is 6.25. Use this factor in this experiment.

Determination of Fat Content

Weigh accurately 3-4g of sample into a thimble lined with a circle of filter paper. Place thimble and contents into a 50ml beaker and dry un an oven for 2 h at $110^\circ C$.

Transfer thimble and contents to extraction apparatus. Rinse the beaker several times with the solvent. Extract the sample contained in the thimble with the solvent in a Soxhlet extraction apparatus for 6-8 h *at a* condensation rate of *at least* 3-6 drops per second. At the completion of the extraction, transfer the fat extract from the extraction flask into a pre-weighed evaporating small beaker (150 250ml) with several rinsing with the solvent. Place the evaporating small

beaker in a fume hood and evaporate off the solvent on a steam bath until no odor of the solvent is detectable. Dry the beaker and contents in an oven for 30 minutes at 100°C. Remove from the extraction flask into a pre-weighed evaporating small beaker in a fume hood and evaporate off the solvent on a steam bath until no odor of the solvent is detectable. Dry the beaker and contents in an oven for 30 minutes at 100°C. Remove from the oven, cool in a desiccator and weigh beaker plus contents and calculate % of crude fat as given below.

$$\text{Lipid}\% = \frac{M_f - M_i}{\text{sample weight by dry basis}} \times 100 \quad \dots\dots\dots(3.6)$$

Where “M_f” is mass of flask and lipid extracted; “M_i” is mass of dried flask.

Determination of Carbohydrate Content

The carbohydrate contents of each of the samples analyzed were determined by difference using the formula below;

$$\text{Percentage carbohydrate} = 100 - (\% \text{ moisture} + \% \text{ Ash} + \% \text{ crude fibre} + \% \text{ crude protein} + \% \text{ fat}). \quad \dots\dots\dots(3.7)$$

3.2.4.2 Functional properties of the flours

Determination of falling number

A 7-gram sample of ground wheat or flour is weighed and combined with 25 ml of distilled water in a glass falling number tube with a stirrer and shaken to form a slurry .As the slurry is heated in a boiling water bath at 100oC and stirred constantly, the starch gelatinizes and forms a thick paste. The time it takes the stirrer to drop through the paste is recorded as the falling number value

Determination of bulk density

The bulk density of each of the flour samples was determined according to the method of Giami et al (1992). A calibrated centrifuge tube was weighed using an analytical balance in each case and the samples were filled up to the 10ml mark. They were then tapped until there were no further changes in volume. The contents were each weighed with the aid of an analytical balance and from the difference in weights, the bulk density of each sample was calculated as;

$$\text{Bulk Density (g/ml)} = \frac{\text{weight of sample (g)}}{\text{Volume of sample (ml)}} \dots\dots\dots(3.8)$$

Determination of swelling index

The swelling index of each flour sample was determined as the ratio of swollen volume of a unit weight of each sample to its initial volume in a graduated measuring cylinder. One gram of each of the flour samples was weighed out with a weighing balance and dispensed or added into a 20ml measuring cylinder in each case. Then, this was followed by the addition of 10ml of distilled water in each measuring cylinder. The volumes of the samples were noted. They were then allowed to stand undisturbed for 1 hour before their volumes were taken again and recorded accordingly. The swelling index of each flour sample was calculated thus; swelling index = V_2/V_1 . Where V_1 = Initial volume occupied by sample and V_2 = Final volume occupied by the sample after swelling.

Determination of dispersability

Dispersibility was determined using the method described by (Kulkarni et al., 1991). Ten grams of the flour sample was weighed into 100ml measuring cylinder, water was added to each volume of 100ml. The set up stirred vigorously and allowed to stand for three hours. The volume of settled particles was recorded and subtracted from 100. The differences reported as percentage Dispersibility.

$$\% \text{ Dispersibility} = 100 - \text{volume of settled particle}$$

3.2.4.3. Dough rheological testing

Farinograph testing

Farinograph testing was carried out on control (wheat flour) and enriched flour blends with the use of a Brabender - Farinograph®-E (AACC 54-21 / ICC 115/1 /ISO 5530-1). Absorption is the amount of water required by a given weight of flour to yield dough of given consistency. The dough development time (DDT) was the time for the dough to reach maximum consistency (peak); stability was the time that the top portion of the curve is above the 500 BU line; mixing

tolerance index (MTI) is the drop in BU from the top of the curve at DDT to the top of the curve 5 minutes after DDT. Dough stability– is the difference in time, to the nearest half-minute, between the time when the curve first intercepts the 500BU line (arrival time) and the time when the curve leaves the 500 BU line. Quality number is the resultant of all farinograph parameter.

3.2.4.4 Evaluation of the characteristics of the breads

The bread characteristics or baking qualities were evaluated by measuring the loaf volume, the loaf weight, the loaf specific volume, the oven spring and the organoleptic properties of the breads.

The loaf volume

The loaf volume of each bread sample was measured 50 minutes after the loaves were removed from the oven by using the rape-seed displacement method as described by (Onwuka, 2005). The weight of the bread samples was weighed with the aid of a weighing balance. This was then followed by taken the volume of the container from the graduation on its body which was recorded as $V_1(\text{cm}^3)$. The container was then filled with about 4–5volume of the loaf sample with the seeds until the seed dropping from a height of $\frac{1}{2}$ foot above the container rim is cut-off such that the seeds formed a Plateau with the rim of the container. They were then poured out, weighed and recorded as $W_1(\text{g})$. The weight of the seeds that filled the contained is equivalent to the total weight of seed that completely occupied the volume of the container. Then, $\frac{1}{3}$ of the volume of the container was filled with the seeds, the loaf in each case laid flat at the centre of the container and then the remaining seeds used to fill up the container to overflow from $\frac{1}{2}$ foot above the container. With a ruler, the seeds above the rim were cut off as they formed, plateau with the container. The seeds displaced by the loaf in each case were collected, weighed and recorded as $W_2(\text{g})$ (this weight of seeds corresponded to the volume of space displaced by the loaf sample in each case placed in the container). The loaf volume for each bread sample was calculated thus;

$$\text{Loaf volume} = \frac{W_2 \times V_1}{W_1} \dots\dots\dots(3.9)$$

Where W_1 = weight of seeds that filled the container, W_2 = weight of seeds displaced by the loaf sample and V_1 – volume capacity of the container.

The loaf weight

The loaf weight for each of the bread samples was determined by weighing directly on a weighing analytical balance.

The specific loaf volume

The specific loaf volume for each sample was determined by dividing the loaf volume of each sample with the corresponding loaf weight thus;

$$\text{Specific loaf volume (cm}^3\text{/g)} = \frac{\text{loaf volume}}{\text{loaf weight}} \dots\dots\dots(3.10)$$

The Oven spring

The oven spring for each bread sample was determined from the difference in height of each dough before and after baking.

3.3 Experimental design and statistical data analysis

3.3.1 Experimental design

Design of Experiments allows to plan in a rational way the minimum set of experiments to obtain the maximum significant information about the effect of a systematic variation of the variables (factors) of interest in a defined experimental domain. In particular, mixture design is suited for those cases in which the object of study is a blend in which all ingredients sum to 100%. In this respect Okafor et al. (2012) reported that not douse more than 25% mushroom flour to breads for fortification purpose. Thus, it was chosen to consider the major ingredients ranges as follows:

WWF (B): From 75 to 100%

MF (A): From 0 to 25% , Where WWF = whole wheat flour and MF = mushroom flour

The experimental mixture design and statistical analysis were performed using Design Expert software version 7 (StatEasy Co., Minneapolis, MN, USA). A D-optimal design consisting of 13 experimental runs, including 3 replications at the center point, was chosen to evaluate the combined effect of 2 independent variables; WWF (B) and MF (A). Based on preliminary experiments, the ranges of the two flours were $0.75 \leq B \leq 1$ and $0 \leq A \leq 0.25$, respectively. The response values were total protein, energy, and overall acceptability. The effect and regression coefficients of individual linear, interactive, and cubic terms were determined. The significance of all terms was judged statistically by a probability (p) of 0.05. The interaction effects of

ingredients on the mixture were determined using a trace plot. The numerical optimization was performed to bases canonical model by setting a response goal area. Desirability was predicted using a contour plot and was calculated by the following equation (Derringer,1980).

$$D = (d_1 \times d_2 \times \dots \times d_n)^{1/n} = (\prod d_i)^{1/n} \dots\dots\dots(3.11)$$

Where D is overall desirability, d is desirability, and n is the number of responses

The formulations were obtained based on a constrained mixture D-optimal design. Table 1 presents sample codes and actual proportion of ingredients used. Totally there were 13 sets of experimental combinations.

3.3.2 Design point selection for blending

According to WHO Global Database on Child Growth, the synthesis of new tissue requires protein and other nutrients. Synthesis also requires considerable amount of energy. The aim is to provide all necessary nutrients so that none limits the rate of recovery. Normal rate of growth of children is such that they gain a weight of 1gram/kg/day by taking 105 kcal/kg/d and 0.78gram of protein /kg/d. To increase this rate of growth by 20 times the normal, the energy and protein intakes need to be increased to 200kcal/kg/day and 5kcal/kg/day, respectively.

Table-3.2 Food items for different population groups (1- 5 years)

Nutrient	WHO
Protein (g/day)	13-40
Energy(kcal/day)	900-1200

Source- FAO/WHO Human energy and protein requirements (2001)

Mixture design is an important methodology for an experiment in which factors are the proportions of the components of a blend and response variables vary as a function of these proportions (Choi 2006). The range of mixture ratios of whole wheat flour and mushroom flour were 75-100% and 0 - 25% (w/w), respectively. The total contents of these flours (sum of all variables) were 100% (w/w), without including the fixed variables. In order to allocate the points for the mixture within the feasible design region, a modified distance design was applied. Thirteen total design points, 5 points for calculating the lack of fit, and 5 replication points (Table 3). In order to remove division error for the order of the actual experimental designs, all

experimental orders were performed at random (Han 1997). According to a mixture design, 3 kinds of responses, protein contents, Energy, and overall acceptability were determined. protein contents ranged from 15.25 to 38.28g/160g, energy from 435.54 to 498.76kcal, and overall acceptability from 4.3 to 8.25 depending upon the mixing ratio.

The nutritional requirement of different groups is also shown in Table 2.5 and this shows the daily requirement of a person. In this Table it shows that children require high amount of protein and as they grow to adult stage the need for protein increases but this is due to the continued movement and work of that group. A dry weight of 150 -250g of the supplementary foods(cereal based product) were calculated and compared to recommended dietary allowance (RDA) for the same age group for children 1 - 5 year Swaminathan (1989) .

Table-3.3 Food items for different population groups

Food	Adult(sedentary)		Adolescent (16 – 18)		Children (years)	
	Male(g/d)	female(g/d)	Male (g/d)	female(g/d)	1-3(g/d)	7-9(g/d)
Cereal	400	300	450	300	150	250

Source: Swaminathan, 1989

Table: 3.4: Blends used for formulation

Run_order	Component A	Component B
	Wheat flour (%)	Mushroom flour (%)
1.	75.00	25.00
2.	100 0.00	0. 00
3.	87.50	12.50
4.	93,69	6.301
5.	81.30	18.69
6.	96.85	3.15
7.	76.15	23.85
8.	84.45	15.55
9.	75.00	25.00
10.	100.00	0.00
11.	75.00	25.00
12.	100.00	0.00
13.	87.50	12.50

3.3.3 Statistical analysis

All data analysis was performed using SPSS (Statistical Package for the Social Sciences v16.0, Chicago, IL, USA). The level of significance among samples was tested with analysis of variance (ANOVA and least significance difference (LSD)).

3.4 Sensory evaluation

Sensory evaluation was conducted by using randomly selected 20 semi-trained panelists from Bahir dar University post graduate students and staff members. The panelists were tasted the samples for textuer, aroma, taste, flavor color and over all acceptability of the bread samples. The samples were presented in identical sample presenting dishes coded with 3-digit random number with a sensory data ballot paper. The panelists were instructed to eat the bread and score each sample using a 9-point Hedonic scale (Larmond, 1977). where 1 = dislike extremely and 9 like extremely.

4. Results and Discussion

4.1 Proximate compositions of the flours and the bread samples

The supplementary bread was developed from different blends of flour of whole wheat and mushroom. The results of the proximate compositions of the whole wheat flour, and mushroom flour showed that the whole wheat flour had the highest moisture content of (12.57%) than that of mushroom flour (11.5%). The results are presented in Table 4.1.

Table 4.1 Proximate compositions of the raw flour samples

Four sample	Parameter on dry basis						
	Moisture content (%)	Ash content (%)	Protein (%)	Fat (%)	Fiber (%)	Carbohydrate (%)	Energy (kcal)
Whole Wheat	12.57±0.21	0.58±0.04	10.77±0.15	1.27±0.06	2.7±0.08	72.09±0.05	342.83±1.24
Mushroom	11.5±0.1	7.6±0.06	31.3±0.6	2.2±0.1	9.76±0.04	37.98±0.61	296.93±0.81

Values are means + SD of three analyse

Moreover, the results of the crude protein contents of the flours from table 4.2 showed that the mushroom flour had the highest crude protein content of (31.3%). Wheat flour had (10.77%) of crude protein. On the other hand, the carbohydrate content of Whole wheat flour was (72.09%) while that of mushroom flour was obtained as (37.98%). The high crude protein content of the mushroom flour was because, mushroom has a lot of protein compared to the sources of the other flours (Okafor, 2012). Also, whole wheat flour is a good source of carbohydrate compared to the sources of the mushroom flours and hence the high carbohydrate content of the mushroom flour. According to Okaka (2005) cereals such as wheat flour are lower in protein and lysine deficient but rich in sulphur containing amino acid, mushroom on the other hand is very rich in lysine with about 36% crude protein and good balance of other essential amino acids, hence the consumption of mushroom flour supplemented bread will mean eating bread with higher protein content and improved protein quality, invariably, a more balanced diet with enhanced nutritional value that would help reduce protein-energy malnutrition.

4.2 Nutritional composition of mushroom enriched breads

The proximate composition of the formulated product was determined to ascertain its overall nutritional content, as shown in Table 4.2.

Table 4.2: Proximate composition of supplementary diets on dry based

Blends /breads (WF: MF)	Components						
	Moisture content (%)	Ash content (%)	Protein (%)	Fat (%)	Fiber (%)	Carbohydrat e (%)	Energy (kcal)
WWFMF1	30.84±0.008 ^a _b	0.88±0.02 ^{hi}	10.21±0 ^e	1.72±0.02 ^{de}	1.59±0.04 ^d	54.76±0.05 ^a	275.4±0.2 ^{ab}
WWFMF2	30.63±0.03 ^{ab}	1.06±0.01 ^g	12.91±0.44 ^d	1.74±0.00 ^{ld} _e	1.98±0.05 ^c	51.69±0.4 ^b	274.04±0.16 ^{de}
WWFMF3	30.43±0.02 ^{ab}	1.27±0.01 ^f	13.85±0.25 ^d	1.75±0.03 ^d	1.76±0.47 ^c	50.93±0.69 ^b	274.91±1.96 ^e
WWFMF4	30.15±0.17 _b	1.56±0.003 ^c	18.3±1.46 ^b _c	1.87±0.02 ^c	2.25±0.01 ^b	45.87±1.39 ^c	273.51±0.77 ^{de}
WWFMF5	30.82±0.02 ^a	1.59±0.002 ^d	19.32±1.14 ^b	1.85±0.03 ^c	2.29±0.02 ^b	44.13±1.08 ^d	270.46±0.15 ^c
WWFMF6	29.63±0.02 ^{bc}	2.15±0.004 ^c	20.42±0.25 ^b	1.87±0.02 ^c	2.32±0.02 ^{ab}	43.62±0.25 ^d	272.96±0.09 ^d
WWFMF7	28.53±0.03 ^d	2.5±0.01 ^b	23.48±2.2 ^a	1.92±0.01 ^b	2.62±0.002 _a	41.00±2.25 ^e	275.17±0.15 ^b
WWFMF8	28.02±0.02 ^d	2.69±0.02 ^a	23.92±0.1 ^a	1.91±0.02 ^b	2.57±0.03 ^{ab}	40.89±0.08 ^e	276.45±0. ^{ab}

Means in the same column with the same superscript are not significantly different at (P< 0.05)

LEGEND

WWFMF1 = 100%Whole wheat flour + 0% Mushroom flour

WWFMF2= 96.8% Whole wheat flour +3.2% Mushroom flour

WWFMF3=93.7% Whole wheat flour +6.3% Mushroom flour

WWFMF4=87.5% Whole wheat flour +12.5% Mushroom flour

WWFMF5 =85.5% Whole wheat flour +14.5% Mushroom flour

WWFMF6 =81.3% Whole wheat flour +19.7% Mushroom flour

WWFMF7 = 76.2% Whole wheat flour +23.8% Mushroom flour

WWFMF8 =75% Whole wheat flour + 25% Mushroom flour

To investigate that whether there exist a significant difference in these proximate analyses among the diet formulations a one – way ANOVA was conducted.

The proximate nutrient composition of the formulated bread was presented in Table 4.2. The results indicated that the protein, fiber, ash and fat values are increased with increasing the substitution of mushroom. The protein and fat content to the blend are mainly provided by mushroom while the main source of carbohydrate is wheat. The results of this project had been shown that mushroom in formulated bread is a good nutritional supplement because of its high protein and fat content for children.

The moisture contents of the bread samples from Table 4.2 there was no significant difference at ($P < 0.05$) from sample (100% wheat bread) with 30.84 ± 0.08 % moisture content down to sample (87.5 with (30.15 ± 0.17) % moisture content. But there was differed significantly in the moisture contents of sample (81.3:18.7%) with (29.63 ± 0.02) % moisture content and sample (75:25%) with (28.02 ± 0.02) % moisture content at ($P < 0.05$). Sample (100:0) had the highest moisture content (30.84 ± 0.08) % while sample (75:25) had the least (27.91 ± 0.18) %. The high moisture contents of the bread samples may be attributed to the water added during the baking process.

Nevertheless, Table 4.2 shows that the Ash contents of the bread samples increased from sample (100% wheat bread) with the least Ash content of (0.88 ± 0.02) % to sample (75:25) with the highest Ash content of (2.69 ± 0.02) %. Although the Ash content of sample (100% wheat bread) differed significantly at ($P < 0.05$) from the rest of the samples but there was no significant difference at ($P < 0.05$) in each other. The Ash content results indicated that as the substitution of mushroom flour was increased, the Ash contents of the bread samples equally increased. This may be due to the high Ash content of the mushroom flour which indicates the high mineral contents in it.

In the same vein, the crude fibre content of the bread samples also increased as the substitution of mushroom flour was increased. Sample (100% wheat bread) had the least crude fibre content (1.59 ± 0.04) % while sample (75:25) had the highest crude fibre content (2.57 ± 0.03) %. There was no significant difference also existed at ($P < 0.05$) in the crude fibre contents of samples (96.8:3.2 %) and sample (93.7:6.3). The fat contents of the bread samples were increased

significantly at ($P < 0.05$) as the substitution of mushroom flour was increased. But generally, the increase in the fat contents of the bread samples was as a result of the added fat during baking and mushroom flour used to improve on the characteristics of the breads. The crude protein contents of the bread samples

4.3 Effect of mushroom enrichment on the functional properties of the whole wheat flour samples.

From the results in Table 4.2 were also very high but decreased significantly at ($P < 0.05$) just like the fat content as the substitution of mushroom flour was increased. The reason for the increase in protein content as the mushroom flour substitution was increased may be attributed to the high protein content of mushroom flour since it had high protein content from the beginning. The general high crude protein content was because of the mushroom flour used as an improver.

Finally, unlike the crude fibre contents of the bread samples, the carbohydrate contents of the breads decreased significantly at ($P < 0.05$) in line with increase in mushroom flour substitution sample (100:0) had the highest carbohydrate content (54.76 ± 0.05)% whereas, sample (75:25) % had the least carbohydrate content (40.89 ± 0.08)%.

The effect of mushroom flour enrichment on the functional properties parameters of whole wheat flour is showed in Table 4.3. The result of the bulk density showed that there was significant difference between all samples ($P < 0.05$). The bulk density of the enriched mushroom flour blends were considerably low in comparison to the bulk density of the sample WWFMF1 (100% whole wheat flour) which had the highest value of 0.72g/ml, which means that whole wheat flour was denser than the mushroom flour. The bulk density is influenced by particle size and the density of the flour and is important in determining the packaging requirement and material handling (Karuna, 1996). Plaami (1997) reported that bulk density is influenced by the structure of the starch polymers and loose structure of the starch polymers could result in low bulk density.

The swelling power of the flour blends decreased significantly ($P < 0.05$) as the proportion of mushroom flour increased, ranged from 3.45-2.95 with the 75: 25 blend having the lowest value in comparison with the control (100% whole wheat flour) having a swelling power of 3.45. The swelling power is an indication of presence of amylase which influences the quantity of amylose

and amylopectin present in the mushroom flour. Moorthy and Ramanujam (1986) reported that the swelling power of flour granules is an indication of the extent of associative forces within the granule. Swelling power is also related to the water absorption index of the starch-based flour during heating (Loss,1981). Therefore, the higher the swelling power, the higher the associate forces. The variation in the swelling power indicates the degree of exposure of the internal structure of the starch present in the flour to the action of water (Ruales,1983)

The dispersability of the flour from the three blends showed that there were no significant difference ($P<0.05$) of the sample WWFMF1(100%whole wheat flour) and WWFMF2(96.8:3.2) blend s. The dispersability of the 100% whole wheat flour was higher than that of the enriched flour blends and this may be due to the fact that whole wheat flour has higher water absorption than the mushroom flour and it can reconstitute in water better than the mushroom flour. However, the values of dispersability were relatively high for all the flour blends hence, they will easily reconstitute to give fine consistency dough during mixing (Adebowale, 2008).

The falling number determines whether the flour can be used for the bread production or for other processing by just determining the falling number. In wheat it used to determine the germination of the wheat. The lower the value of the falling number, the lower the quality of the wheat and if it is bellow the required value, it will not be used for the processing of bread or any other byproduct. Here in Table-4.3 the falling number for WWFMF8(75:25) flour is higher than the whole wheat flour WWFMF1(100:0) . But this does not tell anything about the germination because the enzymatic activity was very low or does not exist in mushroom enriched flour.

Table 4.3: Functional properties of whole wheat flour enriched with mushroom flour.

	Bulk density (g/ml)	Dispersability (%)	Swelling power	Failing number(sec)
WWFMF1	0.72±0.01 ^a	62.5±1.32 ^a	3.45±0.01 ^a	315.33±0.57 ^e
WWFMF2	0.71±0.01 ^b	61.5±0.7 ^a	3.41±0.01 ^b	322.67±0.58 ^e
WWFMF3	0.68±0.001 ^c	59.83±1.04 ^b	3.35±0.00 ^c	322.67±0.51 ^e
WWFMF4	0.65±0.01 ^d	56.67±0.58 ^c	3.3±0.02 ^d	330.33±0.58 ^d
WWFMF5	0.63±0.01 ^e	57.5±0.87 ^c	3.26±0.01 ^f	344.3±0.58 ^c
WWFMF6	0.62±0.01 ^e	55.33±0.58 ^d	3.32±0.01 ^e	361.67±0.58 ^b
WWFMF7	0.58±1 ^g	54±1 ^d ^e	3.01±0.006 ^g	395±0.51 ^b
WWFMF8	0.56±0.01 ^h	53.33±0.58 ^e	2.95±0.001 ^h	405.33±0.57 ^a

Values of the same letter means that they are not significantly different while values of different letters are significantly different

LEGEND

WWFMF1 = 100%Whole wheat flour + 0% Mushroom flour

WWFMF2= 96.8% Whole wheat flour +3.2% Mushroom flour

WWFMF3=93.7% Whole wheat flour +6.3% Mushroom flour

WWFMF4=87.5% Whole wheat flour +12.5% Mushroom flour

WWFMF5 =85.5% Whole wheat flour +14.5% Mushroom flour

WWFMF6 =81.3% Whole wheat flour +19.7% Mushroom flour

WWFMF7 = 76.2% Whole wheat flour +23.8% Mushroom flour

WWFMF8 =75% Whole wheat flour + 25% Mushroom flour

4.4 Physical properties of the bread samples

The results in Table 4.5 showed that the oven spring, loaf volume and specific loaf volume all decreased as the substitution of mushroom flour was increased from 10% up to 50%. The loaf volume and the oven-spring are good indicators of the crumb, how light and airy the interior of the breads are. Little values indicate a dense and compact crumb.((www.thekitchen.com/word-of-month-ove...)). The loaf weights did not follow any trend. Samples WWFMF1 (100% Whole wheat bread) and WWFMF3 (93.7:6.3) had (312.07) and (310.1) gram, respectively each as the highest loaf weight while sample WWFMF2 had the least loaf weight (265.67) gram.

Table 4.4 Effect of mushroom supplementation on bread making characteristics

Sample	Parameters			
	Oven spring(cm)	Volume (cm ³)	Weight (g)	Specific volume (cm ³ /g)
WWFMF1	2.27	1180.68	312.07	3.78
WWFMF2	2.19	993	265.67	3.74
WWFMF3	2.1	1092.33	310.1	3.52
WWFMF4	1.87	1074	292.37	3.67
WWFMF5	1.83	1029	292.37	3.69
WWFMF6	1.67	1055	291.47	3.62
WWFMF7	0.67	634	277.97	2.28
WWFMF8	0.73	651.3	288	2.26

Means in the same column with different letters as superscript are significantly different (p<0.05)

LEGEND

WWFMF1 = 100% Whole wheat flour + 0% Mushroom flour

WWFMF2 = 96.8% Whole wheat flour + 3.2% Mushroom flour

WWFMF3 = 93.7% Whole wheat flour + 6.3% Mushroom flour

WWFMF4 = 87.5% Whole wheat flour + 12.5% Mushroom flour

WWFMF5 = 85.5% Whole wheat flour + 14.5% Mushroom flour

WWFMF6 = 81.3% Whole wheat flour + 17.3% Mushroom flour

WWFMF7 = 76.2% Whole wheat flour + 23.8% Mushroom flour

WWFMF8 = 75% Whole wheat flour + 25% Mushroom flour

4.5 Effect of mushroom substitution with wheat flour on rheological properties of dough

The effect of mushroom enrichment on the rheological properties of Whole wheat flour is summarized in Table 4.3.. The results showed that farinograph data depended on the mushroom flours portions in the mixtures. The water absorption increased when mushroom flours were added to the wheat flour: from 54.1 to 65.27 % with increasing the mushroom flour portion in the mixtures. It is well known that the main dough component in the wheat flour responsible for water absorption is gluten. The mushroom flours are gluten free, but had higher protein content than the wheat flour (30.1 g/100 g) (Table 4.1). The reason for higher absorption ability might be a higher protein content in all investigated flour mixtures. (Ribotta et al 2005), The reason could also be the interaction between mushroom proteins such and wheat gluten which were reported to occur in the composite flour (Fiuro,2000). Besides the higher protein content, a higher content of dietary fibbers in the mixtures might be also responsible for higher absorption properties, as the results show that the content of dietary fibbers in the mushroom flour was almost four times higher (9.76 g/100 g) than in the wheat flour (2.73 g/100 g). Generally, fibbers provide better properties of water absorption and this is mainly due to a higher number of hydroxyl groups which exist in the fibber structure which allows a stronger interaction of water through the hydrogen bonding Rosell et al 2001). The effect of the water absorption value increase was reported when the wheat flour has been supplemented with oyster mushroom flour (Hesham et al 2007).

Table 4.5. Farinograph parameters for whole wheat flour and mushroom enriched flour blends

Samples	Parameter				
	Water absorption (%)	Development time (min)	Stability(min)	Mixing Tolerance index (MTI)	Farinograph quality number(Bu)
WWFMF1	54.3±0.31 ^f	1.57±0.06 ^h	2.47±0.06 ^f	31 ^h	57 ^a
WWFMF2	56.3±0.1 ^e	1.8±0 ^g	2.73±0.06 ^e	141.33 ^g	40±0.00 ^c
WWFMF3	56.43±0.25 ^e	1.97±0.06 ^f	2.83±0.06 ^e	142.67 ^f	37.33±1.15 ^d
WWFMF4	56.8±0.1 ^d	2.17±0.06 ^e	3.07±0.05 ^d	185.3 ^b	36±1 ^e
WWFMF5	59.3±0.3 ^c	2.47±0.05 ^d	3.33±0.06 ^c	218.67 ^a	35±0.00 ^f
WWFMF6	60.13±0.06 ^b	3.7±0 ^c	3.77±1 ^b	165 ^d	31.7±0.06 ^g
WWFMF7	60.47±0.35 ^b	4.13±0.15 ^b	1.87±0.06 ^g	174 ^c	44.67±0.6 ^b
WWFMF8	65.27±0.25 ^a	4.5±0.06 ^a	4.03±0.05 ^a	146.67 ^c	40±0.00 ^c

Values are means of three determinations ± standard deviation. a,b– mean values in columns with different letters differ significantly

LEGEND

WWFMF1 = 100%Whole wheat flour + 0% Mushroom flour

WWFMF2= 96.8% Whole wheat flour +3.2% Mushroom flour

WWFMF3=93.7% Whole wheat flour +6.3% Mushroom flour

WWFMF4=87.5% Whole wheat flour +12.5% Mushroom flour

WWFMF5 =85.5% Whole wheat flour +14.5% Mushroom flour

WWFMF6 =81.3% Whole wheat flour +17.3% Mushroom flour

WWFMF7 = 76.2% Whole wheat flour +23.8% Mushroom flour

WWFMF8 =75% Whole wheat flour + 25% Mushroom flour.

Table 4.4 shows that there were no significant differences in moisture content between all sample. The differences for the dough development time among the flour mixtures with the mushroom flour ranged from 1.6 to 4.5 minute. The dough stability of the mixtures with the mushroom flour portion of 23.8 % was lower (1.8 min) than for the whole wheat flour dough and for the other flour portion.

It is evident that the addition of the protein rich plant flour prolonged the development time and the dough stability. The effect of delaying the dough development time and dough stability by addition most of the investigated mushroom flours was obtained by Nikolić et al. (2013).

The degree of softening of the mixtures with the mushroom flour decreased from 218. to 141.33 BU when the mushroom flour portion in the flour mixture increased, but contrary to the mixtures with the mushroom flour, all values were higher than for the dough made of the wheat flour. The reason for the decrease in the degree of softening might be the destruction and shortening of the fiber in the gluten network (Kulkarni et al.,1991). A different effect of mushroom flours on the degree of softening as a farinograph property was achieved probably due to a different chemical composition of these flours, especially of the protein composition and different nature of interactions of mushroom components with the components of the wheat flour.

The addition of the mushroom flours changed the quality number and group of wheat-mushroom mixtures. The quality number of the mushroom mixtures was higher (35 to 44.6) than the number of the wheat flour (57). The explanation for these results might be the same as the explanation for a different effect of mushroom flours on the degree of softening.

4.6 Sensory evaluation of the bread samples

From table 5, the mean score for sample WWFMF2 in terms of sample WWFMF4 (100% whole wheat bread) with mean score of 8.2. Sample WWFMF8 had the least mean score of 4.55. Although, the mean scores for taste decreased as the level of mushroom flour substitution increased, there was no significant difference in the tastes of the bread samples from std1 (100% wheat bread) up to std4 12.5% addition of mushroom flour (WWFMF4) at ($P < 0.05$). However, this trend changed at 14.5% substitution of wheat flour with mushroom flour (sample WWFMF5).

On the other hand, there was no significant difference ($P < 0.05$) in the flavor of the entire bread samples from WWFMF1 (100% whole wheat bread) down to sample WWFMF4 wheat/mushroom bread though the mean scores varied slightly with sample. The reason why there was no significant difference at ($P < 0.05$) up to WWFMF4 in the flavor of the bread samples could be that the mushroom flour used in each blend as an improver could have masked the odour of wheat flour in each of the various blends.

Also, there was no significant difference in the textures of the bread samples from sample (100% wheat bread) down to sample WWFMF4 at ($P < 0.05$). These results showed that the mushroom flour used as an improver may be responsible for the slight consistency in the texture of the

bread samples. The mean scores of the bread samples decreased as mushroom flour substitution was increased, with sample WWFMF4 having the highest mean score of 8.1 and sample WWFMF7, with the least mean score of 4.6. The mean scores showed that the textures of samples WWFMF1, WWFMF2, WWFMF3 and WWFMF4 were liked very much by the panelists who neither liked nor disliked those of samples WWFMF7 and WWFMF8.

Furthermore, the mean scores for the crust color of the bread samples also decreased as the mushroom flour substitution was increased with samples. There was no significant difference in the crust colors of the bread samples; WWFMF1 (100% wheat bread), WWFMF2, WWFMF3 and WWFMF4 at ($P < 0.05$). This indicated that the crust colors of the breads were almost the same up to 12.5% substitution of mushroom flour for wheat flour in the bread samples. These results showed clearly that the crust colors of the breads were comparable and almost the same as that (100% wheat bread) at ($P < 0.05$). This may be attributed to the maillard reaction brought about by the amino acids from the proteins of the mushroom flour and the wheat flour which reacted with the added sugar to brown the crusts of the breads. The appearance of the breads were different up to 14.5% substitution (WWFMF4), a marked no significant difference in the appearance existed at ($P < 0.05$). The appearance of samples WWFMF5, WWFMF6, WWFMF7 and WWFMF8 had approximately same mean scores of each and this was the least mean score in terms of appearance and had no significant difference in appearance when compared with each other.

Finally, the mean scores for overall acceptability showed that samples WWFMF4 had the highest mean scores. The bread samples up to 14.5% substitution of mushroom flour was acceptable and their characteristics similar to those of WWFMF1 (100% wheat bread). This trend changed remarkably from 18.7% mushroom flour substitution downwards but nevertheless, there was significant difference in the overall acceptability of sample WWFMF5(87.5:12.5) , WWFMF6 , WWFMF7 and sample WWFMF8 at ($P < 0.05$).

Table 4.6: Mean scores of the sensory evaluation of the bread samples made from blends of whole wheat flour and mushroom flour

Samples	Parameter					
	Texture	Taste	Appearance	Color	Flavor	Over all acceptability
WWFMF1	8.05±0.76 ^a	7.95±0.76 ^a	8.07±0.75 ^a	7.95±0.89 ^a	7.95±0.83 ^a	8.05±0.75 ^a
WWFMF2	7.95±0.75 ^a	8.05±0.76 ^a	7.72±0.73 ^{ab}	8.05±0.76 ^a	8.1±0.73 ^a	8.1±0.72 ^a
WWFMF3	8±0.73 ^a	8.05±0.69 ^a	7.28±0.67 ^b	7.95±0.76 ^a	8.2±0.69 ^a	8.1±0.72 ^a
WWFMF4	8.1±0.72 ^a	8.2±0.69 ^a	6.58±0.67 ^c	8.25±0.64 ^a	8.15±0.67 ^a	8.25±0.72 ^a
WWFMF5	5.8±0.69 ^b	5.85±0.67 ^b	5.18±0.75 ^d	5.75±0.72 ^b	5.95±0.69 ^b	5.85±0.67 ^c
WWFMF6	5.75±0.79 ^b	5.8±0.77 ^b	4.97±0.83 ^d	5.7±0.80 ^b	5.9±0.79 ^b	7.05±0.93 ^b
WWFMF7	4.6±4.65 ^c	4.65±0.75 ^c	4.76±0.81 ^d	4.55±0.76 ^c	4.65±0.75 ^c	4.75±0.79 ^d
WWFMF8	4.75±0.85 ^b	4.55±0.76 ^c	4.8±0.89 ^d	4.45±0.76 ^c	4.35±0.49 ^c	4.3±0.47 ^e

Means with the same superscripts within the same column are not significantly different at (P < 0.05)

LEGEND

WWFMF1 = 100% Whole wheat flour + 0% Mushroom flour

WWFMF2= 96.8% Whole wheat flour +3.2% Mushroom flour

WWFMF3=93.7% Whole wheat flour +6.3% Mushroom flour

WWFMF4=87.5% Whole wheat flour +12.5% Mushroom flour

WWFMF5 =85.5% Whole wheat flour +14.5% Mushroom flour

WWFMF6 =81.3% Whole wheat flour +18.7% Mushroom flour

WWFMF7 = 76.2% Whole wheat flour +23.8% Mushroom flour

WWFMF8 =75% Whole wheat flour + 25% Mushroom flour

4.7 Modeling and analysis for selected response

In order to analyze the interaction effect on each flour mixture, modeling was necessary for each response (Yoon,1997). Analysis of the selected models and regressions of the polynomial equations are shown in Appendix. The significance for the selected models was determined by the F-test. Protein, and acceptability were selected to a quadratic non-linear regression model where as energy were selected to a cubic non-linear regression model. The lack of fit test showed the probability values for protein, energy and acceptability 0.265,0.324 and < 0.0724 , respectively. Therefore, the fitness to the preference model was confirmed by these probability values. Each coefficient determined at the predicted canonical equation showed the effect of each flour on each response as a numerical value, which indicated that the interaction effect of each flour could be confirmed, as shown in the quadratic and cubic mode.

Table 4.7. Quality characteristics of bread produced with ‘whole wheat and mushroom flour

Run order	Component		Response		
	Wheat	Mushroom	protein (g)	energy (kcal)	acceptability
	flour (%)	flour (%)			
1.	75.00	25.00	38.18	498.76	4.3
2.	100 0.00	0. 00	16.34	440.06	8.05
3.	87.50	12.50	29.25	437.6	8.25
4.	93.69	6.301	22.16	447.56	8.1
5.	81.30	18.69	32.65	442.91	7.05
6.	96.85	3.15	20.66	438.45	8.1
7.	76.15	23.85	37.87	440.28	4.65
8.	84.45	15.55	30.91	442.24	5.08
9.	75.00	25.00	38.27	497.65	4.3
10.	100.00	0.00	15.87	441.87	8.0
11.	75.00	25.00	38.19	497.52	4.3
12.	100.00	0.00	15.25	441.84	8
13.	87.50	12.50	28.64	435.54	8.25

Analysis of variance for each response showed that a significant effect was found for nutrient content and sensory properties with regard to WWF and MF. To establish predictive models for the bread properties from various level of the WWF and MF the experimental data for each response variable shown in equation in Terms of L_Pseudo Components 1 – 3

$$Y1 = 38.34A + 16.02B + 5.90AB \dots\dots\dots 1$$

$$Y2 = 495.04A + 439.74B - 123.75AB - 173.90AB(A-B) \dots\dots\dots 2$$

$$Y3 = 4.18A + 8.00B + 6.41 AB \dots\dots\dots 3$$

All the independent variables have positive effect on protein, energy and overall acceptability. The interaction terms of WWF (B) and MF (B) in energy shows negative effect on the responses. The cubic interaction terms of all the independent variables shows negative effect in WWF and MF respectively.

4.8 Optimization of mixture ratio for blending flours

The optimum mixture ratio of flours with whole wheat and mushroom flours' was determined using the optimization process suggested by (Derringer and Suich, 1980). The result of the two component map (desirability) is shown in Fig. 4.3. The plot indicated the change in each element through the plot (Hwang ,2008), and it was used to predict final mixture ratios. Protein content were set in range and energy and overall acceptability were set to the maximized values. The proportions of whole wheat and mushroom flours were set at 75-100 and 0-25%, respectively. The plot represented a range of the reiterated graph by model of content and reaction; desirability used the criterion of degree of optimization. From the numerical optimization results, the optimum flour formulation was 19.14% mushroom flour and 80.86% whole wheat flours. The optimum process revealed 81.6% desirability (fig 4.3), and the predicted response values according to this mixture ratio showed that total protein content, energy, and overall acceptability were 34 gm, 455.78kcal and 6.2, respectively (Appendix IV)

Table 4.8. Optimum constraint values obtained using a numerical optimization method

Factor (F) Response (R)	Goal	Lower limit	Upper limit	Solution of numerical optimization
WWF(F)	Inrange	75%	100%	80.86%
MF(F)	In range	0	25%	19.14%
Protein (R)	In range	13g	40g	34g
Energy (E)	Maximum	900Kcal	1200kcal	455.78kcal
Acceptability (R)	Maximum	4.3	8.25	6.2

Design-Expert® Software

Desirability
 ♦ DesignPoints
 Std # 4 Run # 11
 Actual Y: 0.816
 X1: A = 18.6989
 X2: B = 81.3011

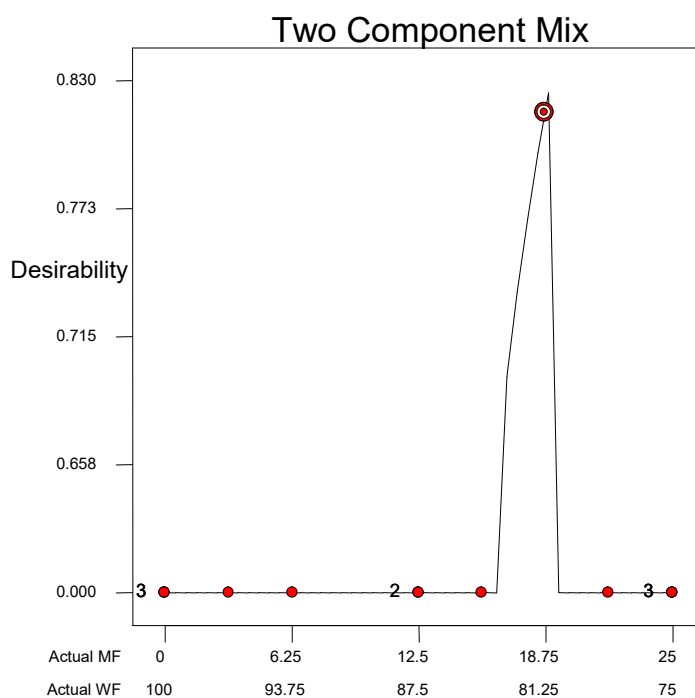


Fig. 4.1. Two component mix plot for the desirability of the optimum result

5. Flow sheet for the production of bread

5.1. Bread-making procedure

The whole wheat flour was then mixed, with oyster mushroom. The composite flours were blended with other baking ingredients in a mixer, kneaded for 12 min into consistent dough and the resulting dough was molded and placed in a pre-oiled baking bowl. The pieces of fermented dough were flattened to be about 20 cm in diameter. The dough was proofed for 45 to 60 min at 35°C and 85% relative humidity and baked in a reel oven for 35 min at 217°C. The loaves of bread were allowed to cool on racks for about 1 h before packaging and

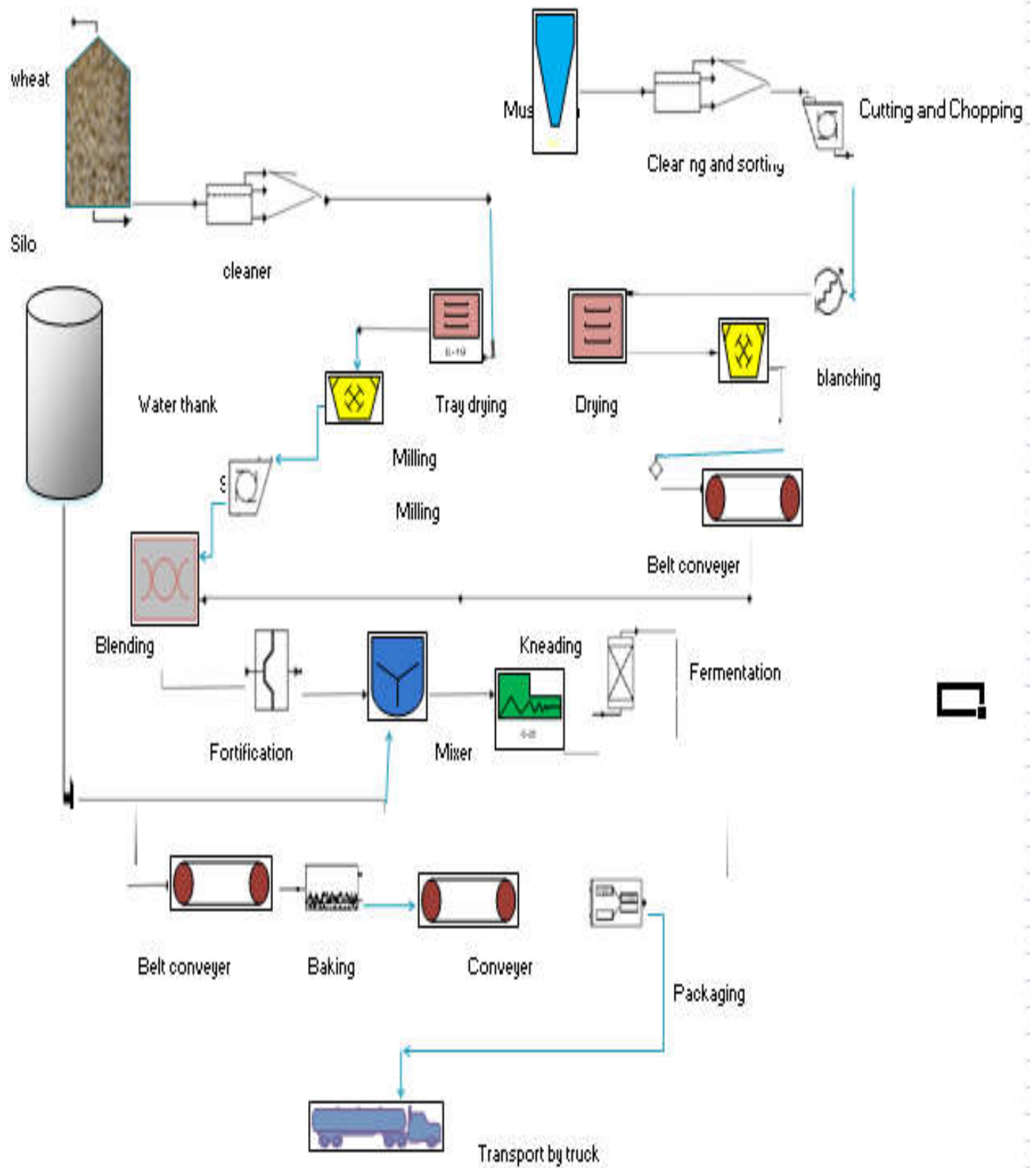


Fig . 5.1 schematic flow diagram for bread production

6. Conclusions and Recommendation

6.1 Conclusion

This study was focused on substituting a part of whole wheat flour (WWF) with mushroom flour (MF), as an attempt to solve the problem in protein energy malnutrition of children from 1-5 years. composite breads with mushroom-flour substitutions were found to be nutritionally superior (have higher protein, fat and crude fibre content) to whole wheat bread.

The farinograph properties depended on the mushroom flour portions in mixtures. The addition of the protein rich flour increased the water absorption value and prolonged the development time and dough stability. The proteins from mushroom were responsible for a higher water absorption value and the lipid content for a high value of the dough development time.

Bread with 25% mushroom flour contains the highest amount of protein, fat and ash but consequently it has the lowest acceptability to the consumers. However, the investigation shows that there was significant improvement in the bread protein content and nutritional quality on addition of mushroom flour. This was evident in the significant increase of 10.21- 23.98% in the crude protein content of fortified bread sample. Also over 50% increase in the ash and crude fiber content was achieved. Acceptable bread that compared favorably well with whole wheat bread were produced with 3.2 and 17.3% supplementation with mushroom flour. However, the acceptability decreased with increase in inclusion of mushroom flour. Bread with 25% mushroom powder was the least acceptable. The daily requirement of protein for children with the age of 1-5 was 13-40g/day and energy was 900-1200kcal, but the optimum energy content of all the formulate bread was 455.78 Kcal which was below than RDA, to achieve this they should be provided with foods from each of the different food groups every day. If a child of age 1-5 eat bread of 160 g which covers the daily requirement of protein and this shows that the protein malnutrition can be reduced by just giving the children the fortified mushroom bread.

The composite breads would serve as functional food because of the high fibre content. However, further research work should be focused on the functional properties and characteristics of bread and how to improve the organoleptic qualities and hence acceptability of mushroom-enriched breads. Public enlightenment on the nutritional benefits of the mushroom-

supplemented functional foods would help to improve the sensory acceptability of the mushroom supplemented bread. There is also the need to adjust the mixing ingredients and baking techniques in order to improve the composite bread qualities. Since mushroom is cheaper and readily available, mushroom enrichment of wheat flour would have little or no effect on the price of the product. Enrichment of mushroom with whole wheat flour can also reduce the problem of malnutrition in places where wheat is consumed as a staple food.

However, the scores for organoleptic attributes like taste, aroma, texture (mouth feel), except for colour were generally inferior to that of whole-wheat bread. Therefore, the mushroom-composite had better overall acceptability scores than the whole-wheat breads.

Mixture D-optimal design was successfully applied to optimize WWF and MF to improve the quality of bread seems to be justified. The two variables employed in the study had a great effect on the quality of bread to meet the nutritional requirement of children under the age of 5 years. Up to a certain limit of MF addition, the energy value decreases while the protein, and overall acceptability increased. Modeling of experimental data allowed the generation of useful equations for general use, to predict the behavior of the system under different factor combinations. The final result of optimization suggested that the optimal ingredient doses to achieve with 80.86% WWF and 19.145% MF for the bread

6.2 Recommendation

Under nutrition in young children is a major public health problem in low- and middle-income countries. In an attempt to address this problem, supplementary feeding is commonly implemented. Supplementary feeding is thought to be beneficial by optimizing the nutritional value of the diet and improving the general health of disadvantaged families. Consumption of bread with addition of other vegetables like mushroom should be encouraged. Policy makers, universities, research institutes and non-governmental organizations, especially those engaged in food security and nutrition issues, should give due emphasis to this technology. Provision of adequate and appropriate supplements to young children prevents malnutrition. Further studies should also be comments to determine the nutrition profile, the anti nutritional factors (if present) in supplemented foods, the essential amino acids profile as well as shelf life studies play a crucial role in maintain a weaning food from food safety point of view. Research is

needed to further define the optimal energy density among both stunted and moderately wasted children. To achieve the nutritional requirements children should be encouraged to enjoy different foods. Therefore, this research recommends not douse more than 20% mushroom flour to breads for fortification purpose. The findings of the present study may help in developing commercial processing technology for effective utilization of mushroom flour especially for manufacturing of breads.

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Appendices

Appendix- I. Sensory evaluation result for different blend for mushroom bread

Questionnaire for sensory evaluation

Name: _____

Date: _____

- ✓ Please look at and taste each sample of bread, based on the information listed out below , in order from left to right as shown on the ballot. Indicate how much you like or dislike each sample by checking the appropriate phrase under the sample code number.
- ✓ Information about organoleptic analysis
 - **Appearance:** Degree of porosity and its uniformity from non uniform to uniform
 - **(Color):** Degree of colour darkness in the crust ranging from pale to dark brown ,how it looks like, if it is appealing to the eyes inviting and bright\
 - **Flavor :**The fundamental taste sensation of the bread refers to the sweet sensation caused in the mouth by contact with the bread due to the sweetening agent
 - **Texture** is the quality of the bread that can be decided by touch, the degree to which it is rough or smooth, hard or soft
 - The **overall acceptance** expresses :how the consumers or panellists accept the product generally.

Sample													
Attributes	1	2	2	4	5	6	7	8	9	10	11	12	13
Texture													
Taste													
Appearance													
Color													
Flavor													
Overall acceptability													

Score the samples based on the ff.

- 1= Dislike extremely 5= neither like nor dislike 7. Like moderately
- 2= Dislike very much 6= Like slightly 8. Like very much

3= Dislike moderately

4= Dislike slightly

9.= like extremely

Comment

Appendix - II: Result of farinograph parameter**Report**

Flour blends	Farinograph parameter					
		Water Absorption(%)	Dough development time (min)	Dough stability (min)	Mixing tolerance index (FU)	Quality number (BU)
75:25	Mean	65.2667	4.5333	4.0333	1.4667E2	40.3333
	N	3	3	3	3	3
	Std. Deviation	.25166	.05774	.05774	1.15470	.57735
	Minimum	65.00	4.50	4.00	146.00	40.00
	Maximum	65.50	4.60	4.10	148.00	41.00
100:0	Mean	58.3333	1.5667	2.4667	31.0000	57.0000
	N	3	3	3	3	3
	Std. Deviation	.30551	.05774	.05774	1.00000	2.00000
	Minimum	58.00	1.50	2.40	30.00	55.00
	Maximum	58.60	1.60	2.50	32.00	59.00
87.5:12.5	Mean	56.8000	2.1667	3.0667	1.8533E2	36.0000
	N	3	3	3	3	3
	Std. Deviation	.10000	.05774	.05774	4.04145	1.00000
	Minimum	56.70	2.10	3.00	181.00	35.00
	Maximum	56.90	2.20	3.10	189.00	37.00
93.7:3.2	Mean	56.4333	1.9667	2.8333	1.4267E2	37.3333
	N	3	3	3	3	3
	Std. Deviation	.25166	.05774	.05774	1.15470	1.15470
	Minimum	56.20	1.90	2.80	142.00	36.00
	Maximum					

		Maximum	56.70	2.00	2.90	144.00	38.00
81.3:18.7	5	Mean	60.1333	3.7000	3.7667	1.6500E2	31.6667
		N	3	3	3	3	3
		Std. Deviation	.05774	.00000	.05774	.00000	.57735
		Minimum	60.10	3.70	3.70	165.00	31.00
		Maximum	60.20	3.70	3.80	165.00	32.00
96.8:3.2	6	Mean	56.3000	1.8000	2.7333	1.4133E2	40.0000
		N	3	3	3	3	3
		Std. Deviation	.10000	.00000	.05774	1.15470	.00000
		Minimum	56.20	1.80	2.70	140.00	40.00
		Maximum	56.40	1.80	2.80	142.00	40.00
76.2:23.8	7	Mean	60.4667	4.1333	1.8667	1.7400E2	44.6667
		N	3	3	3	3	3
		Std. Deviation	.35119	.15275	.05774	2.00000	.57735
		Minimum	60.10	4.00	1.80	172.00	44.00
		Maximum	60.80	4.30	1.90	176.00	45.00
84.5:15.5		Mean	59.3000	2.4667	3.3333	2.1867E2	35.0000
		N	3	3	3	3	3
		Std. Deviation	.30000	.05774	.05774	1.15470	.00000
		Minimum	59.00	2.40	3.30	218.00	35.00
		Maximum	59.60	2.50	3.40	220.00	35.00
75:25		Mean	65.0667	4.5667	4.1000	1.4533E2	40.0000
		N	3	3	3	3	3
		Std. Deviation	.11547	.05774	.10000	2.08167	.00000
		Minimum	65.00	4.50	4.00	143.00	40.00
		Maximum	65.20	4.60	4.20	147.00	40.00

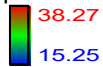
100:0	Mean	58.5333	1.5667	2.4000	30.6667	56.6667
	N	3	3	3	3	3
	Std. Deviation	.05774	.05774	.00000	.57735	1.52753
	Minimum	58.50	1.50	2.40	30.00	55.00
	Maximum	58.60	1.60	2.40	31.00	58.00
75:25	Mean	64.9667	4.4667	4.1333	1.4267E2	39.0000
	N	3	3	3	3	3
	Std. Deviation	.20817	.05774	.11547	.57735	1.00000
	Minimum	64.80	4.40	4.00	142.00	38.00
	Maximum	65.20	4.50	4.20	143.00	40.00
100:0	Mean	58.6333	1.5000	2.4000	30.3333	57.6667
	N	3	3	3	3	3
	Std. Deviation	.23094	.00000	.10000	.57735	.57735
	Minimum	58.50	1.50	2.30	30.00	57.00
	Maximum	58.90	1.50	2.50	31.00	58.00
87.5:12.5	Mean	56.7000	2.1000	3.1333	1.8433E2	36.3333
	N	3	3	3	3	3
	Std. Deviation	.10000	.00000	.05774	2.08167	2.08167
	Minimum	56.60	2.10	3.10	182.00	34.00
	Maximum	56.80	2.10	3.20	186.00	38.00
Total	Mean	59.7641	2.8103	3.0974	1.3369E2	42.4359
	N	39	39	39	39	39
	Std. Deviation	3.23797	1.22320	.72129	6.12034E1	8.73226
	Minimum	56.20	1.50	1.80	30.00	31.00
	Maximum	65.50	4.60	4.20	220.00	59.00

Appendix -III: Result for Response surface plot showing the effect of WWF and MF (in gram) on Protein, Energy and overall acceptability

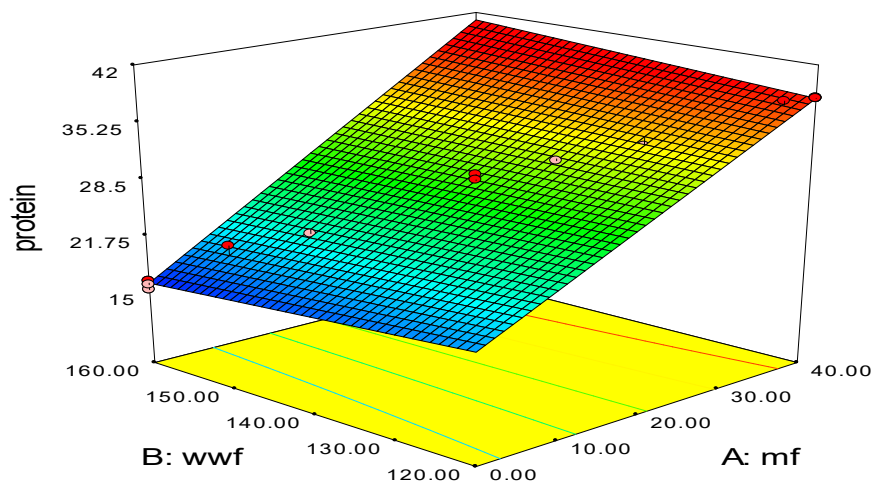
a. Protein

Design-Expert® Software

protein



X1 = A: mf
X2 = B: w w f



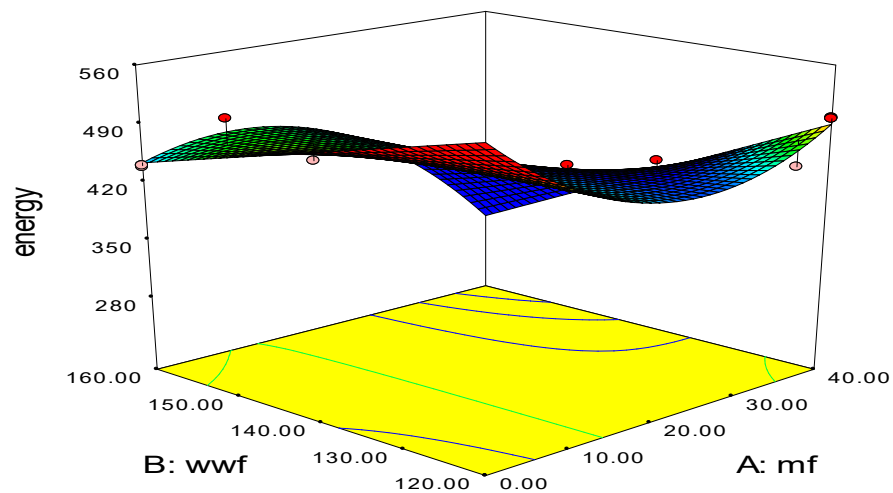
b. Energy

Design-Expert® Software

energy



X1 = A: mf
X2 = B: w w f



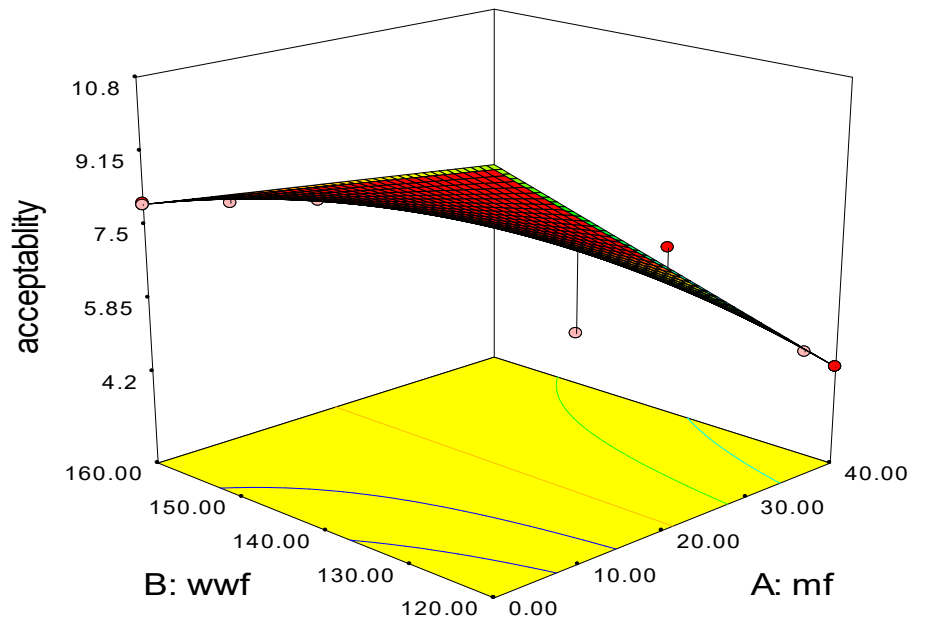
c. Overall acceptability

Design-Expert® Software

acceptability



X1 = A: mf
X2 = B: w w f



Appendix - IX Analysis of variance of the response variables

Response	Source	Sum of square	df	Mean square	F value	Prob>F	R ² (%)
Y1	Model	963.88	2	481.94	750.86	< 0.0001	99.34
	Lack of fit	5.61	5	1.12	6.95	0.076	
	Pure error	0.81	5	0.16			
	Total	970.30	12				
Y2	Model	6924.46	3	2308.15	34.29	0.065	91.96
	Lack of fit	601.64	4	150.41	182.63	< 0.0001	
	Pure error	4.12	5	0.82			
	Total	7530.22	12				
Y3	Model	32.97	2	16.48	50.90	< 0.0001	91.06
	Lack of fit	3.24	5	0.65	1942.00	0.542	
	Pure error	1.667E-003	5	0.000	3.333E-004		
	Total	36.20	12				

ADDIS ABABA UNIVERSITY
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**Development and Characterization of Bread from Mushroom and
Wheat Composite Flour.**

By: Genenu Alemu

Approved by the Examining Board: _____

Chairman, Department's Graduate Committee _____

_____	_____	_____
Advisor	Signature	Date
_____	_____	_____
External Examiner	Signature	Date
_____	_____	_____
Internal Examiner	Signature	Date