EFFECT OF MUSHROOM FLOUR ON PROXIMATE COMPOSITION AND DOUGH RHEOLOGICAL PROPERTIES OF WHOLE WHEAT FLOUR BREAD

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Abstract
This study was focused on substituting a part of whole wheat flour (WWF) with mushroom flour (MF) to determine the effect of MF on rheological properties of dough and proximate composition of bread. Based on the preliminary experiments, the minimum and maximum concentration of WWF and MF considered as 75%-100% and 0%-25% respectively. A D-optimal design consisting of 8 experimental runs was designed. The bread was prepared with WWF substituted with various portions of the MF. The effect of different concentration of mushroom flours supplementation on the proximate components and rheological properties of the bread was determined by one way ANOVA. Crude protein, crude fat, crude fibre, ash and energy 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 were decreased with increased level of MF supplementation from 30.84% to 28.02%, and 54.76% to 40.89% respectively. The result showed that the optimized acceptability of the MF incorporated bread containing WWF 80.86%, MF 19.14%, protein 34g, and energy 455.78kcal. The Protein concentration of the bread was increased as the MF increased, so the bread prepared in present study is suitable for children between the age groups of 1-5 years being not lower than the recommended dietary allowance for this group of children

Keywords: Bread, Mushroom flour, Optimization, Rheological properties, Whole wheat flour.

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1. INTRODUCTION

Bread is an ancient staple, baked, well-known food product widely consumed in the world. Bread is a staple food in many countries of Africa, Europe, Near East, Indian subcontinent, parts of China, and indigenous populations of Australia and the Americas including Ethiopians. It is consumed almost by all people in Ethiopia in both rural and urban areas. The bread making practice and technology is probably one of the oldest technologies known to people (Selomulyo & Zhou, 2007). Refined wheat flour is the major ingredient in bread preparation, whereas yeast, fat, sugar, salt and water are the minor (Badifu et al. 2005). Bread is a staple food and plays a fundamental role in social relations and religious celebrations of many peoples.

Food fortification is very important process, defined as the addition of one or more nutrients rich ingredients together aimed to improve nutritional composition of food; usual goal of food fortification is to control nutrient deficiency problems. In this line, present study is designed for addition MF to increase protein and other nutrient content in wheat bread.

Mushrooms are fruit-bodies; spontaneously grow in nutritious rich lands abundantly (forests and farmlands) after the rainy seasons. The substrates for mushrooms growth include decomposed waste (agro, food, and animal), and soil where rich nutrients are available, mycelium absorb nutrients external digestion. Use of wild mushroom by ethnic groups in Ethiopia shows a clear evidence of its importance since ancient time. Naturally grown mushrooms have been used as food by the *Majangir* tribe and *Wacha* inhabitants in southwestern Ethiopia (Tuno, 2001 and Teferi et al. 2013). Now a day, mushrooms cultivation is going on industrial scale, mushrooms are considered popular food in several countries, mainly in Europe and Asia, including Ethiopia because of their favourite delicacy (Zhu et al. 2011; Kalač, 2010).

Further more, mushrooms have been reported as therapeutic foods; clear reports are available regarding their diseases prevention and wider biological properties such as antibacterial, antimutagenic, anticancer and antiviral activities (Garcia-Lafuentea et al. 2010; Schillaci et al. 2013) due to their superior chemical composition (Manzi et al., 2001). Nutritionally fruiting bodies of the mushrooms contain about 39.9 % carbohydrate, 17.5 % protein, 2.9 % fats, the rest being fiber and minerals on dry weight basis (Demirbaş, 2001). Manzi et al. (2001) have demonstrated that oyster mushrooms are healthy foods, low in calories and in fat, rich in protein, chitin, vitamins and minerals. The authors further reported that *Pleurotus* mushrooms also contain high amounts of γ-amino butyric acid (GABA) and ornithine. Similarly, an extract from *P. ostreatus* appeared to protect major organs such as the liver, heart, and brain of aged rats against oxidative stress (Jayakumar et al. 2007).

Wheat (*Triticum aestivum*) is a grass, originated from the Fertile Crescent region of the Near East, but present days a common crop worldwide. Globally, wheat is the leading source of vegetable protein in human food, holding a higher protein content than maize or rice, the other major cereals (Belderok, 2000). Ethiopia is one of the largest wheat (durum and bread wheat) producers in sub-Saharan Africa. Wheat is one of the major cereal crops in the Ethiopian highlands. About 60% of the wheat area is covered by durum and 40% by bread wheat. Wheat is a major ingredient in foods such as bread, porridge, crackers, biscuits, Muesli, panbreads, pies, pastries, breads, cookies etc. (Giami et al. 2003). The protein content of white wheat flour is low due to separation of germ and bran during milling so, generally, bread prepared with refined wheat flour is naturally low in protein and nutritionally not a balanced diet because it is low in lysine and other essential amino acids. Lysine is the limiting essential amino acid from wheat protein (Hugo et al. 2000; Mamat et al. 2013).

The main objective of this study is to develop the bread with whole wheat flour and mushroom flours for the best nutritional quality of bread.

2. MATERIALS AND METHODS

The study was conducted in different laboratories of Chemical and Food Engineering Departments of Bahir Dar, Addis Ababa University and Kality Food Share Company, Ethiopia. Wheat variety of "Digilu" was obtained from Holetta Agricultural Research Center. Fresh Oyster Mushroom (*Pleurotus ostreatus*) was purchased from commercial mushroom producer in Addis and stored at 3–4°C for 2 days. Sugar, yeast, shortening, salt, and other ingredients were collect from the local market. High density polyethylene bags were used for packaging and storage of samples.

Preparation of Mushroom Flour (MF)

MF was prepared in the laboratory from fresh mushroom using the method described by (Mahedy et al., 1998), the clean and fresh mushrooms were chopped into uniform sized small pieces around 2 cm size with kitchen knife and blanched in hot water at 100°C for three minutes containing 3% table salt and 0.01% citric acid. Then water was drained and mushroom pieces were spread in trays and dried in sunlight to get 9-10% moisture level at 33±2°C for 48hours. After drying, the pieces were allowed to cool to room temperature; the dried mushrooms were ground into powder in a grinder. Then the powder was passed through 0.5mm sieve and packaged in polythene bags and stored at room temperature for further use in the preparation of bread.

Preparation of Whole Wheat Flour (WWF)

Collected wheat sample was manually cleaned to remove contaminants such as sands, sticks, inferior and immature seeds and leaves. Wheat converted to flour in roller mill, the flour was passed through 0.5mm sieve mesh for uniform size particles, the samples were packed in high density polyethylene bags until further use.

Bread Preparation

Bread was prepared by straight dough Method of Chauhan, et al. (1992). For all baking experiments, Dry yeast 1% (Activated), Salt 2%, Sugar 6%, Fat 1% were weighed according to the levels of flour required per treatment and the formulation. The composite flours and baking ingredients were mixed in a mixer, kneaded for 7 minutes into consistent dough and the resulting dough was allowed to ferment for 90min at 30-32^{o}C. The fermented dough was placed in to pre–oiled baking mould (Eissa et al, 2007). The dough was allowed for proving for 60 min and 85% relative humidity and baked in a reel oven for 35 min at 217°C (Okafor et al. 2012). The baked bread loafs were allowed to cool to room temperatures and were packed in high density polyethylene bags and further processed for analysis.

Experimental design

In present experiment mixture design were performed using Design Expert software version 7 (Stat Easy Co., Minneapolis, MN, USA). A D-optimal design consisting of 8 experimental runs was chosen to evaluate the combined effect of 2 independent variables. Based on preliminary experiments, the ranges of the WWF was considered as 75% minimum and 100% maximum, whereas minimum 0% and maximum 25% for MF.

Data collected

Proximate analysis flour and bread sample

The moisture content of the individual flour samples and bread samples was determined using hot air oven method, protein content was determined according to Kjeldahl method of crude protein analysis by using conversion factor as 6.25. Crude fat was determined using soxhlet extraction method and hexane as a solvent; crude fiber was determined by the non-enzymatic gravimetric method, Ash content was determined by official methods (AOAC 2012) with method numbers of 925.10, 979.09, 2003.06, 920.168, 923.03 respectively. The total percentage of carbohydrate content was determined by the difference method as reported by Onyeike et al. (1995). Gross energy was calculated according to the method developed by Osborne and Voogt (1978).

Farinograph testing

Farinograph testing was carried out on control (whole wheat flour) and enriched flour blends with the use of a Brabender - Farinograph®-E (AACC 54-21 / ICC 115/1 /ISO 5530-1). A composite flour sample of 50 grams with the adjusted moisture content of 14% was taken into the farinograph mixing bowl. Water from a burette was added to the flour and mixed to form dough. As the dough is mixed, the farinograph was records a curve on graph paper. The amount of water was added (absorption) affects the position of the curve on the graph paper. If water amount was less, the dough consistency increases and curve moves to the upward. The curve was centred on the 500-Brabender Unit (BU) line ±20 BU by adding the appropriate amount of water and was continued until the curve leaves the 500-BU line. The farinograph was used to determine dough and gluten properties of a flour sample by measuring the resistance of dough against the mixing action of paddles (blades). Farinograph results include water absorption, development time, stability, mixing tolerance index, Farinograph quality number (Bu).The dough development time (DDT) was determined as the time for the dough to reach maximum consistency (peak). Stability was considered the time that the top portion of the curve is above the 500 BU line. The mixing tolerance index (MTI) was considered as the drop in BU from the top of the curve at DDT to the top of the curve 5 minutes after DDT(Wheat Marketing center, 2008).

Statistical analysis

All data analysis was performed using SPSS (Statistical Package for the Social Sciences ver.16.0, Chicago, IL, USA). The level of significance among samples was tested with analysis of variance ANOVA and least significance difference (LSD). To investigate the significant difference in proximate analyses and dough rheological properties among the dough formulations used one – way ANOVA.

3. RESULTS AND DISCUSSION

Proximate compositions of the flours

The results of the proximate compositions of the WWF and MF showed in table 1. WWF had the highest moisture content of 12.57% than that of MF (11.5%). The MF has highest amount of Ash, protein, fiber, but the carbohydrate concentration and energy are rich in WWF.

Moreover, the results of the crude protein contents of the flours from table 1 showed that the MF had the highest crude protein content of 31.3%. WWF had 10.77% of crude protein. The high crude protein content of the MF reported because, mushroom has a lot of protein compared to the sources of the other flours (Okafor et al. 2012). The protein content of the MF is lower than soy bean (36%), but higher than maize (9%) and Beef meat (26%) (USDA, 2017). On the other hand, the carbohydrate content of WWF was 72.09%, while that of MF 37.98%, WWF is a good source of carbohydrate compared to the sources of the MF. According to Okaka (2005) cereals such as wheat is lower in protein and lysine but rich in sulphur containing amino acid and carbohydrates. In contrast, mushrooms are very rich in lysine with 36% crude protein and good balance of other essential amino acids. The fiber content of the MF is higher than that the WWF. The proximate composition of MF results of different researchers (Kalač, 2009; Nikolić et al. 2013; Reguła & Siwulski, 2007; Wani et al. 2010) are in line with the values obtained in present study, they reported that dried oyster mushrooms have 345 Kcal, water content 10.6%, protein 15.7%, fat 2.66%, carbohydrate 64.1% and ash 7.04% which are almost all in line with present study.

Nutritional composition of mushroom enriched breads

The proximate composition of the formulated bread was presented in Table 2. The results indicated that the protein, fiber, ash and fat values were increased with increasing the substitution of MF. The protein and fat content to the blend were mainly provided by MF while the main source of carbohydrate was WWF. The results of this work had been shown that MF in formulated bread is a good nutritional supplement because of its high protein and fat content.

The range of moisture content determined in prepared bread samples as 30.84-28.02%, and it is clear from the table 2 that as the WWF concentration decreased the moisture content was reduced. The moisture contents of the bread samples were no significant difference ($P_{<}$ 0.05) from control sample (bread from 100% WWF). The moisture content of the bread from 100% WWF is about 30.84 ± 0.08%. The high moisture contents of the bread samples may be attributed to the water added during the baking process. The moisture content of the bread in reported study of Mahedy Mahamud et al. (1998) is lower than that of present study. This is may be because of the WWF variation and concentration of MF. As the WWF is the rich source of the carbohydrates and the glutens the water absorption capacity is high, for instance, Bushuk and Hlynka, (1964) reported that water holding properties of wheat starches was 21-44% , whereas gluten is 110%, as the MF is less in the both gluten and carbohydrates, the bread samples which were prepared from that of high concentration of MF are the less in moisture content.

The range of ash composition determined in the different bread samples are ranged from 0.88% to 2.69%. Ash contents directly represents the mineral contents of certain foods, 0.88±0.02% was the ash content of the bread samples prepared from that of 100% WWF. MF concentration increases, the ash content of the bread was also increased. It clearly attributed to that of the high ash continent of the dried MF (Table 1). As shown in table 1 the MF having the highest ash (7.6±0.06%) compared to that of the WWF (0.58 ±0.04). It is very important to increase the mineral components of the foods for better health. The results of ash of present study are followed similar trend of the study reported by Okafor et al., (2012). Although the Ash content of control sample (100% wheat bread) is 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. Mahedy Mahamud et al. (1998) reported that 15% MF supplemented bread flour was showed 2.20% ash, this is slightly lesser then the present study this may be because of the agro geological variations of the raw materials, but the similar trend was reported in the increase of ash concentration.

The ranges of dietary fiber in the prepared bred samples were determined as 1.59±0.04% to 2.57±0.03%. The crude fibre content of the bread samples also increased as the substitution of MF was increased in the prepared bread samples. Bread prepared with 100% WWF had showed the least crude fibre content (1.59±0.04%) while sample with 75% of WWF and 25% MF had the highest crude fibre content of 2.57±0.03 %.There was no significant difference also existed at (P < 0.05) in the crude fibre contents of samples prepared with 96.8% of WWF and 3.2 % of the MF and sample prepared from 93.7% WWF, 6.3% MF. The raise in the fiber content of the bread with the raise in the MF attributed to the high content of the fiber in the MF compared with the WWF (Table 1). Okafor et al. (2012) reported the similar trend in the increase of fiber content as the MF increase, and the fiber value of the prepared bread sample is similar with that of reported study. The fiber content of bread sample of this study is higher than bread prepared from wheat-cassava (Eddy, et al. 2007) in line with bread breadfruit, breadnut and wheat (Malomo et al. 2011) and lesser in wheat and plantain composite of bread (Mepba et al. 2007) and tiger nut-wheat bread samples (Ade-Omowaye, 2008).

Fat is one of the important components in the food and act as energy source, helps to increase the palatability of the foods. The range of the fat determined in the bread samples was 1.72±0.02% to 1.92±0.01%.

The fat content of bread prepared in present study was similar with that of Eddy et al. (2007) (Wheat–cassava bread), Okafor et al. (2012)(Wheat and Nigerian Oyster Mushroom), less than the report of Malomo et al. (2011) (breadfruit, breadnut and wheat).

Table 1. Proximate compositions of the raw flour samples

Flour samples	Proximate components						
	Moisture (%)	**Ash (%)**	**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 presented means ± SD of three analyses

Table 2. Proximate composition of bread samples different concentration of the whole what and mushroom flours

Flours		Proximate composition						
Whole wheat	Mushroom	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Fiber (%)	Carbohydrate (%)	Energy (kcal/ 100 gms)
*100	0	30.84±0.008^{ab}	0.88±0.02^{hi}	10.21±0^{e}	1.72±0.02^{d}e	1.59±0.04^{d}	54.76±0.05^{a}	275.4±0.2^{ab}
96.8	3.2	30.63±0.03^{ab}	1.06±0.01^{g}	12.91±0.44^{d}	1.74±0.00^{1de}	1.98±0.05^{c}	51.69±0.4^{b}	274.04±0.16^{de}
93.7	6.3	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}
87.5	12.5	30.15±0.17^{b}	1.56±0.003^{e}	18.3±1.46b^{c}	1.87±0.02^{c}	2.25±0.01^{b}	45.87±1.39^{c}	273.51±0.77^{de}
84.5	14.5	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}
81.3	18.7	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}
76.2	23.8	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}
75	25	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±0.1^{ab}

*= Control sample (100% WWF)

The values are presented in Mean ± Standard deviation of three observations; Means in the same column with the same superscript are not significantly different at (P < 0.05)

Proteins are very important molecules in the foods, which provide the essential amino acids for the metabolism of living cells. The range of protein present in the bread prepared was 10.21±0% and 23.92±0.1. The lowest protein content (10.21%) was observed in the bead samples prepared 100% WWF. The control bread sample is statistically non-significant ($P < 0.05$) with the other samples (MF incorporated). It is a known fact that WWF is a poor source of the protein and good source of carbohydrate. In contrast, as the MF concentration increased the protein concentration of the bread samples increased. The highest protein content of 23.92 ±0.1% was observed in the bread formulation with the highest concentration of MF (25%). This is attributed to that of rich source of the quality protein in MF. It is conformed from the study that the protein concentration of the bread can improve more than twice by incorporation of 25% of the MF in bread formulation. The protein content of the sample in the present study is highest than the results reported by different researchers (Ade-Omowaye, 2008; Eddy et al. 2011; Mepba et al. 2007; Okafor et al. 2012) who prepared bread with different wheat based composite flours.

Carbohydrates are the major composition of the many plant based foods, and they are the immediate energy sources in the metabolism. The carbohydrate contents of the breads decreased significantly ($P < 0.05$) as MF concentration was increased. The highest amount of carbohydrates (54.76±0.05%) were observed in the bread samples prepared from the 100% WWF where as the lowest (40.89±0.08 %) observed in the breads prepared from highest concentration (25%) of the MF. This trend is because of the wheat is a good source of carbohydrate then mushroom flours (Table 1). The carbohydrate content of the bread in the present study is lower with that of Ade-Omowaye, (2008); Eddy et al. (2007); Mepba et al. (2007); Okafor et al. (2012) , and in line with the study of Malomo et al. (2011), who reported the bread properties with different wheat based composite flours.

The ranges of energy in the bread samples prepared from the composite flours were 276.45±0 to 270.46±0.15 Kcal/100gm. There was no specific trend was observed in effect of MF with that of composite flours. The energy supplied by the bread in the present study is lower than that of Eddy et al. (2007), this may be because of the lower the concentration of the carbohydrates and the fats, which are the major source of the energy, but similar with Malomo et al. (2011) study.

Effect of MF substitution with wheat flour on rheological properties of dough

Table 3 shows the results of rheological properties of dough determined from farinograph method. Absorption is the amount of water required to center the farinograph curve on the 500-Brabender Unit (BU) line. This relates to the amount of water needed for a flour to be optimally processed into end products it is expressed as percentage (Wheat Marketing center, 2008). In the present study, the range of the absorption % is identified as 54.3±0.31to 65.27±0.25. The absorption of the control samples in statistically not significant ($P<0.05$) with the MF incorporated samples. As the MF concentration increase the absorption index also increased.

The absorption of more water during mixing is a typical characteristic of composite starches and proteins (Doxastakis et al. 2002). Different authors mentioned that the dough prepared from composite flours absorbed more water than that made from only wheat flour (Lee, et al. 2001; Morita et al. 2002). It is well known that the main dough component in the WWF responsible for water absorption is gluten. The MF is gluten free, but composed of higher protein content than WWF (30.1 g/100 g) (Table 1). The reason for higher absorption ability might be a higher protein content in all investigated flour mixtures (Ribotta et al. 2005). The other reason may also that the interaction between mushroom proteins and wheat gluten were reported to occur in the composite flour (Eissa et al. 2007). Besides the higher protein content, higher dietary fibers in the mixtures may be responsible for higher absorption properties. As the results show that the content of dietary fibers in the MF was almost four times higher (9.76 g/100 g) than in the WWF (2.73 g/100 g). Generally, fibers provide better properties of water absorption and this is mainly due to a higher number of hydroxyl groups which exist in the fiber 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 (Eissa et al. 2007). Higher water absorptions made dough became sticky; such absorptions resulted in larger loaf volumes. The values of the absorption% were similar with the report of Nikolić, et al. (2013) (wheat-mushroom and soy bean flour), Hong et al. (2005) (wheat and mushroom flour), Luz Fernandez & Berry, (2007) (Wheat and chick pea), Malomo et al. (2011) (breadfruit, breadnut and wheat)

Development time is the time taken from the first addition of water to the time the dough reaches the point of highest torque. During this phase of mixing, the water hydrates the flour components and the dough is developed. The dough development time among the flour mixtures with the MF ranged from 1.6 ±0.06 to 4.5±0.06 minutes. There was no significant different ($p<0.05$) between the development time of control samples with the MF incorporated samples. As the protein rich MF concentration increases the development time also increases. 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 of MF investigated by Nikolić et al. (2013). However, the increase in the amount of MF needs longer dough development time due to the increase in fiber (cellulose) content which requires longer period of time to absorb water (Torbica et al. 2010). The dough development time is relatively higher than the report of Malomo et al. (2011) (breadfruit, breadnut and wheat), but lower then Luz Fernandez and Berry (2007) (chickpea-wheat), Torbica et al. (2010) (rice and buckwheat flour) similar with Nikolić et al. (2013), (Soy–mushroom and Wheat). The variations in the results are because of compositional variations of the flours.

Stability Time is the difference in time between arrival time and departure time. This parameter indicates the time the dough maintains maximum consistency and is a good indication of dough strength. Stability time is expressed in minutes. There was no significant difference ($p<0.05$) between the stability time of control samples (100% WWF) with the MF incorporated samples. The highest stability time of 2.47±0.06 min was observed in the breads prepared from only WWF, in contrast the stability time of 4.03±0.05 was determined in the breads prepared with the composite flour of 25% MF and 75% of WWF. It is evident that the addition of the protein rich plant flour prolonged the development time and the dough stability. The effect of soy flours on delaying the dough development time and dough stability was investigated by Ribotta et al. (2005), stability time of the dough in the present experiment is lower than that of different reported studied (Luz Fernandez & Berry, 2007; Torbica et al. 2010), and similar with Nikolić et al. (2013) report.

Mixing Tolerance Index (MTI) is the difference in BU value at the top of the curve at peak time and the value at the top of the curve 5 minutes after the peak. This indicates the degree of softening during mixing. Mixing tolerance index is expressed in minutes. There was no significant different ($p<0.05$) between the MTI of control samples with the MF added samples. The degree of softening (mixing tolerance index) of the mixtures with the MF decreased from 218 ± 0.44 to 141. 33± 0.22 min when the MF portion in the flour mixture increased, but contrary to the mixtures with the MF, all values were higher than for the dough made of the WWF (31±0.26 min). 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 MF 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 MF components with the components of the WWF.
Farinograph Quality Number (FQN) is a measure for the flour quality. Similar to the valorimeter number, FQN expresses the shape of the farinograph in a single number. The FQN value is a combined value for dough development time, dough stability, and mixing tolerance index (Fu et al., 2008). Therefore, the FQN can represent flour quality in a single value. The addition of the MF changed the quality number and group of WWF-MF mixtures, but there was no significant different ($p<0.05$) between the FQN of control samples with the MF added samples. The quality number of the MF mixtures was higher (31 ±0.06 to 44.6±0.6) than the number of the WWF (57±0.26). These results obtained might be because of different effect of MF on the degree of softening. Weak flours weakens first and rapidly shows a low FQN number, whereas strong flour weakens late and shows a high FQN (Miralbés, 2004).

Optimum point selection for blending

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 change (Choi et al., 2006). The range of mixture ratios of WWF and MF were 75-100% and 0 - 25% (w/w), respectively. The total contents of these flours (sum of all variables) were 100% (w/w). In order to allocate the points for the mixture within the feasible design region, a modified distance design was applied. According to a mixture design, 2 kinds of responses were considered protein content and energy.
In order to analyze the interaction effect on each flour mixture, modeling was necessary for each response (Cornell, 2011). The interaction effects according to the component change were determined using response surface. Protein was selected to a quadratic non-linear regression model whereas energy was selected to a cubic non-linear regression model. The lack of fit test showed the probability values for protein, energy were 0.0265, ≤0.0001, respectively. Therefore, the fitness to the preference model was confirmed by these probability values.
The effects of the two flours on each response were shown in the two component mix. The slope of the graph for each flour represented the degree of influence of each response. Two component mixes showed the protein contents, energy of flour blends for WWF and MF, which were formulated with various mixing ratios. On the basis of the probability value of the model, cubic were generated to show trends for each flour component in the mixture. In the case of WWF (B-B), the protein content increased as the content of MF (B-B) increased. However, as the contents of MF (A-A) increased, protein generally increased (Figure 1). Furthermore, it was shown that the interaction plot of energy was slightly decreased as the contents of MF increased (A-A) (Figure 2).

Table 3. Rheological properties of the dough with different concentration of the whole what and mushroom flours

Flours (%)		Rheological properties				
Whole wheat	Mushroom	Water absorption (%)	Development time (min)	Stability (min)	Mixing Tolerance index (MTI)	Farinograph quality number (Bu)
*100	0	54.3±0.31^{f}	1.57±0.06^{h}	2.47±0.06^{f}	31±0.26^{h}	57±0.26^{a}
96.8	3.2	56.3±0.1^{e}	1.8±0^{g}	2.73±0.06^{e}	141.33±0.22^{g}	40±0.00^{c}
93.7	6.3	56.43±0.25^{e}	1.97±0.06^{f}	2.83±0.06^{e}	142.67±0.5^{f}	37.33±1.15^{d}
87.5	12.5	56.8±0.1^{d}	2.17±0.06^{e}	3.07±0.0^{d}	185.3±0.28^{b}	36±1^{e}
84.5	14.5	59.3±0.3^{c}	2.47±0.05^{d}	3.33±0.06^{c}	218.67±0.44^{a}	35±0.00^{f}
81.3	18.7	60.13±0.06^{b}	3.7±0^{c}	3.77±1^{b}	165±0.26^{d}	31.7±0.06^{g}
76.2	23.8	60.47±0.35^{b}	4.13±0.15^{b}	1.87±0.06^{g}	174±0.25^{c}	44.67±0.6^{b}
75	25	65.27±0.25^{a}	4.5±0.06^{a}	4.03±0.05^{a}	146.67±0.10^{e}	40±0.00^{c}

*= Control sample (100% WWF)
Values are means of three determinations ± standard deviation; letters followed by the numbers in columns with different letters are differ significantly at $p<0.05$

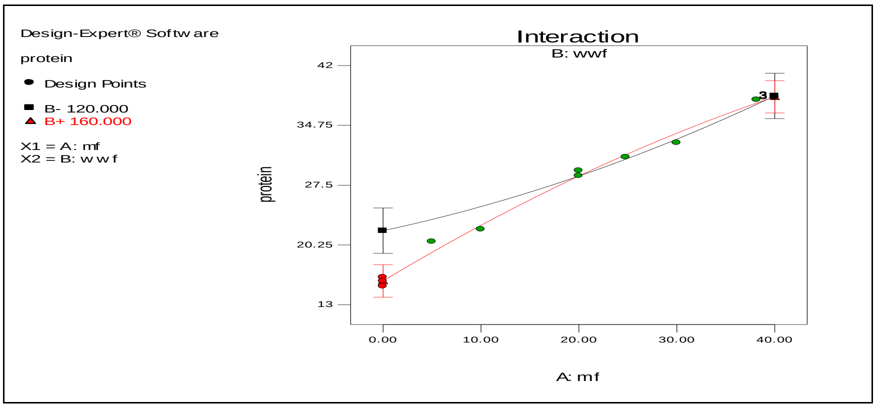

Fig. 1. Optimization plot for protein value in mushroom powder substituted bred

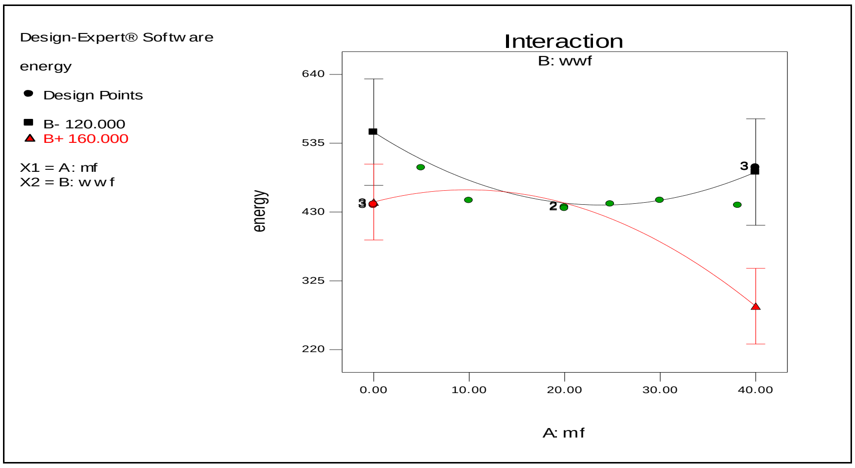

Fig. 2. Optimization plot for energy value in mushroom powder substituted bread

The optimum mixture ratio of flours with WWF and MF was determined using the optimization process suggested by (Derringer, 1980). The plot indicated the change in each element and it was used to predict final mixture ratios. Protein content was set in range and energy was set to the maximized values. The proportions of WWF and MF were set at 75-100 and 0-25%, respectively. From the numerical optimization results, the optimum flour formulation was 19.14% MF and 80.86% WWF. The predicted response values according to this mixture ratio showed that total protein content, energy were 34 %, 455.78kcal respectively.

4. CONCLUSION

Composite breads with MF substitutions were found to be nutritionally superior (have higher protein, fat and crude fibre content) to WWF bread. The farinograph properties depended on the MF 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 MF were responsible for a higher water absorption value and high value of the dough development time. Bread with 25% MF contains the highest amount of Protein, Fat and Ash. However, the investigation shows that there was significant improvement in the bread protein content and nutritional quality on addition of MF. 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.

Therefore, this research recommends not using 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. The final result of optimization suggested that the optimal ingredient concentrations to achieve best nutrition quality bread is with 80.86% WWF and 19.14% MF.

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Conflict of interest:
All the authors showed no conflict of interest

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