

Effect of Refined Milling on the Nutritional Value and Antioxidant Capacity of Wheat Types Common in Ethiopia and a Recovery Attempt with Bran Supplementation in Bread

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Abstract

The effect of wheat flour refined milling on nutritional and antioxidant quality of two types of wheat (hard and soft) grown in Ethiopia was first evaluated. Then a recovery was attempted on bread prepared with the supplementation of the white wheat flour with different levels (0%, 10%, 20% and 25%) of wheat bran. Whole wheat flour (100% extraction) and white wheat flour (68% extraction) were subjected to proximate, mineral and antioxidant analysis. Results indicated that at low extraction rate (68%) the value of protein, fat, fiber, ash, iron, zinc and phosphorous and antioxidant content of the samples were significantly affected (decreased) ($P < 0.05$) by milling. The Total Phenolic Content (TPC) of white wheat flours, which ranged from 3.34 to 3.49 mg GAE/g were significantly lower ($p < 0.005$) than those of whole wheat flours (ranging 7.66 to 8.20 GAE/g). At the concentration of 50 mg/mL, the DPPH scavenging effect of wheat extracts decreased in the order of soft whole, hard whole, soft white and hard white wheat flour, which was 90.39, 89.89, 75.80, and 57.57%, respectively. Moreover, the protein, fat, ash, fiber, iron and zinc contents of the bran supplemented breads increased significantly ($P < 0.05$) with the progressive increase in the bran level of the bread. The highest value for protein, fat, fiber, ash, iron and zinc was 12.04, 2.61, 2.48, 3.27 g/100 g and 4.84 and 2.33 mg/100 g respectively were found in 25% bran supplemented bread. The sensory evaluation of bread showed that all level of supplementation had mean score above 4 on a 7 point hedonic scale on over all acceptances. The results indicated that refined milling at 68% extraction significantly reduces the nutritional and antioxidant activity of wheat flour. Bread of good nutritional and sensory qualities could also be produced from 10% and 20% bran supplementation.

Keywords: Wheat; Whole flour; White flour; Bran; Refined milling; Nutritional; Antioxidant

Introduction

Wheat has accompanied humans since remote times (as far back as 3000 to 4000 BC) in their evolution and development, evolving itself (in part by nature and in part by manipulation) from its primitive form (emmer wheat) into the presently cultivated species [1]. Wheat crop is widely adapted to a variety of environments and is cultivated in tropical, subtropical and temperate areas [2]. It is widely consumed by humans, in the countries of primary production (which number over 100 in the FAO production statistics for 2004) and in other countries where wheat cannot be grown [3]. It also occupies 27 percent of the total cereal production worldwide [1]. It is thus, an important agricultural commodity which is consumed in large amount all over the world among all grains.

Ethiopia is the largest wheat producer in sub-Saharan Africa [4]. Nationally, wheat ranks fourth in total area coverage (1,389, 215.00 ha). It is also third in productivity (after maize and sorghum) among cereals [5]. It is one of the most important crops grown and consumed in Ethiopia both in terms of total production (2.85 million MT in 2010/11) [6] and the proportion of total calories consumed in the country (19.6% of calories consumed) [7].

Wheat possesses several health benefits, especially when utilized as a wholegrain product. According to Kumar [8], wheat provides protection against diseases such as constipation, ischaemic, heart disease, diverticulum, appendicitis, diabetes and obesity. These benefits are attributed in part to the presence of different compounds such as dietary fibers, phytochemicals, proteins, vitamins and minerals [9].

Whole wheat grain consists of bran, germ and endosperm. When refined, only carbohydrate rich endosperm is retained. This results in a big loss of many nutritionally valuable biochemical compounds such

as dietary fiber, vitamins, minerals and antioxidant compounds which play an important role in reducing cardiovascular disease (CVD) [10]. When white flour is produced, many important nutrients and fibre are removed, because these components are mainly located in bran and germ [11]. Wheat bran is rich in protein (~14%), carbohydrates (~27%), minerals (~5%) and fat (~6%) [12]. In addition, bran is the main by-product produced by milling. Wheat bran is a most important fiber source which is inexpensive and available. It is a good source of not only dietary fiber but also for other major nutrients. The loss of vitamins and minerals in the refined wheat flour has led to widespread prevalence of constipation and other digestive disturbances and nutritional disorders [8].

Milling is the critical process affecting the concentrations of nutrients in wheat-derived food products. The outer parts of the kernel, especially the aleurone layer and the germ are richer in minerals. Conventional milling reduces nutritional content in flour and concentrates them in the milling residues [13]. White flour with the extraction rate 68% meaning up to 32% of the original grain is not in the flour. Whole grain flour includes all parts of the seed and is 100%

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extraction. Milling of wheat into highly refined flours not only preclude considerable amounts of nutrients from human consumption, but the remaining flours have a much poorer nutritive value than flour made from whole wheat.

Over the past twenty years, wheat production and consumption have both increased in Ethiopia [14]. On top of this wheat flour covers a substantial proportion of the population. According to the survey conducted in Ethiopia, 28% of consumers purchase flour and flour products. It is estimated that wheat flour reaches about 22 million people [15]. However, no published information is available regarding the effect of refining on nutritional and antioxidant capacity of wheat that are commonly grown in Ethiopia though it is widely distributed and consumed. The nutritional value and antioxidant properties of wheat grain are significantly influenced by soil type and richness, growing temperatures, moisture levels, other climatic differences, and genotype [16].

It is therefore, very important to understand the nutritional value of wheat grown in Ethiopia and evaluate after the effect of refined milling. In addition, it is necessary to find a way to improve the nutrient quality of the refined wheat flour products without compromising the palatability of the product. Hence, in this study an attempt was also made to recover the nutrients through supplementation of bran in wheat bread making.

Materials and Methods

Samples

Hard wheat (*Kubsa*) and soft wheat (*ET-13*) samples were obtained from Kebron food complex (Oromia region) and Wedera farmers cooperative (Debrebrhan), respectively in Ethiopia. Bran sample was obtained from Universal Food Complex (Addis Ababa, Ethiopia) (Figure 1).

Milling of wheat

The amount of water required for tempering was calculated according to AACC [17]. One kilogram of each sample from both soft and hard wheat were cleaned and tempered separately to 14% moisture level and kept for 6 and 24 h, respectively at ambient temperature in a closed plastic jar. After tempering, wheat samples were milled at the extraction rate of 68% based on the capacity of the milling machine and 100% by using Buhler Automatic mill (Deutschland). The milling of flour was conducted at Kokeb Flour and Pasta Factory and extraction rate was calculated according to Slavin [18].

Formulation of bread

Flour blends were prepared by mixing wheat flour with wheat bran in the proportions of 100:0, 90:10, 80:20 and 75:25 (wheat flour to bran) using homogenizer and 100% white wheat flour was used as control. The formulation was made based on the preliminary test. The four flour samples were packaged in black low density polyethylene bags and stored in plastic containers at room temperature from where samples were taken bread production.

Processing of bread

Bread was prepared by partially replacing the wheat flour with 0%, 10%, 20%, and 25% of bran sample. The dough was prepared based on the method described by Hertzberg [19] with some modification by weighing 400 g of wheat flour, 8 g sugar, 4 g salt, 8 g of oil and 1.6 g yeast. A mixer (Linkrich-B15) was used to mix the ingredients in order to homogenize the mixture for 30 min and then water was added to

the dough until the desired consistency was achieved. The dough was weighed and divided into 3 equal portions for replications. These were placed in baking pans and left for 1 h. Then they were transferred into an oven pre-heated to about 180 - 250°C and allowed to bake for 20 min. The baked products were left to cool.

Nutritional analysis of wheat flour, bran and bread

All samples were analyzed for moisture, crude protein, crude fat, crude fiber and total ash according to their respective standard methods as described below.

Determination of moisture content: Dishes used for the moisture determination were first dried at 105°C for 1 hour in drying oven. It was then transferred to the desiccators, cooled for 30 minutes, and weighed. The prepared samples were mixed thoroughly and about 5 g of the flour samples were transferred to the dried and weighed dishes. The dishes and their contents were placed in the drying oven and dried for 3 hrs at 105°C, and then the dishes and their contents were cooled in desiccators to room temperature and reweighed [20].

Determination of total ash content: Total ash was determined according to AOAC [20]. The porcelain dishes used for the analysis were placed into a muffle furnace for 30 min at 550°C. The dishes were removed and cooled in desiccators for about 30 minutes to room temperature; each dish was weighed. About 2.5 g of the flour and bread samples were added into each dish. The dishes were placed on a hotplate under a fume hood and the temperature was slowly increased until smoking ceases and the samples become thoroughly charred. The dishes were placed inside the muffle furnace at 550°C for 5 hours and removed from the muffle and cooled in the desiccator and weighed. The amount of total ash was calculated by using the following formula. Weight of total ash was calculated by difference and expressed as percentage of sample.

Determination of crude protein: Protein ($N \times 6.25$) was determined by the Kjeldahl method [20]. All nitrogen is converted to ammonia by digestion with a mixture of concentrated sulfuric acid containing copper sulfate and potassium sulfate as a catalyst. The ammonia released after alkalization with sodium hydroxide is steam distilled into boric acid and titrated with hydrochloric acid.

Determination of crude fat content: Crude fat was determined by exhaustively extracting a known weight of sample in diethyl ether (boiling point, 55°C) in a soxhlet extractor [20]. The ether was evaporated from the extraction flask. The amount of fat was quantified gravimetrically and calculated from the difference in weight of the extraction flask before and after extraction as percentage.

Determination of crude fiber content: 1 g of sample (W_1) and 1 g of celite (sand) was weighed into a crucible and placed the crucible in Fibertec hot extraction and added 150 ml of hot 0.64 N of sulfuric acid [20]. Add 2 - 4 drops of n-octanol to prevent foaming and boiled for



Figure 1: Hard wheat, bran and soft wheat.

10 min. In step 2 of hot extraction add 150 ml of 0.556 N NaOH and put 2 - 4 droplets of n-octanol and boil it for 10 min. In both process washing with hot deionized water was applied. Evaporate solvent, dry the crucibles at 130°C for 2 hours and cooled in desiccator and weigh. Ash the sample in the crucible at 550°C for 3 hours and cooled down and weigh.

Determination of mineral contents

Determination of Fe, and Zn: Iron and zinc were determined according to the standard method of AOAC [20] using flame Atomic Absorption Spectrophotometer. Ash was obtained from dry ashing of the samples. The ash was wetted completely with 5 ml of 6 N HCl, and dried on a low temperature on hot plate. A 7 ml of 3 N HCl was added to the dried ash and heated on the hot plate until the solution just boiled. The ash solution was cooled to room temperature in a hood and was filtered using filter paper (Whatman 45). A 5 ml of 3 N HCl was added into each crucible dishes and was heated until a solution boiled then cooled and filtered into the flask. The crucible dishes are again washed three times with deionized water, the washing was filtered into a flask. Then the solution was cooled and diluted to 50 ml with deionized water. A blank was prepared by taking the same procedure as the sample.

Determination of phosphorus: Phosphorus was determined using the molybdovanadate method [21]. Briefly, 5 ml of aliquot was pipetted from the sample digest into a 100 ml volumetric flask. Ten ml of the molybdate and vanadate solutions was added to the samples and the standards and made up to volume with distilled water. After 10-30 minutes color developed and measured on the absorbance of the blank, sample and standards by spectrophotometer at a wavelength of 460 nm.

Antioxidant capacity determination

Sample extraction: Samples were extracted based on the procedures as outlined by Woldegiorgis [22]. The powdered wheat samples were homogenized and weighed in to ten gram was then extracted by stirring with 100 ml of methanol at 25°C at 150 rpm for 24 h using an incubator shaker (ZHWY-103B) and then filtered through Whatman No. 1 filter paper. The residue was then extracted with two additional 100 ml portions of methanol as described above. The combined methanolic extracts were evaporated at 40°C to dryness using a rotary evaporator and re-dissolved in methanol at a concentration of 50 mg/ml and stored at 4°C for further use.

Determination of free radical scavenging activity: The hydrogen atoms or electrons donation ability of the corresponding extracts and some pure compounds were measured from the bleaching of purple colored methanol solution of DPPH [23]. Antioxidant activity of the methanol extracts was determined by DPPH radical scavenging method as described by Woldegiorgis [22]. A 0.004% solution of DPPH radical solution in methanol was prepared and then 2 ml of DPPH solution was mixed with 1 ml of various concentrations (0.1-50 mg/ml) of the extracts in methanol. Finally, the samples were incubated for 30 min in the dark at room temperature. Scavenging capacity was read spectrophotometrically by monitoring the decrease in absorbance at 517 nm. Ascorbic acid was used as a standard and mixture without extract was used as the control. Inhibition of free radical DPPH in percent (I%) was then calculated.

Total phenolics determination: Phenolic compounds concentration in the wheat was estimated with Folin-Ciocalteu reagent according to the Singleton and Rossi method [24] as described by

Woldegiorgis [22]. One milliliter of sample (5000 µg) was mixed with 1 ml of Folin and Ciocalteu's phenol reagent. After 3 min, 1 ml of saturated sodium carbonate (20%) solution was added to the mixture and adjusted to 10 ml with distilled water. The reaction was kept in the dark for 90 min, after which the absorbance was read at 725 nm. Gallic acid was used to construct the standard curve (5-80 µg/ml). The results were mean values ± standard error of mean and expressed as mg of gallic acid equivalents/g of extract (GAEs).

Sensory evaluation of bread

Sensory evaluation was conducted for the freshly baked breads by 30 semi-trained panelists consisting of students male and female aged from 23-43 years old from Food Science and Nutrition Center of Addis Ababa University. The samples were presented randomly in identical containers, coded with three digit numbers. The sensory was conducted using a seven point hedonic scale. Where 1=dislike very much, 2 = dislike moderately, 3 = dislike slightly, 4 = neither like nor dislike, 5 = like slightly, 6 = like moderately and 7= like very much. The sensory attributes which were evaluated were taste, odour, colour, texture and overall acceptability. Those samples were considered as acceptable which their average score for the overall acceptability were greater than 4 which mean neither like nor dislike [25].

Statistical analysis

The data were subjected to analysis of variance (ANOVA) and Duncan's multiple range tests were used for mean separation at $p < 0.05$. Linear regression analysis was used to calculate IC50 value. Pearson correlation between DPPH scavenging (%) and total phenolic content was considered at $p < 0.05$.

Results and Discussion

Proximate composition of wheat, wheat bran and bread

The mean value for moisture, crude protein, crude fat, total ash and crude fiber of wheat bran, wheat flour (hard and soft), white wheat flour (hard and soft) and bran supplemented bread are presented in Tables 1-3. The mean values for moisture contents of different whole wheat and white wheat flours are presented in Table 1. It ranged from 10.48 to 12.30%. The highest moisture level, 12.30%, was found in hard white wheat flour. The moisture content varies significantly between whole and white flour and between hard and soft white flour ($P < 0.05$). The increment on moisture content of both soft and hard white wheat as compared to the whole wheat flour could be due to the addition of water during the tampering process to facilitate milling of wheat which resulted in retaining more water in refined wheat flour than whole wheat flour.

There is also a significant difference ($P < 0.05$) on total ash contents of all flour samples (Table 1). The result indicated that highest ash content ranged from 1.62 to 1.41%. The highest ash content (1.62%) was found in hard whole wheat flour where as the soft white flour showed the lowest (0.38%) ash content. The results were comparable to Azizi [26] values obtained from different extraction rate of wheat flour which ranged 1.51% to 0.54% ash content at 93% and 70% extraction rate respectively.

The result for crude fat content is shown in Table 1 and the values showed significant difference ($P < 0.05$) between whole wheat flours and white wheat flours. The fat content decreased in white wheat flour. The highest fat content, 1.83%, was found in whole wheat flour (100% extraction rate); whereas, the lowest, 1.32%, was found in white wheat flour (low extraction rate). The high percentage of fat in whole wheat

Parameters	Wheat samples			
	HWF	HWWF(refined)	SWF	SWWF(refined)
Moisture (%)	10.75 ± 0.38 ^a	12.30 ± 0.09 ^c	10.48 ± 0.10 ^a	11.60 ± 0.23 ^b
Ash (%)	1.62 ± 0.03 ^d	0.65 ± 0.01 ^b	1.41 ± 0.07 ^c	0.38 ± 0.07 ^a
Fat (%)	1.82 ± 0.04 ^b	1.43 ± 0.18 ^a	1.78 ± 0.10 ^b	1.32 ± 0.11 ^a
Protein (%)	14.40 ± 0.30 ^d	11.91 ± 0.087 ^c	9.11 ± 0.12 ^b	7.13 ± 0.06 ^a
Fiber (%)	2.6 ± 0.08 ^b	0.42 ± 0.06 ^a	2.5 ± 0.08 ^b	0.36 ± 0.07 ^a

HWF- Hard whole wheat flour, HWWF- Hard white wheat flour (refined), SWF -Soft whole wheat flour, SWWF- Soft white wheat flour (refined).

Data are average of triplicate ± SE .Mean value with different superscript in the same rows are significantly different (P<0.05)

Table 1: Proximate composition of wheat.

Parameter	Composition (g/100g)
Protein	15.26 ± 0.35
Fat	3.12 ± 0.7
Fiber	9.97 ± 0.27
Ash	4.5 ± 0.16

Table 2: Proximate composition of wheat bran.

flour is because wheat germ is ground along with endosperm during milling [27].

The results of this study also indicated that the protein contents for all flours varied significantly (P<0.05). The protein contents decreased with in both hard and soft white wheat flour; at the same time there was significant difference between hard and soft whole wheat flour. The highest protein content found on hard whole wheat flour was 14.40%; whereas the protein content of soft whole wheat flour was 9.11%. The result of this study was in line with the value of hard and soft whole wheat reported by Blakeney [28].

The mean value for fiber content of hard whole wheat, hard white wheat, soft whole wheat and soft white wheat flour are 2.6, 0.42, 2.5 and 0.36 g/100 g, respectively (Table 1). The result of this study showed that there is significant difference (P<0.05) between whole wheat flour and white wheat flour. Both hard and soft whole wheat flour exhibited high crude fiber content (2.6 and 2.5%). The white wheat flour showed less fiber content because wheat bran was removed during milling process which decreased the amount of fiber in flour. The result of this study is in agreement with Azizi [26], who reported crude fiber in the range of 0.30 to 2.24% white wheat and whole flour respectively.

The proximate composition of wheat bran samples are given in Table 2. Wheat bran was found to contain highest amounts of crude protein, fat, fiber and ash with mean values of 15.26%, 3.12%, 9.97%, and 4.45% respectively (Table 2). The objective of milling is to separate the bran and germ from the starchy endosperm so that the endosperm can be ground into flour. The aleurone layer, which is rich in protein, minerals and vitamins, usually breaks away with the outer layer of the bran in the milling process, thus, contributing significantly to the nutritional quality of the bran fraction [29].

Proximate composition of different bran supplemented bread and control were also analyzed for proximate composition. The mean values for moisture contents of the bread samples are presented in Table 3 which ranged from 30.92 to 32.83%. The highest moisture level, 32.83%, was found in 25% bran supplemented bread. The moisture content of the control bread decreased from the three samples bread significantly (P<0.05).

The statistical analysis for crude protein is presented in Table 3. The mean value for protein content of all the study samples of bread ranged

from 9.42 for control to 12.04 for WBB (75:25). The protein contents for three of the breads (0%, 10%, 20% bran supplemented bread) varied significantly (P<0.05). However, there was no significant difference between 20% and 25 % bran supplemented bread. The result of protein contents are in agreement with the findings of Butt [30] who reported an increase contents of protein with an increase in bran proportion.

The result of this study indicate that crude fat showed significant difference (P<0.05) among all breads. The fat content increased with an increase in bran level. The highest fat content, 2.61%, is found in 25% bran supplemented bread, whereas, the lowest, 1.56%, was found in the control bread. The increase in fat content is because of the germ which is grounded along with bran and endosperm during milling, results in bread with higher fat content than the control bread [27].

The mean values of crude fiber contents of different bread samples are given in Table 3. The statistical analysis showed significant (P<0.05) effect on the quantity of crude fiber. The crude fiber contents ranged from 0.38% to 3.27%. The 25% bran supplemented bread exhibited the highest crude fiber (3.27%), whereas, the control bread contain the lowest crude fiber (0.38%). The crude fiber increased with an increase in bran supplementation rate. The control showed less fiber contents because the bread was made from refined bread with no addition of bran.

Ash is the mineral residue remaining after a sample has been completely oxidized in a manner such that all organic volatile material is driven off, while preventing any mineral from being lost [29,30]. Ash varied significantly among all the bread (Table 3). The statistical analysis showed significant (P<0.05) effect on total ash contents. The results indicate that ash content ranged from 1.38 to 2.48%. The highest ash content (2.48%) was found in 25% bran supplemented bread, whereas the control showed the lowest (1.38%) ash content. The addition of 10 to 25% wheat bran to the bread increased the ash content.

Mineral content of wheat flour and bread

The mineral content of the whole and white flour samples are shown in Table 4. According to the results of this study, the iron content level in hard and soft whole wheat flour is significantly different (p<0.05) from hard and soft white wheat flour. The iron content of whole wheat flour ranged from 2.95 to 4.15 mg/100 g whereas the iron content of the white wheat flour ranged from 2.51 to 3.35 mg/100 g.

Dewettinck [31] reported that the iron content of the whole wheat was (1-5 mg/100 g) which is in agreement with the result of this study. However, the level of iron in the white flour decreases significantly. The milling process removes many important nutrients when white flour is produced. The bran and the germ are relatively rich in minerals and the milled products contain less of these than the original grain. As a result of milling the palatability is increased but the nutritional value of the products is decreased [32].

Zinc content varied significantly among all whole wheat and white wheat flour (Table 4). The zinc content of the whole wheat ranged from 3.59 to 2.47 mg/100 g. However, the white flour contained in the range of 0.58 to 1.39 mg/100 g. The highest Zn content 3.59 mg/100 g was found in hard whole wheat flour. This study showed that low rate of extraction of wheat reduce the zinc content of the wheat significantly (P<0.05). The hard whole wheat flour zinc content was 3.59 mg/100 g where as milling reduced the zinc content to 1.39 mg/100 g. Whereas in case of soft wheat the decrease was from whole wheat to white wheat flour 2.47 to 0.58 mg/100 g of zinc respectively. According to Lopez [33] 80% of the total amounts of minerals are concentrated in the

Samples	Moisture %	Protein %	Fat %	Ash %	Fiber %
Control(WFB)	30.92 ± 0.38 ^a	9.42 ± .22 ^a	1.56 ± .006 ^a	1.38 ± 0.10 ^a	0.38 ± 0.02 ^a
WF:BR (90:10)	33.14 ± 0.26 ^b	10.70 ± 0.55 ^b	2.14 ± 0.032 ^b	2.04 ± 0.32 ^b	2.13 ± 0.05 ^b
WF:BR (80:20)	33.24 ± 0.69 ^b	11.66 ± 0.89 ^c	2.36 ± 0.04 ^c	2.29 ± 0.04 ^c	3.11 ± 0.06 ^c
WF:BR (75:25)	32.83 ± 0.58 ^b	12.04 ± 0.84 ^c	2.61 ± 0.06 ^d	2.48 ± 0.02 ^d	3.27 ± 0.008 ^d

WFB-(white wheat flour bread)- control, WF:BR 90:10, 10% bran supplemented bread ,WF:BR 80:20- 20% bran supplemented bread, WF:BR 75:25 - 25% bran supplemented bread

Data are average of triplicate ± SE. Mean value with different superscript in the same column are significantly different (P<0.05).

Table 3: Proximate composition of Bran supplemented bread and control (Dry weight basis).

Wheat samples	Mineral content mg/100 g		
	Iron	Zinc	Phosphorous
HWF	4.15 ± 0.12 ^c	3.59 ± 0.063 ^d	337.99 ± 0.56 ^d
HWWF	2.51 ± 0.16 ^a	1.39 ± 0.036 ^b	144.69 ± 0.61 ^b
SWF	3.35 ± 0.17 ^b	2.47 ± 0.04 ^c	313.98 ± 1 ^c
SWWF	2.95 ± 0.26 ^a	0.58 ± 0.01 ^a	77.03 ± 0.51 ^a

HWF- hard whole wheat flour, HWWF- Hard white wheat flour, SWF -soft whole wheat flour. SWWF-Soft white wheat flour

Mean value with different superscript in the same column are significantly different (P<0.05).

Table 4: Mineral composition of whole and refined wheat flour.

aleurone layer of pericarp (bran) which was removed during milling process while only 20% minerals are present in endosperm.

The mean total content of phosphorous on hard whole and soft whole wheat flour was 337.99 and 313.98 mg/100 g respectively however, there was significant decrease of phosphorous content (P<0.05) on the refined milled product of hard and soft white wheat flour which was 144.69 and 77.03 mg/100 g respectively. Wheat is one of the cereals which are classified as rich sources of phosphorous. The result of the whole wheat flour was in line with the finding of Dewettinick [31] which reported phosphorous from 200-1200 mg/100 g.

The replacement effect of different levels of wheat bran on the mineral content of bread is shown in Table 5. All the mean value for iron content varied among the bread samples. Results showed that mineral progressively increased when levels of bran were increased. Control contained 1.98 mg/100 g iron and at the level of 25% bran replacement, the value increased to 4.84 mg/100 g of iron. The result of this study was in agreement with the study of Butt [30].

The replacement effect of different levels of wheat bran on the zinc content of bread is shown in Table 5. Results showed that zinc progressively increased when levels of bran were increased. The result ranged from 0.93 to 2.33 mg /100 g of zinc. The value of zinc at the bran supplementation of between 20 and 25% was not significantly different (P>0.05).

Micronutrient malnutrition greatly increases mortality and morbidity rates, diminishes cognitive abilities of children, lowers labor productivity and reduces the quality of life for all those affected. Deficiency of micronutrients, such as iron and zinc, is critical and major problem. It could be concluded that the addition of bran improves the nutritional quality of bread and could be a means of providing adult their daily requirements of iron and zinc. Although supplementation of bran improves the mineral content of the bread due to increase of phytic acid content, the availability of these minerals may be reduced. Therefore, such issues would need further evaluation.

Antioxidant capacity of wheat

The percentage yields of extracts were 7.58% w/w (Hard refined wheat), 8.2% w/w (Hard whole flour wheat) and 7.7% w/w (Soft refined wheat flour) and 7.3% w/w (soft whole flour wheat).

It has been recognized that the total phenolic content of plant extracts is associated with their antioxidant activities due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers. Total phenolic content (TPC) was expressed as milligrams of Gallic acid equivalent (GAE) per gram (mg/g) of dry flour samples. As shown in Table 6, the total phenolic content (TPC) in whole wheat was highest. The TPC of white wheat flours (refined), which ranged from 3.34 to 3.49 mg GAE/g which were significantly lower (p<0.005) than those of whole wheat flours (range 7.66 to 8.20 mg GAE/g). However, the mean content did not vary much between whole hard and soft wheat type. Also there was no significant variation between soft and hard white wheat flour. The difference in the total phenolic content between whole and white wheat flour could be due to the process of milling. Research found antioxidants in wheat concentrated mostly in the aleurone layer of bran with some in the pericarp, nuclear envelope and germ [34,35].

The ability of wheat extracts to quench reactive species by hydrogen donation was measured through the DPPH radical scavenging activity test. The antioxidants can react with DPPH, a violet colored stable free radical, converting it into a yellow colored α,α -diphenyl- β -picrylhydrazine. The discoloration of the reaction mixture can be quantified by measuring the absorbance at 517 nm, which indicates the radical-scavenging ability of the antioxidant. The antioxidant capacity whole and refined wheat was measured as the DPPH scavenging activity.

The DPPH radical scavenging effects of wheat methanol extracts was shown in Figure 2. As the concentration of sample increased, the percent inhibition of DPPH radical also increased [36]. At the concentration of 50 mg/mL, the scavenging effect of ascorbic acid, and wheat extracts, on the DPPH radical scavenging decreased in the order of L- ascorbic acid > soft whole > hard whole > soft white > hard white wheat flour, which were 92.53, 90.39, 89.89, 75.80, 57.57 % respectively. Therefore, the percentage of DPPH radical scavenging capacity of soft whole and hard whole wheat extracts are comparable with commercial antioxidants, L-ascorbic acid at concentration of 50 mg/mL. This suggested that whole wheat contain compounds that can donate electron/hydrogen easily and stabilizes free radicals.

The IC₅₀ values of all the extracts were calculated from plotted graph of percentage scavenging activity against concentration of the extracts (Figure 2). The lower the IC₅₀ value, the higher is the scavenging potential. The IC₅₀ values ranged from 10.56 mg/mL for whole wheat extracts to 41.25 mg/mL for hard white wheat extracts. Strongest scavenging activity (lower IC₅₀ values) was recorded for whole hard and soft wheat extracts which appeared more than four times stronger than that of hard white flour and two times stronger than that of soft white wheat extracts. IC₅₀ value of ascorbic acid well known antioxidant was relatively more pronounced than that of the extracts (Figure 2). The results of this study demonstrate that the antioxidant content of wheat has been affected by the refined extraction/milling process. According

Samples	Iron mg/100g	Zinc mg/100g
Control (WFB)	1.98 ± 0.056 ^a	0.93 ± 0.064 ^a
WF:BR (90:10)	2.34 ± 0.159 ^b	1.697 ± 0.108 ^b
WF:BR (80:20)	2.95 ± 0.0753 ^c	2.28 ± 0.131 ^c
WF:BR (75:25)	4.83 ± 0.074 ^d	2.33 ± 0.066 ^c

WFB- (white wheat flour bread)- control, WF:BR 90:10, 10 % bran supplemented bread ,WF:BR 80:20- 20% bran supplemented bread, WF:BR 75:25 - 25% bran supplemented bread.

Data are average of triplicate ± SE Mean value with different superscript in the same column are significantly different (P<0.05)

Table 5: Mineral analysis of bread (Dry weight basis).

Sample	Yield % (g/100g)	Total Phenolics (mg GAE/g)
HWF (whole)	8.2	7.66 ± 0.70 ^b
HWWF(refined)	7.58	3.49 ± 0.86 ^a
SWWF (refined)	7.7	3.34 ± 0.14 ^a
SWF(whole)	7.3	8.20 ± 0.35 ^b

HWF- hard whole wheat flour, HWWF- Hard white wheat flour (refined), SWF -Soft whole wheat flour. SWWF-Soft white wheat flour (refined)

Data are average of triplicate ± SE. Values in the same column with different superscript are statistically significant (p< 0.05).

Table 6: Percent yield and Total Phenolics Content.

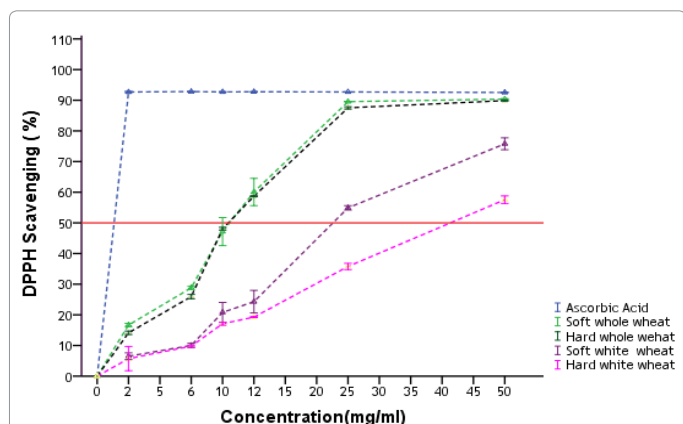


Figure 2: DPPH radical scavenging activity (%) of wheat extracts and standard (Values are average of triplicate measurements (mean ± SEM)).

to Fikreyesus [37] the DPPH (IC₅₀) for whole wheat flour is 15.56 which is different from the result obtained in this research. It is known that the antioxidant properties of wheat grain are significantly influenced by the genotype and environmental conditions [16]. To the best of our knowledge, there are no or few studies conducted on antioxidant content of Ethiopian wheat and particularly on comparison of antioxidant content on the whole and white wheat flour.

A relationship between phenolic content and antioxidant activity was extensively investigated, and both positive and negative correlations were reported. Bakchiche [38], Petra [39] and many other research groups stated that there was a positive correlation. However, a few evidences of no significant correlation were confronted [40]. In this study, the dependence of DPPH scavenging activity (%) in relation to the total phenolic content was also evaluated. The total phenolic content correlated significantly with DPPH scavenging activity (R²=0.637, p<0.05). Thus the phenolics from the wheat extracts showed a good hydrogen-donating capacity, as well as high reactivity to free radicals, leading to the stabilization and termination of the radical chain reactions.

Sensory analysis of the bread

The sensory attributes of bread made from bran (Figure 3) using different ratio were evaluated using 7-point hedonic scale at Addis Ababa University; Center for Food Science and Nutrition by semi-trained panelists of first year M.Sc program students of Food Science and Nutrition stream and the mean scores of evaluated sensory attributes were presented in Table 7.

Taste is an important parameter when evaluating sensory attribute of food. The product without acceptable good test is likely to be unacceptable by consumers. The observed mean score of taste in experimental bran supplemented bread ranged from 4.43 to 5.93 (Table 7). Control (100% white wheat flour bread) had the highest mean score in taste (5.93) followed by 10% bran supplemented bread (5.93). The 100% white wheat flour (control) bread had significant difference (p<0.05) with 10% bran (5.26), 20% bran bread (4.80) and 25% bran bread (4.43). The control bread had significant difference (p<5) from three of the bran supplemented bread (10%, 20%, 25%). The 10% bran supplemented bread scores >5 indicating that it is moderately likable by panelists and 20% and 25% bran supplemented bread were rated as neither like nor dislike by the panelists (scored as 4.80 and 4.43 respectively). This is due to the addition of the bran to the bread.

The mean score of odor of bread ranged from 4.76 to 5.53 (Table 7). Most of the samples were similar in odor while only 25% supplemented bread significantly (P<0.05) decreased. Three of the samples were liked moderately whilst the 25% bran supplemented bread was rated as neither like nor dislike.

As is shown in Table 7 colour of bread had low score as a result of increasing the level of wheat bran. The colour of control and 10% supplemented bread were similar in appearance while 20% and 25% bran supplemented bread were decreased (4.76 and 4.13) significantly (p<0.05). The results indicated that no significant difference (p>0.05) were observed by panelists between the control and 10% supplemented bread. The texture of the control and 10% bran supplemented bread were relatively most preferred (liked moderately) by the panelists. While the bread prepared from increasing level of bran supplement from 25% were scored as 4.5.

Generally, among the bread products, the control was highly acceptable by the panelists, with a score of 5.93. Next to this the 10% and 20% bran supplemented bread scored, 5.43 and 4.96 respectively. These two are liked moderately. The result obtained for 25% bran

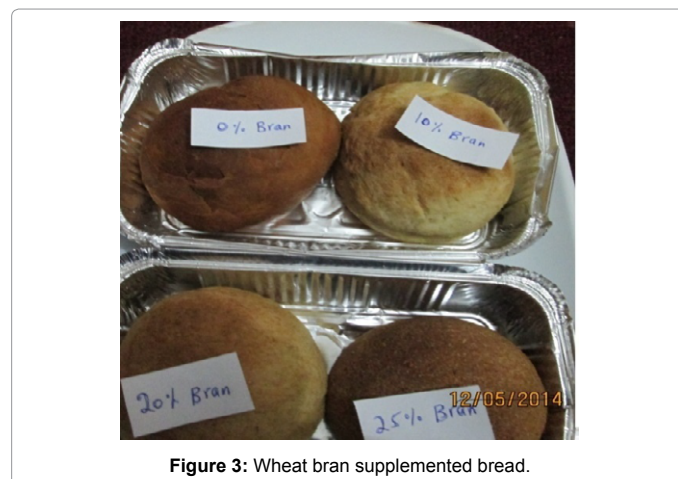


Figure 3: Wheat bran supplemented bread.

Sample	Test	Odour	Colour	Texture	Overall acceptability
Control (WFB)	5.93 ± 0.14 ^c	5.53 ± 0.21 ^b	5.93 ± 0.18 ^c	5.70 ± 0.19 ^b	5.93 ± 0.11 ^c
WF:BR (90:10)	5.26 ± 0.16 ^b	5.43 ± 0.18 ^b	5.46 ± 0.14 ^c	5.30 ± 0.13 ^b	5.43 ± 0.15 ^b
WF:BR (80:20)	4.80 ± 0.24 ^b	5.33 ± 0.23 ^b	4.76 ± 0.22 ^b	4.60 ± 0.20 ^a	4.96 ± 0.16 ^b
WF:BR (75:25)	4.43 ± 0.30 ^a	4.76 ± 0.22 ^a	4.13 ± 0.24 ^a	4.36 ± 0.23 ^a	4.50 ± 0.22 ^a

WFB-(white wheat flour bread) - control, WF:BR 90:10, 10% bran supplemented bread ,WF:BR 80:20- 20% bran supplemented bread, WF:BR 75:25 - 25% bran supplemented bread.

Data are average of triplicate ± SE Mean value with different superscript in the same column are significantly different (P<0.05).

Table 7: Sensory characteristics of wheat bran supplemented Bread.

supplemented bread was significantly different from the previous two. The latter was neither like nor disliked by the panelists and it scored 4.5. In relation to this Lazardiou [25] reported that those samples were considered as acceptable which their average score for the overall acceptability were greater than 4 which mean neither like nor dislike. Thus, whole wheat flour with high extraction rate (100%) needed to be given high emphasis by consumers because of its nutritional and antioxidant capacity of the product. Commercialization of wheat bran as a value added food ingredient to benefit from its high nutrition content.

Conclusion

Wheat and wheat products are important staple foods that are commonly consumed in Ethiopia. Consumption of whole grains as part of the diet is recommended for health reasons because they are good source of minerals, fibers, protein and antioxidants. There are no studies on the effect of refining on the nutritional content and antioxidant capacity of wheat grown in Ethiopia. This study showed that wheat extraction/refining at the lower rate have significantly reduces the proximate composition as well as the antioxidant content of wheat in both hard and soft wheat samples.

Addition of wheat bran to white wheat flour improves the nutritional value of the bread. Based on obtained results, the incorporation of wheat bran in the ratio of 10 to 20% showed better sensory acceptability though the proximate composition and mineral content increased at 25% bran supplemented bread. This indicates that bread of good nutritional and sensory qualities could be produced from 10% and 20% bran supplementation. The result of this study also indicated that wheat bran as a good source of minerals and fibers and can be used to supplement bread.

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