RESEARCH ARTICLE

Effects of milling and baking processes on bread quality: A case in Kamise, Ethiopia

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Milling and baking are processes which determine bread quality. Proximate compositions of wheat grains and flour samples from one central milling factory and bread samples from different bakeries in Kamise, Ethiopia were determined using standard methods in the laboratory. The fat and ash contents decreased by milling wheat grains; whereas, the moisture, protein and fat contents of the wheat flours increased in bread samples after baking. Highest amounts of Mg (243.25 \pm 2.5 mg/kg) and Ca (350.40 \pm 2.2 mg/kg) were found in the bread; while lowest concentrations of Mg (81.25 \pm 0.15

mg/kg) and a higher Ca concentrations ($110.75 \pm 0.2 \text{ mg/kg}$) were observed in flour samples. Significantly highest amount of iron was obtained in the grains than in the flour and bread samples; indicating loss of iron in milling and baking processes. Lead was found on bread samples than in the grain or flour samples, which show the presence of contamination during baking processes. The variations between samples due to milling and differences in baking processes could be as a result of differences in water used, additives and fermentation parameters. The evaluation of milling and baking processes in affecting nutrient composition are important to control the nutritional quality of bread.

Keywords: Bread; Food analysis; Food quality; Wheat grains

INTRODUCTION

Cereals crops are one of the most important agricultural commodities in Ethiopia, where wheat is the second important cereal consumed next to maize. With regard to the human diet, the major cereals consumed include wheat (Triticum), rice (Oryza sativa), oats (Avena sativa), barley (Oldeumvulgare), rye (Secalecereale), corn (Zea mays) and millet (Panicum miliaceum). From these varieties, wheat is the most common and widely consumed cereal crops in the world [1].

Wheat provides nutrients and essential elements (such as Fe, Se, Co, Cu and Zn) which are vital to our biological functions at optimal levels but also hazardous to our well-being in excess concentrations [2]. It can also contain some inherently toxic heavy elements (such as As, Hg, Pb and Cd) which are rated in the priority lists from human exposure risk point of view [3]. The presence of trace essential and non-essential elements in wheat-based foods depend up on many factors, such as biological factors, environmental conditions, and methods of production and processing. Processes such as milling, baking, and equipment such as machine types have considerable effects on the levels of some trace metals and hence nutritional quality of bread and flour [4].

Wheat milling involves processes of cleaning, tempering, or conditioning in water to toughen the bran in order to reduce fragmentation when it is removed [5]. The processes of drying and conditioning with steam resulted in minerals migration into the endosperm which may increase the content of certain minerals in the flour. In the process of milling, the flour released from the endosperm can be separated into different grades or streams, according to fineness which possesses different mineral and protein contents [6]. The implication is that milling is the critical process affecting the concentration of nutrients in wheat-derived food products [7].

Bread making processes such as fermentation, mixing and baking also influence the nutritional quality of bread, for example; physicochemical changes such as evaporation of water, volume expansion, and protein denaturation take place during baking affects bread quality. Therefore, baking is also considered as an important step in bread making process which needs attention to control bread quality.

The quality of bread is influenced not only by grain quality from the farm but also from the milling and baking processes in bread making, which indicated that nutritional quality of the bread could be improved by way of constant monitoring and improving related processes. Based on these premises, this study make its concern in determining proximate composition and levels of some essential and toxic elements (such as Mg, Ca, Fe, Co, Cu, Cd, and Pb) present in wheat grain, flour, and bread samples in Kamise Ethiopia to evaluate bread quality for human consumption and study the effect of milling and baking processes on bread quality.

MATERIALS AND METHODS

Reagents and materials

Analytical grade concentrated nitric acid (69-70%, INDENTA Chemical-India), perchloric acid (65%, Neolab Life science), and hydrogen peroxide (30%, Bululux) were used for sample digestion. Standard solutions were prepared *via* dilution from standard solutions for Inductively Coupled Plasma Optical Emission Spectrometry; ICP-OES (Optima Perkin Elmer, ICP8000) to analyze the trace metals in each sample. Deionized water was used for solution preparation.

Sampling and sample preparation

The grain and flour samples were collected from a central wheat flour provider factory in Kamise town, Ethiopia and the bread samples were purchased from bakeries in the town, which used flours produced by this factory. These samples were transported to the laboratory for immediate preliminary treatment and subsequent analysis using the method adapted from previous literature, which was based on the wet digestion [8]. To select an optimum condition for digestion procedure, parameters like digestion time, reagent volume, and digestion temperature were optimized by varying one parameter at a time while keeping the others constant. From the optimization procedures, an acid volume of 7 mL mixture of HNO₃, HClO₄, and H₂O₂ in 3:2:2 volume ratio at temperatures 112,110, and 104°C for grain, bread, and flour respectively for 3 hrs were found to be the

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optimal condition to digest 1 gm of samples. Three replicate digestions were made for each sample together with blanks for every experiment. The spiked samples were also prepared in an identical way.

Sample measurement and analysis

The proximate analysis of the samples (moisture, protein, and fat) was performed using the official methods of analysis of AOAC International. The nitrogen content of the samples was determined by Kjeldhal method. The elemental analysis was measured by ICP-OES (Perkin Elmer Optima 8000). A calibration curve was constructed using a standard mix solution containing all analyzed elements. The element contents were calculated by using standard curves and the final concentrations were expressed as mg/Kg. The mean and standard deviation were calculated for each experimental parameter. Differences among the samples were determined for each element by Analysis of Variance (ANOVA).

Method validation

The method of detection (MDL) and Limits of Quantification (LOQ) for all the metals have been calculated from the response of several replicates of the calibration reagent blank using a standard formula. The data revealed that the values for MDL have ranged 0.003-0.063, while the corresponding LOQ value ranged from 0.01 to 0.21. This indicated that the instrument used was in good sensitivity for analysis.

RESULTS

Protein, moisture, fat and ash content in samples of wheat grain, flour and bread

The proximate composition of all samples is presented in Table 1. The protein contents in samples are ranged from 11.03-13.52%, both lowest and highest value were observed in bread samples B1 and B4 respectively. The fat content ranged from 0.50-1.50% where the maximum fat content, was found in wheat grain whereas, the lowest in bread samples. The mean value for moisture contents of different samples ranged from 10.06-11.8%. The highest moisture level 11.80% was found in wheat flour. The ash content (0.80-1.60) obtained in this work was comparable to 1.7 to 2.6% reported for the ash content of cookies enriched moringa leaf powder.

TABLE 1
The mean levels of proximate composition in the wheat whole grain and wheat refined flour samples from the factory and wheat refined flour bread samples from different bakeries in the bread supply system in Kamise in Ethiopia

Samples per 100 g	Moisture (g)	Fat (g)	Protein (g)	Ash (g)	
WWG	10.06 ± 0.00	1.50 ± 0.00	11.73 ± 0.25	1.60 ± 0.00	
WRF	11.80 ± 0.00	1.00 ± 0.00	11.20 ± 0.25	0.80 ± 0.00	
WRFB					
B1	11.40 ± 0.28	0.50 ± 0.00	11.03 ± 0.00	0.80 ± 0.00	
B2	10.80 ± 0.00	0.50 ± 0.00	13.04 ± 0.37	1.20 ± 0.00	
В3	11.40 ± 0.00	0.50 ± 0.00	12.87 ± 0.24	1.20 ± 0.00	
B4	11.40 ± 0.00	1.00 ± 0.00	13.52 ± 0.19	1.20 ± 0.00	
B5	11.20 ± 0.00	0.50 ± 0.00	13.21 ± 0.62	1.60 ± 0.00	
Note: MANC: Mast Crain.	MDC: Wheet Defined Claure	nd WPER: Wheat Refined Flour P	rood, D. Dokom,		

Note: WWG: Wheat Grain; WRF: Wheat Refined Flour; and WRFB: Wheat Refined Flour Bread; B: Bakery

Level of metals in wheat grain, flour and bread

Essential metals (Mg, Ca, Fe, Co and Cu: The result of the analyses of macro metals (Mg and Ca) is presented in Table 2. The mean concentration of Mg and Ca increased in bread than flour and grain. Compare to grain, flour showed higher concentration of Ca and lower Mg. The mean concentration of Mg in the samples of study ranges from 81.25 to 243.25 mg/kg. The maximum Mg concentration level was observed in bread samples

samples (B2), while the lowest concentration was determined for flour sample. The mean concentration of Ca for all samples of study varies from 103.75 to 350.40 mg/kg. The highest concentration was observed on bread samples (B5) while the lowest level of Ca was observed for wheat grain samples. Statistical analysis revealed significant differences between the bread samples.

TABLE 2
The mean levels of minerals and toxic metals in the wheat whole grain and wheat refined flour samples from the factory and wheat refined flour bread samples from different bakeries in the bread supply system in Kamisee in Ethiopia

Samples per kg	Mg (mg)	Ca (mg)	Fe (mg)	Co (mg)	Cu (mg)	Cd (mg)	Pb (mg)
WWG	116.25 ^b	103.75 ^a	15.64 ^d	0.05 ^a	5.66 ^e	0.33 ^a	2.25 ^{a,b}
WRF	81.25 ^a	110.75 ^a	13.05 ^b	0.13 ^a	3.06 ^b	0.33 ^a	1.48 ^a
WRFB							
B1	217.25 ^f	292.32 ^c	15.10 ^c	ND	4.14 ^d	0.33 ^a	5.73 ^c
B2	243.25 ^g	327.12 ^d	13.06 ^b	0.08 ^a	3.88°	0.33 ^a	3.75 ^b
В3	181.25°	235.75 ^b	12.55 ^a	ND	2.38 ^a	0.33 ^a	2.55 ^{a,b}
B4	209.75 ^e	292.80 ^c	12.71 ^a	ND	3.67 ^c	0.33 ^a	2.25 ^{a,b}

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B5	200 ± 0.5 ^d	350.40 ^e	15.88 ^d	0.16 ^a	3.46 ^c	0.33 ± 0.0^{a}	1.67 ^a

Note: (1) Values in the same column that are followed by different letters (a-g) imply significant difference at p ≤ 0.05 by Tukey HSD tests. (2) B1 to B5 represents the wheat bread samples (taken from sample sites of Bakery 1-5)

The average concentration of Fe metal in the study samples ranged from 12.55-15.88 mg/kg. The relative comparison among the samples shows Fe level decreased from grain to flour samples whereas; the trend from flour to bread samples shows inconsistent variations. B3 and B4 showed lower Fe value in bread than flour.

The Co content was determined ranging from ND level to a maximum of 0.16 mg/kg. Its concentration was observed lowest in grain (0.05 mg/kg) and highest in bread (0.16 mg/kg). Its concentration level was found bellow detection limit of the method reported as ND for bread samples B1, B3 and B4. Based on the net average of samples, their relative concentration shows grain

| Specific or concentration of 0.05, 0.12 and 0.13 mg/kg.

Copper was determined with a concentration of 2.38-5.66 mg/kg where the maximum level was observed in grain sample and the lowest level was seen in one of bread sample B3. The ANOVA result showed a significance difference between grain, flour and bread.

Toxic metals (Cd and Pb): The levels of toxic metals (Cd and Pb) have been investigated in all samples and the result is presented in Table 2. Cadmium concentration was observed with constant concentration of 0.33 mg/kg through the triplicate measurement for all samples where the observed difference was claimed insignificant based on Tukey HDS test at 95% confidence level.

The mean concentration of Pb was observed ranging from 1.48 mg/Kg in flour to 5.73 mg/kg in bread sample (B1). ANOVA test implies insignificant different among the samples, except for bread sample (B1), where it is significantly higher than the rest. The general Pb level trend is flour<grain

stream of 1.48<2.25<3.20 mg/Kg.

DISCUSSION

Effects of bread making processes on proximate compositions and levels of metals

Milling effect (wheat grain and flour): As this study revealed, milling process reduced levels of metals (typically Mg, Fe, Cu, and Pb) in wheat flour samples. The concentration levels of Mg, Fe, Cu, and Pb reduced from their original concentration of 116.25, 15.64, 5.66, and 2.25 mg/kg in grain to concentration of 81.25, 13.05, 3.06, and 1.48 mg/kg in flour samples. This is expected since the first stages of milling include cleaning and removing the dust, de-hulling, and then washing with water [9]. In addition, the process of milling like; drying and conditioning also cause mineral migration of metals in to the endosperm which is the main component of flour [10]. This process critically affects the concentration of nutrients in wheat derived products and reduces the nutrient content of flour by concentrating metals in residue. Usually the metal concentration in wheat grain is expected to be higher than its values in flour samples by the factor of 5 to 10 times [11]. On the other hand, the process of milling in the study sample increased the levels of Ca and Co from 103.75 mg/kg and 0.05 mg/kg in grain to 110.75 mg/kg and 0.13 mg/kg in flour respectively. However, this rising was not found statistical significant in Ca.

The proximate composition result showed a decrease of protein, fat and ash when grain processed to flour. Milling is a process of converting grain to flour, during this process bran and germ two main part of grain with high content of protein and fat will be separated from the grain [12]. In a study conducted by the ash content and protein reduced from 1.26 and 10.6 to 0.50 and 9.52 respectively. Therefore, the lower value of ash and protein in the present study is expected and agrees with different studies [13]. The moisture content was higher in flour than in the grain; this increment on

moisture content of flour compared to grain could be due to the addition of water during milling [14].

Baking effect: Baking processes vary in fermentation and baking condition such as time, temperature and additives in the dough mixture. As observed from the current study, process of baking affects the final product. Even though all bakeries used similar flour the result of bread analysis showed different results with a significant increase of Mg and Ca in all bread types [15]. Significant increment was observed in magnesium content of bread where, flour concentration (81.25 mg/kg) was increased with lower value 181.25 mg/kg (B3) and higher 243 mg/kg (B2) which showed an increase of two to three times. Correspondingly Ca level of bread increased from 110.75 mg/kg to 350.40 mg/kg and there was a difference not only between bread and flour but within the bread too which varied from 235.75 mg/kg to 350.40 mg/kg. Therefore, the results show baking increased the levels of Mg and Ca significantly. This could be the water and additives which all bakers used during baking.

The Fe content of bread varied with in breads from 12.55 mg/kg to 15.88 mg/kg, the results also reveals significant different between flour and bread content of Fe. The level is expected to decrease in bread since baking process use water however, the increase result in some bread indicate that Fe content of bread is influenced by baking process. In addition, content of Cu also showed different results between bread sample where value decreased and some bread content increased than the flour and Co was also detected in two bread samples.

Toxic metals Cd didn't show any change during baking which remained 0.33 mg/kg in all sample of bread and flour this indicated that baking in this study didn't affect the Cd content. However, the other toxic metal in this study Pb showed a significance difference during baking and the amount increased from 1.48 mg/kg (flour concentration) to 5.73 mg/kg (B1) even the other four bread sample also show higher value than the flour this indicated that baking materials and additive need serious evaluation.

Several factors like baking equipment and utilities used during the process may contribute to the metals level presence in bread samples. Hence, levels of metals usually increase in breads due to baking process than their levels in wheat flour samples [16]. From the results of this study it can be also concluded that most mineral contents compared in bread after baking was found high. Previous study argue metal introduction during bread making may arise from a number of sources which include bioaccumulation in the raw materials used, water used by the bakeries, baking fuel types and metal pans used for baking. In the vicinity of current study, many bakeries observed using cultural method of making bread. The locally molded metal pan was commonly used by the bakeries. These used materials and poor safety in the process may have contributed to metals level in bread samples collected from these bakeries.

Proximate results also showed variation after baking compared with the flour. Protein and ash value increased after baking whereas fat and moisture decreased. The increment on moisture content of flour and bread compared to grain could be due to the addition of water during milling and baking [17].

Comparison of proximate compositions and metal levels of wheat and wheat bread in literature

The levels of Ca concentration determined in bread were higher than the reported values in Ethiopia, Turkey and Poland [18]. But its concentration in flour was lower compared to previous study Rennan et al., and Tejera et al. However the Mg result agreed with results found in Bangladish. Similarly as can be seen from Table 3 the mean concentration of Mg content, in bread and flour sample were lower than in and higher than in.

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TABLE 3 Comparisons of the chemical and nutritional information in Wheat Grains (WG), Wheat Refined Flours (WRF), and Wheat Refined Flour Breads (WRFB) in the literature

Sample	Origin	Nutrients (mg/Kg)							
		Mg	Са	Fe	Со	Cu	Protein	Fat	
WG	Argentina			43.5	0.05	3.79			
	Ethiopia						10.6-11.53		
	Ethiopia						7.13-14.4	1.32-1.82	
	Ethiopia			10.64	0.35	1.72			
	Iran				ND	0.5			
	Ethiopia	116.25	103.75	15.64	0.05	5.66	11.73	1.5	
lour	Brazil	346	170.4	30		1.9			
	Ethiopia						9.45-11.07		
	Bangladesh	218.70-358.40	50.00-200.30				8.67-12.47	0.89-1.39	
	Spain	226.40-226.7	179.43-200.6	8.17-8.63					
	ES						Aug-16		
	Ethiopia	81.25	110.75	13.05	0.13	3.06	11.2	1	
Bread	Nigeria						9.5	1.3	
	Gahana				0.08	0.36			
	Ethiopia		11.05-22.00	1.16-12.52			8.01-14.34	0.41-1.87	
	Turkey	324	225	16.9		2.7	11.31	1.08	
	Poland	20.6	9.4	1.9		0.3			
	Ethiopia	181.25-243.25	235.75-350.40	12.55-15.88	ND-0.16	2.38-4.14	11.03-13.52	0.5-1.00	
ote: ND: No	t detected								

The concentration of Fe in this study is significantly higher than the result obtained by in wheat grains, wheat flour, and wheat bread samples respectively. However, it is lower than the concentration obtained by in grain and flour.

The average result of Co metal obtained in wheat grain were lower compared to previous reported values and agreed with the values by. On the other hand, the concentration of copper in this study was higher than previous studies in different countries in grain in flour and in bread.

Although processing does not alter its concentration, Cd level is found higher than the results of similar study as well as limit of standard values set by the Ethiopia standard and FAO/WHO. In addition the values of Pb in wheat grain, flour and bread were higher than the reported values [19]. The study results were also higher than tolerable limit for toxicity of Pb set by Ethiopian standard, Codex and recommended dietary allowance for wheat flour and bread as well as the RDA value set by commission reg. (EC) for wheat grain, flour and bread level of Lead.

The reported protein level in grain and flour were higher than Bekele and Shimelis but within the range of results obtained by Genet et al., and Saeid et al., as well as limit recommended by Ethiopian standard for wheat flour. On the other hand protein content in breads corresponds to previous reported levels and higher than reported value of.

The value of fat in wheat grain, flour and bread results in the current study lie in the range of previously reported work by respectively [20].

The moisture content of bread was lower than the previous study and level set by Ethiopian standard which was 12.16-28.38% and 13% respectively. However, the flour moisture content was in the range of previously reported values. The ash content of the flour is slightly higher than the standard value of 0.60% which is recommended by Ethiopian standards.

CONCLUSION

Based on the above results, it could be concluded that milling and baking had a noticeable effect on the bread quality. The milling processes decreased contents of Fe, Cu, protein and fat levels, while the baking processes increased contents of Mg, Ca, and ash levels in bread making. The proximate composition and metal analysis results revealed differences after milling the grain and baking; which could be due to water used, additives and fermentation parameters in the process of baking. The toxic metal assessment for Pb in bread found higher than the maximum permissible limit. Hence, special caution is necessary when selecting the ingredient used during the baking and milling process in order to maintain health and nutritional quality.

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