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Nutritional Analysis of Wheat Flour at Hyderabad for Detection of Essential and Toxic Metals

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ABSTRACT

Wheat flour is basic diet in Asian countries. Quality of wheat flour and milling process has been changing day by day which have profound impact on nutrition value of wheat flour. **Objective:** To carry out Nutritional assessment of whole and refined wheat flour grinded locally at 13 mills of Hyderabad to determine presence of essential and toxic metals. **Methods:** Whole and refined wheat flour were randomly collected from 13 flour mills of Hyderabad for determination of moisture, ash, fat, fiber, carbohydrates, proteins, essential (Fe, Zn and Mn) and Toxic (Cd, Cr, Pb and Cu) metals with standard scientific methods. **Results:** High moisture has been recorded in F.M.13 mill in whole and refined flour as 12.5% and 11.8% respectively where as low moisture 7.1% has been found in whole flour in F.M.10 mill and 7.4% in refined flour in F.M.01 mill. F.M.04 contains high Iron in whole and refined wheat flour as 0.91±1.1 and 0.74±0.5mg/kg respectively. Zinc content has been high in F.M.10 and F.M.11 as 9.95±5.6mg/kg and 8.66±5.1mg/kg respectively. Cadmium has been high in F.M.09 as 0.06±0.01mg/kg in refined flour whereas Lead has been high in F.M.09 as 0.28±0.13mg/kg in whole wheat flour. **Conclusions:** Carbohydrates have been high whereas fiber and protein has been low in refined flour. Fe, Zn and Mn has been significantly low whereas Cd, Pb, Cr and Cu has been significantly high in refined wheat flour. It is concluded that consumption of whole wheat flour is better than refined wheat flour.

INTRODUCTION

Human beings have been using cereal grains as food since birth. Corn, Oat, Barley seeds are grown worldwide, however; wheat grains are popular worldwide due to flavor/taste, multiple uses (bakery items etc) and availability in the market. In Asia, wheat grains (Triticum aestivum) are grinded to make flour for meals or bakery items and refined flour etcetera [1]. In ancient times wheat was grinded in small grinders at home after proper washing and cleaning. With advancement; large flour mills were installed to meet bulk supply and demand of the market [1-3] and elaborate procedures of washing, drying, cleaning/ segregation of rotten wheat were cut short while compromising the quality of flour. From nutritional point of view; our diet should ideally contain 50% carbohydrates (glucose and fiber) in wheat flour [4]. Short shelf life of whole wheat flour introduced refined form of flour in the market by removing extra fiber from whole grain flour thereby increasing its shelf life and making it easy to digest by all ages. It gained rampant popularity in market due to easy making of bakery items; however; less fiber and more percentage of glucose proved it harmful for diabetic and obese people. Discreet use of bleach was also noticed with few mills to improve colour and rihiology of dough and enhance its shelf life [5]. Heavy metal accumulation in soil and wheat crops can lead to insoluble complexes that hinder fertilizer uptake. The growing need for food safety has raised concerns about pesticides, heavy metals (Pb, Cd, Cu, Zn, and Co), and toxins [6, 7]. However, refined flour

becomes more vulnerable to contamination during grinding process due to chemical treatment and might cause disorders [8, 9]. In this research work wheat flour samples have been collected for analysis of nutritional assessment and presence of essential and toxic metals to determine quality of wheat flour.

METHODS

A cross sectional analytical study was conducted at Institute of Biochemistry, University of Sindh, Jamshoro from June 2022- June 2023. Initially survey of shops and consumers was carried out and it was found that 13 mills have been supplying two types of wheat flour at shops of Hyderabad and adjoining areas. A sample each of whole and refined flour was collected during three different stages i.e. grinding, packing and storage, of all 13 mills (3x13=39 samples each of whole and refined flour). Samples were quantitatively tested for moisture, ash, total carbohydrates, total protein, iron (Fe), zinc (Zn), manganese (Mn), cadmium (Cd), cromium (Cr), lead (Pb) and copper (Cu). Flour mills were coded as Gul Star flour mill (FM.01), Hameed flour mill (FM.02), Geo flour mill (FM.03), Sukkur flour mill (FM.04), Jubilee flour mill (FM.05), Al. Mustafa flour mill (FM.06), Hyderabad flour mill (FM.07), Sun Shine flour mill (FM.08), Syed flour mill (FM.09), Super Shine Roller flour mill (FM.10), Ghori flour mill (FM.11), Aghaz flour mill (FM.12) and Al.Noor Roller flour mill (FM.13). **Wheat Flour Samples:** 500g samples of whole and refined flour were collected in polyethylene bags from each mill during grinding, packing and storage (3 x 13=39+39=78). Labeled all samples and brought them to laboratory for nutritional assessment and metal analysis. **Moisture Content:** Took 5g of each sample (whole and refined flour) in Petri dish and carried out weight on digital machine. Covered the sample in aluminum foil and put it in oven at 130°c for 120mins. Sample was repeatedly heated after every 10mins until final weight became constant [10]. Moisture (%)= Obtained weight/total weight of sample x 100. **Ash Content:** 5g of each sample was put in crucible and placed in furnace at 580°c for two hours. Carried out its weight on normalization and repeated the procedure until complete carbon was burnt out of it [10]. Ash (%)=Weight of Ash/total weight of sample x 100. **Total Fat:** Soxhlet apparatus has been used for determination of total fat [10]. Fat (%)= Obtained weight/total weight of sample x 100. **Total Fiber:** McCleary 2023 has been used for the determination of fiber [11]. Fiber (%)= Obtained weight/total weight of sample x 100. **Analysis of Carbohydrates:** Took 5g of each sample in conical flask and added 5ml of 2.5 N HCl in it. Took the mixture and placed it on hot water bath for 3hrs. Cooled it and added 0.1ml normal sodium bicarbonate until sparkle ended. Diluted it with 100ml distilled water and filtered it. **Procedure:** Took 1ml of sample and 4ml of Anthrone

solution and had put it on boiling water bath for 10mins. Checked; absorption of sample on double beam spectrophotometer at 630nm. Determined standard of different concentrations and found concentration of total carbohydrates against standard curve [12]. **Analysis of Proteins:** 5g of each sample was taken in conical flask. Added 10ml phosphate buffer (pH from 7.4-7.6) in it and put it in shaking water bath overnight. Centrifuged the solution, filtered it and filtrate was collected, diluted with 10ml distilled water and kept it for boiling water bath for 10mins. Added 0.1ml of Folin coicaltau and kept the solution at room temp for 30-60mins. Checked; absorption at 750nm with double beam spectrophotometer [13]. **Analysis of Metal Sample Preparation:** 1g of grinded whole and refined flour was put in a beaker and added 2M H_2SO_4 and HNO_3. Placed beaker on hot plate at 100°c for 2hrs and covered it with glass plate to prevent any kind of evaporation. Added 50ml distilled water and filtered it. Filtrate was used for metal analysis. Atomic absorption spectrometry was used for metal analysis, wavelength of metals; Cadmium (228.8nm), Lead (217nm), Chromium (357.9nm), Copper (324.7nm), Iron (248.3nm), Zinc (213.9nm) and Maganese (279.8nm) have been used [14]. Mean ± SD of carbohydrates, protein, Fe, Zn and Mn, Cd, Cr, Pb and Cu have been determined by descriptive analysis. p-values have been calculated by Independent samples t-test. (Note: a sample each of whole and refined flour collected during three different stages i.e. grinding, packing and storage, of all 13 mills (3x13=39 samples each of whole and refined flour) and analyzed. Obtained results have been used to find mean and standard deviation and p-values and <0.05 p-value has been considered statistically significant. Data entry and analysis has been done with SPSS version 22.0 [15].

RESULTS

Table 1 shows percentage of moisture, ash, fat and fiber and Mean ± SD of carbohydrates and protein of 13 mills. F.M.12 has lowest ash content of 0.69% and F.M.01 has 0.98%. Carbohyderates have been high in whole flour in F.M.04 and F.M.08 as 73.26±5.4g/100g and 73.43±3.1g/100g whereas in refined flour F.M.04 and F.M.09 have 80.95±4.6g/100g and 80.24±6.5g/100g respectively. Protein content has been found high in whole flour in F.M.11 and F.M.13 as 10.19±1.9g/100g and 10.11±1.9g/100g respectively. High protein was also found in refined flour in F.M.11 and F.M.13 as 8.78±1.5g/100g and 8.84±1.3g/100g respectively.

Table 1: Nutritional Evaluation of Whole and Refined Wheat Flour at Hyderabad, Sindh

Mills	Moisture (%)	Ash (%)	Fat (%)	Fiber (%)	Carbohydrate (g/100g) Mean ± SD	Protein (g/100g) Mean ± SD
F.M.01	8.7ᵃ	1.32ᵃ	0.7ᵃ	0.82ᵃ	68.46 ± 4.4ᵃ	9.21 ± 0.9ᵃ
	7.4ᵇ	0.98ᵇ	0.8ᵇ	0.79ᵇ	73.672.0ᵇ	8.08 ± 1.7ᵇ
F.M.02	10.0ᵃ	0.84a	0.6ᵃ	1.16ᵃ	66.06 ± 7.9ᵃ	8.24 ± 0.8ᵃ

	10.8b	0.74b	0.8b	0.93b	69.28 ± 3.1b	7.75 ± 0.6b
F.M.03	9.5a	0.95a	0.9a	0.7a	70.52 ± 5.7a	9.42 ± 1.6a
	9.2b	0.8b	1.2b	0.69b	75.86 ± 6.4b	8.79 ± 1.1b
F.M.04	8.5a	0.9a	1.18a	0.86a	73.26 ± 5.4a	9.93 ± 1.34a
	8.1b	0.76b	1.6b	0.81b	80.95 ± 4.6b	8.12 ± 1.2b
F.M.05	11.4a	0.84a	0.7a	0.69a	72.03 ± 7.2a	8.25 ± 3.3a
	11.0b	0.78b	0.9b	0.65b	76.57 ± 4.6b	8.51 ± 0.7b
F.M.06	9.6a	1.16a	0.9a	0.73a	68.72 ± 8.7a	8.66 ± 0.7a
	9.0b	0.79b	1.21b	0.59b	73.0 ± 5.7b	7.96 ± 1.0b
F.M.07	8.5a	0.88a	0.6a	0.87a	69.63 ± 6.3a	9.66 ± 2.8a
	8.4b	0.75b	0.81b	0.83b	67.9 ± 3.1b	8.41 ± 1.8b
F.M.08	7.5a	1.16a	0.75a	0.92a	73.43 ± 3.1a	7.89 ± 1.5a
	7.6b	0.79b	0.8b	1.02b	77.95 ± 74.86b	8.64 ± 0.5b
F.M.09	9.0a	0.98a	1.31a	0.76a	71.79 ± 4.9a	8.65 ± 2.3a
	9.6b	0.86b	1.05b	0.75b	80.24 ± 6.5b	7.11 ± 2.0b
F.M.10	7.1a	1.41a	0.75a	1.26a	72.49 ± 7.9a	8.72 ± 0.9a
	8.6b	0.96b	1.1b	0.85b	75.62 ± 4.8b	7.99 ± 1.5b
F.M.11	10.2a	0.92a	0.71a	0.82a	70.69 ± 5.1a	10.19 ± 1.9a
	10.4b	0.78b	0.75b	0.75b	73.64 ± 2.8b	8.78 ± 1.5b
F.M.12	12.2a	0.9a	0.72a	1.35a	65.41 ± 6.6a	8.25 ± 1.0a
	11.2b	0.69b	0.8b	0.91b	69.67 ± 4.3b	8.32 ± 1.2b
F.M.13	12.5a	0.86a	1.15a	1.0a	72.78 ± 3.7a	10.11 ± 1.9a
	11.8b	0.71b	1.47b	0.89b	76.1 ± 5.9b	8.84 ± 1.3b

a Whole Wheat Flour   b Refined Wheat Flour

Note: A sample each from three different stages of 13 mills (Sample Size n=13). Number of data points including replicates =3x13=39 samples each of whole and refined flour.

Table 2 shows mean, standard deviation, minimum, maximum and p value of carbohydrates and protein of 13 mills.

Table 2: Statistical Assessment of Carbohydrates and Protein Concentration in Whole and Refined Wheat Flour at Hyderabad, Sindh (n=39)

Nutritional Assessment	Min–Max (g/100g) Mean ± SD	Min-Max (g/100g)	p-Value
Carbohydrates	70.41 ± 2.6a	65.41-73.43a	0.01
	74.65 ± 4.02b	67.9-80.95 b	
Protein	9.01 ± 0.79a	7.89-10.19a	0.02
	8.25 ± 0.49b	7.10-8.84 b	

a Whole Wheat Flour (n=13)   b Refined Wheat Flour (n=13)

 *Note: A sample each from three different stages of 13 mills (Sample Size n=13). Number of data points including replicates =3x13=39 samples each of whole and refined flour. Independent sample t-test has been used for p value and <0.05 has been considered statistically significant.

Table 3 shows Fe, Zn and Mn in whole and refined flour in 13 Mills at Hyderabad.

Table 3: Determination of Essential Metals in Whole and Refined Wheat Flour at Hyderabad, Sindh

Mills	Iron (mg/Kg) Mean ± SD		Zinc (mg/Kg) Mean ± SD		Manganese (mg/Kg) Mean ± SD	
F.M.01	0.62 ± 0.5a	0.39 ± 0.4b	6.27 ± 3.1a	5.52 ± 2.5b	9.34 ± 5.6a	7.98 ± 4.3b
F.M.02	0.52 ± 0.4a	0.41 ± 0.3b	5.83 ± 2.7a	4.35 ± 2.1b	6.89 ± 3.6a	6.35 ± 2.8b
F.M.03	0.71 ± 0.4a	0.71 ± 0.4b	6.35 ± 3a	6.54 ± 3.1b	7.61 ± 3.2a	7.19 ± 3.5b
F.M.04	0.91 ± 1.1a	0.9 ± 1.1b	7.11 ± 3.5a	6.71 ± 3.5b	5.59 ± 1.3a	6.11 ± 1.9b
F.M.05	0.73 ± 0.6a	0.72 ± 0.4b	8.01 ± 4.2a	7.45 ± 3.1b	6.66 ± 2.4a	6.95 ± 2.5b
F.M.06	0.49 ± 0.3a	0.35 ± 0.3b	6.94 ± 3.5a	7.81 ± 4.2b	8.48 ± 4.9a	7.1 ± 3.6b
F.M.07	0.61 ± 0.4a	0.43 ± 0.6b	8.16 ± 4.7a	7.68 ± 3.9b	10.18 ± 6.1a	9.34 ± 5.4b
F.M.08	0.51 ± 0.4a	0.66 ± 0.4b	9.45 ± 5.8a	8.35 ± 4.5b	8.04 ± 4.3a	7.18 ± 3.6b
F.M.09	0.6 ± 0.5a	0.51 ± 0.42b	7.46 ± 4.3a	6.53 ± 3.9b	7.69 ± 3.8a	8.04 ± 4.3b
F.M.10	0.62 ± 0.5a	0.5 ± 0.6b	9.95 ± 5.6a	8.52 ± 4.6b	9.93 ± 5.6a	9.39 ± 5.5b
F.M.11	0.46 ± 0.4a	0.37 ± 0.4b	8.89 ± 5.01a	8.66 ± 5.1b	9.62 ± 5.9a	8.88 ± 5.1b
F.M.12	0.52 ± 0.4a	0.46 ± 0.5b	6.88 ± 3.4a	6.34 ± 3.3b	8.81 ± 4.5a	8.42 ± 3.7b
F.M.13	0.55 ± 0.5a	0.74 ± 0.5b	8.56 ± 4.1a	8.13 ± 4.4b	7.56 ± 4.2a	8.1 ± 3.8b

a Whole Wheat Flour   b Refined Wheat Flour

Note: A sample each from three different stages of 13 mills (Sample Size n=13). Number of data points including replicates =3x13=39 samples each of whole and refined wheat flour.

Table 4 shows toxic metals (Cd, Pb, Cr and Cu) in 13 Mills.

Table 4: Cadmium, Lead, Chromium and Copper in Whole and Refined Wheat Flour at Hyderabad, Sindh

Mills	Cadmium (mg/Kg) Mean ± SD	Lead (mg/Kg) Mean ± SD	Chromium (mg/Kg) Mean ± SD	Copper (mg/Kg) Mean ± SD
F.M.01	0.02 ± 0.02a	0.19 ± 0.20a	0.32 ± 0.05a	0.28 ± 0.2a
	0.03 ± 0.01b	0.23 ± 0.13b	0.18 ± 0.05b	0.32 ± 0.3b
F.M.02	0.04 ± 0.03a	0.24 ± 0.12a	0.25 ± 0.14a	0.26 ± 0.1a
	0.04 ± 0.02b	0.26 ± 0.12b	0.27 ± 0.22b	0.24 ± 0.2b
F.M.03	0.03 ± 0.01a	0.18 ± 0.14a	0.20 ± 0.07a	0.27 ± 0.2a
	0.02 ± 0.01b	0.24 ± 0.16b	0.19 ± 0.14b	0.28 ± 0.2b
F.M.04	0.03 ± 0.01a	0.20 ± 0.22a	0.26 ± 0.21a	0.23 ± 0.1a
	0.03 ± 0.01b	0.28 ± 0.12b	0.31 ± 0.13b	0.22 ± 0.3b
F.M.05	0.02 ± 0.03a	0.24 ± 0.13a	0.23 ± 0.22a	0.28 ± 0.2a
	0.03 ± 0.02b	0.22 ± 0.17b	0.21 ± 0.05b	0.30 ± 0.3b
F.M.06	0.03 ± 0.01a	0.27 ± 0.11a	0.25 ± 0.14a	0.24 ± 0.2a
	0.05 ± 0.02b	0.33 ± 0.11b	0.31 ± 0.20b	0.26 ± 0.3b
F.M.07	0.03 ± 0.02a	0.23 ± 0.12a	0.24 ± 0.11a	0.23 ± 0.1a
	0.03 ± 0.01b	0.26 ± 0.14b	0.21 ± 0.11b	0.27 ± 0.2b
F.M.08	0.01 ± 0.01a	0.27 ± 0.14a	0.16 ± 0.21a	0.27 ± 0.2a
	0.05 ± 0.02b	0.28 ± 0.15b	0.17 ± 0.12b	0.29 ± 0.2b
F.M.09	0.02 ± 0.01a	0.28 ± 0.13a	0.28 ± 0.13a	0.31 ± 0.2a
	0.06 ± 0.01b	0.24 ± 0.14b	0.26 ± 0.11b	0.32 ± 0.2b
F.M.10	0.03 ± 0.02a	0.23 ± 0.21a	0.24 ± 0.12a	0.32 ± 0.2a
	0.05 ± 0.01b	0.27 ± 0.11b	0.25 ± 0.23b	0.31 ± 0.2b
F.M.11	0.04 ± 0.01a	0.25 ± 0.22a	0.14 ± 0.05a	0.23 ± 0.1a
	0.03 ± 0.01b	0.28 ± 0.12b	0.21 ± 0.05b	0.32 ± 0.3b
F.M.12	0.02 ± 0.01a	0.26 ± 0.31a	0.22 ± 0.13a	0.32 ± 0.3a
	0.04 ± 0.01b	0.28 ± 0.1b	0.27 ± 0.22b	0.28 ± 0.2b
F.M.13	0.04 ± 0.02a	0.28 ± 0.21a	0.20 ± 0.11a	0.26 ± 0.2a
	0.03 ± 0.01b	0.32 ± 0.05b	0.18 ± 0.05b	0.29 ± 0.2b

a Whole Wheat Flour   b Refined Wheat Flour

Note: A sample each from three different stages of 13 mills (Sample Size n=13). Number of data points including replicates =3x13=39 samples each of whole and refined flour.

Table 5 showed mean, standard deviation, minimum, maximum and p value of iron, zinc, manganese, cadmium, lead, chromium and copper of 13 mills.

Table 5: Statistical Assessment of Fe, Zn, Mn, Cd, Pb, Cr and Cu in Whole and Refined Wheat Flour at Hyderabad, Sindh (n=13*)

Metals	(mg/Kg) Mean ± SD	(mg/Kg) Min-Max	p-Value
Iron *15mg/Day	0.6 ± 0.11[a]	0.461-0.89[a]	0.03
	0.50 ± 0.13[b]	0.36-0.74[b]	
Zinc *10-15mg/Day	7.68 ± 1.28[a]	5.83-9.95[a]	0.04
	7.12 ± 1.27[b]	4.35-8.66[b]	
Manganese *12.2mg/Day	8.18 ± 1.41[a]	5.59-10.19[a]	0.03
	7.77 ± 1.1[b]	6.11-9.42[b]	
Cadmium *1.50µg/Day	0.02 ± 0.004[a]	0.016-0.027[a]	0.04
	0.03 ± 0.003[b]	0.021-0.031[b]	
Lead *0.30mg/Day	0.241 ± 0.034[a]	0.173-0.281[a]	0.02
	0.263 ± 0.038[b]	0.204-0.332[b]	
Chromium **1.50µg/Day	0.22 ± 0.045[a]	0.145-0.285[a]	0.02
	0.24 ± 0.048[b]	0.177-0.31[b]	
Copper ***2-3mg/Day	0.276 ± 0.027[a]	0.235-0.321[a]	0.04
	0.287 ± 0.031[b]	0.224-0.327[b]	

*Note: A sample each from three different stages of 13 mills (Sample Size n=13). Number of data points including replicates =3x13=39 samples each of whole and refined flour

 aWhole Wheat Flour (n=13) bRefined Wheat Flour (n=13) **FAO/WHO Tolerable limit [14]. ***copper [15]. Independent sample t-test has been used for p value and <0.05 has been considered statistically significant.

DISCUSSION

Moisture in flour is very important for bakers and millers. In this research; moisture content has been 7-10% which is good for long storage of flour. All 13 mills have shown <1% of moisture which indicates good quality of flour. Whole and refined flour contains less ash percentage in mill flour which indicate low quantity of essential minerals. Czaja T et al., in 2020 and Liu Y et al., in 2023 also reported that moisture and ash were key parameters of nutritional assessment [16, 17]. In current analysis both types of flour contain low percentage of fat which is good as it delays rancidity [1-4]. Almost all mills have been found with low fiber in both types of flour. Nowadays mill owners are selling fiber separately at high cost. Meng Y et al., in 2023 reported that wheat fiber have been good in diet for human health as it lowers glucose level [5]. Carbohydrates are important compound of wheat flour; however; their excessive use especially glucose can cause Diabetes and Obesity etcetera. Mean of all 13 mills shows less proteins and high carbohydrates in refined flour. In developed countries; sell of flour without adding essential metals is not allowed; however; this aspect is neglected in our country. Iron, zinc and manganese are essential nutrients of human metabolism and their deficiency can cause severe disorders. Sample from F.M.04 contained high Iron in whole flour and refined flour as 0.91±1.1 and 0.74±0.5mg/kg respectively. Zinc content has been high in F.M.10 and F.M.11 9.95±5.6mg/kg and 8.66±5.1mg/kg respectively. F.M.10 contained high Mn in both types of flour as 9.93±5.6mg/kg and 9.39±5.5mg/kg. Cadmium has been high in refined flour

in F.M.09 as 0.06±0.01mg/kg. Lead has been high in whole flour in F.M.09 as 0.28 ± 0.13mg/kg. Copper has been high in whole flour as 0.32±0.3mg/kg in F.M.12 and F.M.01 contained high copper i.e. 0.32 ± 0.3mg/kg in refined wheat flour. All mills contained statistical significantly low Fe (p-0.03), Zn (p-0.04) and Mn (p-0.03) in refined flour than whole flour. Jiang Z et al., in 2023 observed that biofortified of wheat with Fe and Zn was useful for human diet [2]. Wheat flour samples produced in Corum had higher Pb concentrations (2.009, 1.617, 1.574, 2.201, and 1.915 mg/kg) as compared to guideline values of 0.2 mg/kg. whereas no statistical difference has been found in concentrations of zinc and cadmium in wheat of similar and different cultivation lands [18]. In 16 different districts of Shanghai city, various mycotoxins and heavy metals have been studied in rice, maize, soybean and wheat flour [19]. These metals accumulate in our body cells after exposure; get attached to the cells membrane, carry out mimic reaction and produce toxicity [20].

CONCLUSIONS

Refined flour has slightly higher amount of carbohydrates and lower amount of proteins than whole flour. However, overall difference in values has been not more than few grams. Four toxic metals i.e. Cd, Cr, Pb, and Cu have been found in more concentration in refined flour than whole flour; whereas essential metals i.e. Fe, Zn, and Mn have been found high in whole flour than in refined flour. However, obtained results were well within maximum food limits laid down by FAO/WHO. It is concluded that whole wheat flour is better than refined wheat flour.

Authors Contribution

Conceptualization: SM
Methodology: SM
Formal analysis: SM, AMS
Writing, review and editing: MM, SH

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

All the authors declare no conflict of interest.

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