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ESTIMATION OF TRACE METAL CONTENTS IN LOCALLY-BAKED BREADS

N. KHALID and *S. REHMAN

Chemistry Division, Directorate of Science, PINSTECH, P. O. Nilore, Islamabad, Pakistan

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In order to establish base line levels, estimation of some essential trace metals (Cu, Fe, Mn & Zn) has been conducted in four brands of fifteen locally baked breads of Rawalpindi /Islamabad area employing Atomic Absorption Spectrophotometry (AAS). The samples were digested in a mixture of nitric acid and perchloric acid and the analysis was done with air-acetylene flame. The reliability of the procedure employed was verify by analyzing Standard Reference Material, i.e., wheat flour (NBS-SRM-1567) for its Cu, Fe, Mn and Zn contents which were in good agreement with the certified values. The results revealed that brown breads contained higher amount of Fe 177.3 $\mu g\ g^{-1}$ and Zn 19.27 $\mu g\ g^{-1}$ while levels of Cu 21.90 $\mu g\ g^{-1}$ was found higher in the samples of plain bread. The determined metal concentrations in the bread samples were compared with the reported values for other countries. The effect of kneading/baking/slicing processes on the concentration levels of these metals was also studied. The daily intake of these metals through this source was calculated and compared with the recommended dietary allowance.

Keywords: Trace metals, AAS, Locally baked breads, Pollution, Dietary allowance

1. Introduction

Cu, Fe, Mn and Zn are essential elements for the maintenance of good health. These are necessary for the activity of several enzymes which are involved in energy changes and protein formation in the body. These essential trace metals form compounds with proteins which have a wide range of catalytic functions. In humans Cu, Fe, Mn and Zn accomplish decisive functions to maintain human health. Deficiency or excess of any of these elements leads to adverse pathological conditions. In recent years due to industrial development and urbanization the environment, soil, underground and surface water, the agricultural raw materials are getting polluted by trace metals. Food safety to these metals is of major concern because of environmental pollution [1]. These metals find their way into the human beings through different food chain systems and food thus becomes the ultimate source of metal intake in to the human body. It is, therefore, important to monitor various food commodities of daily consumption for their metal contents to evaluate the extent of pollution and essential metals to assess their adequacy/inadequacy for human beings. The bread is one of the items of daily diet. The determination of trace elements in bread samples has made an important contribution to environmental sciences and toxicology. Trace elements are though present in small concentrations in food samples but for long time has an effect on the human health. Trace elements though required in smaller quantities are to be taken in diet as they are required by the body for specific functions but taking them in excess causes adverse effects on human health [2]. Keeping in view the large scale production and consumption of bread it was desired to determine some of the essential metals in various brands of bread in order to assess the daily intake of these metals through this source. Different analytical techniques [3-10] have reported the metal contents at trace level in food items but the Atomic Absorption Spectrophotometry (AAS) is one of the preferred techniques due to its rapidness, high sensitivity, economical and specificity. The present study deals with the determination of some essential metals in various brands of breads available in Rawalpindi/Islamabad in order to see that whether the levels of these metals are adequate for health, employing Atomic Absorption Spectrophotometry technique.

2. Experimental

2.1. Sampling and Sample Preparation

Fifteen samples of locally baked breads (four brands) were purchased from different bakeries of Rawalpindi/Islamabad. These samples were oven dried at 60 ^{0}C to constant weights. The dried samples were ground to fine powder in an agate mortar to obtain adequate granulometry for the analysis. The ground samples were thoroughly mixed to get homogeneous and representative samples. The homogenized and dried samples were stored in air tight plastic containers till required for analysis.

* Corresponding author : sohailarahman@gmail.com

Table 1. Instrumental parameters used for the measurement of Cu, Fe, Mn and Zn.

Parameters	Fe	Cu	Mn	Zn
Lamp current (mA)	12.5	7.5	7.5	5.0
Resonance abs. line (nm)	248.3	324.8	279.5	213.9
PMT Voltage (V)	400	330	400	430
Width of slit (nm)	0.2	1.3	0.4	1.3
Type of burner	S	S	S	S
Burner height (mm)	7.5	7.5	7.5	7.5
Oxidant (air) flow (L min^{-1})	15.0	15.0	15.0	15.0
Fuel (C_2H_2) flow (L min^{-1})	1.8	2.0	2.0	1.8

S = Standard

2.2. Reagents

Stock solutions (1000 mg L^{-1}) of metals were individually prepared by dissolving appropriate amounts of metal oxides (Johnson Matthey Chemical, Limited) in minimum amount of distilled nitric acid [11] and heated nearly to dryness and were then diluted to the desired volume with the addition of water. Fresh standard solutions were prepared by appropriate dilution of these stock solutions immediately before use. Glassware was cleaned by overnight soaking in nitric acid (1:1) followed by multiple rinsing with water. Analytical reagent grade perchloric acid (70%) and distilled nitric acid were used for the digestion of the samples. Distilled and deionized water was used throughout.

2.3. Instrumentation

The atomic absorption spectrophotometric measurements were made with Hitachi Model Z-2000 polarized Zeeman instrument, which was coupled with a microprocessor-based data-handling facility and a printer. A water-cooled, premix, fish-tail type burner, having a 10 $\times$ 0.05 cm^2 slot, was used for the air-acetylene flame. Single element hollow cathode lamps of Cu, Fe, Mn and Zn were utilized as radiation sources.

2.4. Procedure

About 500 mg of dried sample was taken in triplicate in 50 mL flask fitted with an air condenser of about 30 cm length and 5.0 mL of nitric acid was added to it. This mixture was heated at 60 ^{0}C for 30 min. After cooling, 2.0 mL of perchloric acid was added and heated again at 250 ^{0}C with occasional shaking till white fumes evolved. The clear solution obtained after digestion was cooled and transferred into a 10 mL measuring flask and the volume was made up with water. A blank was prepared under identical conditions. The solutions were aspirated into an air-acetylene flame in the order of blank, standards, sample blank and samples, using the optimized instrumental parameters given in Table 1. The signal evaluation was made by subtracting the signal of blank from the signals of the samples [7].

3. Results and Discussion

The concentration of some essential metals i.e., Cu, Fe, Mn and Zn was determined in fifteen different types of locally baked breads (plain, brown, milky and butter) consumed by the residents of Rawalpindi and Islamabad area. The quantification of the metals was made by employing AAS using air-acetylene flame. Before analysis the samples were oven dried and the average moisture contents were found to be 33.1 $\pm$ 2.9 %. All the reported values are on dry weight basis and are the average of at least triplicate independent determinations.

The analytical quality of the method used was checked by analyzing NBS Standard Reference Material i. e., wheat flour (NBS-SRM-1567) for its Cu, Fe, Mn and Zn contents under identical experimental conditions. The results of the analysis are given in Table 2 along with the certified values, which are in good agreement with each other indicating the reliability of the method used.

Table 2. Concentration of trace metals in NBS Standard Reference Material Wheat Flour-1567.

Element	Measured Concentration ($\mu g\ g^{-1}$)	Certified value ($\mu g\ g^{-1}$)
Cu	1.9 ± 0.1	2.0 ± 0.3
Fe	19.1 ± 1.6	18.3 ± 1.0
Mn	7.7 ± 0.5	8.5 ± 0.5
Zn	10.2 ± 0.6	10.6 ± 1.0

Table 3. Concentration of trace metals ($\mu g\ g^{-1}$) in locally baked breads.

Type (Brand)	Cu	Fe	Mn	Zn
Plain Bread (4)				
Range	7.8-49.9	29.5-150.7	4.6-6.2	11.9-14.7
Mean	21.90	75.18	5.46	12.77
SD	14.41	42.13	0.64	1.03
Brown Bread (4)				
Range	2.1-18.5	11.6-184.2	3.1-15.3	13.6-24.9
Mean	8.0	177.3	9.9	19.27
SD	8.22	162.3	6.22	5.65
Butter Bread (3)				
Range	9.95-11.45	28.5-36.9	15.8-16.69	ND
Mean	10.55	32.9	16.33	-
SD	0.75	4.2	0.19	-
Milky Bread (4)				
Range	2.7-38.1	37.2-70.5	4.5-18.9	11.0-16.1
Mean	20.3	52.35	8.15	13.62
SD	14.56	14.17	7.17	2.12

ND= not detected

The determined concentration of essential trace metals (Cu, Fe, Mn and Zn) in the samples of locally baked breads are summarized in the form of range, mean and standard deviation, in Table 3. The determined values of copper in samples of plain, brown, milky and butter breads ranged from 7.8-49.9 (Mean 21.9), 2.1-18.5 (Mean 8.0), 9.95-11.45 (Mean 10.55) and 2.7-38.1 (Mean 20.3) $\mu g\ g^{-1}$ respectively. The corresponding range for iron from 29.5-150.7 (Mean 75.18), 11.6-184.2 (Mean 177.3), 28.5-36.9 (Mean 32.9) and 37.2-70.5 (Mean 52.35), that of manganese was from 4.6-6.2 (Mean 5.46), 3.1-15.3 (Mean 9.9), 15.8-16.69 (Mean 16.33) and 4.5-18.9 (Mean 8.15). The determined concentration of zinc in samples of plain, brown and milky breads ranged from 11.9-14.7 (Mean 12.77), 13.6-24.9 (Mean 19.27), and 11.0-16.1 (Mean 13.62) $\mu g\ g^{-1}$ respectively.

The data in Table 3 revealed that the mean concentration of copper was maximum in samples of plain breads and minimum in brown breads. The mean concentration of iron was maximum in samples of brown breads and minimum in butter bread. The mean concentration of Mn was found maximum in butter breads while minimum in plain breads, similarly the mean concentration of zinc was maximum in brown breads and found minimum in plain breads whereas it was not detected in butter bread samples.

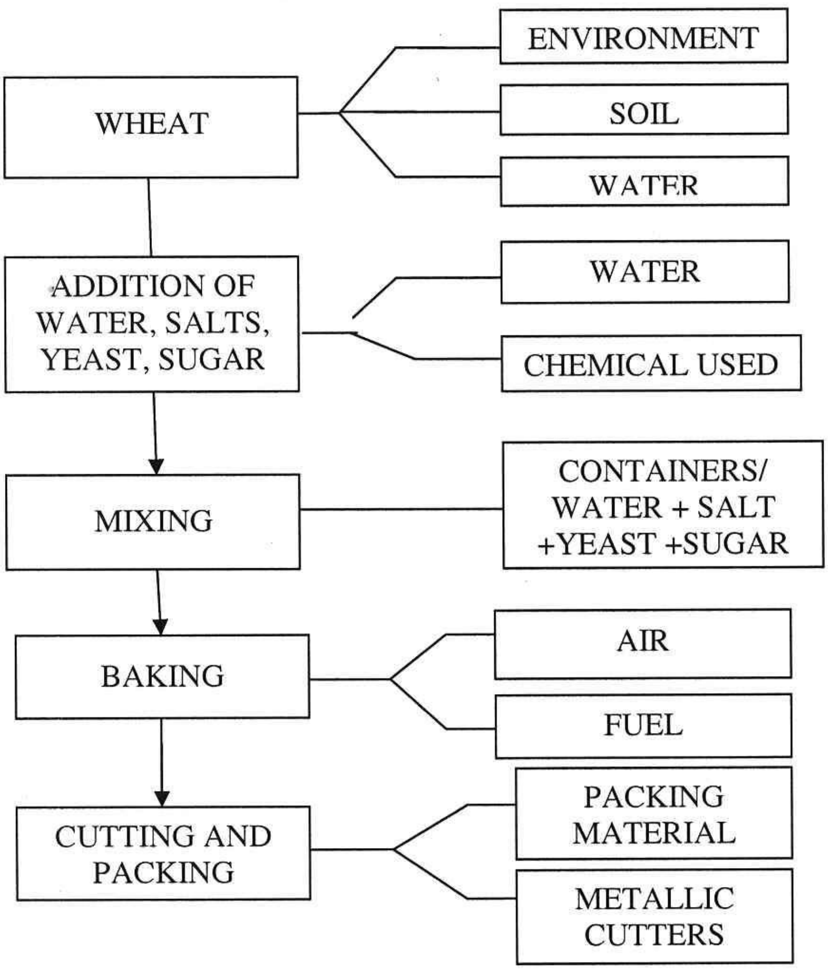

Figure 1. Sources of contamination in bread.

Moreover the data in Table 3 shows that the determined average concentration of Cu in plain breads is significantly higher than the reported values for USA (0.9-3.4 $\mu g\ g^{-1}$) [12], Brazil (2.1-2.5 $\mu g\ g^{-1}$) [13] and Iraq (0.35-0.81 $\mu g\ g^{-1}$) [14]. The average concentration of Fe in breads is almost equal to the upper range of Brazil (37-60 $\mu g\ g^{-1}$) [13] and Iraq (49.8-59.21$\mu g\ g^{-1}$) [14]. The average manganese contents are within the reported ranges for Brazil (6.4-8.8 $\mu g\ g^{-1}$) [13], USA (4.7-24.5 $\mu g\ g^{-1}$) [12] and almost half than that of Iraq (11.09-11.4 $\mu g\ g^{-1}$) [14]. The average determined concentration of zinc is in the reported range of Iran (10.42-14.25 $\mu g\ g^{-1}$) [15], and is higher than the reported values of USA (7.2 $\mu g\ g^{-1}$) [16], UK (5-6 $\mu g\ g^{-1}$) [17], Egypt (8.2 $\mu g\ g^{-1}$) [18], Brazil (9.7-10 $\mu g\ g^{-1}$) [13], Nigeria (2.93 $\mu g\ g^{-1}$) [19] and Iraq (7.89-8.2 $\mu g\ g^{-1}$) [14].

Brown flour is usually rich in Fe, Cu and Zn while white flour contains lesser amount of these metals than the brown flour. This is mainly due to the facts that the white flour contains mainly endosperm while brown flour contains variable levels of germ as well as endosperm [14]. The germ contains higher levels of Fe and Zn [20], therefore the concentration of Fe in flours is highly affected by the milling process [15].

Intoxications of trace metals are normally linked with one of three patterns of occurrence, environmental pollution, accidental inclusion during processing and contamination at processing or storage stage [21]. Various sources of metal contamination into the breads have been shown in Figure 1.

The effect of kneading/baking/slicing processes on the concentration levels of the metals determined was also checked by analyzing these

Table 4. Effect of kneading/baking/slicing on the concentration of trace metals

	Concentration ($\mu g\ g^{-1}$)			
	Cu	Fe	Mn	Zn
Wheat grains	4.9	16.54	35.2	33.5
Breads	21.6	63.5	6.6	14.5

Table 5. Recommended daily intake of essential metals (mg) through breads [18-19].

Age groups	Body Wt.	Intake of			
	(kg)	Cu	Fe	Mn	Zn
Children	12	60.2*	0.21	15.0*	35.12*
Recommended dietary allowances per kg body wt.		50-100*	1.7	2.5-75*	0.5
Adults	70	1.47	5.04	0.37	0.85
Recommended Dietary Allowances		2-5	8-18	0.5-5	8-15

* Amount in µg.

metals in the samples of wheat grains and then flour of that grains usually used in making of breads. The results have been reported in Table 4, which shows significantly higher concentration of Cu and Fe in the final products as compared to the wheat grains. This can be explained on the basis of contamination during the machining and addition of water, salt and other additives during the manufacturing processes involved. Similar observations have been reported by Gholem Reza et al. [15]. The concentration of Mn and Zn were higher in the wheat grains as compared to the finish products whereas Zn was not detected in butter bread. This could probably be explained on the basis of losses of these metals during machining. Similar results have also been reported by Zook et al. [16] and Kashlan et al. [17].

The daily intake of Cu, Fe, Mn and Zn by the adults as well as children through breads was also calculated. These calculations were based on the assumption of daily intake of four slices of bread (100 grams) per person per day, whereas half of this quantity was used for children. The mean concentration of these metals in plain bread samples were used for such calculations. The results are reproduced in Table 5 alongwith the recommended dietary allowances [18-19]. The data in Table 5 revealed that the said amounts of breads provide appreciable amounts of Cu, Fe and Mn towards daily recommended allowances for adults, whereas the intake values of Cu and Mn for children are sufficient for their daily requirements. The daily intake of Zn through this source is much lower as compared to the recommended allowances for both children and adults. It is, therefore, suggested that other food items should also be included in the diet pattern especially for children, in order to fulfill the dietary requirements of such essential metals.

4. Conclusion

The present study has shown the applicability of Atomic Absorption Spectrophotometry (AAS) for the determination of essential trace metals (Cu, Fe, Mn & Zn) in bread samples. The concentration of essential trace metals in locally baked breads of Rawalpindi/ Islamabad area is documented. Breads provide substantial amounts of Cu, Fe and Mn towards daily recommended allowances for adults. It can be concluded that kneading/ baking/slicing have a significant effects on the levels of Fe and Cu. The processing sometime reduced the levels of trace metals considerably depending on the type and stages of it, as seen in case of Mn and Zn. During bread processing, the possible sources of pollution are metal surfaces in contact with the bread dough, air and environment.

5. Recommendations

Since metals are products of environmental pollution resulting from various industrial activities, the ultimate goals should be reducing metals exposure or to interrupt the possible pathways into food.

References

[1] B. Demirozu and I. Saldamli, Turkish J. Eng. Environ. Science **26 (**2002) 361.

[2] R. Gholam J. Khaniki, M. Yunesian, A.H. Mahvi and S.H. Nazmara, J. Agri. Soc. Sci. **1** (2005) 301.

[3] M. Lugowska, E. Stryjewska and S. Rubel, J. Electroanal. Chem. Interfacial Electrochem. **226** (1987) 263.

[4] E. Stryjewska, S. Rubel, A. Hension and G. Henrion, Fresenius Z. Anal. Chem. **327** (1987) 679.

[5] K. Ikebe and R. Tanaka, Shokuhin Eiseigaku Zasshi **24** (1983) 282.

[6] P. N. Elene, S. E. Fábio, T. Luciano and D. Tatiana, Food Chem. **112** (2009) 727.

[7] N. Khalid, S. Rahman, R. Ahmad and I.H. Qureshi, Intern. J. Environ. Anal. Chem. **28** (1987) 133.

[8] A. M. Islam, A. Kar, S. K. Diswas, D.A. Hadi, and A.H. Khan, J. Radioanal. Nucl. Chem. (Articles) **97** (1986) 113.

[9] M. Wasim, N. Khalid, A. Asif, M. Arif and J. H. Zaidi, J. Radioanal. Nucl. Chem. **292** (2012) 1153.

[10] L. Perring, D. Andrey M. Basic-Dvorzak and D. Hammer, J. Food Compos. Analysis **18** (2005) 655.

[11] R. P. Maas and S. A. Dressing, Anal. Chem. **55** (1983) 808.

[12] E. G. Zook, F. E. Greene and E. R. Morris, Cereal Chem. **47** (1970) 720.

[13] V. A. Maihara and M. B. A. Vasconcellos, J. Radioanal. Nucl. Chem. **122** (1988) 161.

[14] I. Jawad and S. H. Allafaji, Aust. J. Basic Appl. Sci. **6**, No. 10 (2012) 88.

[15] R. Gholam and J. Khaniki, Pak. J. Nutrition **4** (2005) 294.

[16] R. C. Hoseney, Principle of Cereal Science and Technology. American Association of Cereal Chemists Inc, St Paul., Minnesota. 2nd Edition, USA (1994).

[17] N. B. Kashlan, V. P. Stnivastava, N.A. Mohanna, Y. K. Motawa and M. S. Mameesh, Food Chem. **39**, (1991) 205.

[18] WHO, Technical Report Series 880: (1995).

[19] B. Welz and M. Sperling, Atomic Absorption Spectrometry. Wiley-VCH, Weinheim (1999).

[20] L. Klaus and L. Robert, Mineral Composition of U.S. and Canadian Wheat and Wheat Blends, J. Agric. Food Chem. **25** (1987) 806.

[21] C. F. Moffat and K. J. Whittle, Environmental Contaminants in Foods. Academic Press, London (1999).