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TRACE METALS DETERMINATION IN CHICKEN EGGS

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To evaluate the adequacy for essential metals and to get an estimate of toxic metals variation in poultry farm and domestic chicken eggs, concentrations of those trace metals which are problematic with neutron activation analysis i. g., Cu, Fe, Mn, Zn, Pb and Cd were carried out using atomic absorption spectrophotometry. The samples were digested with $HNO_3 - HClO_4$ mixture. Median concentrations of Cu, Fe, Mn and Zn in the yolk of poultry farm eggs were found to be 3.6, 101.2, 1.7 and 42.3 $\mu g\ g^{-1}$ respectively. As expected the concentrations of these elements were much lower in egg albumen. On the other hand the median concentrations of Pb (0.130 $\mu g\ g^{-1}$) and Cd (0.049 $\mu g\ g^{-1}$) were higher in egg albumen as compared to egg yolk. Almost similar pattern was observed from the analytical data of domestic chicken eggs. The results obtained were compared with the reported results from the literature. Using the average concentrations, daily intake values of these elements were calculated for poultry farm eggs and were found to be well below the WHO recommended/permissible values for essential and toxic metals.

Keywords : Trace metals, Chicken eggs, Atomic absorption spectrophotometry

1. Introduction

Global awareness about the adverse effects of trace metals in the human biosystem and environment is rapidly increasing due to the enhanced industrialisation and urbanization. The ingestion of metals into human body is mainly through different food chains. Food being the major and constant source of metal uptake into the human body, therefore, it is important to monitor the essential and toxic metals in different food articles. Such study will help to establish baseline levels of essential and toxic metals in consumable items. These data will also help to establish the adequacy of the essential metals and degree of contamination of toxic metals through various sources. Chicken eggs is one of the items of regular daily consumption, therefore, it is important to determine their trace metal contents.

For such studies, various analytical methods have been reported in the literature such as, ion selective electrode [1], spectrometry [2], fluorometry [3], neutron activation analysis [4], voltametry [5, 6] and atomic absorption spectrometry [7–11], All these techniques have their own merits and demerits, but atomic absorption spectrometry is one of the preferred techniques for heavy metals due to its precision at ultratrace level, simplicity, rapidity, high sensitivity, specificity [12] and availability in almost every laboratory due to medium cost.

The present study deals with the determination of some essential and toxic trace metals in poultry farm and domestic chicken eggs using atomic absorption spectrophotometric technique. It is also the continuation of the studies of our group for monitoring the trace metals in complete range of food articles [13–15] and integrated diet [16] for the inhabitants of Rawalpindi/Isamabad area, to establish the baseline levels of trace metals. Some of the elements selected in the present study are those which could not be analysed or problematic with neutron activation analysis (NAA).

2. Experimental

2.1. Instrumentation

All the absorption measurements were made with a polarised Zeeman atomic absorption spectrophotometer from Hitachi model Z–8000, which was coupled with a graphite furnace, a microprocessor and a built in printer. A water

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cooled premix, fish-tail type burner having a 10 x 0.5 cm^2 slot, was used for the air-acetylene flame. Hollow cathode lamps of appropriate elements used as radiation sources were from Hitachi, Japan.

2.2. Reagents

Stock solutions (1000 mg L^{-1}) of the desired metals were prepared by dissolving appropriate weights of the specpure metals or their oxides from Johson & Matthey, in minimum amount of distilled nitric acid [17] and making up the volume with water. Standard solutions for the construction of calibration curves were prepared by appropriate dilution of these stock solutions. Fresh working standards were prepared immediately before use. Glassware was cleaned by soaking in nitric acid (1+1) for four hours and subsequently rinsed several times with deionized water. Analytical reagent grade perchloric acid and distilled nitric acid were used for the digestion of the samples.

2.3. Sampling and sample preparation

The samples of poultry farm and domestic chicken eggs were randomly collected from different markets and poultry farms of Rawalpindi/Islamabad area. Normally the eggs were boiled for five minutes and the albumen and yolk were separated. The average weights of poultry farm and domestic chicken eggs were found to be 53.3 ± 1.2 and 45.1 ± 2.5 g respectively. The average weights of the albumen, yolk and shell of poultry farm were found to be 30.6 ± 1.6, 15.5 ± 0.9 and 7.2 ± 0.4 g, and of domestic chicken eggs were 24.5 ± 1.3, 13.8 ± 0.7 and 6.8 ± 0.3 g respectively. Before the analysis all the samples were oven dried at 60 °C to a constant weight and the moisture contents in the albumen were determined to be 85.13 ± 1.72 and in yolk of poultry farm chicken eggs 49.93 ± 0.51% respectively. The corresponding moisture contents of domestic chicken eggs were 84.93 ± 1.76 and 48.76 ± 0.45 % respectively. Before analysis dried samples of albumen and yolk were ground to fine powder with grinder having teflon coated blades to avoid the elemental contamination and thoroughly mixed to get homogeneous samples. Homogenized samples were stored in precleaned and air tight plastic containers for analyses.

2.4. Procedure

About 0.5 g portion of the prepared sample was transferred into the flask fitted with a 30 cm long air condenser and 5 ml of distilled nitric acid was added to it. The mixture was heated at about 70 °C for 40 minutes. After cooling the mixture, 2 mL of perchloric acid (70%) was added and then heated at 280 °C with occasional shaking till white fumes evolved. The resultant clear solution was cooled and transferred into a 25 mL volumetric flask and the volume was made up with distilled and deionised water. This sample solution was analysed by atomic absorption spectrophotometry. A blank was also prepared and analysed under identical experimental conditions. Minimum of three absorption readings were recorded for each solution and the mean values of the absorption signals were used for subsequent calculations. The absorption signals were evaluated by subtracting the value of the blank from the signal of the sample.

3. Results and Discussion

Concentration of some of the essential (Cu, Fe, Mn, Zn) and toxic (Pb and Cd) metals were determined in egg albumen and egg yolk of 162 poultry farms and domestic chicken eggs using atomic absorption spectrophotometry. Complete digestion was achieved with a mixture of nitric and perchloric acid. The concentrations of Cu, Fe, Mn and Zn were determined using an air-acetylene flame, whereas graphite atomisation technique was used for the measurement of lead and cadmium. The optimised instrumental parameters and furnace conditions used are given in Table 1. For the optimisation of the instrumental parameters and furnace programmes the criterion of maximum signal-to-noise ratio was adopted. All the calculations were made on dry weight basis and the reported values are the average of triplicate independent determinations.

The accuracy of the procedure used was checked by analysing the NBS standard reference material wheat flour (SRM–1567) under identical experimental conditions. The results obtained are tabulated in Table 2 alongwith the certified values, which are in good agreement with each other.

The determined concentration ranges along with the average and median values of the analysed elements in poultry farm and domestic chicken eggs, are reported in Tables 3-4. A comparison of the reported metal contents in chicken eggs from different countries has been made in Table 5. The average concentrations of heavy metals in total egg were calculated by using the determined average concentration of metals in

Table -1. Instrumental conditions used for the determination of trace metals in chicken eggs.

Analytical conditions	Cu	Fe	Mn	Zn	Pb	Cd
Lamp current (mA)	7.5	12.5	7.5	10.0	7.5	7.5
Wavelength (nm)	324.8	248.3	279.5	213.8	283.3	228.8
Slit width (nm)	1.3	0.2	0.4	1.3	1.3	1.3
Burner height (mm)	7.5	7.5	7.5	7.5	-	-
Oxidant gas pressure (kg cm^{-2})	1.6	1.6	1.6	1.6	-	-
Fuel gas pressure (kg cm^{-2})	0.3	0.3	0.3	0.2	-	-
Carrier gas flow (mL $min.^{-1}$)	-	-	-	-	100	100
Sample volume (μL)	-	-	-	-	10	10
Heating programme:						
Drying temp. (^{o} C) time (s)	-	-	-	-	80-120 30	80-120 30
Ashing temp. (^{o} C) time (s)	-	-	-	-	400 30	300 30
Atomizing temp. (^{o} C) time (s)	-	-	-	-	2100 7	1700 7
Cleaning temp. (^{o} C) time (s)	-	-	-	-	3000 3	2600 3

Table 2. Determined concentrations of trace metals (μg g^{-1}) in SRM Wheat Flour (NBS-1567).

Metal	Concentration	
	Certified	Experimental
Cu	2.1 $\pm$ 0.21	2.0 $\pm$ 0.17
Fe	14.1 $\pm$ 0.56	14.3 $\pm$ 0.43
Mn	9.4 $\pm$ 0.94	9.5 $\pm$ 0.32
Zn	11.6 $\pm$ 0.35	11.2 $\pm$ 0.22
Pb*	20.0 $\pm$ 2.1**	23.2 $\pm$ 3.4
Cd*	26.0 $\pm$ 2.1	26.4 $\pm$ 2.7

* Concentration in ng g^{-1}

** Not certified but reported

Table 3. Determined concentration of trace metals ($\mu g\ g^{-1}$) in poultry farm chicken eggs.

Metal	Albumen			Yolk		
	Range	Average	Median	Range	Average	Median
Cu	0.3 - 2.2	1.2 ± 0.4	1.2	2.9 – 4.6	3.6 ± 0.5	3.6
Fe	0.6 - 7.8	3.9 ± 2.1	4.0	79.6-146.2	109.0 ± 20.5	101.2
Mn	0.1 - 0.6	0.2 ± 0.1	0.2	1.5 - 2.2	1.8 ± 0.3	1.7
Zn	0.7 - 6.8	2.4 ± 2.2	2.1	34.0-48.2	41.1 ± 4.6	42.3
Pb*	49-306	139.8 ± 73	130	29.0-173	86.1 ± 37.2	85.5
Cd*	30.4-81.4	53.0 ± 14.2	49.3	13.6-60	33.3 ± 12.8	31.1

*Concentration in $ng\ g^{-1}$

Table 4. Determined concentration of trace metals ($\mu g\ g^{-1}$) in domestic chicken eggs.

Metal	Albumen			Yolk		
	Range	Average	Median	Range	Average	Median
Cu	0.4-3.0	1.7 ± 0.7	1.7	2.2-3.9	3.1 ± 0.6	3.0
Fe	2.9-21.7	9.8 ± 5.6	8.7	85.0-194.6	148.5 ± 40.6	164.1
Mn	0.1-3.9	0.7 ± 1.1	0.3	0.8-3.0	1.6 ± 0.6	1.5
Zn	0.1-1.9	0.6 ± 0.4	0.6	33.4-61.3	44.4 ± 10.1	40.6
Pb*	93.5-247	177.2 ± 49.4	176.2	60.0-199.5	112.9 ± 49.3	95.0
Cd*	27.0-64	47.2 ± 12.7	45.5	18.4-55.3	38.5 ± 12.3	32.6

* Concentration in $ng\ g^{-1}$

Table 5. Concentration of trace metals in chicken eggs ($\mu g\ g^{-1}$) from different countries.

Metal/country	Cu	Fe	Mn	Zn	Pb*	Cd*
Pakistan (Present work)	2.71	70.16	1.21	26.8	105.9	40.6
Pakistan [4]	–	79	1.2 – 2.5	57	–	–
China [8], [11]	0.56-0.64	20.99-26.8	–	10.96-12.33	210	–
Canada [18]	0.66	21.5	0.29	14.6	10	10
Germany [21]	–	–	–	–	75	25
Czechoslovakia [20]	–	–	–	–	370	10
Spain [19]	–	–	–	54.0	43-135	3.3-5.6

* Concentration in $ng\ g^{-1}$

Table 6. Estimated daily intake of trace metals through farm eggs (mg/person)

Metal	Intake value	Requirement/Tolerance level
Cu	0.07	2.0 - 5.0
Fe	1.72	08.0 - 18.00
Mn	0.03	0.5 - 5.0
Zn	0.66	08.0 - 15.0
Pb*	2.60	429
Cd*	1.00	55-70

* Intake expressed in μg

both the portions of egg on dry weight basis. The calculated average concentrations of Cu, Fe, Mn and Zn in poultry farm chicken eggs were found to be 2.7, 70.2, 1.2 and 26.8 μg g^{-1} respectively. The corresponding concentrations in the domestic chicken eggs were 2.6, 100.9, 1.3 and 29.4 μg g^{-1} respectively. The determined concentration of copper was about four times higher than that of Canada (0.66 μg g^{-1}) [18] and China (0.56-0.64 μg g^{-1}) [11]. The determined iron contents were almost similar to the reported value for Pakistan (79 μg g^{-1}) [4] and were higher than the reported values for Canada (21.5 μg g^{-1}) [18] and China (20.99-26.78 μg g^{-1}) [11]. The concentration of manganese was in the reported range for Pakistan (1.2 – 2.5 μg g^{-1}) [4] and was higher than those of Canada (0.29 μg g^{-1}) [18]. The zinc contents were higher than the reported values for Canada (14.6 μg g^{-1}) [18] and China (10.96-12.33 μg g^{-1}) [11] but were lesser than the reported values for Pakistan (57 μg g^{-1}) [4] and Spain (54 μg g^{-1}) [19].

The determined average concentration of lead in the whole egg of poultry farm and domestic chicken were found 105.9 and 134.9 ng g^{-1} respectively. The concentration of lead was in the reported range for Spain (43-135 ng g^{-1}) [19], lower than the reported values for China (210 ng g^{-1}) [8] and Czechoslovakia (370 ng g^{-1}) [20], and higher than the reported values for Canada (10 ng g^{-1}) [18], and Germany (75 ng g^{-1}) [21]. The higher concentration of lead in eggs could be due to the use of contaminated feeds. The principal source of environmental lead is the use of leaded fuel in the automobiles. It is, therefore, utmost important to protect the environment from such pollutants. The determined average concentration of cadmium in the whole eggs of poultry farm and domestic chicken were 40.6 and 41.5 ng g^{-1} respectively. The cadmium contents are higher than the reported values for Canada (10 ng g^{-1}) [18], Germany (25 ng g^{-1}) [21], Czechoslovakia (10 ng g^{-1}) [20] and Spain (3.3-5.6 ng g^{-1}) [19].

Perusal of data in Tables 3 and 4 reveals that the concentrations of iron and lead in the domestic chicken eggs were relatively higher as compared to poultry farm chicken eggs which could probably be due to the use of different feeds of poultry farm and domestic chickens. The data also show that the egg yolk contained higher concentrations of Fe, Cu, Mn and Zn whereas higher concentration of Pb and Cd were observed in egg albumen. Similar findings have been reported by Ziqiang et al., [6]. This could probably be due to the comparatively larger ionic radii of Pb^{+2} and Cd^{+2}, which inhibit their migration / transference from albumen to yolk.

The daily intake of heavy metals through poultry farm chicken eggs was calculated on the basis of consumption of two eggs per person per day and the results are reported in Table 6 alongwith the recommended and tolerance levels of WHO [22-24]. The average concentration of each metal in the whole egg was used for such calculations. The estimated daily intake of Cu, Fe, Mn and Zn through poultry farm chicken eggs was found to be 0.07, 1.72, 0.03 and 0.66 mg which correspond to 3.5, 22.5, 6.0 and 8.25% to their minimum recommended requirements respectively. This indicates that the chicken eggs are inadequate as a source of Cu, Fe, Mn and Zn, therefore, the consumers should not rely on this source of essential trace metals only and other food commodities which are enriched with respect to these essential metals must be included in the diet pattern. The daily intake values of Pb and Cd through chicken eggs were found to be 2.6 and 1.0

μg respectively, which are much lower than the permissible daily intake values of these elements.

4. Conclusion

Concentrations of trace metals in the farm and domestic chicken eggs were determined by employing atomic absorption spectrophotometry to establish base line levels and the degree of contamination of these elements through different sources. Concentration of Cu, Fe, Mn and Zn were higher in egg yolk whereas the concentration of Pb and Cd were higher in egg albumen. The estimated daily intake values of these metals were much lower than the recommended dietary requirements and tolerance levels of WHO.

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