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Heavy Metal Content of Hard Biscuits Produced in Turkey

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Because of the negative and positive roles of the heavy metal ions in the human body, the trace heavy metal analyses are an important part of the analytical chemistry. Some of metals including Ca, Co, Cu, Cr, Fe, Mg, Mn, Mo, Na, and Zn are essential and some of them including Ag, Al, As, Ba, Be, Cd, Ni, Pb, Sb, Sn, Sr, Ti, Tl, U, and V are non-essential for human body (Smith and Arsenault 1996, Soto-Quintana *et al.*, 2003). It is necessary to investigate the levels of heavy metal ions in food samples due to the main source of the traces metal ions for human body is foods (Fernandez-Caceres *et al.*, 2001, Milacic and Kralj 2003). In the determination of the heavy metal ions in food samples, flame atomic absorption spectrometry (FAAS) is generally the main instrument, due to its simplicity, low cost and accuracy (Welz and Sperling 1999, Milacic and Kralj 2003). For FAAS determinations, the physical state of the sample should be liquid. Prior to sensitive flame atomic absorption spectrometric determination of heavy metals in solid samples including foods, a digestion procedure is necessary. Because of high sample-processing time, costs and hazards, instead of these methodologies, the many researchers have preferred microwave digestion procedures. In wet digestion processing, large volumes of concentrated acids in open beakers over heat are necessary. Microwave digestion of food samples including wheat (Doner and Akman 2000, Adams *et al.*, 2003), edible mushroom (Tuzen *et al.*, 2003), vegetables (Borkowska-Burnecka *et al.*, 2000), black tea (Fernandez-Caceres *et al.*, 2001, Narin *et al.*, 2003) for trace heavy metal analysis are very popular prior to their FAAS determinations. The hard biscuits are consumed as an important food especially for children. Various studies have been performed for the traces heavy metal contents of hard biscuits produced around the world (Vinas *et al.*, 1993, Tinggi *et al.*, 1998, Onianwa *et al.*, 2001, Sebecic *et al.*, 2002).

In the present work, iron, zinc, manganese and copper contents of twenty kind of hard biscuit samples produced in Turkey were determined by flame atomic absorption spectrometry after microwave digestion.

MATERIALS AND METHODS

All reagents were of analytical reagent grade unless otherwise stated. Double deionised water (Milli-Q Millipore 18.2 MΩ/cm) was used for all dilutions.

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Table 1. Operating conditions for microwave digestion system

	Program 1		Program 2		Program 3	
Steps	Time (min)	Power (W)	Time (min)	Power (W)	Time (min)	Power (W)
1	2	250	4	250	6	250
2	2	0	4	400	6	400
3	6	250	6	500	8	550
4	5	400	-	-	8	250
5	8	550	-	-	-	-
6	8	Vent	8	Vent	8	Vent
Total time (min)	31		24		36	

H_2SO_4, HNO_3 and H_2O_2 were of suprapure quality (E. Merck). All the plastic and glassware were cleaned by soaking in dilute nitric acid (1+9) and were rinsed with distilled water prior to use. The standard solutions of analytes for calibration procedure were produced by diluting a stock solution of 1000 mg/l of the all the investigated element supplied by Sigma.

A Perkin Elmer AAnalyst 700 model AAS with deuterium background corrector was used in this study. All measurements were carried out in an air/acetylene flame. The operating parameters for working elements were set as recommended by the manufacturer. Milestone Ethos D closed vessel microwave system (maximum pressure 1450 psi, maximum temperature 300 °C) was used. Teflon reaction vessels were used all the digestion procedures. The reaction vessels were cleaned using 5 ml of concentrated nitric acid before each digestion.

Wet digestion of hard biscuit samples was performed using a mixture of HNO_3:H_2O_2 (6:2) (8.0 ml for a 1.0 g sample). This mixture was heated up to 130 °C for 4h. After cooling, 5 ml of distilled water was added to the sample and mixed. The residue was filtered through blue band filter paper. Then the sample was diluted to 10 ml with distilled water. Blank digestions were also carried out in the same way. The analyte levels in the final solutions were determined by FAAS.

For microwave digestion, the standard reference materials (SRM) and hard biscuit samples were accurately weighed around 0.10 mg and 1.00 g, respectively. The samples were transferred to Teflon vessels. Then acid mixtures were added to samples. The microwave digestion programs given in Table 1 were applied. After digestion program completed, the residue was filtered through blue band filter paper. Then the sample was diluted to 10 ml with distilled water. The metal determinations were performed by FAAS. A blank digest was carried out in the same way for each digestion. All sample solutions were clear.

RESULTS AND DISCUSSION

The detection limit based on 3σ of the reagent blank and quantitation limit based on 10σ of the reagent blank for flame AAS are calculated for each analyte ions.

The detection limits for iron, zinc, manganese and copper were 0.29 mg/l, 0.15 mg/l, 0.12 mg/l and 0.14 mg/l, respectively. The quantitation limit for Fe, Zn, Mn and Cu were 0.97 mg/l, 0.50 mg/l, 0.40 mg/l and 0.47 mg/l, respectively. The contamination was not a problem in the microwave digestion procedure, because of the level of the analyte ions in the blank digest were close to detection limits of analytes.

Three different microwave program given in Table 1 were applied to a reference material (SRM 1573a) with HNO_3:H_2O_2 (6:2) mixture. Total digestion times of the programs are: 31 minutes for Program 1, 24 minutes for Program 2 and 36 minutes for Program 3. For Program 2, the values for analytes were found lower than certified values of the SRM 1573a. This point may source from low digestion time of the Program 2. Program 1 provided the most consistent recovery data for the reference standard material.

In order to compare the results found by wet and microwave digestion procedures, SRM 1573a-reference standard was digested both wet and microwave procedures. For microwave digestion, the condition given in Program 1 was used with HNO_3:H_2O_2 (6:2) mixtures. The relative standard deviations were less than 10 % for all the investigated elements. T-test was used in this study ($p<0.05$). The comparison of wet and microwave digestion methods showed no statistically significant differences in results (Table 2). The recovery values for the wet and microwave digestions for SRM 1573a were quantitative (>95%). Also the comparison of wet and microwave digestion procedures were performed by analyzing a hard biscuit sample with the same conditions. The results for this study are given in Table 3. The two digestion methods both (wet and microwave) gave acceptably consistent and reliable results for the all analyte species. The microwave digestion of the hard biscuit samples was easier than the wet digestion procedure.

Table 2. The traces metal contents of Tomato Leaves (SRM 1573a) reference material as µg/g, N=4

Analyte	Certified Value	Wet Digestion	Microwave digestion
Cu	4.7±0.1	4.8±0.3	4.5±0.3
Fe	368±7	360±10	348±32
Mn	246±8	238±15	231±21
Zn	30.9±0.7	31.5±1.5	29.3±2.7

Table 3. Comparison of trace metal contents in a hard biscuit sample using wet and microwave digestion methods (as µg/g), N=5

Element	Wet Digestion	Microwave digestion
Cu	2.7±0.2	2.9±0.1
Fe	31.3±2.9	29.5±2.3
Mn	11.7±1.2	12.4±0.8
Zn	12.8±1.2	13.4±1.1

According to light of the these results, the concentration of copper, iron, manganese and iron ions in the some biscuit samples produced and marketed in our country have been analyzed by flame atomic absorption spectrometry after microwave digestion. Relative standard deviations (RSD) were calculated from pooled data for method. In the precision test, the average RSD % for all analytes are in the range of 1-10 % (n=18) for method. The results, which were repeated in triplicate, were given in Table 4.

The range for copper concentrations was found as <1-4.2 μg/g. The mean copper content of the samples was 1.9±0.9 μg/g. The lowest level of copper was found Firm B- for baby and Firm B- Bar cracker with cheese samples, while the highest in Firm A- form. The mean level of copper in hard biscuit samples from India were given as 1-7 μg/g by Semwal *et al.* (1996). In a report, copper concentration of hard biscuit samples from Nigeria has been given as 1.93 μg/g by Onianwa *et al* (2001). The range for present study for copper levels is agreed with the results given for hard biscuit samples from around the world.

The mean concentration of zinc in the biscuit samples was found 7.6±3.4 μg/g (minimum: 3.1 μg/g (in Firm B- As cracker), maximum: 16.1 μg/g (in Firm A- Form Cracker)). The level of zinc in the samples was generally in the lower range of other studies. Semwal *et al.* (1996) have been reported the range of zinc in biscuit samples from India as 8.2-25.5 μg/g. The levels of zinc in biscuits from Nigeria have been reported by Onianwa *et al* (2001) as 6.87 μg/g.

The levels of manganese were in the range of 5.2-12.4 μg/g (mean: 7.8±2.1 μg/g). The highest and lowest levels of manganese were found in Firm B- Altinbasak and Firm B- As cracker, respectively. Tinggi *et al.* (1998) have been reported that manganese concentrations in the various biscuit samples in the range of 1.4-22.0 μg/g. The range of manganese in the some biscuit samples from Croatia was given as 3.76-16.37 μg/g (Sebecic *et al.*, 1998). The concentrations of manganese ranged from 3.5-10.4 μg/g in 32 kinds of hard biscuits produced in India by Semwal *et al.* (1996).

The lowest concentrations of iron were found in Firm B- Bar cracker with cheese (6.9 μg/g). The highest iron concentration was in Firm A- Burcak sample (31.4 μg/g) (Table 1). The mean concentration of iron was 20.0±8.3 μg/g. Semwal *et al.* (1996) have been reported the range of the iron concentration in the hard biscuits as 38-230 μg/g. Fe contents in seven biscuit samples range from 9.32 up to 24.80 μg/g have been reported by Sebecic *et al.* (1998).

The experimental results for the reference standard material (SRM 1573a) for both wet and microwave digestions were in agreement with the certified values. The proposed microwave digestion method was precise and accurate. The advantages of the present method are its simplicity, low cost, high speed of sample attack and rapid calibration. The microwave digestion procedure was the best because of more accurate with respect to both time and recovery than wet digestion.

Table 4. The levels of investigated ions in the hard biscuit samples

		Concentration (μg/g)			
Sample	Properties	Cu	Zn	Mn	Fe
Firm A- Bar cracker	Form Bran	2.9±0.1	11.5±0.9	6.4±0.6	26.3±2.5
Firm B- Bar cracker	Wheat flour	1.6±0.1	6.7±0.2	8.9±0.5	13.4±1.1
Firm C- Bar cracker	Wheat flour	1.7±0.1	7.6±0.6	8.4±0.6	17.1±1.5
Firm A- Bar cracker	Wheat flour	1.4±0.1	6.3±0.5	8.2±0.7	14.1±1.3
Firm D- Bar cracker	Wheat flour	1.6±0.1	5.6±0.5	8.5±0.8	23.3±2.1
Firm A- Bar cracker	Wheat flour with cheese	1.3±0.1	5.8±0.5	8.4±0.7	14.2±1.3
Firm B- Bar cracker	Wheat flour with cheese	<1	5.7±0.3	5.5±0.4	6.9±0.5
Firm B- As cracker	Wheat flour	1.8±0.1	3.1±0.2	5.2±0.4	11.9±1.1
Firm B- Petit Beurre	Wheat flour	1.2±0.1	5.7±0.4	7.9±0.6	12.7±1.2
Firm A- Petit Beurre	Wheat flour	1.1±0.1	4.8±0.3	7.0±0.5	11.1±0.9
Firm C- Petit Beurre	Wheat flour	1.1±0.1	5.3±0.5	7.6±0.7	11.0±0.8
Firm A- Yulafli	Wheat flour	3.1±0.2	8.7±0.7	8.5±0.7	28.3±2.4
Firm A- Burcak	Wheat flour	2.8±0.2	8.2±0.7	4.8±0.4	31.4±3.1
Firm B- Altinbasak	Oat and bran	2.9±0.2	13.4±1.2	12.4±1.1	29.5±2.2
Firm B- for baby	Wheat flour	<1	3.2±0.2	3.0±0.3	24.6±2.2
Firm D- Petit Beurre	Wheat flour	1.1±0.1	4.9±0.3	7.0±0.6	27.0±2.7
Firm A- Form Cracker	Bran	4.2±0.3	16.1±1.4	10.1±1.0	35.4±3.2
Firm A- Form Cracker	Bran with lemon	2.5±0.2	12.4±1.1	8.8±0.8	12.8±1.1
Firm E- Petit Beurre	Wheat flour	2.1±0.1	8.7±0.7	7.8±0.6	22.9±2.1
Firm E- Petit Beurre	Wheat flour with cheese	2.0±0.2	8.0±0.6	10.6±0.9	24.5±2.2

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