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# **Comparison of Digestion Procedures on Commercial Powdered Soup Samples for the Determination of Trace Metal Contents by Atomic Absorption Spectrometry**

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#### **ABSTRACT**

The concentrations of copper, manganese, zinc, iron and aluminum in commercial powdered soup samples produced in Turkey were determined using flame and graphite furnace atomic absorption spectrometry. We compared wet, dry and microwave digestion procedures for the digestion of the soup samples. The microwave digestion procedure for the soup samples was demonstrated to be most effective due to its simplicity and quick results. The accuracy of the method was checked against a standard reference material (SRM 8418 Wheat Gluten). Contents of investigated trace metals in soup samples ranged between 0.41 and 4.78 μg/g for copper, 1.29 and 49.4 μg/g for manganese, 1.26 and 22.5 μg/g for zinc, 4.62 and 61.7 μg/g for iron, and 6.86 and 547.7 μg/g for aluminum. The results were compared with values reported in the literature.

Key words: powdered soup, metal, human health, atomic absorption spectrometry, microwave digestion

# **INTRODUCTION**

Trace metals such as copper, iron, and zinc, among others, while considered essential for normal body functions, are toxic at high concentrations. Moreover, the difference between the ranges of what is essential and what is toxic for the body is often very small. Also certain metal ions, e.g., aluminum and lead, play toxic roles in biochemical reactions on our body. Due to the dual positive and negative roles and the toxicity of trace metals in terms of both human health and the environment, we performed for this study an analysis of trace metal levels in various environmental and food samples<sup> $(1-5)$ </sup>. Accurate and adequate food composition data are invaluable to estimate the intake adequacy of essential nutrients and assess exposure risks from the consumption of toxic non-essential metals $<sup>(6)</sup>$ . The main sources of trace ele-</sup> ments in foods include soil, agricultural practices, manufacturing processes and environmental sources such as traffic pollution and industrial activities.

The dried foods sector, which includes the commercial powdered soup industry, holds an important place in our country and around the world $^{(7-12)}$ . Soups represent groups of dried foods and play an important role in people' s diet $(13,14)$ . The consumption of powdered soups is high in Turkey. For example, a traditional Turkish soup called tarhana is prepared by mixing wheat flour, yoghurt, yeast and a variety of cooked vegetables, salt and spices. Tarhana soup is especially important in our country in the diets of children and the elderly<sup> $(15)$ </sup>. According to our survey of relevant literature, while many studies have been done on the organic components of powdered soups<sup>(8-10,16)</sup>, few<sup>(13,16)</sup> were found that address the trace metal contents of commercial powdered soups.

Wet and dry ash procedures represent the two main methods by which traces metals are digested in food samples. However, these procedures are slow and time-consuming, result in analyte loss, are complicated in nature, and frequently run a high risk of contamination. The wet digestion procedure requires the heating of a large volume of concentrated acid in an open beaker. The digestion of food samples using microwaves in trace metal analysis prior to their detection by AAS or ICP-MS has become very popular during the past decade<sup> $(17-19)$ </sup>. Microwave digestion is simple, rapid and reliable for the digestion of a variety of sample matrices. It is a closed system with considerably reduced chemical use and considerably reduced potential hazards, as well as costs. Microwave digestion techniques are widely applied to decompose such food items such as tea<sup>(20)</sup>, mushrooms<sup>(19,21)</sup>, honey<sup>(22)</sup>, wheat<sup>(23)</sup>, fish<sup>(24)</sup>, medicinal herbs<sup>(25)</sup> as well as  $others<sup>(26)</sup>$ .

In this study, the authors compare the efficacy of differing digestion procedures (wet and dry ashing and microwave digestion) on commercial soup samples. The contents of copper, manganese, zinc, iron and aluminum in soup samples produced in Turkey were determined by flame and/ or graphite furnace atomic absorption spectrometry after microwave digestion.

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# **MATERIALS AND METHODS**

#### I. *Apparatus*

A Perkin Elmer Analyst 700 atomic absorption spectrometer equipped with graphite furnace and flame unit was used in the experiments. A deuterium background corrector was used for background corrections. The operating parameters for the elements were set as recommended by the manufacturer (Table 1). For flame measurements, a 10-cm long slot-burner head, a lamp and an air-acetylene flame were used.

Argon was used as the inert gas for graphite furnace measurements. Pyrolytic-coated graphite tubes (Perkin Elmer part no. B3 001264) with a platform were used for aluminum determinations by GFAAS. Samples of 20 μL plus 5 µL of 10000 mg/L  $Mg(NO_3)$  as matrix modifier during the study were injected into the graphite furnace using a Perkin Elmer AS-800 autosampler. The signals were measured as peak areas. We defined absolute sensitivity by the characteristic mass of each element, giving a peak absorbance of 0.0044 and 17 pg for Al. During our analyses, the internal argon flow rate through the graphite tube was 250 mL/min; gas flow was interrupted during atomization. Sample volume, ramp and hold times for the drying, ashing, atomization and cleaning temperatures were optimized prior to analysis in order to obtain the maximum absorbance with minimum background.

A Milestone Ethos D closed vessel microwave digestion system (maximum pressure 1450 psi, maximum temperature 300°C) was used. Teflon reaction vessels were used in all digestion procedures. Reaction vessels were cleaned with 5 mL of concentrated nitric acid before each digestion.

All reagents were of analytical reagent grade unless otherwise stated. Double distilled deionised water (Milli-Q Millipore 18.2 M $\Omega$ -cm resistivity) was used for all dilutions.  $HNO<sub>3</sub>$  and  $H<sub>2</sub>O<sub>2</sub>$  were of suprapure quality (E. Merck, Darmstadt). All plastic and glassware were cleaned by soaking in diluted  $HNO<sub>3</sub>$  (1+9, v/v) and rinsed with distilled water prior to use. The element standard solutions used for calibration were prepared by diluting stock solutions of 1000 mg/L of each element supplied by Sigma.

#### III. *Sampling*

Sixteen different powdered soup samples representing two different manufacturer brands were purchased from local markets in Kayseri and Tokat, Turkey during December 2004. The samples were preserved in their original packaging and stored at 4~5°C until analysis. Samples were dried at 105°C for 2 hr before taking weight measurements.

#### IV. *Digestion Procedures*

Three different procedures (dry, wet and microwave) were applied to digest the commercial powdered soup samples. The procedures for each are noted below.

#### (I) *Dry Ashing*

One gram of powdered soup sample was placed into a porcelain crucible. Furnace temperature was slowly increased from room temperature to 450°C over a 1-hr period. The sample was ashed for about 8 hr, until a white





Element	Certified value $(\mu g/g)$	Found by dry ashing $(\mu g/g)$	Recovery $(\%)$	Found by wet ashing $(\mu g/g)$	Recovery $(\%)$	Found by microwave digestion $(\mu g/g)$	Recovery $(\%)$
Cu	5.94	$5.35 \pm 0.48^a$	$90 \pm 8$	$5.64 \pm 0.32$	$95 \pm 5$	$5.78 \pm 0.20$	$97 \pm 3$
Zn	53.8	$51.1 \pm 4.5$	$95 \pm 8$	$52.9 \pm 3.6$	$97 \pm 7$	$53.3 \pm 2.5$	$99 \pm 5$
Mn	14.3	$13.2 \pm 1.2$	$92 \pm 8$	$13.8 \pm 1.1$	$97 \pm 8$	$14.1 \pm 0.9$	$99 \pm 6$
Fe	54.3	$51.6 \pm 4.8$	$95 \pm 9$	$52.3 \pm 4.4$	$96 \pm 8$	$53.9 \pm 2.6$	$99 \pm 5$
A1	10.8	$9.3 \pm 0.9$	$86 \pm 8$	$9.9 \pm 0.9$	$91 \pm 8$	$10.3 \pm 0.5$	$95 \pm 5$

**Table 2.** Trace metal contents in SRM 8418 Wheat Gluten reference material ( $\mu$ g/g, n = 4)

 $a<sup>a</sup>$ Data presented as mean  $\pm$  standard deviation.

(or grayish) ash residue was obtained. The residue was dissolved in 5 mL of  $HNO<sub>3</sub>$  (25%, v/v) and the mixture, when necessary, was heated slowly to dissolve the residue. The solution was transferred to a 10-mL volumetric flask and made up to volume. Blank digestions were also carried out in the same way.

#### (II) *Wet Ashing*

Wet digestion of soup samples was performed using an oxi-acidic mixture of  $HNO<sub>3</sub>/H<sub>2</sub>O<sub>2</sub>$  (2/1) (12 mL for a 1.0 g sample). This mixture was heated at 60°C for 4 hr to complete dryness, and then brought to a volume of 10 mL with deionized distilled water. Blank digestion was also carried out in the same way.

#### (III) *Microwave Digestion*

One gram of soup sample was digested with 6 mL of HNO<sub>3</sub> (65%) and 2 mL of H<sub>2</sub>O<sub>2</sub> (30%) in a microwave digestion system and diluted to 10 mL with deionized distilled water. A blank digest was carried out in the same way.

All digested sample solutions were clear. Digestion conditions for the microwave system were applied as follows: 2 min for 250 W, 2 min for 0 W, 6 min for 250 W, 5 min for 400 W, 8 min for 550 W, and vent for 8 min.

#### **RESULTS AND DISCUSSION**

Detection limit was defined as the concentration corresponding to three times the standard deviation of ten blanks. Detection limit values for elements (as measured in milligrams per liter) in flame AAS were found to be 0.004 for Cu, 0.009 for Zn, 0.008 for Fe, 0.007 for Mn. Aluminum was determined with graphite furnace AAS.

In order to compare dry, wet and microwave digestion procedures, SRM 8418 Wheat Gluten - standard reference material was digested by each. Results are given in Table 2. We found dry ash recovery values to be consistently lower than those obtained in wet digestion. Microwave digestion generally achieved the highest recovery values for investigated metal ions in the SRM 8418 Wheat Gluten standards.

**Table 3.** Comparative efficacy of recovering trace metals in brand A lentil soup using dry ashing, wet ashing and microwave digestion processes,  $n = 3$ 

Element	Dry ashing $(\mu g/g)$	Wet ashing $(\mu g/g)$	Microwave digestion $(\mu g/g)$
Cu	$2.70 \pm 0.27$	$2.90 \pm 0.26$	$3.07 \pm 0.25$
Zn	$13.1 \pm 1.3$	$13.5 \pm 1.2$	$13.9 \pm 1.1$
Mn	$3.99 \pm 0.35$	$4.14 \pm 0.39$	$4.43 \pm 0.23$
Fe	$27.6 \pm 2.5$	$28.8 \pm 2.3$	$29.5 \pm 1.7$
A1	$36.9 \pm 3.4$	$38.7 \pm 3.1$	$39.4 \pm 2.7$

We also compared microwave digestion results against certified values using a *t*-test at a 95% confidence limit (C.L.). We found strong correlation between certified values and our own test values for certified analyte ions obtained through microwave digestion.

All three procedures were checked by running recovery studies. We performed digestions on lentil soup samples (brand A), into which 10 µg of each analyte (for aluminum: 1 µg) was added. Recovery values exceeded 95% for all procedures, with relative standard deviations less than 10% for all investigated elements.

To compare performance measures of the three digestion procedures, lentil soup (brand A) was digested by all three. Results are shown in Table 3. The results achieved by the microwave digestion procedure were superior to the other two, providing a greater degree of accuracy both in terms of time and recovery. We evaluated a conventional microwave oven digestion method for sample digestion prior to determining the level of analytes in soup samples for sample dissolution in closed vessels.

After results were obtained, we analyzed the copper, manganese, zinc, iron and aluminum concentrations in the domestically (Turkey) manufactured commercial powdered soup samples by atomic absorption spectrometry after microwave digestion. All samples were analyzed in quadruplicate. Results are shown in Table 4. All metal concentrations were determined on a wet weight basis.

Copper is recognized as being both vital to and toxic for many biological systems, based on ingested amounts. Copper can enter the food chain through crop soil mineralization, food processing procedures and environmental contamination (e.g., copper-based pesticides, which are in common use in some countries $(6)$ . Copper concentrations identified ranged from  $0.41 \mu g/g$  in tripe soup (brand A) and  $4.78 \mu g/g$  in lentil soup (brand B), respectively. The level of copper in brand A cream of chicken soup fell below the AAS detection limit. Olivares *et al.*(27) have been reported copper concentrations of powdered chicken soup from Chile at 0.70 µg/g. Mean copper levels in cooked vegetable and lentil soups from Kuwait were reported at 127 µg/g by Dashti *et al.*(28). Leblanc *et al.* reported that the mean value of copper in cooked soups from France was 0.70  $\mu$ g/g<sup>(29)</sup>.

Zinc is an essential trace element for many biological functions, including proper immune functions $(30)$ . As can be seen in Table 4, we found the lowest zinc content  $(1.26 \mu g/g)$ in brand A cream of chicken soup and the highest content (22.5  $\mu$ g/g) in brand A Tarhana soup. The level of zinc in powdered chicken soup produced in Chile was reported as 8.25  $\mu$ g/g<sup>(27)</sup>. Zinc levels in vegetable soups manufactured in Egypt were reported at 32.0 µg/g by Hussein and Brugge $man^{\tilde{3}1)}$ 

Foods generally containing high levels of manganese include whole grains, nuts, seeds, legumes, pineapple, tea, and leafy green vegetables<sup>(32)</sup>. However, differing levels of manganese in the soil impact the level of manganese in foods. High-tech farming and lime added to soil can lower the manganese levels in certain foods. Manganese levels can also be affected by food processing procedures. The range of manganese levels detected in the soups analyzed fell between 1.29  $\mu$ g/g, for brand A tripe soup, and 49.4  $\mu$ g/g, for brand b tripe soup (all values in wet weight). Manganese fell below the AAS detection limit in brand A's creamof-chicken and cream-of-mushroom soups. The level of manganese ranged between 1.2 and 2.5  $\mu$ g/g<sup>(28)</sup> in cooked soup samples from Kuwait and was reported (by Leblanc *et*   $aI^{(29)}$  at 0.97 µg/g in cooked soups from France.

Iron is an essential element for life and for our diets $(33)$ . Low iron levels can cause anaemia. The lowest detectible iron level was found in brand A's tripe soup  $(4.62 \text{ µg/g})$  and the highest was found in brand A's lentil powder with noodle soup (Ezogelin) (61.7  $\mu$ g/g). The concentration of iron fell below AAS detection limits in brand A's cream-of-chicken and cream-of-mushroom soups. The average iron content of chicken soup was reported at 4.21  $\mu$ g/g<sup>(27)</sup>.

Aluminum is widespread throughout the natural environment and is present in the air, water, plants and, consequently, throughout the food chain<sup> $(34)$ </sup>. Main sources of aluminum in our body include consumed foods and water. Aluminum in food occurs naturally and also through its addition through food additives and by contact with aluminum utensils and containers $(35)$ . The minimum and maximum aluminum levels found in our study were 6.86 µg/g in brand A's cream of mushroom soup and  $547.7 \mu g/g$  in brand A's tarhana soup*,* respectively. Aluminum levels in the investigated soup samples were higher than other analyte metals. The source of aluminum may be the materials in which the soups were packaged. Aluminium in the various soup samples from Kuwait ranged between 3.0 and 5.0  $\mu$ g/g<sup>(28)</sup> and mean aluminium levels in cooked noodle soup with chicken and cooked chicken-vegetable soup from Granada-Spain were reported as 23.97  $\mu$ g/g and 19.54  $\mu$ g/g, respectively<sup>(36)</sup>

**Table 4.** Copper, zinc, manganese, iron and aluminum levels detected in powdered soup samples  $(\mu g/g)$ 

Soup sample	Cu	Zn	Mn	Fe	Al
Brand (A) Cream of vegetable	$0.65 \pm 0.05$	$5.91 \pm 0.40$	$2.47 \pm 0.15$	$7.51 \pm 0.62$	$31.3 \pm 2.1$
Brand (B) Cream of vegetable	$1.86 \pm 0.12$	$8.58 \pm 0.62$	$5.22 \pm 0.34$	$11.1 \pm 0.9$	$15.2 \pm 1.3$
Brand (A) Lentil soup	$3.07 \pm 0.25$	$13.9 \pm 1.1$	$4.43 \pm 0.23$	$29.5 \pm 1.7$	$39.4 \pm 2.7$
Brand (B) Lentil soup	$4.78 \pm 2.39$	$21.1 \pm 1.5$	$6.76 \pm 0.53$	$31.6 \pm 2.9$	$246.4 \pm 19.5$
Brand (A) Lentil powder with noodle (Ezogelin)	$4.05 \pm 0.32$	$15.7 \pm 1.3$	$7.41 \pm 0.63$	$61.7 \pm 4.2$	$229.1 \pm 10.3$
Brand (B) Lentil powder with noodle (Ezogelin)	$4.50 \pm 0.27$	$15.7 \pm 1.2$	$6.94 \pm 0.50$	$27.6 \pm 2.4$	$20.1 \pm 1.8$
Brand (A) Tripe	$0.41 \pm 0.03$	$6.84 \pm 0.55$	$1.29 \pm 0.11$	$4.62 \pm 0.35$	$9.70 \pm 0.76$
Brand (B) Tripe	$1.16 \pm 0.10$	$5.89 \pm 0.36$	$49.4 \pm 2.5$	$40.6 \pm 3.2$	$25.1 \pm 1.7$
Brand (A) Peasant soup with yoghurt (Yayla)	$1.14 \pm 0.10$	$9.52 \pm 0.65$	$3.85 \pm 0.30$	$8.88 \pm 0.76$	$16.9 \pm 1.4$
Brand (B) Peasant soup with yoghurt (Yayla)	$1.15 \pm 0.10$	$6.49 \pm 0.43$	$5.38 \pm 0.32$	$6.44 \pm 0.47$	$17.1 \pm 1.2$
Brand (A) Cream of chicken	BDL <sup>a</sup>	$1.26 \pm 0.10$	<b>BDL</b>	<b>BDL</b>	$12.9 \pm 0.9$
Brand (B) Cream of chicken	$0.67 \pm 0.05$	$5.37 \pm 0.48$	$3.21 \pm 0.20$	$7.82 \pm 0.60$	$12.6 \pm 1.1$
Brand (A) Wheat flour (Tarhana)	$3.81 \pm 0.25$	$22.5 \pm 1.8$	$18.2 \pm 1.3$	$27.4 \pm 2.4$	$547.7 \pm 32.3$
Brand (B) Wheat flour (Tarhana)	$2.86 \pm 1.90$	$12.8 \pm 1.1$	$10.5 \pm 0.9$	$22.4 \pm 1.9$	$27.1 \pm 2.5$
Brand (A) Cream of mushroom	$2.83 \pm 2.38$	$4.71 \pm 0.35$	<b>BDL</b>	<b>BDL</b>	$6.86 \pm 0.47$
Brand (B) Cream of mushroom	$1.37 \pm 0.12$	$6.71 \pm 0.52$	$3.64 \pm 0.26$	$11.9 \pm 0.9$	$24.9 \pm 2.2$

a Below detection limit.

The United States Food and Drug Administration (FDA) recommends daily copper, iron, manganese, zinc and aluminum allowances of 3 mg, 18 mg, 10 mg, 50 mg and 8 mg, respectively(37-39). Considering that a single service portion of cooked soup is prepared using approximately 20 g of powdered soup, analyte element levels in the soup samples examined fall below FDA recommended allowances.

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