Determination of Trace Elements in Human Milk, Cow's Milk, and Baby Foods by Flame AAS Using Wet Ashing and Microwave Oven Sample Digestion Procedures

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ABSTRACT

Three sample digestion procedures using dry and wet ashing and microwave oven are compared for the flame atomic absorption spectrometry analysis of human milk, cow's milk, and baby food samples. Copper, Mn, Zn, and Fe were determined in the digestion solutions. Various acid mixtures were examined in conventional wet digestion using a hot plate. It was found that the mixture of HNO_3/H_2O_2 (1:1) was simple to use and provided best results in comparison to either $HNO_3/HClO_4$ or HNO_3 in wet ashing procedure.

The analytical parameters show that the microwave oven digestion procedure provided best results as compared to the wet ashing procedure. Microwave sample digestion is an accurate, simple, and fast method for the flame AAS determination of Cu, Mn, Zn, and Fe in human milk, cow's milk, and baby formulas, except for the determination of Fe in rice flour and baby biscuits.

INTRODUCTION

Human milk, under non-pathological states, is a well-balanced food that provides the infant with the essential elements. The element concentration ranges in human milk are generally used as a reference in manufacturing infant formulas (1). In addition, studies on the total content of the essential elements in human milk are useful to evaluate the ideal food for the first months of an infant's life.

While Cu and Zn are essential elements for infant development, they can also become toxic when taken in excess. Toxicity or the amount of an element required to maintain an infant's health varies from element to element. Milner (2) reported that the concentrations of Zn, Mn, Fe, and Cu are higher in infant formulas than in human milk, while cow's milk has a lower Cu content. Thus, infants who are provided a strictly cow's milk diet may develop Cu deficiency and anemia. Farida et al. (3) reported that Zn intake from breast milk is inadequate during the weaning period, especially when weaning foods are introduced at an early stage. A study by Perrone et al. (4) shows that infants are at risk of developing iron deficiency if weaning foods are introduced before the infant reaches one year of age.

In ashing procedures, sample digestion is often the most time-consuming step and involves potential problems such as incomplete dissolution, precipitation of insoluble analytes, contamination, and loss of some volatile elements. In order to prevent loss of elements, closed digestion bombs are used. However, this procedure requires a prolonged time for complete dissolution.

In recent times, both commercial domestic microwave ovens (5) and commercial microwave ovens equipped with temperature and pressure regulators have become widely used because sample dissolution is faster and prevents analyte loss as well as sample contamination (6–7). On the other hand, commercial microwave ovens equipped with temperature and pressure regulators are very expensive. Therefore, an examination of using a domestic microwave oven for this purpose is very important.

In our laboratory, a flame atomic absorption spectrometry (FAAS) method was used for the determination of trace metals in foods consisting of different matrices (8–11). Although considerable information is available with regard to trace element concentrations in milk and baby foods (12–13), an accurate, reliable, and fast method for the determination of these elements is needed for diagnostic purposes.

In this study, Zn, Cu, Mn, and Fe were determined in human milk, cow's milk, and baby foods such as ready-powdered baby formula, baby biscuits (commercially available), and rice flour (home-made) using the classic wet ashing or the domestic microwave oven method as the sample digestion methods. The digestion solutions were then analyzed by flame atomic absorption spectrometry.

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EXPERIMENTAL

Instrumentation

A Model ATI UNICAM 929 (UNICAM, England) flame atomic absorption spectrometer, equipped with ATI UNICAM hollow cathode lamps, was used for the sample analysis. The optimum instrumental conditions are given in the Table I. Slotted Tube Atom Trap (STAT) was used for improving the sensitivity of Cu by FAAS. A domestic microwave oven (Kenwood, UK) and specially made Teflon® bombs were used for the digestion procedure.

Reagents and Standard Solutions

The metal stock solutions (1000 mg L^{-1}) were prepared from their nitrate salts (Merck, Darmstadt, Germany). Nitric acid (65%, Merck), hydrogen peroxide (35%, Merck), and perchloric acid (70%, Merck) were used for the sample digestions.

Digestion of Samples by Wet Ashing

Human milk samples were provided by Firat University and the local state hospitals. The cow's milk samples were collected in precleaned polyethylene bottles. Approximately 10 mL of cow's milk was accurately measured into Pyrex® vessels, 5 mL of HNO_3/H_2O_2 (1:1) was added; the mixture was then dried on a hot plate with occasional stirring. The same procedure was repeated using 2.5 mL of HNO_3/H_2O_2 (1:1). Then, 2.0 mL of 1.0 mol L^{-1} HNO_3 was added to the residue and centrifuged to obtain a clear solution.

The same procedures and volumes were applied to the new 10-mL sample of the same cow's milk by using HNO_3 instead of HNO_3/H_2O_2 (1:1).

A 0.75-g sample of ready-made powdered infant formula (Humana 3) was accurately weighed into Pyrex vessels, 3.0 mL of concentrated HNO_3/H_2O_2 (1:1) was added, and the mixture was dried on a hot plate with occasional stirring. The same procedure was repeated using 1.5 mL of HNO_3/H_2O_2 (1:1). Then, 2.0 mL of 1.0 mol L^{-1} HNO_3 was added to the residue and centrifuged to obtain a clear solution.

The same procedures and the same volumes described above for infant formula were also applied to the new 0.75-g sample of the same infant formula (Humana 3) by using HNO_3 instead of HNO_3/H_2O_2 (1:1).

In addition, the mixture of $HNO_3/HClO_4$ (1:1) was used as the digesting reagent instead of HNO_3/H_2O_2 for the new sample of the same infant formula, using the same procedures and volumes described above. Then, 3.0 mL and 1.5 mL of $HNO_3/HClO_4$ (1:1) were added to 0.75 g of the same infant formula in the first and second step described above, respectively. The blank digests were carried out in the same way.

Digestion of Samples by Microwave Oven

Different baby foods such as the ready-made powdered infant formula, rice flour (home-made), and baby biscuits (commercial) were digested by using a microwave oven as follows: Approximately 0.5 g of the sample was accurately weighed into Pyrex vessels. Then, 2.0 mL of concentrated HNO_3/H_2O_2 (1:1) was added and the mixture placed into a water bath at 70°C for 60 min with occasional stirring. After adding 2.0 mL of HNO_3/H_2O_2 (1:1) diluted with water (1:1), the mixture was transferred into a Teflon bomb. The bomb was closed, placed inside the microwave oven, and microwave radiation was carried out for 4 min. After a 4-min cooling period, the mixture was dried on a hot plate. Then, 2.0 mL of 1.0 mol L^{-1} HNO_3 was added and the mixture transferred to a Pyrex tube.

In addition, 5.0 mL each of cow's milk and human milk were accurately measured into Pyrex vessels, separately. Then, 2.5 mL of concentrated HNO_3/H_2O_2 (1:1) was added to the milk sample. The mixture was placed into a water bath at 70°C for 60 min with occasional stirring. After adding 2.0 mL of HNO_3/H_2O_2 (1:1), the mixture was transferred into a Teflon bomb. The bomb was closed, placed inside the microwave oven, and microwave radiation was carried out for 4 min. After a 4-min cooling period, the mixture was dried on a hot plate. Then, 1.5 mL of 1.0 mol L^{-1} HNO_3 was added and the mixture transferred to the Pyrex tube.

After centrifugation, the clear solutions were analyzed by FAAS. Blank digests were carried out using the same procedures.

TABLE I
Instrumental Operating Parameters for FAAS

Parameter	Cu	Mn	Zn	Fe
Wavelength (nm)	324.8	279.5	213.9	248.3
HCL Current (mA)	4.5	11.5	9.5	15
Acetylene Flow Rate (L/min)	0.5	0.5	0.5	0.5
Air Flow Rate (L/min)	4.0	4.0	4.0	4.0
Slit (nm)	0.5	0.2	0.5	0.2

RESULTS AND DISCUSSION

Calibration curves were obtained using Cu solutions of 0.025, 0.05, 0.1, 0.2, 0.4, and 0.8 mg L^{-1}; Mn and Zn solutions of 0.1, 0.2, 0.4, 0.8, and 1.0 mg L^{-1}; and Fe solutions of 0.25, 0.5, 1.0, 2.0, and 3.0 mg L^{-1}. The curves obtained were linear in the concentration range described above and the equations of the curves were as follows:

Copper

$Y = 205 X + 7$
$R^2 = 0.9994$
using STAT-FAAS

Manganese

$Y = 136.8 X + 4.8$
$R^2 = 0.9997$

Zinc

$Y = 327 X + 6.6$
$R^2 = 0.9998$

Iron

$Y = 69.8 X + 0.4$
$R^2 = 1.0$

Accuracy

The accuracy of the method was studied by examining the Standard Reference Material (Tomato Leaves-1573a). The results are given in Table II. It can be seen that the recovery values were 100% for Cu, 94% for Zn, 90% for Mn, and 80% for Fe. In addition, the accuracy of the method was studied by examining the recovery of the metals from cow's milk and baby formula samples fortified with different amounts of Cu, Mn, Zn, and Fe. The following metal amounts were added:
20 ng/mL of Cu and Mn, 200 ng/mL of Fe and 2000 ng/mL of Zn for cow's milk; and
500 ng/g of Cu and Mn, 30 000 ng/g of Fe and Zn for ready-made powdered baby formula (Humana 3).
After digestion by microwave oven, the recoveries were found to be at least 95% for all metals.

The standard additions method was used to investigate possible interferences caused by the matrix. The slopes of the calibration curves for all studied elements were compared with the slopes obtained by the standard additions method. The slopes of the calibration curves were found to be the same as those

TABLE II
Comparison of Digestion Methods for Reference Material, Cow's Milk, and Baby Formula and the Effect of Acid Mixtures Using Wet Digestion (n=5)

Sample		(Watt)	Cu	Mn	Zn	Fe
Cow's milk (ng/mL)						
	HNO_3		55±4	18±1.4	2650±210	375±25
	HNO_3/H_2O_2		60±5	20±1.6	2800±205	350±26
Our domestic microwave oven		360 W	61±5	18±1.6	2750±225	390±30
		450 W	45±4	20±1.5	2900±230	381±28
Ready-made powdered baby formula, Humana 3 (mg/kg)						
Wet ashing	HNO_3		5.2±0.4	0.33±0.02	44±4	51±4
	HNO_3/H_2O_2		5.0±0.4	0.36±0.03	50±5	63±5
	$HNO_3/HClO_4$		4.5±0.4	0.35±0.02	48±4	61±5
Dry ashing	Humana 3		5.0±0.3	0.30±0.02	45±4	59±5
	SMA		3.5±0.2	1.1±0.07	31±2	64±5
ETHOS plus MILESTONE microwave oven	Humana 3		4.5±0.4	0.36±0.02	45±4	65±6
	SMA		3.4±0.2	1.1±0.1	32±3	69±6
Our domestic microwave oven						
	Humana 3	360 W	5.0±0.4	0.36±0.02	50±4	63±5
		450 W	4.9±0.4	0.40±0.03	47±4	61±6
	SMA	360 W	3.5±0.2	1.2±0.08	32±2	68±5
		450 W	3.2±0.2	1.0±0.07	30±2	67±6
Reference material (Tomato Leaves 1573a)						
Certified value (mg kg^{-1})			4.7±0.14	246±8	30.9±0.7	368±7
Our domestic microwave oven		360 W	4.7±0.4	211±15	28±2	280±19
		450 W	4.7±0.4	221±17	29±2	295±21

obtained with the standard additions method. In other words, all standard additions curves were parallel to the calibration curves; the results indicate an absence of chemical interferences. Thus, calibration with aqueous standards was valid.

The possibility of sample contamination was studied by subtracting the values obtained for the blanks. Adsorption loss can be excluded as the procedure was followed in exactly the same way as described above, using the same glassware and the same reagents throughout. The results showed that there was no contamination or adsorption loss.

On the other hand, Standard Reference Material Tomato Leaves 1573a, powdered baby formulas (Humana 3 and SMA), and cow's milk were digested by using a microwave oven at the different powers of 360 and 450 W. As can be seen from Table II, significantly higher Cu levels were obtained by using 360 W for cow's milk. For other matrices, the obtained concentrations of all elements at 360 W were generally higher or close to the concentrations at 450 W using the microwave oven.

The baby formulas (Humana 3 and SMA) were also digested using a commercial microwave oven equipped with temperature and pressure regulators (MILESTONE ETHOS Plus with MPR-300/125 medium pressure rotor). The obtained results were found to be very close to the results obtained with the domestic microwave oven (Table II).

The analytical parameters obtained in the recovery assays using microwave oven digestion of cow's milk and human milk and the powdered baby's formula samples showed that the method is simple and fast for the determination of Cu, Mn, Zn, and Fe. However, the Fe determination in rice flour and baby biscuits was not good. This is attributed to the fact that the rice flour and baby biscuit samples were not sufficiently digested by the microwave oven method for Fe determination.

Comparison of Acid Mixtures With Ashing Methods

The determination of Cu, Mn, Zn, and Fe in cow's milk and powdered baby formula (Humana 3) using different acid mixtures in the wet ashing method are given in Table II. It can be seen that the analyte concentrations of the baby formula when using HNO_3/H_2O_2 (1:1) is higher in comparison to using either $HNO_3/HClO_4$ (1:1) or HNO_3. For cow's milk, the analyte concentration when using HNO_3/H_2O_2 (1:1) is slightly higher in comparison to using HNO_3, except for the Fe concentration. Iron concentrations in the HNO_3/H_2O_2 (1:1) digestions are slightly lower than in HNO_3 digestions. Therefore, HNO_3 had to be used as the wet digestion reagent for iron determination in milk samples.

In addition, the powdered baby formulas (Humana 3 and SMA) were digested by dry ashing at 480°C for 4 h and the results are given in Table II. It was found that the examined metal levels in both baby formulas were lower or equal to the levels of the microwave or wet ashing methods.

Table III shows that the Cu concentrations in all examined samples using the closed-vessel microwave oven method are very close to the Cu concentrations in the classic wet ashing method (at least 95% recovery). Particularly in the rice flour samples, slightly higher Cu levels were obtained when the microwave oven was used. For Mn concentrations, the evaluations described above for Cu were valid.

Table IV shows that the Zn concentrations in the samples using closed-vessel microwave oven were very close to the Zn concentrations obtained in the classic wet ashing method, except for the infant formulas (87% recovery).

In comparing the wet ashing and microwave methods, it was found that for the rice flour and baby biscuit samples, higher Zn levels were obtained with the microwave oven (Table IV). Although the Zn concentrations obtained for the baby formulas using wet ashing were slightly higher than the Zn concentrations obtained using microwave oven, these differences were not significant. For Fe concentrations, acceptable recoveries were obtained (90% recovery) using microwave oven in comparison to wet ashing, except for the rice flour and the baby biscuit samples.

Metal Concentrations in Baby Foods With Different Matrices

The concentration ranges of the elements considered in human milk and cow's milk were within the ranges reported in the literature (13). The values in Tables III and IV were obtained by using HNO_3/H_2O_2 (1:1) for wet ashing and 360 W for microwave oven. The observed metal concentrations using microwave oven can be summarized as follows.

The Cu content of the samples (Table III) ranged from 19–21 ng mL^{-1} for cow's milk, 200–300 ng mL^{-1} for human milk, and 0.8–3.6 mg kg^{-1} for the powdered baby formulas. The Cu content in the examined cow's milk was at the lower end of the range as reported in the literature. It can be seen that the Cu content in the human milk samples was approximately 10-fold higher than in cow's milk. However, even this concentration level would make the daily intake much lower than the recommended value of 0.5–1.0 mg/day due to an average production of 700 mL of human milk

TABLE III
Comparison of Cu and Mn Concentrations in Baby Foods Using Wet Ashing (HNO_3/H_2O_2 (1:1)) and Microwave Oven (at 360 W) [human milk (n= 3); for other samples (n= 5)]

Samples	Cu Wet Ashing	Cu Microwave	Mn Wet Ashing	Mn Microwave
Cow's milk 1 (ng/mL)	23±1.7	21±1.5	20±1.6	21±1.6
Cow's milk 2 (ng/mL)	22±1.6	21±1.6	21±1.7	20±1.5
Cow's milk 3 (ng/mL)	20±1.5	19±1.5	20±1.5	19±1.5
Cow's milk 4 (ng/mL)	20±1.5	20±1.5	22±1.5	23±1.6
Cow's milk 5, ng/ml	25±1.6	20±1.5	19±1.6	20±1.5
Cow's milk 6, ng/ml	60±5.0	61±4	20±1.6	18±1.5
Human milk 1(ng/mL)l (11 days*)	290±15	275±15	14±1.3	14±1.3
Human milk 2 (ng/mL) (12 days*)	325±17	300±16	15±1.2	15±1.2
Human milk 3 (ng/mL) (24 days*)	265±15	240±15	14±1.2	14±1.2
Human milk 4 (ng/mL) (24 days*)	280±14	260±14	15±1.3	14±1.2
Human milk 5 (ng/mL) (41 days*)	230±16	200±15	13±1.2	13±1.1
Baby foods:				
Rice flour (mg/kg)	0.98±0.06	1.1±0.06	8.7±0.5	10.5±0.6
Baby biscuit (mg/kg)	0.53±0.04	0.51±0.03	4.1±0.2	3.9±0.2
Powdered baby formula, SMA (mg/kg)	3.6±0.11	3.5±0.2	1.0±0.1	1.2±0.08
Powdered baby formula, Humana 3 (mg/kg)	5.0±0.3	5.0±0.3	0.36±0.02	0.36±0.02
Powdered baby formula, Guigoz (mg/kg)	3.7±0.19	3.6±0.21	0.3±0.03	0.29±0.03

*The number of days for human milk represents the days since the birth of the infant when the milk was sampled.

TABLE IV
Comparison of Zn and Fe Concentrations in Baby Foods Using Wet Ashing (HNO_3/H_2O_2 (1:1)) and Microwave Oven (at 360 W) [human milk (n= 3); other samples (n= 5)]

Samples	Zn Wet Ashing	Zn Microwave	Fe Wet Ashing	Fe Microwave
Cow's milk 1 (mg/L)	2.8±0.13	2.7±0.13	0.19±0.02	0.17±0.02
Cow's milk 2 (mg/L)	2.9±0.13	2.8±0.12	0.22±0.02	0.18±0.02
Cow's milk 3 (mg/L)	2.8±0.13	2.8±0.13	0.20±0.02	0.17±0.02
Cow's milk 4 (mg/L)	3.0±0.16	2.9±0.13	0.18±0.02	0.16±0.02
Cow's milk 5 (mg/L)	2.7±0.17	2.6±0.12	0.21±0.02	0.18±0.02
Cow's milk 6 (mg/L)	2.8±0.19	2.8±0.15	0.35±0.02	0.39±0.03
Human milk 1 (mg/L) (11 days*)	3.3±0.018	3.1±0.13	0.52±0.03	0.46±0.03
Human milk 2 (mg/L) (12 days*)	3.5±0.20	3.3±0.22	0.52±0.03	0.45±0.03
Human milk 3 (mg/L) (24 days*)	1.8±0.014	1.6±0.11	0.60±0.03	0.56±0.03
Human milk 4 (mg/L) (24 days*)	1.5±0.01	1.3±0.09	0.72±0.04	0.66±0.04
Human milk 5 (mg/L) (41 days*)	1.5±0.01	1.3±0.10	0.58±0.03	0.51±0.03
Baby foods:				
Rice flour (mg/kg)	9.4±0.5	9.8±0.5	4.2±0.3	3.4±0.2
Baby biscuit (mg/kg)	4.1±0.3	4.5±0.3	5.0±0.3	3.3±0.2
Powdered baby formula, SMA(mg/kg)	33±3	32±2	69±6	68±5
Powdered baby formula, Humana 3 (mg/kg)	50±4	50±3	63±5	63±4
Powdered baby formula, Guigoz (mg/kg)	39±2	37±2	59±3	55±3

*The number of days for human milk represents the days since the birth of the infant when the milk was sampled.

within 24 h during the first year of lactation (14).

The Mn concentrations of the examined samples (Table III) ranged from 19–23 ng mL^{-1} for cow's milk, 13–15 ng mL^{-1} for human milk and 0.20–1.05 mg kg^{-1} for powdered baby formulas. As reported in the literature (15), the Mn levels in human milk were lower than in cow's milk.

The Fe content of the examined samples (Table IV) ranged from 0.16–0.18 mg L^{-1} for cow's milk, 0.45-0.66 mg L^{-1} for human milk, and 54-55 mg kg^{-1} for the powdered baby formulas. The cow's milk contained a low Fe concentration, similar to Cu. In addition, it is reported that the fractional absorptions of Fe, Zn, and Mn are better from human milk than from cow's milk. Based on these facts, infants provided solely with a diet of cow's milk may become Fe deficient and develop anemia.

The Zn content of the samples (Table IV) ranged from 2.6–2.9 mg L^{-1} for cow's milk, 1.3–3.1 mg L^{-1} for human milk, and 26–37 mg kg^{-1} for the powdered baby formulas.

CONCLUSION

This study shows that closed-vessel microwave digestion of the samples and determination of the elements by FAAS is accurate, rapid, and simple. The analytical parameters obtained make this method suitable for the determination of Cu, Mn, Zn, and Fe in human milk and cow's milk and various baby foods. We found that there are no safety concerns when using a domestic microwave oven at the studied conditions because of the predigestion of the sample in a water bath for 1 h and using the Teflon bomb (durable to 360°C).

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