

# Optimization of microwave assisted digestion procedure for the determination of zinc, copper and nickel in tea samples employing flame atomic absorption spectrometry

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## Abstract

The present paper describes the development of a microwave assisted digestion procedure for the determination of zinc, copper and nickel in tea samples employing flame atomic absorption spectrometry (FAAS). The optimization step was performed using a full factorial design ( $2^3$ ) involving the factors: composition of the acid mixture (CMA), microwave power (MP) and radiation time (RT). The experiments of this factorial were carried out using a certified reference material of tea GBW 07605 furnished by National Research Centre for Certified Reference Materials, China, being the metal recoveries considered as response. The relative standard deviations of the method were found below 8% for the three elements. The procedure proposed was used for the determination of copper, zinc and nickel in several samples of tea from Turkey. For 10 tea samples analyzed, the concentration achieved for copper, zinc and nickel varied at 6.4–13.1, 7.0–16.5 and 3.1–5.7 ( $\mu\text{g g}^{-1}$ ), respectively.

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## 1. Introduction

Tea is one of the most popular beverages in the world. Various kinds of teas including black, green and herbal etc. are consumed at the high ratios. The chemical composition of tea leaves and manufactured tea are very complex and consists of tanning substances, flavonols, alkaloids, proteins and amino acids, enzymes, aroma-forming substances, vitamins and trace elements [1–4]. The medical community recognizes tea as a drink that may offer several health benefits [5,6]. The regular consumption of teas can contribute to the daily dietary requirements of traces heavy metals including zinc, copper, manganese, iron, etc. The main sources of trace metals to plants including tea samples are their growth media, i.e., nutrient solutions or soils [7,8]. Other sources

of trace metals include insecticides, herbicides and fertilizers that may be absorbed through the leaves of tea. The content of heavy metals in tea samples have also received great interest [8,9].

A wide range of digestion methods for plants have been published such as dry ashing, wet ashing with different mixtures of reagents or conventionally heating procedures, microwave dissolution and acid bomb digestion, etc. [10–13]. These methods generally show both good accuracy and precision. The wet and dry ashing digestion procedures are generally slow and time consuming. Depending on analysis task, several factors should be considered for sample digestion. These may include level of contamination, sample homogeneity, completeness of digestion, reproducibility, suitability of the analytical technique employed, time needed for sample preparation, economic aspects including labour and reagent consumption, equipment cost, etc. [10,11].

Microwave digestion offers many advantages over conventional digestion procedures used for food analysis. Microwave

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extraction using diluted acids in a closed high-pressure polytetrafluoroethylene (PTFE) lined vessel at temperatures above the boiling point of nitric acid is a simple alternative to sample preparation and its use is increasing. Diluted mineral acid solutions can more intensely absorb microwave energy, owing to their water content. These features reduce acid consumption, contamination, and preparation time. Microwave digestion is usually complete within 1 h. Microwave digestion programs can be operated unattended and are easily transferred to other laboratories. The microwave digestion was preferred because this procedure is more proper with respect to both time and recovery than wet digestion. However, the mass of the analytical portion must be carefully selected to prevent excessive pressure during the digestion. Each food matrix may require a different microwave program due to their properties. Microwave digestion procedures have been developed as a rapid and reproducible sample preparation method for a great variety of complex matrices.

Procedures for optimization of factors by multivariate techniques have been encouraged, as they are faster, more economical and effective, and allow more than one variable to be optimized simultaneously. Chemometric techniques have been used for optimization of analytical methods. These techniques allow the simultaneous study of several control variables, which are faster to implement and more cost-effective than traditional univariate approaches [14–16]. Among these techniques, the most popular is the two-level full factorial, in which every factor is experimentally studied at only two levels. These design structures are based on full two-level factorial design by center point replication and inclusion of an axial portion. Several digestion procedures for natural samples have been optimized using this chemometric tool [17–21].

The goal of the present study is to optimize a microwave-assisted digestion method for the extraction of copper, nickel and zinc from tea samples by means of a multivariate approach (i.e., experimental design). Analytical solutions were analyzed by flame atomic absorption spectrometry (FAAS). Accuracy was demonstrated with analysis of a standard reference material.

## 2. Experimental

### 2.1. Instrumentation and apparatus

A Perkin-Elmer Analyst 700 atomic absorption spectrometer with deuterium background corrector was used in this study. The operating parameters for working elements were set as recommended by the manufacturer. The elements were determined by using air–acetylene flame.

Table 2

Factors and levels used in the factorial design for microwave digestion

Variable	Low (–)	Central point	High (+)
Composition of the acid mixture (CMA)	HNO <sub>3</sub> :H <sub>2</sub> O <sub>2</sub> :H <sub>2</sub> O (3 mL:1 mL:4 mL)	HNO <sub>3</sub> :H <sub>2</sub> O <sub>2</sub> :H <sub>2</sub> O (4 mL:2 mL:2 mL)	HNO <sub>3</sub> :H <sub>2</sub> O <sub>2</sub> (6 mL:2 mL)
Microwave power, W (MP)	400	450	550
Microwave time, min (MT)	4	6	8

Amount of sample, 0.2 g.

Volume of mixture, 8.0 mL.

Table 1

Heating program for the digestion procedure

Step	Time (min)	Power (W)
1	2	250
2	2	0
3	6	250
4	5	400
5	8	550
Ventilation	5	0

The microwave digestions were carried out in an Ethos D (Milestone, Sorisole, Italy) with maximum pressure 1450 psi and maximum temperature 300 °C. Heating program for the digestion procedure is given in Table 1.

### 2.2. Standard reference materials and reagents

All reagents used the presented work were of analytical reagent grade unless otherwise stated. Double deionised water (Milli-Q Millipore 18.2 MΩ cm<sup>-1</sup> conductivity) was used for all dilutions. HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> were of suprapure quality (E. Merck). All the plastic and glassware were cleaned by soaking in dilute HNO<sub>3</sub> (1 + 9) and were rinsed with distilled water prior to use. The standard solutions of analytes used for calibration were produced by diluting a stock solution of 1000 mg l<sup>-1</sup> of the given element supplied by Sigma and Aldrich, USA.

The calibration curves for analyte ions were drawn after setting various parameters of FAAS including wavelength, slit width, lamp current at an optimum level. The precision of the method was investigated by using the model solutions containing the spiked elements on the optimal conditions of the method (Table 1).

The accuracy of the analytical technique was checked by analyzing GBW 07605 Tea certified reference material from National Research Centre for Certified Reference Materials, China.

### 2.3. Sampling

Tea samples were purchased from supermarkets from Kayseri and Tokat-Turkey during 2005.

### 2.4. Microwave digestion

After the optimization of the digestion conditions, the selected factors (the optimum conditions) were applied to an oven-dried tea samples. About 0.2 gram was digested with 6 ml

Table 3  
Results of the factorial design

Experiment	CMA	MP	MT	Zinc recovered	Copper recovered	Nickel recovered	Average recuperation
1	-1	-1	-1	4	35	31	35
2	1	-1	-1	75	75	71	73
3	-1	1	-1	47	46	43	45
4	1	1	-1	91	86	95	91
5	-1	-1	1	44	44	44	44
6	1	-1	1	79	76	76	77
7	-1	1	1	51	55	53	53
8	1	1	1	99	98	99	99
9(C)	0	0	0	62	56	65	61
10(C)	0	0	0	65	56	66	62
11(C)	0	0	0	64	57	67	62

of concentrated HNO<sub>3</sub> (Suprapure, Merck) and 2 ml of concentrated H<sub>2</sub>O<sub>2</sub> (Suprapure, Merck) in microwave digestion system. A blank digest was carried out in the same way (digestion conditions for microwave system are given in Table 2).

### 3. Results and discussion

#### 3.1. Determination of the experimental conditions for microwave digestion

The optimization of the experimental conditions of digestion procedure was performed using a full two-level factorial (2<sup>3</sup>) design with three replicates of central point, involving the factors: composition of the acid mixture (CMA), microwave power (MP) and radiation time (RT). The levels and experimental domain of each factor are summarized in Table 3. The experiments executed for this design and their results are presented in Table 4.

All the experiments were performed using a certified reference material of tea sample and the responses used for evaluation of factorial design were: zinc recovery, copper recovery and nickel recovery. It can be seen in Table 5 that the recoveries for all the three metals are always maximum in the conditions of the experiments 4 and 8. Considering it, the average recovery was calculated and later it was used for evaluation of the factorial design. The results of this multivariate technique presented by

Table 4  
Analysis of variance

	SS	df	MS	F	P
Curvatr.	15.612	1	15.61	19.76	0.0470
(1) CMA	3288.605	1	3288.60	4162.79	0.0002
(2) MP	420.500	1	420.500	532.28	0.0019
(3) MT	98.000	1	98.000	124.05	0.0080
1 by 2	50.000	1	50.000	63.29	0.0154
1 by 3	2.880	1	2.880	3.646	0.1964
2 by 3	1.445	1	1.445	1.829	0.3088
Lack of Fit	3.645	1	3.645	4.614	0.1648
Pure error	1.580	2	0.790		
Total SS	3882.267	10			

SS, sum of squares; DF, degrees of freedom; MS, mean squares; P level, probability level.

Pareto chart (as Fig. 1) demonstrated that all the three factors: CMA, MP and RT and the interaction CMA X MP are significant at the levels studied.

The Pareto chart shows also that the factors CMA and MP have effects positive and higher than the factor RT. This explains why the results of the experiments 4 and 8 have maximum recovery for all metals. The analysis of variance (ANOVA) for the results of the factorial design, as Table 4 shows also the significance of the factors CMA, MP and RT and of the interaction CMA X MP. In this table can be seen also that the linear model

Table 5  
Addition-recovery test for a black tea sample from Black Sea (N=4)

Element	Added (μg/g)	Found (μg/g)	Recovery (%)
Cu	–	9.5 ± 0.5	–
	5.0	14.4 ± 0.9	97
	10.0	19.2 ± 1.1	97
	20.0	29.1 ± 1.7	98
Zn	–	8.3 ± 0.7	–
	5.0	13.1 ± 1.0	97
	10.0	17.9 ± 1.1	97
	20.0	27.8 ± 1.9	98
Ni	–	4.2 ± 0.2	–
	2.0	6.1 ± 0.4	96
	4.0	8.0 ± 0.6	96
	8.0	12.0 ± 0.9	97

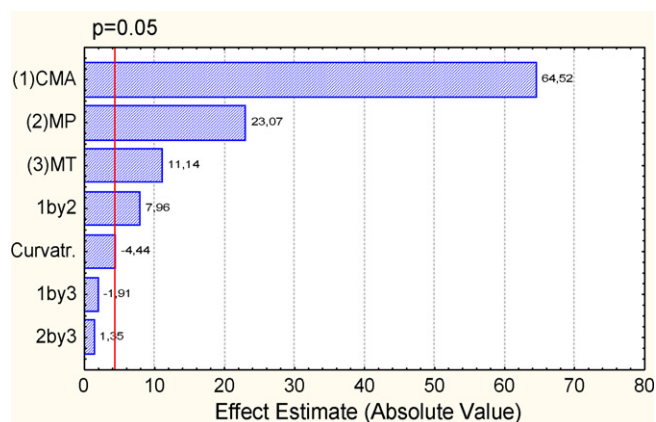


Fig. 1. Pareto chart.

established by three factors and the response (average recovery) does not suffer from lack of fit. Considering all these observations, the conditions chosen for the digestion procedure coincide with the established conditions by experiment 8, which has all the factors at maximum level. This way, the digestion procedure recommends a composition of acid mixture of 6 ml of concentrated HNO<sub>3</sub> and 2 ml of concentrated H<sub>2</sub>O<sub>2</sub>, microwave power of 550 W and the radiation time of 8 min.

### 3.2. Analytical features

The accuracy of the microwave digestion procedure for tea sample was confirmed by analysis of the GBW 07605 tea reference standard material. The achieved results were 16.9 ± 0.8, 26.0 ± 1.3 and 4.5 ± 0.3 (microgram per gram), for copper, zinc and nickel, respectively. The certified values are 17.3 ± 1.0 μg g<sup>-1</sup> for copper, 26.3 ± 0.8 μg g<sup>-1</sup> for zinc, and 4.6 ± 0.3 μg g<sup>-1</sup> for nickel. This results show that the validation of the presented procedure.

The precision was also evaluated. Portions of 0.2 g of a black tea sample containing copper, zinc and nickel with a concentration of 9.5, 8.3 and 4.2 (microgram per gram), respectively were digested and these metals were determinate. The precision expressed as relative standard deviations were 6.1 for copper, 7.8 for zinc and 6.0 for nickel (%) (*N* = 7).

Tests of addition/recovery were performed on a tea sample from Black Sea region of Turkey. The procedure was applied to this sample with addition of different amounts of each analyte ions. The results are listed in Table 5. It can be seen that the recoveries obtained for analytes are sufficient (96–98%) and the performance of the method is very good for determination of these metals in tea samples.

### 3.3. Application

The microwave digestion procedure proposed was applied for determination of copper, zinc and nickel in several tea samples from Turkey. The results are shown in Table 6. The concentration achieved for copper varied at 6.4–13.1 μg g<sup>-1</sup>. The copper level in tea samples from Turkey and United Kingdom were reported as 16.5 μg g<sup>-1</sup> [9] and 21.0 μg g<sup>-1</sup> [22], respectively. The range for zinc in the tea samples was found at 7.0–16.5 μg g<sup>-1</sup>. Marcos

et al. [24] have been reported that mean zinc concentrations in tea samples as 28.2 μg g<sup>-1</sup>. The concentration achieved for nickel varied at 3.1–5.7 μg g<sup>-1</sup>. The mean concentration of nickel was 23.3 μg g<sup>-1</sup> by Colak et al. [9]. Marcos et al. in 1998 have reported the range of the nickel concentration in the tea samples as 2.89–22.6 μg g<sup>-1</sup> [24]. The ranges for the analytes for present study are agreed with the results given for tea samples [9,22–24]. The levels of the investigated metal ions studied are compared well with levels in tea samples from other parts of the world. The study will be valuable in the appraisal of trace elements in black tea and be helpful for people who like drinking tea. The results obtained for investigated analytes were acceptable to human consumption at nutritional and toxic levels.

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Table 6  
Determination of copper, zinc and nickel in the tea samples from Turkey (μg/g, *N* = 3)

Samples	Copper	Zinc	Nickel
Bud Black tea	13.10 ± 0.80	9.26 ± 0.73	4.27 ± 0.26
Black tea (Brand A)	12.10 ± 0.50	7.01 ± 0.30	5.70 ± 0.38
Black tea (Brand B)	9.12 ± 0.45	8.31 ± 0.55	3.81 ± 0.23
Black tea (Brand C)	7.24 ± 0.38	10.50 ± 0.71	4.10 ± 0.32
Ceylon tea	10.80 ± 0.70	16.50 ± 1.40	3.52 ± 0.21
Mulberry tea	12.40 ± 1.11	11.50 ± 0.91	3.31 ± 0.18
Blackberry tea	8.73 ± 0.52	13.40 ± 1.21	3.10 ± 0.26
Green tea (Brand A)	6.39 ± 0.26	8.52 ± 0.55	4.52 ± 0.37
Green tea (Brand B)	9.84 ± 0.67	14.80 ± 1.31	3.93 ± 0.25
Herbal tea	12.61 ± 1.20	8.83 ± 0.74	3.18 ± 0.20

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