

# Direct Determination of Lead, Cadmium, Zinc, and Copper in Honey by Electrothermal Atomic Absorption Spectrometry using Hydrogen Peroxide as a Matrix Modifier

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Electrothermal atomic absorption spectrometric procedures for the rapid determination of copper, zinc, lead, and cadmium in honey are proposed. The samples are dissolved in a medium containing 10% v/v concentrated hydrogen peroxide and 1% v/v concentrated nitric acid and directly introduced in the electrothermal atomizer. In this way, even with no previous mineralization of the samples, organic matter is efficiently destroyed during the heating cycle, thus avoiding the buildup of carbonaceous residues inside the atomizer and permitting low background signals to be obtained. Calibration is carried out using aqueous standards. The metal contents of honey samples of different origin obtained by using the proposed procedures agree with those obtained by means of a lengthy conventional procedure based on a previous mineralization stage.

**Keywords:** *Lead; cadmium; zinc; copper; AAS; ETAAS; honey*

## INTRODUCTION

Honey is a liquid (or semiliquid) product made up of about 80% solids. It contains a complex mixture of carbohydrates, namely, fructose (25–45%), glucose (25–37%), maltose (2–12%), and sucrose (0.5–3%) with traces of many other sugars depending on the floral source (Clarke, 1995). The product is used worldwide as a basic foodstuff, either by direct ingestion or as a sweetener in a variety of foodstuffs. The mean content of mineral substances in honey has been calculated to be 0.17% although this can vary within a wide range (White, 1979). Some of the components of this mineral fraction can be determined by using flame atomic absorption spectrometry (Petrovic et al., 1994; Rodríguez-Otero et al., 1992; Salinas et al., 1994; Sauri-Duch and Hernández-Chávez, 1994) but others, including toxic metals, require a more sensitive analytical technique. Electrothermal atomic absorption spectrometry (ETAAS) is a suitable technique for such purposes and the literature contains several reports to this end (Bloc and Walter, 1989; Stein and Umland, 1986; Voget and Baudisch, 1983). However, conventional ETAAS procedures are not without their problems because if a previous time-consuming mineralization stage is carried out, there are risks of contamination or analyte loss as a result of prolonged sample manipulation. If a mineralization stage is not carried out, problems appear as a result of the high amount of organic compounds introduced into the atomizer.

To overcome these difficulties and to obtain reliable data rapidly with no previous mineralization stage, advantage may be taken of recent advances in ETAAS methodology. For example, a suitable way might be to dissolve honey samples directly in water and then apply a heating program in which an air-ashing stage is included. Such a technique has proved suitable for destroying organic matter when dealing with slurries prepared from samples of a high organic matter content (Ebdon et al., 1990). However, one drawback of such a procedure is that the useful life of expensive graphite

atomizers will almost inevitably be shortened. An alternative way to destroy the organic matter might be to include in the solution an oxidant agent whose decomposition during the heating cycle would provide an oxidizing environment. Research along this line has been carried out in our laboratory and the technique has proved successful when dealing with slurries prepared from foods and biological samples (López-García et al., 1996; Viñas et al., 1994a,b, 1995a,b). No damage to the graphite atomizers has been noted in such cases.

This paper discusses procedures for determining lead, cadmium, copper, and zinc in honey samples of different origins using ETAAS with no previous mineralization stage. The addition of hydrogen peroxide to the solution of honey in water considerably decreases the background value and the buildup of carbonaceous residues inside the atomizer. Because sample manipulation is minimal, contamination problems are decreased and reliable data can be obtained more rapidly than with the more conventional method based on a previous mineralization step.

## EXPERIMENTAL PROCEDURES

**Instrumentation.** A Perkin-Elmer Model 1100B atomic absorption spectrometer equipped with deuterium-arc background correction and an HGA-400 electrothermal atomizer were used. Measurements were performed using hollow cathode lamps operated at 283.3 nm, with a current of 10 mA and a bandwidth of 0.7 nm for lead; 228.8 nm, 10 mA, and 0.7 nm for cadmium; 213.9 nm, 10 mA, and 0.7 nm for zinc; and 324.8 nm, 15 mA, and 0.7 nm for copper. Argon was used as the inert gas at 300 mL/min except in the atomization step, during which the flow was stopped. A miniflow of 200 mL/min was maintained during zinc atomization to decrease sensitivity. Wall atomization with pyrolytic graphite-coated graphite tubes (Perkin-Elmer, ref B013-5653) was used for Cu. Platform atomization with pyrolytic graphite platforms (Perkin-Elmer, ref B012-1092) was used for Zn, Pb, and Cd. Background-corrected integrated absorbance was used as the analytical signal. A Branson 4 ultrasonic bath with a constant power of 14 W was also used.

**Reagents.** High-quality water obtained using a Milli-Q system (Millipore) was used throughout. Preliminary experiments for cadmium and zinc determinations showed severe contamination problems arising from micropipet tips and the

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**Table 1. Furnace Programs<sup>a</sup>**

step	parameter	element			
		lead	cadmium	zinc	copper
drying	temp, °C	400	400	400	200
	ramp, s	10	10	10	5
	hold, s	20	20	20	20
calcination	temp, °C	1200	650	1100	1100
	ramp, s	5	1	1	1
	hold, s	20	25	20	10
atomization	temp, °C	1800	1600	1700	2100
	ramp, s	0	0	0	0
	hold, s	3 <sup>b</sup>	4 <sup>b</sup>	3 <sup>c</sup>	3 <sup>b</sup>
cleaning	temp, °C	2650	2650	2650	2650
	ramp, s	1	1	1	1
	hold, s	3	3	3	3

<sup>a</sup> All temperatures and times quoted are values set on the HGA-400 power supply. <sup>b</sup> The internal gas flow was stopped. <sup>c</sup> The internal gas flow was reduced to 200 mL/min.

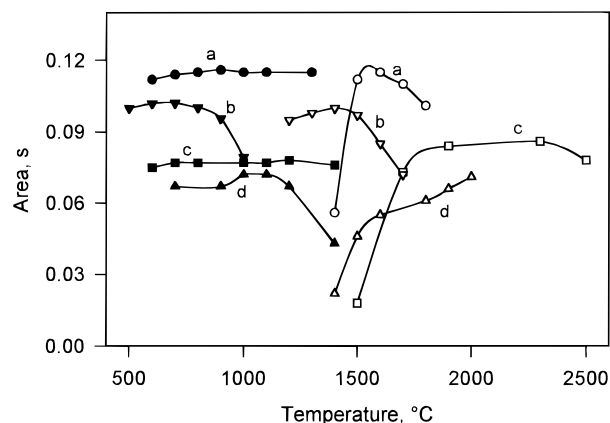
vessels used to store solutions. Consequently, all glassware, plasticware, and storage bottles used were previously immersed for several hours in a solution containing 10% v/v concentrated hydrochloric acid and 20% v/v concentrated nitric acid. Stock standard solutions of lead, cadmium, zinc, and copper (1000 µg/mL) were obtained from Panreac (Spain) and Fisher Scientific and diluted as necessary to obtain working standards. Ammonium dihydrogenphosphate, concentrated hydrochloric acid (32% w/v), and concentrated hydrogen peroxide (33% w/v) were obtained from Fluka. Concentrated nitric acid (65% w/v) and other chemicals used were obtained from Merck.

**Procedures.** The honey samples were purchased from a supermarket and maintained in tightly closed vessels in the absence of light. To decrease viscosity and obtain homogeneous samples, the honeys were lightly heated in a water bath before the solutions were prepared. Solutions were prepared by weighing amounts of honey of 1.0–1.5 g for Cu, Cd, and Pb and 0.1–0.2 g for Zn in plastic vessels and then adding 250 µL of nitric acid, 2.5 mL of concentrated hydrogen peroxide (warning: suitable precautions must be adopted to avoid burns when working with this chemical), and 25 mg of ammonium dihydrogenphosphate for Cd, Pb, and Zn, before making up to a final volume of 25 mL with water. The solutions were sonicated for 5 min and then, while being magnetically stirred, 20-µL aliquots were taken and injected into the furnace. The optimized heating programs are given in Table 1. Calibration was performed with aqueous standards to which 1% v/v concentrated nitric acid, 50% v/v concentrated hydrogen peroxide, and 25 mg of dihydrogenphosphate for Cd, Pb, and Zn were also added, making up to a final volume of 25 mL with water. The standards used for cadmium determination also contained 2% w/v fructose. Background-corrected peak area was used as the analytical signal.

The samples were previously analyzed for comparison purposes. Portions of 1 g were heated at 450 °C for 16 h in platinum crucibles. The ashes were treated with 4 mL of concentrated nitric acid, heated to dryness, and then again treated with 2 mL of nitric acid and 1 mL of concentrated perchloric acid, before being heated to near dryness. Finally, the resulting solution was diluted to 10 mL with water. Aliquots of 20 µL were injected directly into the furnace. For the measurement of zinc, a 10-times dilution was necessary before the sample was injected.

## RESULTS AND DISCUSSION

As expected, the preliminary experiments confirmed that the atomization of solutions obtained by directly dissolving honey samples in water rapidly led to the accumulation of carbonaceous residues inside the atomizer and high background levels. The addition of hydrogen peroxide to the solutions considerably alleviated both problems. No deleterious effects on the atomization profiles or the peak areas due to the



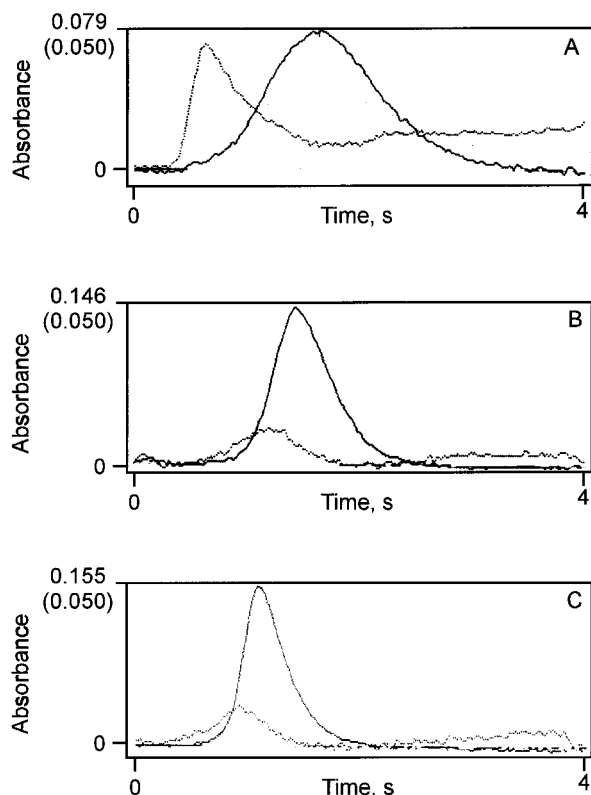
**Figure 1.** Optimization of the calcination (filled symbols) and atomization (hollow symbols) temperatures. A solution containing 0.5% w/v honey was used for zinc (curve a). Solutions containing 4% w/v honey were used for cadmium, copper, and lead (curves b, c, and d, respectively). Standard solutions of cadmium were added to increase the signal.

analytes were observed even when 50% v/v concentrated hydrogen peroxide was included in the solutions. However, since no advantages were noted when more than 10% v/v of the chemical was present, this percentage was routinely used throughout the work. An additional decrease in background was observed when nitric acid was also incorporated in the solutions. The amount of acid to be used was studied in the 0.1–2% v/v concentrated nitric acid range and 1% v/v was found to be suitable. The joint use of nitric acid and hydrogen peroxide in the dissolving medium kept background levels low, allowing reliable correction by the deuterium device. Manual cleaning of the atomizer was only required occasionally.

Dihydrogenphosphate was used as a chemical modifier for lead, cadmium and zinc determination (Tsalev et al., 1990). It was found that by incorporating 0.1% w/v of this chemical in the solution, premature losses of these analytes prior to atomization were avoided. In addition, peak profiles were delayed meaning that atomization took place in a more reliable isothermal environment.

**Optimization of the Heating Program.** Platform atomization was used for lead, cadmium, and zinc. Since the lead and, particularly, cadmium contents of the samples studied were low, the heating programs were optimized by using solutions containing 4% w/v honey. In the case of cadmium, the signals were very low and 1 ng/mL of a standard solution was also incorporated in the solutions being atomized so that signals suitable for optimization could be obtained. Solutions containing 0.5% w/v honey were used to optimize the heating program used for zinc. Because of the high sugar content and the presence of hydrogen peroxide, the drying stage involved the risk of sample loss due to sputtering; this step was optimized by carefully watching not only for external signs of sputtering but also the inside of the atomizer with the aid of a small mirror. The best results were obtained by programming a 400 °C drying temperature on the HGA-400 power supply and using 10 and 20 s for the ramp and holding times, respectively.

Variations of the analytical signals for the analytes used when both the calcination and the atomization temperatures were modified are shown in Figure 1. Lead and zinc peak areas were constant for calcination temperatures up to 1200 and 1300 °C, respectively. The



**Figure 2.** Atomization profiles of cadmium. (A) and (B) a standard solution containing 1 ng/mL cadmium in the absence and presence of 2% w/v fructose, respectively. (C) A solution containing 4% w/v honey plus 1 ng/mL cadmium.

atomization temperatures leading to maximum signals and well-defined atomization profiles were 1800 °C for lead and 1700 °C for zinc. For aqueous cadmium, the analytical signal was constant for calcination temperatures up to 1000 °C. Conversely, premature volatilization of cadmium was observed when the solutions containing honey were atomized, the signal decreasing above 800 °C. A calcination temperature of 650 °C held for 25 s was selected. The atomization temperature leading to the maximum signal for cadmium was 1600 °C. On the other hand, the cadmium atomization profiles obtained from solutions containing honey were notably narrower than those obtained for aqueous standards. This suggests a matrix effect, which might invalidate the direct and simplest calibration using aqueous standards. The inclusion of a cooling stage in the heating program, after calcination and before atomization, did not solve this problem, which was solved by simply adding fructose to the cadmium aqueous standards. As can be seen in Figure 2, in which the atomization profiles are shown, the addition of this carbohydrate to the aqueous standards produced a very similar profile to that shown by the solution containing honey. The incorporation of 2% w/v fructose was sufficient for this purpose.

Preliminary experiments for the optimization of the heating program for determining copper showed nearly identical results when wall atomization was used instead of platform atomization, and so, to reduce costs, wall atomization was used for this element. The heating program was optimized by using a solution containing 4% w/v honey. The optimal conditions for the drying stage were obtained by programming 200 °C as the drying temperature and using 5 and 20 s as the ramp and hold times, respectively. Variations of the copper signals with different calcination and atomization tem-

**Table 2. Calibration Parameters**

parameter	element			
	lead	cadmium	copper	zinc
slope (mL/ng)	0.006	0.15	0.013	0.007
intercept	0.004	0.005	0.006	0.026
regression coefficient	0.9998	0.9994	0.9998	0.9999
detection limit (ng/mL)	1.4	0.05	0.6	3.7 <sup>a</sup>
quantitation limit (ng/mL)	3.4	0.09	1.1	5.7 <sup>a</sup>

<sup>a</sup> The detection and quantitation limits and the slope for zinc considerably improve if the miniflow condition is avoided.

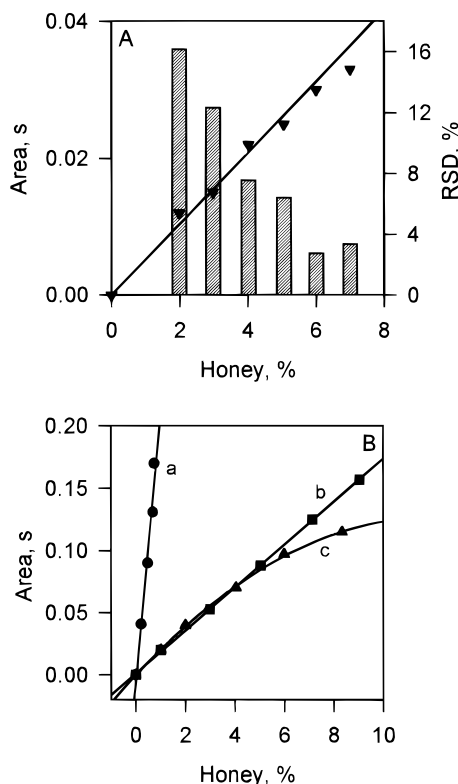
peratures are also shown in Figure 1 and values of 1100 and 2100 °C were selected, respectively. A cleaning step at 2650 °C was also included. Table 1 summarizes the optimized programs.

An additional problem was found in regard to zinc determination. Because of the high sensitivity of ETAAS and the relatively high level of the analyte in the samples, very high signals, exceeding the linear response range, were found. To decrease sensitivity, a miniflow of 200 mL/min purge gas was maintained during atomization. To check that this did not significantly affect reproducibility, 10 successive injections of a solution containing 0.5% w/v honey were carried out. The relative standard deviation (RSD) obtained was  $\pm 3.2\%$ . This way of reducing sensitivity is more suitable than the use of solutions containing very low amounts of honey, because the latter increases the risk of unreliable results due to sample heterogeneity.

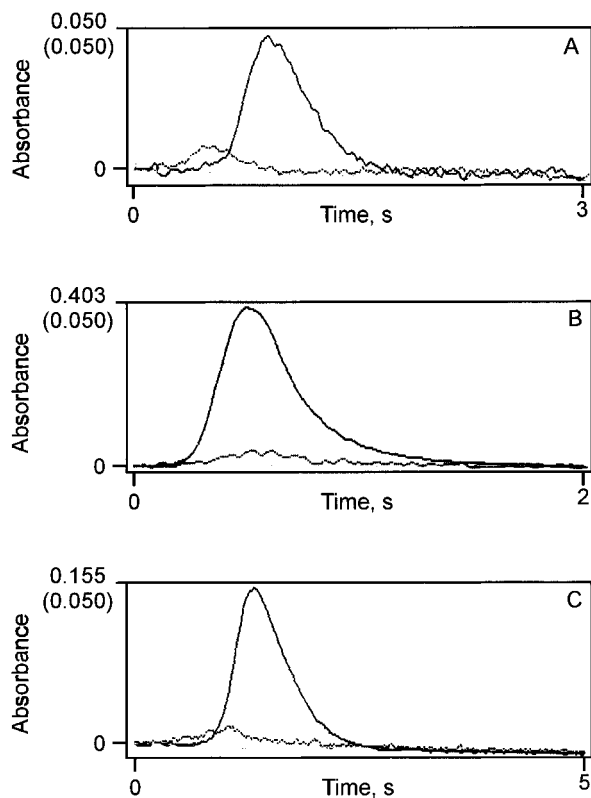
**Calibration and Results.** *Influence of the Honey Percentage.* Calibration graphs were produced by plotting concentration against the background-corrected peak area and were linear over the range 0–25 ng/mL for Pb and Zn, 0–20 ng/mL for Cu, and 0–1 ng/mL for Cd. Table 2 shows the equations obtained for the calibration graphs and the regression coefficients. The detection limits were calculated on the basis of  $3\sigma$  and the quantitation limits on the basis of  $10\sigma$ . The precision of the procedure was studied using solutions prepared with honey percentages in the 1–7% w/v range. Figure 3A shows the linear concentration range for cadmium. The bar graph shows the relative standard deviations for 10 determinations of each solution. The RSD values were below  $\pm 5\%$ , except in the case of very diluted solutions giving peak areas below 0.02 s. The optimal percentages were in the 3–6% range. When solutions more concentrated than 5% w/v honey were used, residues remained in the tube despite the presence of hydrogen peroxide. Similar results were obtained for lead and copper (Figure 3B). For routine purposes a 4% w/v solution is recommended. For zinc, linearity was obtained between 0.2 and 0.8% w/v honey, a 0.5% w/v solution being recommended.

Figure 4 shows the absorbance–time profiles (solid lines) for lead, copper, and zinc obtained in the honey analysis. These profiles were similar to those found for aqueous standards. The background signals were low (broken lines). To check that calibration using aqueous standards was valid, several samples containing different amounts of honey were prepared and standard additions graphs obtained. Each graph was constructed with five points and each point was measured three times. The results indicate that the slopes were very similar (Table 3), confirming the absence of a matrix effect and validating the direct calibration with aqueous standards.

The reliability of the proposed procedure was checked by analyzing the metal contents in several honey



**Figure 3.** (A) Influence of the honey percentage on the signal of cadmium. The bar graph indicates the RSD values. (B) Influence of the honey percentage on the signal of zinc, copper, and lead (curves a, b, and c, respectively).



**Figure 4.** Atomization profiles of lead, copper, and zinc obtained from solutions containing 4, 4, and 0.4% w/v honey, respectively.

samples of different type and origin. Table 4 shows the results obtained for lead, zinc, and copper contents using the method proposed and the values found using the conventional method based on calcination and acid

**Table 3. Slopes of Standard Additions Calibration Graphs**

element	sample	slope (mL/ng)
copper	aqueous standards	0.013
	lemon honey (4%)	0.012
	flowers honey (6%)	0.011
lead	aqueous standards	0.006
	wood honey (4%)	0.005
	zinc	0.007 <sup>a</sup>
zinc	aqueous standards	0.006 <sup>a</sup>
	propolis honey (0.3%)	0.006 <sup>a</sup>
	propolis honey (0.5%)	0.006 <sup>a</sup>
cadmium	aqueous standards	0.152
	lemon honey (4%)	0.151

<sup>a</sup> The low slopes for zinc are due to the miniflow condition.

**Table 4. Results for the Analysis of Honey Samples<sup>a,b</sup>**

honey <sup>c</sup>	lead ( $\mu\text{g/g}$ )	zinc ( $\mu\text{g/g}$ )	copper ( $\mu\text{g/g}$ )	cadmium (ng/g)
lemon	$0.27 \pm 0.02$ ( $0.26 \pm 0.02$ )	$1.28 \pm 0.06$ ( $1.25 \pm 0.03$ )	$0.09 \pm 0.01$ ( $0.08 \pm 0.01$ )	$1.2 \pm 0.1$
lemon	$0.03 \pm 0.01$ ( $0.02 \pm 0.01$ )	$1.55 \pm 0.09$ ( $1.53 \pm 0.02$ )	$0.06 \pm 0.01$ ( $0.06 \pm 0.01$ )	not detected
flowers	$0.07 \pm 0.01$ ( $0.07 \pm 0.01$ )	$2.52 \pm 0.05$ ( $2.47 \pm 0.10$ )	$0.16 \pm 0.01$ ( $0.17 \pm 0.01$ )	$1.6 \pm 0.1$
propolis	$0.04 \pm 0.01$ ( $0.04 \pm 0.01$ )	$3.20 \pm 0.21$ ( $3.15 \pm 0.15$ )	$0.27 \pm 0.01$ ( $0.25 \pm 0.01$ )	$1.8 \pm 0.05$
wood	not detected (not detected)	$1.99 \pm 0.10$ ( $1.98 \pm 0.13$ )	$1.32 \pm 0.03$ ( $1.25 \pm 0.03$ )	$1.2 \pm 0.1$
eucalyptus	$0.07 \pm 0.01$ ( $0.08 \pm 0.01$ )	$1.59 \pm 0.06$ ( $1.60 \pm 0.07$ )	$0.25 \pm 0.02$ ( $0.23 \pm 0.01$ )	$5.4 \pm 0.3$

<sup>a</sup> Mean  $\pm$  SD ( $n = 9$ ). <sup>b</sup> Values in brackets are those obtained by using a previous mineralization stage. <sup>c</sup> Indication given in the label of the commercial product.

dissolution of the samples. Good concordance was obtained in the values found using both procedures. On the other hand, very low contents ranging from 1.2 to 5.4 ng/g were obtained for cadmium (Table 4), while no reproducible results suitable for comparison purposes were found by using the mineralization procedure because of contamination problems leading to very high RSD values. This confirms one of the advantages of the methodology discussed here. The importance of the elimination of the perchloric acid digestion on both the sample preparation time and the safety of the operating conditions should also be noted.

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