Methods for the Determination of Arsenic, Cadmium, Copper, Lead, and Tin in Sucrose, Corn Syrups, and High-Fructose Corn Syrups by Inductively Coupled Plasma Atomic Emission Spectrometry

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This paper demonstrates the determination of arsenic, cadmium, copper, lead, and tin in sucrose, corn syrups, and high-fructose corn syrups by inductively coupled plasma atomic emission spectrometry (ICP-AES). Sample digestion by both open vessel/hot plate and closed vessel/microwave techniques is reported. Open vessel digestion was suitable for the simultaneous determination of cadmium, copper, and lead. Average recoveries (five different sample types) for the open vessel procedure were 98%, 88%, and 93% for cadmium, copper, and lead, respectively, at 0.10 μg/g. In contrast, microwave digestion yielded average recoveries (five different sample types) of 92%, 83%, 89%, 85%, and 88%, respectively, for arsenic, cadmium, copper, lead, and tin at 1.0 μg/g. Method detection limits were lower with open vessel digestion versus microwave digestion because of sample volume reduction. Sample introduction included microporous membrane desolvation, which assisted digestion and matrix normalization.

Keywords: *Trace metal analysis; sweeteners; plasma atomic emission spectrometry*

INTRODUCTION

High-fructose corn syrup (HFCS), corn syrup, and sucrose are classified as "generally recognized as safe" (GRAS) food ingredients (*Federal Register*, 1994). Annual consumption is in excess of 100 million pounds for each, which places them among the top 25 used food ingredients. Because of this high usage, concern exists about the contribution of these ingredients to the total dietary intake of trace metals that may be present as contaminants. Specific attention has focused on lead because of increasing knowledge of adverse health effects from lead at decreasing levels of exposure (Davis et al., 1993). However, these ingredients also serve as potential sources of exposure to other metals, such as arsenic, cadmium, copper, and tin, which occur at various levels in the environment (Berman, 1980; Kubota et al., 1992; Lee, 1972).

Although numerous methods are available for the determination of metals in foods, most procedures involve flame atomic absorption spectroscopy (FAAS), electrothermal/graphite furnace atomic absorption spectroscopy (ETV-AAS or GFAAS), inductively coupled plasma atomic emission spectroscopy (ICP-AES), or neutron activation analysis (Chang et al., 1995). For the determination of trace metals in sweeteners, efforts have been limited to either GFAAS (Miller-Ihli, 1994; Miller-Ihli and Greene, 1993) or electroanalytical techniques (Khoulif et al., 1993; Hissong, 1992). The greatest progress with respect to collaborative method evaluation has occurred only with GFAAS (Miller-Ihli, 1995).

GFAAS procedures have focused on the determination of lead in sucrose and HFCS (Miller-Ihli, 1994; Miller-Ihli and Greene, 1993). Method detection limits of 0.9 and 3.3 ng/g have been demonstrated. With the first method, samples were directly decomposed in the furnace ashing step, whereas a partial off-line digestion was employed with the second method. As a consequence of the off-line digestion, a second sample dilution was required, resulting in the higher detection limit. With either procedure, concentrations were determined by comparison to external standards prepared in dilute acid. Analysis precision for spiked samples was slightly better with the off-line digestion procedure, but overall the percent relative standard deviation was <10%. Method performance for other metals was not provided.

Lead and cadmium levels of 20 and 3.5 ng/g, respectively, have been measured in beet syrups by anodic stripping voltammetry (Khoulif et al., 1993). Quantitation was based on the method of standard addition, and the measurement precision was reported as 15%. Attempts to extend the method to the determination of copper failed because of organic impurities in the syrup sample. With refined sugar, a cleaner sample matrix, the method was applicable to the determination of cadmium, copper, lead, and zinc. Lead levels of 1 ng/g in HFCS have been determined by Osteryoung square wave stripping voltammetry (Hissong, 1992). Quantitation required the method of standard addition, but the relative standard deviation was <5%. Extension of this method to other metals was not reported.

Reports concerning the determination of arsenic and tin in sweeteners are rare; however, general methods for foods have been offered (Capar, 1990; Cervera and Montoro, 1994; Martin et al., 1994). For arsenic, recommended procedures involve digestion with nitric acid followed by hydride generation with atomic absorption spectrophotometry detection (Capar, 1990; Cervera and Montoro, 1994). With the hydride generation step, detection limits are reduced from the micrograms per gram range to subnanograms per gram levels (Ingle, 1988). For the determination of tin, it is recommended that samples be digested with nitric acid and hydrochloric acid followed by treatment with potassium chloride prior to analysis by FAAS (Capar, 1990; Martin

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et al., 1994). With this approach, detection limits in the low micrograms per gram range are possible (Ingle, 1988).

In this paper, we present methods based on open (hot plate) and closed vessel digestion for the determination of arsenic, cadmium, copper, lead, and tin in sucrose, corn syrup, and HFCS with ICP-AES detection. The general objective was to develop methods for 1.0 g sample sizes and to then determine method detection limits. Ultrasonic (USN) sample introduction was utilized instead of pneumatic sample introduction to provide lower instrument detection limits (Conver and Koropchak, 1995). Microporous membrane desolvation (MMD) was also used to assist digestion/matrix normalization of organic samples (Allen et al., 1996). This paper concludes with spike and recovery experiments for sucrose and two different types of corn syrups, carbon-filtered and ion-exchanged, and two different compositions of HFCS, one at 40+% fructose and one at 50+% fructose.

EXPERIMENTAL PROCEDURES

Instrumentation. The initial plasma system used was a Thermo Jarrell Ash 61E (Franklin, MA). During the course of this work, the ICP-AES system was upgraded to a Polyscan 61E which simply added a sequential monochromator to the existing optical system (polychromator), extending the analyte line selection capabilities. The analyte lines used with the polychromator were 193.7 nm for As(I), 228.8 nm for Cd(I), 324.7 nm for Cu(I), 220.3 nm for Pb(II), and 189.9 nm for Sn(II). With the polychromator system, Cd was not analyzed if the sample had known levels of As to avoid spectral overlap with the As(I) line at 228.812 nm. After the upgrade, Cd was analyzed with the sequential monochromator at the 226.5 nm (II) line, while the other metals were simultaneously determined with the polychromator. Because sensitivity differences between the two cadmium lines were not significant, efforts to detail which line was used have been minimized. Reports involving "absolute" simultaneous determinations, all metals with the same sample, will be indicated. Otherwise, samples were segmented with respect to arsenic and cadmium.

The plasma, auxiliary and carrier gas flow rates used were 14, 1.0, and 0.9 L/min, respectively. The applied power was 1.15 kW, and the plasma zone monitored was 15 mm above the upper load coil. Sample introduction to the ICP-AES instrument was accomplished by a CETAC 5000 AT ultrasonic (USN) nebulizer (Omaha, NE). The liquid flow rate ranged from 1.4 to 1.6 mL/min and was controlled by a Gilson, Minipuls 2 peristaltic pump (Middleton, WI). The spray chamber and condenser temperatures were 140 and 5 °C, respectively. The microporous membrane desolvator (MMD) was the MDX-100 (CETAC). The desolvation cell temperature was 160 °C, and the sweep gas flow rate was 1.25 L/min.

Reagents and Glassware. All glassware was scrupulously cleaned and acid-soaked prior to use. All oxidizing agents were of trace metal grade, and NIST certified standards were used to prepare stock solutions, which were then used to prepare working standards. All element standards below 1 μg/mL were prepared fresh daily. Unless otherwise indicated, all reagent percentages reflect volume-to-volume dilutions. The reagent grade (stock) hydrogen peroxide (H_2O_2) was 30% (w/v). Sucrose was purchased from Fluka (Ronkonkoma, NY) and was of reagent grade. Corn syrup and HFCS samples were provided by various members of the Corn Refiners Association. Throughout this paper, corn syrup A denotes a carbon-filtered syrup and corn syrup B indicates a syrup purified by ion exchange. During sample introduction of corn syrup A or digests of syrup A, sodium emission in the plasma was visually evident, and in contrast the introduction of corn syrup B did not visually alter the plasma. HFCS A represents the syrup composed of 40+% fructose, and HFCS B represents the syrup composed of 50+% fructose.

Table 1. Microwave Digestion Procedure

Program A. Predigestion					
stage	1	2	3	4	5
power (W)	346	346	346	346	346
pressure (kPa)	138	276	552	896	1172
temp (°C)	70	80	90	100	105
run time (min)	10	10	10	10	10
time at parameter (min)	3	3	3	3	3
Program B. Final Digestion					
stage	1	2	3	4	5
power (W)	346	346	346	346	346
pressure (kPa)	138	276	620	1034	1310
temp (°C)	65	85	110	140	160
run time (min)	10	10	10	10	10
time at parameter (min)	3	3	3	5	10

Open Vessel Digestion. Sample aliquots (4 mL) were taken from stock sample solutions prepared at concentrations representing 0.25 g/mL sweetener in 1% nitric acid (HNO_3). The aliquots were placed in 50 mL Erlenmeyer flasks. Samples were then spiked with the metals of interest from a multielement standard. Digestion was performed at 90 °C on a Thermolyne Type 2200 hot plate. Oxidizing agents were added in a stepwise fashion to avoid vigorous reactions and minimize analyte loss. Specifically, 1.0 mL of 16 M HNO_3 was added and allowed to react until all evidence of digestion (gas evolution) had ceased. On average, this required 20 min. Next, 4 mL of 30% (w/v) H_2O_2 was added and allowed to react for approximately 30 min. This was then followed by an additional 5 mL of 30% (w/v) H_2O_2. After digestion ceased, the hot-plate temperature was increased to 120 °C and the samples were evaporated to near dryness (volume < 0.5 mL), cooled, and transferred to a final volume of 10 mL using 1% HNO_3. During sample evaporation, samples should be periodically checked to avoid losses due to spattering.

Closed Vessel Digestion. Closed vessel (microwave) digestion was accomplished using a MDS 2000 system (CEM Corp., Matthews, NC) equipped with advanced composite vessels. Similar to open vessel digestion, sample aliquots (4 mL) were obtained from stock sample solutions prepared at concentrations representing 0.25 g/mL sweetener in 1% HNO_3 and then spiked with the metals of interest from a multielement standard. To ensure digestion, it was important that the spike aliquot not exceed 1.0 mL. Following the addition of the spike, 10 mL of 16 M HNO_3 was added to the vessels. The vessels were then sealed and placed in the microwave. The microwave program is summarized in Table 1. To digest 1.0 g of sweetener, two programs consisting of five stages each were required. At the end of the first program (predigestion), the vessels were cooled and then vented via the rupture membranes. Prior to proceeding with the second program (final digestion), the rupture membranes were replaced with new ones. After the second program, the vessels were cooled to room temperature and vented and the digests were transferred to a final volume of 25 mL using 10% HNO_3.

SAFETY NOTE: Both pressure and temperature control were employed with both programs. The power setting was based on the manufacturer's literature regarding the number of vessels and the sample type (organic versus inorganic). Chemical differences among the different types of sweeteners preclude simultaneous digestion. If only pressure control is available, a suggested predigestion program consists of using set points of 138, 450, 760, 970, and 1170 kPa with 3 min holds in the first four stages and a 10 min hold at 1170 kPa. With this program, pressure over-run occurred in stage 2, but control in the other stages was maintained. Final digestion must be tailored to the actual sweetener, but if the above predigestion program is followed, the likelihood of vessel overpressurization is reduced. Attempts were made to allow the samples to predigest prior to their being sealed and placed in the microwave. However, at times up to 1 h, the extent of digestion at room temperature was minimal. Allowing the vessels to sit overnight did allow for some digestion, but the extent of digestion varied from vessel to vessel.

Recovery Experiments and Numerical Protocol. Unless otherwise indicated, the number of replicates (n) used to

Table 2. Spike and Recovery Experiments for Open Vessel Digestion

sweetener	cadmium	copper	lead
A. Results for Samples Spiked at 0.10 μg/g			
sucrose	103 (3.9)[a]	88 (8.0)	85 (30)
corn syrup A	88 (1.1)	85 (7.1)	87 (5.7)
corn syrup B	100 (2.0)	92 (8.7)	104 (14)
HFCS A	98 (3.1)	93 (2.2)	87 (25)
HFCS B	100 (*)	81 (11)	104 (10)
B. Results for Samples Spiked at 0.20 μg/g			
sucrose	96 (0.5)	96 (*)	84 (14)
corn syrup A	84 (*)	84 (*)	94 (*)
corn syrup B	93 (1.6)	93 (5.4)	92 (7.7)
HFCS A	89 (4.5)	90 (17)	95 (10)
HFCS B	94 (3.2)	85 (8.2)	82 (3.7)

[a] Numbers in parentheses represent the relative standard deviation expressed as a percent. (*) denotes samples that were analyzed in duplicate.

calculate recovery was 3. In general, four exposures representing 5 s integrations were used to define the average total signal intensity. Background noise was determined from 20 replicates of the appropriate external standard (1% HNO_3 for the open vessel procedure and 10% HNO_3 for the closed vessel digestion). All calibration curves were initially based on five standards, including the blank, and the concentrations used ranged from 1 to 100 ng/mL. Calibration curves were modified as necessary to provide optimal performance through the concentration range of interest. Modification was limited to standard omission at either the high or low end of the initial calibration curve. The instrument detection limit was calculated on the basis of 3 times the standard deviation of the appropriate blank intensity. Method detection limits were calculated relative to the initial sample size and corrected for any dilutions.

RESULTS AND DISCUSSION

The need to digest the sweeteners prior to analysis by ICP-AES has been previously demonstrated (Allen et al., 1996). The prominent arsenic line suffers spectral overlap with carbon, and chemical interferences limit the determination of cadmium, copper, and lead. As the magnitude of the interference effects varies from element to element and with matrix concentration, method development by either wavelength correction techniques or internal standards would not be practical. In addition, continuous introduction of samples containing 0.1 g/mL sweetener periodically plugged the spray chamber to condenser union (U-tube), which would impede quantitation efforts based on the method of standard addition. As a result, some type of sample pretreatment is required.

Open Vessel Digestion. Results from the spike and recovery experiments for cadmium, copper, and lead are summarized in Table 2. Results for samples spiked at 0.20 μg/g were collected simultaneously while those at 0.10 μg/g were segmented to allow spiking with arsenic. Average recoveries were 94%, 89%, and 91%, respectively, for cadmium, copper, and lead. Recovery appeared to be independent of sample type. In general, the relative standard deviation ($n = 3$) was <10% for cadmium and copper. In contrast, the percent standard deviation for lead was usually higher. Average method detection limits ($n = 6$) were 20 ng/g for lead, 7 ng/g for copper, and 8 ng/g for cadmium. The higher instrument detection limit for lead may explain the higher standard deviation. Both arsenic and tin were tested but produced erratic recoveries.

The determination of sample blank/background levels required the determination of reagent blank levels.

Table 3. Spike and Recovery Experiments for Closed Vessel Digestion

sweetener	arsenic	cadmium	copper	lead	tin
A. Results for Samples Spiked at 0.5 μg/g					
sucrose	92 (6.0)[a]	90 (1.7)	88 (4.6)	100 (8.1)	103 (4.9)
corn syrup A	95 (10)	89 (5.0)	94 (1.4)	96 (12)	86 (4.9)
corn syrup B	86 (5.2)	86 (2.8)	94 (0.8)	74 (13)	94 (3.2)
HFCS A	96 (2.6)	87 (1.6)	83 (6.0)	82 (2.5)	86 (1.7)
HFCS B	92 (14)	87 (4.6)	94 (0.7)	97 (21)	91 (3.2)
B. Results for Samples Spiked at 1.0 μg/g					
sucrose	89 (6.5)	84 (1.6)	86 (2.3)	88 (2.0)	91 (4.7)
corn syrup A	91 (5.8)	82 (2.0)	90 (0.9)	80 (6.3)	89 (2.5)
corn syrup B	91 (4.4)	83 (0.9)	92 (0.2)	84 (6.3)	93 (1.6)
HFCS A	94 (8.2)	82 (3.3)	83 (3.1)	82 (3.8)	85 (1.6)
HFCS B	93 (3.2)	86 (2.6)	92 (0.3)	90 (0.8)	84 (1.5)

[a] Numbers in parentheses represent the percent relative standard deviation.

None of the sweeteners demonstrated detectable cadmium levels with testing on duplicate days ($n = 6$). The highest level of cadmium measured in a single day ($n = 3$) was 8 ng/g in corn syrup A. The highest level of copper was measured in the sucrose. For duplicate day testing ($n = 6$), the average background level of copper was 46 ng/g sucrose with a pooled standard devation of 13%. The highest background lead level was found in HFCS B, which is surprising as all HFCSs are ion-exchanged. The measured lead level was 24 ng/g for HFCS B, and the pooled standard deviation ($n = 6$) was 31%. All other samples produced blank levels at or below the method detection limit.

Closed Vessel Digestion. Results from spike and recovery experiments with the closed vessel digestion procedure are summarized in Table 3. Absolute simultaneous detection occurred for corn syrup A and both HFCSs. Average recoveries were 92% for arsenic, 86% for cadmium, 90% for copper, 87% for lead and 90% for tin. The relative standard deviation for both spike levels was generally <10% and did not exhibit variation with respect to sample type. Average method detection limits were 200 ng/g for arsenic, 25 ng/g for cadmium, 25 ng/g for copper, 175 ng/g for lead, and 75 ng/g for tin.

The highest detected background level for copper was 95 ng/g in sucrose, with a relative standard deviation of 21%. This value is roughly double the value determined by the open vessel procedure, but as the samples were taken from different manufacturing lots, no correlation can be offered. For the other metals, none of the levels appeared above the reagent blank. On the basis of method detection limits, this agrees favorably with the background values established by the open vessel procedure.

Comparisons and Comments. The open and closed vessel procedures demonstrated comparable recoveries and precisions for cadmium, copper, and lead. This may be surprising as closed vessel procedures are generally associated with higher recovery and improved precision because of reduction in analyte loss and more uniform digestion (Kingston and Jassie, 1988). However, because of the vigorous nature of the digestion, analyte losses during venting and during sample transfer along the pressure seals of the vessel caps were problematic. Also, the large sample size and the diluted sample matrix may have caused the digestion efficiency to be lower than normal with the microwave. Method detection limits were lower with the open vessel procedure, but through sample evaporation, lower values could be obtained with the closed vessel procedure.

Other procedures, both open and closed vessel, could easily be developed. In addition, it should be possible to develop procedures that would eliminate the need for the membrane desolvator. Toward this endeavor, current experiments suggest that driving the digestion/decomposition reaction to completeness is required. For example, using 9 mL of H_2O_2 versus 8 mL with the open vessel procedure improved the recovery for lead (spiked at 0.20 μg/g) in sucrose from 46% to 74%. However, the additional time required for the digestion procedure, the increased likelihood of contamination, the increased cost of reagents, and the increased reagent blank levels eventually off-set the cost of employing the membrane desolvator. Also, the feasibility of completely digesting larger sample sizes ($\geq$1.0 g) by microwave processes is questionable (Kingston and Jassie, 1988). Without the membrane desolvator, recoveries for sucrose (spiked at 1.0 μg/g) with the microwave procedure were 38%, 52%, 77%, 19%, and 55%, respectively, for arsenic, cadmium, copper, lead, and tin.

Method verification with a standard reference material was not pursued because of the critical nature of the digestion process, which would make selection of an acceptable surrogate difficult. Equally, confirmation of results via an independent method would be time-consuming and very difficult if it was desired to confirm the blank values. However, the fact that element-to-element variability, indicated through recovery and precision, was not signifcant and the fact that variance among the sweeteners was minimal lend credibility to this method.

In summary, either open or closed vessel digestion can be used to determine cadmium, copper, and lead in sucrose, corn syrup, and HFCS by ICP-AES detection. With closed vessel digestion, it is possible to extend performance to arsenic and tin. In accordance with ICP-AES detection, simultaneous or rapid sequential detection of the metals is achieved. Membrane desolvation was used to aid matrix normalization and demonstrated the most dramatic effect with the microwave procedure.

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