Sample Preparation for Arsenic, Copper, Iron, and Lead Determination in Sugar

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Two sample preparation methods were modified and optimized for the determination of As, Cu, Fe, and Pb in white sugar using atomic absorption spectrometry (AAS). Analyses were carried out using hydride generation AAS (HG-AAS) for As and flame AAS (FAAS) for Cu, Fe, and Pb. White sugar samples were prepared by dry ashing of the samples with H_2SO_4 and $Mg(NO_3)_2$. Recoveries for As, Cu, Fe, and Pb were 87.6, 92.8, 95.8, 91.1% respectively,in ashing with H_2SO_4. They were 95.1, 92.4, 97.4, and 91.1%, respectively, in ashing with $Mg(NO_3)_2 \cdot 6H_2O$. When small quantities of spikes were used, 94.7 and 95.3% recoveries of arsenic were found for the procedures applying H_2SO_4 and $Mg(NO_3)_2$, respectively.

Keywords: *Flame atomic absorption spectrometry; white sugar; trace elements; arsenic; copper; iron; lead*

INTRODUCTION

In recent years, there has been increasing interest in determining the concentration levels of heavy metals in various food products. For example, white sugar, which is known to be one of the purest food substances produced on an industrial scale, contains some metal impurities depending on the quality of sugar beet or cane used in its production technology. The allowable trace metal contaminations of white sugar have been described by national and international white sugar standards. Among them, the Codex Alimentarius Commission (1990) states the maximum allowable level as 1 mg kg^{-1} for As, Cu, and Pb.

Various sample preparation techniques are available presently in the determination of trace metals in organic matrix. Some of them are wet ashing (Lew, 1972; Koster et al., 1981; Huijbregts et al., 1985; Sang et al., 1975; Baetz and Kenner, 1973; Nieuwenhuize and Poley, 1989), yeast fermentation (Morris et al., 1976), oxygen bomb ashing (Fujivara and Narasaki, 1968), fusion (Bowen, 1968), resin preconcentration (Carpenter and Bichsel, 1969), and some dry ashing methods such as dry ashing with H_2SO_4 and dry ashing with $Mg(NO_3)_2$.

Dry ashing without ashing aids was used to determine Cu and Fe by FAAS in white sugar by Silva (ICUMSA, 1990) and in process juices by Sang et al. (1975) as the sample preparation method. Lew (1972) used dry ashing with H_2SO_4 for Cu, Fe, and Pb determinations in granulated sugar, while Koster et al. (1981) used a similar method for Cu and Pb determinations in various foodstuffs by FAAS.

Samples were prepared using dry ashing with the $Mg(NO_3)_2$ method of Dalton and Malonoski (1969) for Cu and Pb determination in meat and meat products using FAAS, and the method of Leblanc and Jackson (1973) was used for As determinations in marine fish using molecular spectrometry. As, Cu, Fe, and Pb determinations in food and biological material using FAAS were performed according to the method of Friend et al. (1977), and As in foodstuffs was determined using HG-AAS according to the procedure of Siemer et al. (1976). In another study, dry ashing with $Mg(NO_3)_2$ and MgO was used to determine As in diet samples using molecular spectrometry (Hundley and Underwood, 1970). A similar method was accepted by AOAC (1990) for As determination in fodder using molecular spectrometry. This method was also used with some modifications to determine As in white sugar using electrothermal atomization AAS (ETA-AAS) by Thielecke and Hackman (ICUMSA, 1986) and by Fernandez Gil and Fernandez Alvares using HG-AAS (ICUMSA, 1990).

The International Commission for Uniform Methods of Sugar Analysis (ICUMSA), an international organization interested in the improvement of analytical methods related to the sugar industry, is seeking to establish some methods for As, Cu, Fe, and Pb determinations in white sugar. FAAS, a well-known method in trace metal determination, is a potential candidate for such studies. That is why flame and hydride generation AAS have been chosen as the analyzing techniques in the present study.

In trace element determinations of the organic substances, sample size usually is in the range of 0.1−10 g. In sugar analysis, however, larger sample sizes should be used, as the trace element concentrations are very low relative to the detection limits of FAAS. Therefore, new procedures must be developed in which at least 100 g of the sugar can be processed. The amount of chemicals should be kept at minimum levels to prevent dilution. On the basis of this conclusion, in this study dry ashing methods with the ashing aids H_2SO_4, $Mg(NO_3)_2$, and a $Mg(NO_3)_2$ + MgO mixture were investigated.

When MgO + $Mg(NO_3)_2$ dry ashing was applied, the ash obtained contained a large amount of solid residue,

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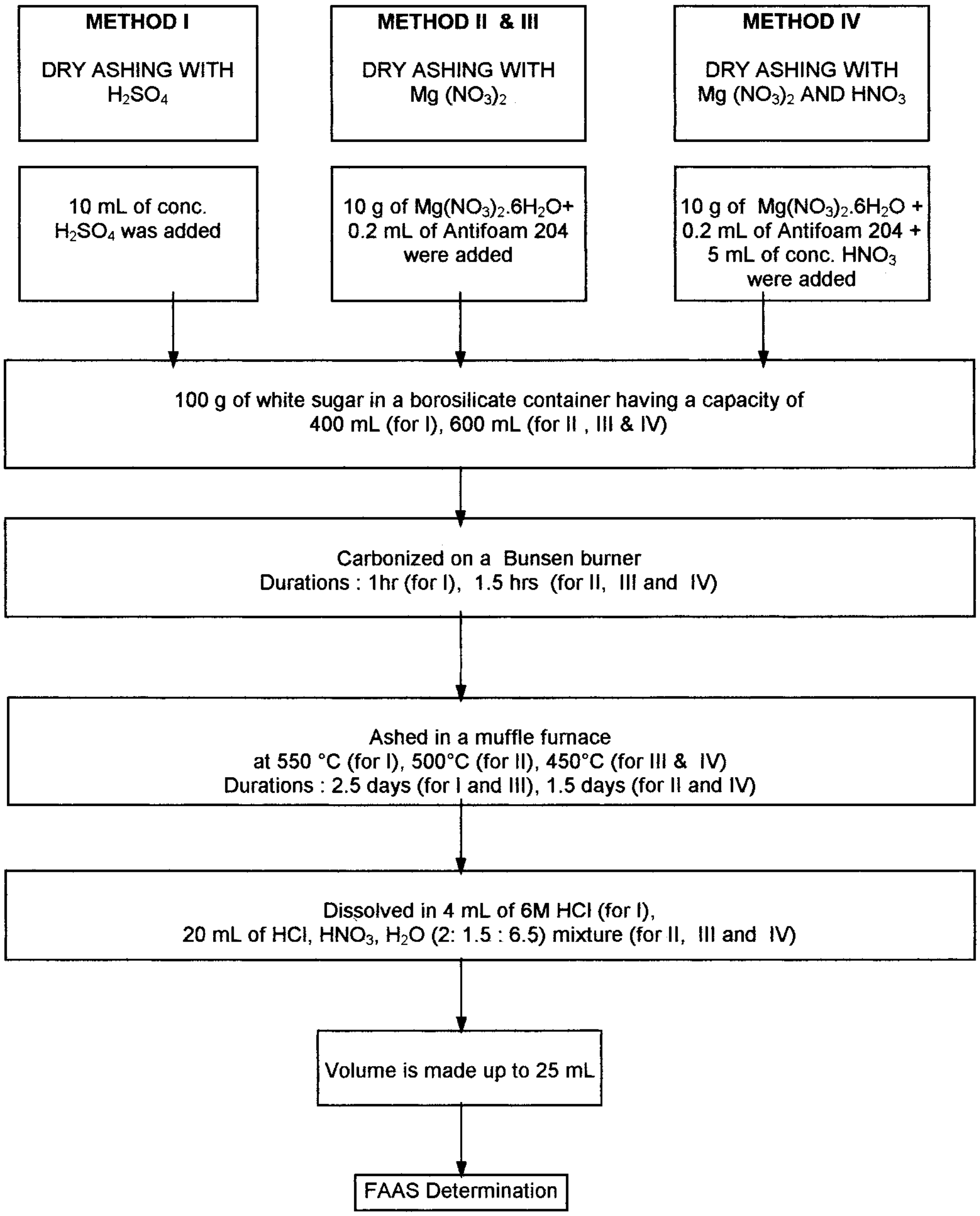

Figure 1. Sample preparation procedures.

which could only be dissolved using a large volume of acid. However, this lowers the concentrations of the elements, below the detection limits of FAAS.

In this study, among the methods mentioned above, those with H_2SO_4 and $Mg(NO_3)_2$ usage were modified to obtain a new sample preparation procedure for the determination of As, Cu, Fe, and Pb utilizing flame and hydride generation AAS.

The methods of Lew (1972), Feinberg and Ducauze (1980), and Koster et al. (1981) involving H_2SO_4 usage and the methods of Dalton and Malonoski (1969), Leblanc and Jackson (1973), Friend et al. (1977), and Siemer et al. (1976) involving $Mg(NO_3)_2$ usage were adapted to obtain one sample preparation method for the determination of As, Cu, Fe, and Pb by flame and hydride generation AAS.

EXPERIMENTAL PROCEDURES

Apparatus. A Varian Model AA-1475 atomic absorption spectrophotometer, equipped with a Model 65 vapor generation accessory was used throughout the study. Instrumental conditions of the measurement are given in Table 1.

Table 1. Instrumental Conditions in AAS

	As	Cu	Fe	Pb
lamp current, mA	10	4	5	15
wavelength, nm	193.7	324.7	248.3	217.0
slit width, nm	1.0	0.5	0.2	1.0
fuel, L/min	1.5	1.5	1.5	1.5
oxidant, L/min	15	15	15	15
nebulizer uptake rate, mL/min	0	10	10	10
background correction	on	off	off	off
height of the optical path from the burner, mm	20	8	8	8

Table 2. Effect of Applied H_2SO_4 Concentration in the Determination of Cu, Fe, and Pb [$x \pm s$ (n), mg/kg] by Dry Ashing with H_2SO_4

H_2SO_4 concn	Cu	Fe	Pb
10% (v / v)	0.028 ± 0.006 (4)	1.74 ± 0.10 (4)	0.030 ± 0.010 (4)
concentrated	0.033 ± 0.004 (6)	1.62 ± 0.14 (6)	0.032 ± 0.010 (6)

Reagents. All of the reagents used were of analytical grade unless otherwise stated. Arsenic(III) and arsenic(V) stock solutions were prepared from their oxides, whereas in the preparation of the other stock solutions the wire, powder, or

Table 3. Results of As, Cu, Fe, and Pb Determinations [$x \pm s$ (n), mg/kg] in White Sugar by AAS following Dry Ashing Methods

dissolution method	As	Cu	Fe	Pb
dry ashing with H_2SO_4 (method I in Figure 1)	0.0041 ± 0.0008 (7)	0.020 ± 0.004 (7)	1.79 ± 0.18 (7)	0.045 ± 0.020 (7)
dry ashing with $Mg(NO_3)_2$ (method II in Figure 1)	0.0080 ± 0.0022 (8)	0.032 ± 0.006 (8)	1.41 ± 0.08 (8)	0.055 ± 0.016 (8)
dry ashing with $Mg(NO_3)_2$ (method III in Figure 1)	0.0089 ± 0.0018(8)	0.035 ± 0.009 (8)	1.65 ± 0.16 (8)	0.048 ± 0.021 (8)
dry ashing with $Mg(NO_3)_2 + HNO_3$ (method IV in Figure 1)	0.0089 ± 0.0004 (6)	0.034 ± 0.007 (5)	1.65 ± 0.23 (6)	0.060 ± 0.013 (6)

Table 4. Recoveries of As, Cu, Fe, and Pb in White Sugar Using Concentrated H_2SO_4 and $Mg(NO_3)_2$ by AAS

	As		Cu		Fe		Pb	
dissolution method	added, mg/kg	% recovery, $x \pm s$ (n)	added, mg/kg	% recovery, $x \pm s$ (n)	added, mg/kg	% recovery, $x \pm s$ (n)	added, mg/kg	% recovery, $x \pm s$ (n)
dry ashing with H_2SO_4 (method I in Figure 1)	1.000	88.4 ± 7.4 (4)	1.00	93.6 ± 0.6 (4)	10.00	96.3 ± 1.2 (4)	1.00	91.4 ± 2.4 (4)
	10.000	86.8 ± 2.2 (4)	2.00	92.1 ± 3.4 (4)	20.00	95.2 ± 0.6 (4)	2.00	90.7 ± 2.2 (4)
X_R[a]		87.6 ± 5.2 (8)		92.8 ± 2.4 (8)		95.8 ± 1.0 (8)		91.1 ± 2.2 (8)
dry ashing with $Mg(NO_3)_2$ (method II in Figure 1)	10.00	94.6 ± 4.2 (4)	2.00	93.6 ± 2.9 (4)	10.00	96.2 ± 1.7 (4)	2.00	90.5 ± 3.4 (4)
	25.00	95.6 ± 1.6 (4)	4.00	91.3 ± 0.7 (4)	25.00	98.6 ± 0.8 (4)	4.00	91.7 ± 2.3 (4)
X_R[a]		95.1 ± 3.0 (8)		92.4 ± 2.3 (8)		97.4 ± 1.7 (8)		91.1 ± 2.8 (8)
dry ashing with $Mg(NO_3)_2$ (method III in Figure 1)	25.00	96.6 ± 1.5 (8)	4.00	92.6 ± 3.0 (8)	25.00	98.3 ± 6.8 (8)	4.00	92.3 ± 3.2 (8)

[a] X_R, average of recoveries obtained by different amount of analyte on sample.

Table 5. Recoveries of As in White Sugar Using Concentrated H_2SO_4 and $Mg(NO_3)_2 + HNO_3$ Mixture by AAS

	As	
dissolution method	added, mg/kg	% recovery, $x \pm s$ (n)
dry ashing with H_2SO_4 (method I in Figure 1)	0.010	94.7 ± 6.2 (6)
dry ashing with $Mg(NO_3)_2 + HNO_3$ (method IV in Figure 1)	0.010	95.3 ± 2.9 (6)

granulated forms of copper, iron, and lead metals were used. Intermediate stock solutions were prepared daily. Double-distilled deionized water was used for dilutions.

Sample. Medium-quality white sugar obtained from the Ankara Sugar factory was mixed thoroughly and used in all experiments. Color type, color in solution, and conductivity ash were taken into account in the selection of the white sugar sample. The purpose of the selection of medium quality is to find a suitable sample preparation method that can be applicable to most of the white sugar produced in the world.

Sample Preparation Procedure. White sugar samples were prepared by using the dry ashing with H_2SO_4 (method I) and the modifications of the dry ashing with $Mg(NO_3)_2$ methods (methods II−IV). Details of the procedures are given in Figure 1.

Analysis of Samples. Samples obtained from preparation procedures were directly aspirated to the air−acetylene flame of the AAS instrument for Cu, Fe, and Pb determinations. The hydride generation accessory of the AAS intrument was used for As determination.

For hydride generation, 10 mL of sample was mixed with 10 mL of 6 M HCl and 1 mL of 20% KI (w/v) in plastic bottles. After 60 min, solutions were poured into the reaction vessel of the vapor generation accessory. After 25 s of mixing, one pellet of $NaBH_4$ (∼0.4 g) was dropped into the solution in the reaction vessel, which was kept in a nitrogen atmosphere. Absorption of the analyte atom generated was measured from the peak height, as the hydride was sent into the hot quartz tube.

RESULTS AND DISCUSSION

Various sources of error are to be considered in the dry ashing studies (Bock and Mainz, 1979), such as losses due to volatilization, reaction with crucible, incomplete dissolution of ash, and long ashing times. Dry ashing methods used were evaluated with these particular sources of errors in mind.

Dry Ashing with H_2SO_4. This sample preparation method has already been used in the analysis of white sugar for the determination of Cu, Fe, and Pb (Lew, 1972). A study was undertaken to investigate the possibility of using dry ashing with the H_2SO_4 method for As determination, with some modifications as follows:

In previous studies (Bock and Mainz, 1979) the sulfate ash method was carried out in platinum dishes, which are quite expensive and limited in size. Borosilicate containers (400 and 600 mL in volume), which seem to be more advantageous, were used in this study.

The Association of Official Analytical Chemists (AOAC) approves the use of 10% (v/v) H_2SO_4 in the official sulfated ash method for sugars. When the AOAC procedure (AOAC, 1990) was applied, the preashing time, which is the time elapsed when the samples were ashed on a Bunsen burner, was too long, and the method was tedious and required too much attention. Therefore, here, the use of concentrated H_2SO_4 instead of 10% H_2SO_4 was tried to shorten the span of preashing. The results of analyses of white sugar samples in terms of Cu, Fe, and Pb concentrations ashed by 10% H_2SO_4 and concentrated H_2SO_4 are given in Table 2. Since there is no big difference between the mean values of these sets, concentrated H_2SO_4 seems to perform as well as 10% H_2SO_4. Modifications in container size and material and in H_2SO_4 concentration decrease the preashing time from 6 to 1 h.

The concentrations of As, Cu, Fe, and Pb determined in white sugar using dry ashing with H_2SO_4 are given in Table 3.

Recovery studies were carried out by adding a known amount of the analyte prior to dry ashing. The average recoveries, calculated by taking the concentrations in Table 3 as the endogenous analyte concentrations in the absence of the spike, are shown in Table 4.

Dry Ashing with $Mg(NO_3)_2 \cdot 6H_2O$. This is the proposed method for As and Pb determination in an organic matrix (Bock and Mainz, 1979), but it has not been applied for sugar analysis previously.

The main drawback in the application of this method for sugar analysis is excessive frothing of the sample at the beginning of the ashing procedure, at the preashing step, unless a lengthy low-temperature charring is used. In this study some modifications in the method have been made to overcome this problem.

During the process, aqueous (Leblanc and Jackson, 1973) or alcoholic solution (Friend et al., 1977) of $Mg(NO_3)_2$ is usually added to assist oxidation of the organic samples. The concentration of the $Mg(NO_3)_2$ solution does not affect frothing, but the use of a concentrated solution decreases the preashing time. Hence, solid $Mg(NO_3)_2 \cdot 6H_2O$ is directly added to the sample. However, the degree of foaming was affected by the relative mass of $Mg(NO_3)_2$ used. Therefore, a minimum possible amount of $Mg(NO_3)_2 \cdot 6H_2O$ [10% (w/w)] was used. In addition, the use of a liquid antifoaming reagent, Antifoam 204 (Sigma A6426), for smooth ashing was also investigated. The volume optimization of Antifoam 204 was performed by visual observation of the froth level in the beaker throughout the ashing cycle. The results of the recovery studies are also given in Table 4. Similar calculations for this method were carried out as in the H_2SO_4 case.

The final modification was the use of concentrated HNO_3 together with $Mg(NO_3)_2 \cdot 6H_2O$ (method IV in Figure 1). This decreases the span of whole ashing time for the mentioned process by ~45% without causing any losses due to volatilization. The results of the analyses carried out under these conditions are given in Table 3. As seen, the results are in good agreement at the 95% confidence level (Student's *t* test).

Another recovery study was carried out for the most volatile element, arsenic, by spiking very small quantities of the metal on white sugar using the final modifications of two methods (methods I and IV). Results are shown in Table 5.

CONCLUSION

Until recently, only spectrophotometric methods were accepted as the standard methods for As, Cu, and Pb determinations in white sugar. However, at the 1990 ICUMSA meeting, a need for further investigation for the use of AAS in such determinations was emphasized.

A wet ashing method of Miller-Ihli employing ETA-AAS (ICUMSA, 1994) was proposed for Pb determination in sugars at the 1994 ICUMSA meeting. However, considering the expense and difficulty in applying electrothermal AAS in routine analyses, the present study, which employs FAAS, an easier and cheaper technique, has been undertaken for establishing a standard sample preparation technique for trace element determinations in white sugar. Another reason for the selection of this technique is to determine As, Cu, Fe, and Pb using only one sample preparation method. During the investigations, dry ashing instead of wet ashing methods were preferred, due to the restrictions in the detection limits of FAAS.

Among the known dry ashing methods, dry ashing using H_2SO_4 has already been used for Cu, Fe, and Pb determinations (Lew, 1972) in white sugar, but it had not been tried for the determination of As. Application of this technique (method I) gave surprisingly satisfactory results.

Another dry ashing technique tested in this study was dry ashing with $Mg(NO_3)_2$. This is an approach suggested for all volatile element determinations in the literature (Bock and Mainz, 1979), but it had not been applied in white sugar analyses previously. The final modification of this technique involving HNO_3 usage (method IV) was also found successful in the determinations.

Both of the dry ashing techniques, that is, dry ashing with H_2SO_4 and dry ashing with $Mg(NO_3)_2$, turned out to be very efficient for the determination of As, Cu, Fe, and Pb in white sugar by FAAS. So far, these two methods seem to be the only ones available for these determinations using a single ashing procedure. Between these two, the ash produced in the $Mg(NO_3)_2$ method is difficult to handle and the duration of digestion is longer compared to that of H_2SO_4 applications.

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ABBREVIATIONS USED

AAS, atomic absorption spectrometry; AOAC, Association of Official Analytical Chemists; ETA-AAS, electrothermal atomization atomic absorption spectrometry; FAAS, flame atomic absorption spectrometry; HG-AAS, hydride generation atomic absorption spectrometry; ICUMSA, International Commission for Uniform Methods of Sugar Analysis.

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