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Food Chemistry 74 (2001) 527–531

Food Chemistry

www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods Section

Determination of mercury in refined beet sugar by anodic stripping voltammetry without sample pretreatment

Daniel Sancho, Luis Debán*, Francisco Barbosa, Rafael Pardo, Marisol Vega

Departamento de Química Analítica, Facultad de Ciencias, Universidad de Valladolid, 47005 Valladolid, Spain

Received 27 October 2000; received in revised form 3 March 2001; accepted 3 March 2001

Abstract

Total mercury has been analysed in refined beet sugar at the μg kg^{-1} level by differential-pulse anodic stripping voltammetry (DPASV) at a rotating gold disk electrode (RGDE). DPASV measurements of mercury are based on its accumulation onto the RGDE as a Hg–Au amalgam and followed by the reoxidation of Hg(0) to Hg(II). Measurements were directly made on untreated sugar solutions. The DPASV procedure was compared with cold-vapour atomic absorption spectrometry carried out at a flow-injection mercury system when applied to digested sugar samples. The DPASV method has been demonstrated to perform conveniently for metal determination in beet sugar of good quality (low metal concentration) produced by Spanish sugar refineries. Mercury concentrations below 5 μg kg^{-1} were found in all commercial beet sugar samples analysed, which are much lower that the maximum permissible contents derived from the provisional tolerable weekly intake recommended by the World Health Organization.

Keywords: Food quality; Sugar; Saccharose; Mercury; Cold-vapor atomic absorption spectrometry; Flow injection mercury system; Stripping voltammetry

1. Introduction

The mercury burden of the environment derives predominantly from natural sources, but human activities such as mining, chloroalkali industry, combustion of fossil fuels, organomercury fungicides, or use of mercury-based electric devices can also increase the levels of this toxicant (WHO, 1989).

Because mercury is widely spread in the environment, the risk derived from its possible incorporation into the human diet during food production must not be despised. In fact, except in the case of local mercury contamination where air and water can contribute largely to the mercury intake, the body burden of mercury in humans is predominantly caused by the diet (WHO, 1990, 1991).

Reported daily food intakes of mercury cover the range 3.0–25 μg, with a median value around 15 μg (Fergusson, 1990). The provisional tolerable weekly intake of mercury as recommended by the World Health Organization (WHO) is 0.3 mg. This amount is equivalent to 5 μg per kg body weight (FAO/WHO, 1973, 1999), or assuming an average dry matter intake of 400 g day^{-1}, a total mercury content of the dry diet close to 0.1 mg kg^{-1} dry matter.

Within the requirements of total quality in the sugar industry, the absence of toxic substances in the final product is compulsory. Therefore, analytical procedures that allow detecting and quantifying toxic species at trace levels must be developed and validated. The goal is to ensure, with a reasonable certainty, that the refined sugar does not contain these species, or at least that the contents are lower than the maximum tolerated levels. In the case of mercury, there is not a specific regulation, but it can be assumed that total mercury in refined sugar should not exceed the maximum permissible level of the dry diet (ca. 0.1 mg kg^{-1}), which is much lower than the level of 1 mg kg^{-1} established by the Spanish legislation (adapted from the Council Directive, 1973) for other toxic elements such as lead and arsenic in sugar (Real Decreto, 1987).

The International Commission for Uniform Methods of Sugar Analysis (ICUMSA) has not yet proposed a standardized procedure for mercury determination in refined sugar. However, the analytical procedures most frequently used for the determination of total mercury

* Corresponding author. Fax: +34-983-423013.
E-mail address: luisdeba@qa.uva.es (L. Debán).

0308-8146/01/$ - see front matter
PII: S0308-8146(01)00177-7

at trace levels in food are based on cold vapour atomic absorption spectrometry (cold vapour AAS) (Clevenger, Smith, & Winefordner, 1997). In these methods, mercury [as mercury(II)] is reduced to vapour Hg(0) usually with tin(II) in acidic medium (AOAC, 1990; Aduna, Alegría, Barberá, Farré, & Lagarda, 1997) or with sodium borohydride in alkaline medium (Navarro, López, Sánchez, & López, 1991; Bortoli, Gerotto, Marchiory, Muntau, & Rehnert, 1995). Recent advances in instrumentation allow to use the cold vapour AAS technique in conjunction with a flow injection system resulting in faster determinations and more accurate results (Aduna et al., 1997; Bortoli et al., 1995).

Cold vapour AAS techniques require a previous digestion step in order to convert all mercury species in soluble mercury(II) and also to eliminate the possible matrix effect caused by the reducing organic matter present in the sample, which is usually accomplished by microwave assisted digestion with oxidizing reagents such as nitric acid and/or hydrogen peroxide (Aduna et al., 1997; Navarro et al., 1991; Schnitzer, Soubelet, Testu, & Chafey, 1995). Sample digestion steps are time-consuming and can result in sample contamination or loss of the very volatile mercury, so the use of an analytical method not requiring such a previous step could be of great interest.

Stripping voltammetry at gold electrodes is a feasible option for the determination of total mercury in foodstuffs at ultratrace levels (Clevenger et al., 1997; Scholz, Nitschke, & Henrion, 1987; Viltchinskaia, Zeigman & Morton, 1995). The method benefits from the high sensitivity that allows to reach very low detection limits with out added preconcentration steps, since the preconcentration is performed in situ. Complex food matrixes (i.e. protein containing products) need to be digested prior to the measurement in order to release complexed mercury, but in simple matrixes such as refined sugar this step could be avoided with the subsequent reduction of analysis time, risk of sample contamination and loss of analyte.

In this paper, the electrochemical determination of mercury in undigested sugar samples by differential-pulse anodic stripping voltammetry (DPASV) at a rotating gold disk electrode (RGDE) is proposed, and the performance of this method compared with that of cold-vapour AAS. The effect of saccharose on the analytical signal is investigated and the means to circumvent this interference are presented.

2. Materials and methods

2.1. Apparatus and reagents

Stripping voltammetric experiments were carried out with a Metrohm (Herisau, Switzerland) E746 VA Trace Analyzer and a Metrohm 747 VA multimode electrode equipped with a rotating gold disk electrode (RGDE) as working electrode, a platinum rod as auxiliary electrode and a $Ag/AgCl,KCl_{sat}$ reference electrode.

Cold-vapour AAS measurements were performed with a Perkin-Elmer (Norwalk, CT, USA) flow injection mercury system (FIMS) instrument equipped with a FIMS-400 unit and a programmable sample dispenser.

Sugar samples were digested in a Milestone (Sorisole, Italy) MLS 1200 microwave oven. pH measurements were made with a Metrohm Model 654 pHmeter.

Standard solutions of mercury were prepared daily by dilution of Panreac (Barcelone, Spain) AAS standard (1000 mg l^{-1}). Saccharose standard solutions were prepared by dilution of extrapure saccharose (Merck, Darmstadt, Germany) and stored at 4°C.

DPASV reagents: the supporting electrolyte was prepared mixing 0.448 g of KCl, 0.372 g of Na_2AEDT and 22 ml of $HClO_4$, and diluting to 1 l with deionized water.

FIMS reagents: tin(II) sulfate solution was prepared by adding 25 g tin(II) sulfate to 250 mL of 0.25 M H_2SO_4. Hydroxylamine reagent was obtained dissolving 12 g of sodium chloride and 12 g of hydroxylamine sulfate in water and diluting to 100 ml.

Glassware and plastic containers were soaked in 2 M nitric acid for 24 h and then rinsed thoroughly with water. All reagents used were of analytical grade. Ultrapure water, obtained by using a Barnstead (Dubuque, IA, USA) Nanopure II water purification system, was used throughout.

2.2. Procedures

2.2.1. Mercury determination by FIMS

Five millilitres of concentrated nitric acid and 10 ml of 40% m/v hydrogen peroxide were added to 0.5 g of saccharose dissolved in 5 ml of water. This solution was placed in a 100-ml PTFE vessel and introduced for 20 min in the microwave oven for digestion of organic matter. The power of the magnetron was linearly increased from 250 to 600 W during the process. After digestion of organic matter, the resulting solution was diluted to 25 ml with water. A reagent blank was conducted simultaneously. To assess that all mercury was present as mercury(II), 5% m/v potassium permanganate was added to the digest to persistent purple colour. The excess of permanganate was removed by adding hydroxylamine reagent until the solution was colourless.

Mercury(II) was then reduced to the elemental state by addition of 10 ml of the tin(II) reagent and vaporized from the solution in the FIMS closed system. The mercury vapour passed through a cell positioned in the light beam path (253.7 nm) of the atomic absorption spectrophotometer. Metal contents were obtained from the calibration plot (absorbance vs. concentration) by interpolation.

2.2.2. Mercury determination by DPASV

This determination was carried out in non-digested saccharose samples. Two grams of untreated saccharose (or sugar where indicated) were dissolved in 25 ml of supporting electrolyte and placed in the voltammetric cell. The solution was deareated by purging it with water-saturated nitrogen for 10 min. The potential was set to 0.4 V for 180 s, whilst the solution was stirred at 2000 rpm. Then the stirrer was stopped and the electrode potential set at 0.4 V for another 30 s, thereafter the potential was scanned towards more positive values at a scan rate of 20 mV/s, and using a superimposed differential-pulse of 50 mV of amplitude. A mercury stripping peak was registered at about 0.64 V (vs Ag/AgCl,KCl_{sat} reference electrode) and its current used as a measure of mercury concentration. The standard additions method was used to calibrate the DPASV sensitivity and to check linearity of response.

The electrode processes can be written as follows:

$$\text{Deposition on the RGDE at } +0.4 \text{ V}: Hg^{2+}Au + 2e^- = Hg(Au)$$

$$\text{Anodic stripping}: \; Hg(Au) = Hg^{2+} + Au + 2e^-$$

2.2.3. Cleaning of the RGDE

The surface of the RGDE was cleaned and activated after each measurement by polishing the gold disk with alumina powder onto a polish cloth. This operation was then repeated without the powder until the electrode showed a mirror-looking surface. The electrode was then rinsed with water. To reactivate the electrode surface, the RGDE was immersed in a solution containing the supporting electrolyte and 0.01 M nitric acid, and successive polarization cycles from 2 V (30 s) to 0 V (10 s) were applied for 15 min.

3. Results and discussion

3.1. Effect of saccharose concentration

The influence of saccharose concentration on the DPASV peaks of mercury is depicted in Fig. 1. Increasing amounts of extrapure saccharose were added to 25 ml of supporting electrolyte containing 100 μg l^{-1} Hg(II). The DPASV peak current corresponding to the reoxidation of amalgamated mercury was registered in the conditions described above. The peak current was found to decrease to 50% in the presence of about 120 g l^{-1} saccharose, and was completely suppressed at saccharose concentrations higher than 600 g l^{-1}. This interference is likely due to the increase of the viscosity of the solution with increasing sugar concentration, which results in a more difficult diffusion of the metal towards the electrode surface (the diffusion coefficient decreases; Khoulif, Jambon, Chatelut, & Vittori, 1993), and hence, in a decrease of the peak current. Peak potential was also found to slightly shift towards more negative values with increasing sugar concentration, but the variation over the whole range of concentrations tested (0–700 g l^{-1}) was only of 10 mV.

The matrix effect caused by saccharose can be overcome using the standard additions method. The sensitivity of DPASV in non-digested solutions of 80 g l^{-1} saccharose was calculated from the slope of the regression line obtained by the standard additions method and was 15 nA μg^{-1} for a deposition period of 60 s.

3.2. Comparison of analytical methods

Aliquots of 25 ml of 80 g l^{-1} extrapure saccharose solutions were spiked with mercury at concentrations ranging from 10 to 80 μg metal kg^{-1} saccharose. Mercury was then determined by FIMS in digested samples, and by DPASV in non-digested solutions, as described in the Procedures section. Experimental results are compiled in Table 1.

To assess the accuracy of the analytical procedures, linear regression procedures were applied by plotting the measured metal concentrations vs. added metal. Table 2 shows the calculated slope (p), intercept (q), and determination coefficient (r^2) of the regression line for each procedure. If the method tested is not affected by bias, added and measured concentration must coincide, i.e. the regression line will have a zero intercept and a slope of 1. As random errors can occur, slope and intercept of the regression line have confidence intervals that are expressed as $p \pm ts_p$ and $q \pm ts_q$, respectively. In these equations, p and q represent the slope and intercept of the calibration line, and s_p and s_q their respective standard deviations. t is the Student's statistical parameter for

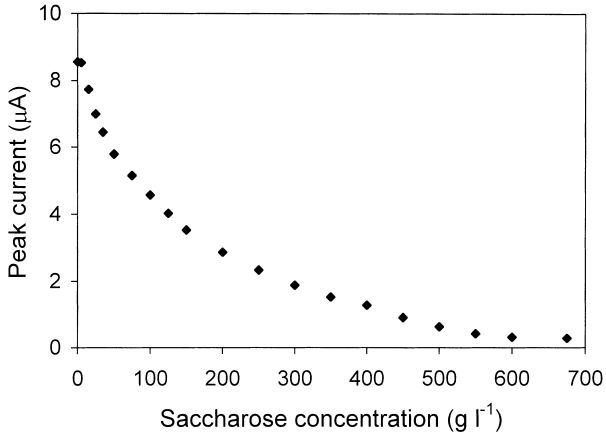

Fig. 1. Effect of the concentration of extra-pure saccharose on the DPASV peak current of 100 μg l^{-1} mercury.

$n-2$ degrees of freedom and 95% confidence level, where n is the number of points of the regression line. In this case, $n=6$ so t is 2.776.

The validation of the linear models was carried out by means of an analysis of the residuals and an F test. A normal distribution of the residuals (not shown) with zero mean was found in all the cases, which demonstrates the goodness-of-fit of the linear regression models.

Further assessment of the validity of the linear model was achieved by means of the F test of linearity. An analysis of variance of the regression lines was carried out, and the F variance ratio calculated as *mean squares of the regression/mean squares of the residual* (Table 2). F values were, in all cases, much higher than the critical value ($F_{(1,4;95\%)}$ is 7.709), thus confirming the validity of the linear models for the concentration range tested. The high values found for r^2 also confirm this affirmation.

As can be seen in Table 2, the intercepts of the regression lines obtained for the tested procedures do not differ significantly from zero, whereas the slopes do differ significantly from 1, i.e. its theoretical value is not within the confidence interval, thus pointing to the existence of bias. FIMS measurements of mercury in digested samples showed to be affected by bias as the slope is significantly lower than 1, therefore hindering the accurate determination of this element in sugar due to possible losses of mercury during sample pretreatment. DPASV measurements of mercury in non-digested sugar solutions halso proved to be also affected by systematic errors. The slope of the regression line differs significantly from 1 ($P>1$) which suggests that a bias that increases with increasing mercury concentration occurs, probably due to the contamination of the gold electrode by mercury accumulation in spite of the systematic cleaning.

None of the two tested procedures is sufficiently accurate. However, because the intercepts do not differ significantly from zero, the systematic errors of these methods at low mercury concentrations can be neglected. Since the levels of mercury to be determined in refined beet sugar samples are expected to be usually very low, both methods could be applied to this product.

We have preferred the DPASV method because it is faster since no sample pretreatment is necessary, thus avoiding sample contamination from reagents or losses of mercury during digestion. Moreover, for high mercury concentrations in sugar, close to the maximum permissible contents derived from the provisional tolerable weekly intake recommended by WHO (0.1 mg kg^{-1} dry matter; Section 1) it is advisable to use the method yielding values in excess to ensure that the maximum tolerated intake of the metal is not exceeded.

3.3. Performance characteristics of the DPASV procedure

The limit of detection of the proposed procedure for mercury in sugar samples was calculated as $\bar{x}_b + 3\sigma_b$, where $\bar{x}_b$ and σ_b are the average concentration and the standard deviation, respectively, of five determinations of a blank of 80 g l^{-1} extra-pure saccharose. The calculated value was 0.5 μg kg^{-1} sugar.

The precision and repeatability of the procedure were investigated by measuring the concentration of mercury in three different samples of refined sugar. Five replications were carried out and average values ($\bar{x}$), their confidence intervals ($ts/n^{1/2}$) and the relative standard deviations ($100s/\bar{x}$ were calculated (Table 3). The low standard deviations obtained ($<10\%$) indicate the good repeatability of the procedure. It can thus be concluded that low levels of mercury can be directly determined in untreated sugar solutions by DPASV.

3.4. Determination of trace metals in refined beet sugar samples

The proposed procedure was applied to the determination of mercury in 10 refined sugar samples supplied

Table 1
Determination of Hg in standard solutions of 80 g l^{-1} extra-pure saccharose by FIMS (digested samples) and DPASV (non-digested samples)

Hg added/μg kgM^{-1}	Hg measured/μg kg^{-1}	
	FIMS (digested)	DPCSV (non-digested)
0	0	0
10	8	10
20	17	20
30	25	34
40	34	45
50	42	55

Table 2
Investigation of the occurrence of systematic errors by linear regression of the analytical procedures tested ($F_{(1,4,P=0.05)}$ is 7.709; $t_{(4,P=0.05)}$ is 2.776)

Regression parameter	FIMS (digested)	DPASV, (undigested)
Slope ($p\pm ts_p$)	0.846±.019	1.126±0.079
Intercept ($q\pm ts_q$)	−0.143±0.588	−0.809±394
Determination coefficient (R^2)	0.9997	0.9974
Variance ratio (F; linearity test)	14 603	1563

Table 3
Repeatability of DPASV determination of mercury in untreated sugar samples ($n=5$)[a]

Sample	Hg concentration (μg kg^{-1} sugar)	RSD (%)
a	1.2±0.10	6.7
b	0.9±0.06	5.6
c	0.6±0.06	8.3

[a] Value of the Student's variable t for 4 degrees of freedom and $P=0.05$ is 2.776.

by different Spanish sugar beet refineries. In seven samples, the average mercury concentration ($n = 5$) was below the detection limit of the procedure. Only in three samples was mercury detectable, but its concentration was lower than 2 μg kg^{-1} (Table 3).

Finally, it can be deduced from these results that total mercury contents in refined white sugar produced in Spanish sugar refineries is much lower than the maximum permissible contents derived from the provisional tolerable weekly intake recommended by the WHO (estimated in 0.1 mg kg^{-1} ingested dry matter).

Acknowledgements

The authors wish to thank the Centro Tecnológico Azucarero (CTA) and Azucarera Ebro Agrícolas S.A. for financial support, and the Instrumental Techniques Laboratory (LTI) of the University of Valladolid for allowing us to use the FIMS instrument.

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