

than by other previously used preparation techniques. Also, because exposure to containers and the environment is minimal in preparation B, contamination is minimal. Because of these factors, this preparation is preferred to preparation A as a method for orange juice.

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Determination of Lead, Cadmium, and Zinc in Sugar

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A new technique for eliminating matrix interference in the determination of lead, cadmium, and zinc in sugars by flameless atomic absorption is described. Yeast fermentation converts the sucrose to ethanol and carbon dioxide, both of which are easily volatilized. Reproducible results for these elements are obtained with this technique. Relative standard deviation for lead and cadmium was less than 10%, and for zinc less than 17%. Using this yeast fermentation technique, it was found that all three elements were present in raw and refined sugars at levels well below limits recommended by regulatory agencies.

Interest in the levels of heavy metals in various food products has increased greatly in the last few years. As analytical techniques become more sensitive, lower limits for toxic metals are often established and it becomes necessary to reevaluate many products. Atomic absorption spectrophotometry has been the method of choice for these studies. Manning (1973) reported the analysis of lead in milk using the graphite furnace and a single point calibration method. Huffman and Caruso (1974) used a tantalum ribbon and a carbon rod atomizer to determine lead in milk. Roschnik (1973) used flame atomic absorption to determine lead in ashed foods. Several studies of heavy metal constituents in molasses and other sugars have been conducted. Morriss and Nicol (1974) reported a direct atomic absorption method for trace constituents in molasses, using flame techniques and the method of additions. They successfully analyzed for copper, zinc, cadmium, lead, and iron at the high levels present in molasses. In 1972, Pommez and Clarke (published 1975) reported the results of a survey of trace elements in a raw

and a refined sugar. The technique was extended by Clarke et al. (1973) to cover raw and refined sugars and the refinery liquors after various stages of the refining process. The graphite furnace was used to determine a number of heavy metals in sugar solutions and in refinery liquors. No matrix interferences were observed for iron, copper, manganese, chromium, and silver; however, a serious interference, attributed to the organic matrix in which the element was originally present, was encountered for lead. Yeast fermentation of the sugar to eliminate this matrix interference is reported in this paper.

EXPERIMENTAL SECTION

Apparatus. The equipment consisted of a Perkin-Elmer Model 306 atomic absorption spectrophotometer, equipped with the HGA 2000 graphite furnace, a Model 56 recorder, and a deuterium background corrector. Details of the graphite furnace have been given by Kahn (1973).

Reagents. Standard solutions were prepared fresh daily from 1000-ppm commercial stock solutions. The sugars were obtained from the refinery, along with process liquors from various stages of the refining process. The liquors were all about 60% solids by weight.

Procedure. The yeast fermentation technique of Roberts and Rowland (1969) was used to break down all

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Table I

| Operating conditions | Element | | |
|----------------------------|------------------|-----------------|-----------------|
| | Pb | Cd | Zn |
| Dry | 30 sec at 100°C | 30 sec at 100°C | 30 sec at 100°C |
| Char | 60 sec at 400°C | 60 sec at 400°C | 60 sec at 400°C |
| Atomize | 10 sec at 2000°C | 8 sec at 1600°C | 8 sec at 2000°C |
| λ , nm | 217.7 | 228.8 | 214.0 |
| Sensitivity, pg/0.0044 abs | 8 | 0.9 | 0.9 |

of the sucrose to carbon dioxide and ethanol, both of which would be removed prior to the charring step. Approximately 5 g of sugar (10 g of sugar liquor) was weighed into a 150-ml beaker. The sugar was dissolved in about 35 ml of deionized water, and the pH adjusted to 4.5–5 with 10% acetic acid. About 0.25 g of baker's yeast was added to each solution. The mixtures were placed in a 40°C oven overnight to complete fermentation. The solutions were transferred quantitatively to 50-ml volumetric flasks and brought to volume with deionized water. Yeast was removed by centrifugation and the clear supernatant decanted. Yeast blanks, containing no sugar, were also prepared.

The operating conditions used to determine each element are given in Table I. The deuterium background corrector was used. Spectral band width was 0.7 nm, and nitrogen was the purge gas. Except in the cadmium determination, nitrogen flow was not interrupted during the atomization stage.

The concentration of metals in the sugar samples was determined both by the method of additions and from a calibration curve prepared using aqueous standards. At least three determinations were made on each sample.

Sample standard deviation and relative standard deviation were calculated by standard statistical equations found in Laitinen (1960).

Recovery levels were determined by adding known amounts of lead, cadmium, and zinc prior to yeast fermentation. The average recovery was found to be $97 \pm 7\%$ (mean \pm standard deviation).

RESULTS AND DISCUSSION

In the determination of metals using the graphite furnace for flameless atomic absorption, the sample of 5–50 μ l is injected into a graphite tube with an Eppendorf microliter pipet. The sample is then successively dried, charred, and atomized. The difficulties encountered in determining lead, previously reported by Clarke et al. (1973, 1974), were twofold. First, the results were surprisingly high and exhibited little variation from the raw sugar through the various refining stages to the refined sugar. The values obtained for one such series of sugars ranged from a low of 0.51 ppm to a high of 0.87 ppm of lead. No trend was observed. Second, when the method of additions was used, practically no increase in absorbance was observed, even when fairly high levels of lead were added.

Lead, cadmium, and zinc are very volatile metals and the charring temperature must be kept below 500°C to avoid loss of element. As a consequence, the organic matrix was not completely destroyed during the charring stage, and the deuterium background corrector could not entirely compensate for the large amount of smoke from the residual organic matter. Attempts to eliminate background absorption by varying the charring and atomizing conditions and the pH of the solution were unsuccessful.

Table II. Amounts Found^a

| Sugar | Lead | | Standards | | Cadmium | | Zinc | |
|-----------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| | Additions | Standards | Additions | Standards | Additions | Standards | Additions | Standards |
| | ppm | rsd, % | ppm | rsd, % | ppm | rsd, % | ppm | rsd, % |
| Raw | 0.102 | 4.5 | 0.089 | 6.0 | 0.012 | 9.2 | 0.100 | 5.9 |
| Liquor A ^b | 0.030 | 6.7 | 0.023 | 5.0 | 0.008 | 5.3 | 0.010 | 16.1 |
| Liquor B ^b | 0.026 | 4.1 | 0.023 | 8.3 | 0.002 | 7.7 | | |
| Refined | 0.034 | 3.2 | 0.030 | 2.4 | 0.002 | 2.0 | | |

^a A minimum of three replicate determinations on each sample. ^b Liquor A is from an earlier stage in the refining process than liquor B.

Suppression of the lead signal by organic matrix was observed by Huffman and Caruso (1974) in the analysis of lead in evaporated milk. These workers were unable to quantify the lead loss and used a pre-ashing step to remove the organic matrix. They postulate that the lead is lost mechanically during destruction and volatilization of the organic matrix during the ashing step. Manning (1973) did not report similar difficulties in his study of lead in milk. Morris and Nicol (1974) did report that the atomization signal in lead determinations was suppressed due to the presence of the molasses matrix and stated that a pre-ashing step appeared necessary. Although they were able to determine lead, cadmium, and zinc directly on molasses solutions using the flame and the method of additions, the detection limits were quite high (0.2 ppm for cadmium and zinc and 0.4 ppm for lead). Detection limits for the determination of lead, cadmium, and zinc using the graphite furnace are much lower. Kahn (1973) gives them as 1×10^{-6} μ g/ml for cadmium, 6×10^{-5} μ g/ml for lead, 6×10^{-7} μ g/ml for zinc based on 100- μ l samples.

The results obtained on a raw sugar, two processing liquors, and a refined sugar analyzed for lead, cadmium, and zinc are summarized in Table II. The table shows the concentration of element and the relative standard deviation (rsd) for each sample. For lead, results for the method of additions were 10 to 20% higher than those from the calibration curve from aqueous standards.

For the cadmium determinations, the same results were obtained from the method of additions and the calibration curve. The relative standard deviation was less than 10%.

The relative standard deviation was slightly higher for the zinc determination, the maximum being 16% and the minimum 6%. Absorbances of the liquor from a more advanced stage of the refining process (liquor B) and the refined sugar were below that of the blank. These are reported as having no detectable zinc. Like cadmium, the same results were obtained from the method of additions and the calibration curve using aqueous standards.

The levels of lead, cadmium, and zinc in a total of 16 different sugars and sugar liquors from three refineries which were determined utilizing this fermentation technique were reported by Clarke et al. (1974). In all cases, the amounts of lead and cadmium were found to be well below any levels of concern, either to the industry or regulatory agencies. The nutrient metal zinc (15 mg U.S. Recommended Daily Requirement) is also at low levels in refined sugar, from below 0.001 to 0.2 ppm.

CONCLUSION

The fermentation technique described produces a solution which is relatively free from interferences in the determination of lead, cadmium, and zinc in sugars by flameless atomic absorption. In the determination of lead, there is a slight interference which can be compensated

for by using the method of additions. In the determination of cadmium and zinc, no interferences were noted and comparison with aqueous standards is satisfactory.

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National Marine Fisheries Service Preliminary Survey of Selected Seafoods for Mercury, Lead, Cadmium, Chromium, and Arsenic Content

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A preliminary screening study was conducted on mercury, lead, cadmium, chromium, and arsenic contents of 34 commonly consumed seafoods. A total of 334 samples were analyzed and, in general, 10 samples per species were obtained from a location. More than 96% of the samples fell below the FDA guideline of 0.5 ppm for mercury, with a mean level of 0.13 ppm. Mean lead values among species tended to be rather uniform and only a few species averaged higher than 0.6 ppm. No species mean exceeded 1 ppm. Cadmium content of most seafoods averaged less than 0.2 ppm. Chromium was present at less than 0.4 ppm in all species. Arsenic levels showed the widest variation and were higher for some species than the other elements tested. Some possible relationships between elements within each species were encountered, but the data were too limited to draw firm conclusions.

Historically, the levels of trace elements found in foods have been reported and, with few exceptions, accepted as natural constituents of the foods. Within the past 30 years, however, evidence has been obtained to support the conclusion that the trace element content of certain foods, especially fish, is often directly related to man-induced wastes discharged into streams, lakes, and the oceans.

One of the first indications was provided in the early 1950's by Japanese scientists who reported the deaths of 43 people who had consumed fish caught in Minimata Bay. These fish were found to contain abnormally high levels of mercury which had been discharged into the Bay by a chemical manufacturing plant (Irukayama et al., 1961). Swedish scientists (Westoo, 1967) also noted the buildup of mercury in freshwater fish with the result that in the

1960's their Government banned the use of alkyl mercury in agriculture and restricted other uses of mercury.

In 1969 Canadian scientists (Wobeser et al., 1970) announced that some of their freshwater fish were contaminated with mercury. Subsequently, Lake St. Clair, then other lakes and areas in the St. Lawrence system, and a few scattered areas were closed to commercial fishing.

Later that year, an announcement was made to the press that some canned tuna fish was found to exceed the Food and Drug Administration's interim guideline of 0.5 ppm of methylmercury. Public concern in the United States mounted and this concern led inevitably to some misleading statements and erroneous allegations. It became clearly important that a study be performed to determine the trace element contents of fish consumed in the United States. Accordingly, this paper reports the mercury, lead, cadmium, chromium, and arsenic contents of frequently consumed seafoods.

SAMPLES

Sampling Plan. This survey was conducted to obtain data on mineral concentrations in fish eaten in the United States; therefore, no attempt was made to follow a formal statistical sampling plan. Preferably, the fish were to be collected over a narrow time span; however, all were not to be harvested at the same time.

Collectors at four National Marine Fisheries Service Technology Centers or Laboratories were requested to obtain eight lots of each of the seafoods assigned to them in quantities sufficient to yield eight 2-lb samples of edible

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