Heavy Metals in Whole Kernel Dent Corn Determined by Atomic Absorption[1]

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ABSTRACT

Whole kernel corn was wet-ashed with nitric and perchloric acids, and the resultant salts were dissolved in dilute acid. Concentration levels in corn samples were determined for zinc, manganese, copper, lead, cadmium, and chromium by flame atomic absorption. Because this technique is not sensitive enough to measure the small quantities of mercury present, an oxidation procedure was developed that decomposed organic material in corn at a temperature of 70°C. The mercury was retained effectively in an aqua regia solution until the elemental mercury was de-emanated for measurement of the vapor by a nonflame atomic absorption technique. The aqua regia solution ensured that mercury was completely solubilized. In addition, mercury standards prepared in a 10% aqua regia medium have proved to be stable at low concentrations for extended periods. Mean concentration values for the seven metals studied in 11 different corn samples ranged from a high of 23 γ per g. for zinc to a low of approximately 0.0024 γ per g. for mercury.

Corn provides food for man and feed for animals. Although its major market is livestock feeding, human consumption has increased, especially with the introduction of new breakfast foods and snack items. Indirectly through meat products and more directly through cereal products, mineral constituents inherent in corn are important nutritionally. Also significant are minor metal elements that occur in corn grain in the parts-per-million range and that serve beneficially as micronutrients. Such metals as lead, mercury, and cadmium have well-known toxic effects in the body. Reliably measuring the concentration of seven trace metals in whole kernel corn involved the development of decomposition methods so that individual elements in corn samples could be determined by either sensitive flame or nonflame atomic absorption.

Breck (1) reviewed the application of atomic absorption for the analysis of various elements in plant tissues. In extensive studies wheat, flour, bread, and millfeeds were analyzed for mineral constituents (2,3). Zook et al. (4) used atomic absorption and colorimetry to measure magnesium and eight trace mineral elements in wheat, wheat products, flours, and flour products. They dry-ashed 55-g. samples before they extracted the minerals with HCl. With shredded wheat, they reported respective recoveries for lead and cadmium of 89 and 92%. With white flour they recovered 90% of the tin. Losses of approximately 10% could result from volatilization of the metals in dry-ashing procedures as reported by Gorsuch (5) and by Russell (6).

The most critical phase in determining heavy metals occurs in the decomposition of the corn sample. Organic material must be destroyed but without any loss of the trace metal to be determined. Moreover, because of the small quantities of lead, cadmium, chromium, and mercury in corn and because of the ever-present small quantities of these same elements in the environment, contamination of a sample from external sources is also a major problem.

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Cadmium has been analyzed in foods of plant and animal origin (7), and chromium, in plant and biological materials (8) by atomic absorption after wet oxidation. Frei (9) determined copper, cobalt, and nickel in oats by thin-layer chromatography (TLC) but also after wet-ashing samples. To determine the mineral constituents in corn and wheat germ a wet-ashing procedure was coupled with atomic absorption (10,11).

In our latest work, decomposition of intact whole kernel corn was preferred so as to measure inherent quantities of the metals and also to avoid contamination from grinding apparatus. Grinding and other pretreatment processes tend to elevate the metal values of samples and blanks. Our approach was to measure the concentration of six metals by flame atomic absorption after wet-ashing 10-g. samples of whole kernel corn with nitric and perchloric acids. However, flame atomic absorption is not sensitive enough to measure concentrations of mercury that occur in corn. Therefore, for mercury we developed a decomposition procedure wherein 10 g. of whole kernel corn was ashed in nitric acid, followed by digestion in aqua regia at a temperature of 70° C. Mercury determination was then made by sensitive nonflame atomic absorption that measured the elemental mercury vapor.

To determine total mercury in pulp and paperboard, Lee and Laufmann (12) used a wet-oxidation procedure wherein aqua regia was reacted with 1-g. samples to break down inorganic forms of mercury, as well as organic mercurials, to form soluble mercuric compounds. In another wet-oxidation procedure for mercury, Malaiyandi and Barrette (13) decomposed cereal flours (corn, oats, and wheat) with a sulfuric-nitric mixture in the presence of small quantities of vanadium pentoxide. In other methods for the determination of mercury in food products (14–16), including grain and wheat samples, decomposition was accomplished with nitric, sulfuric, and perchloric acids before the mercury was measured by flameless atomic absorption.

MATERIALS AND METHODS

Corn Samples

Six whole kernel corn samples (approximately 1 lb. each) originating from six different locations were submitted in plastic bottles by the American Corn Millers Federation through A. W. Kleinschmidt, J. R. Short Milling Co., Kankakee, Ill. Five more yellow corn samples examined represented five varieties, all different from the dent samples supplied by the Federation. Two samples originated from corn breeders in Illinois, two came from the University of Illinois, Urbana, and the fifth came from the Peoria area.

Apparatus

Flame atomic absorption measurements for zinc, manganese, copper, lead, cadmium, and chromium were made with a Varian Techtron AA120 atomic absorption spectrophotometer with an acetylene-air combustion mixture. Single-element hollow cathode lamps were used at the recommended current rating. We could have also used this same instrument to measure mercury by a nonflame atomic absorption technique, but we decided against converting it from one technique to another periodically. Instead, we used a Coleman MAS-50 mercury analyzer with a 14.5-cm. pathlength absorption cell. Absorption data

were recorded on a 10-in. Beckman recorder. For mercury aeration vessels, borosilicate glass, 300-ml. BOD bottles were selected as a group to conform to better than a 1% volume tolerance. Previously decomposed corn samples and standards were transferred to individual BOD bottles to get rapid, successive absorption measurements.

Reagents

Reagent-grade chemicals were used in the preparation of solutions, and double-distilled water was used for all dilution purposes as mentioned in the procedures.

Distilled Nitric Acid. Concentrated nitric acid was distilled from an all-glass, two-piece apparatus, composed of a Friederichs condenser and a 3-liter, flat-bottomed flask connected by a standard taper joint. A similar, all-glass apparatus served to redistill distilled water.

Stannous Chloride Solution. To 50 g. $SnCl_2 \cdot 2H_2O$ was added 100 ml. concentrated HCl; the mixture was warmed to dissolve the stannous chloride, and finally diluted with water up to 500 ml.

Aqua Regia Solutions. Two concentrations of aqua regia solutions were prepared. In a hood, 250 ml. distilled concentrated HNO_3 was cautiously mixed with 250 ml. concentrated HCl. After the evolution of gases subsided, 500 ml. water was added, and this stock solution was designated 50% aqua regia. A more dilute 10% aqua regia solution was prepared, as needed, from this stock solution.

Antifoam Solution. To 33 g. of Dow Corning antifoam "B" was added 100 ml. water with stirring. This prepared solution was always shaken before use.

Calibration and Standardization

For Flame Atomic Absorption. Solutions were prepared immediately before

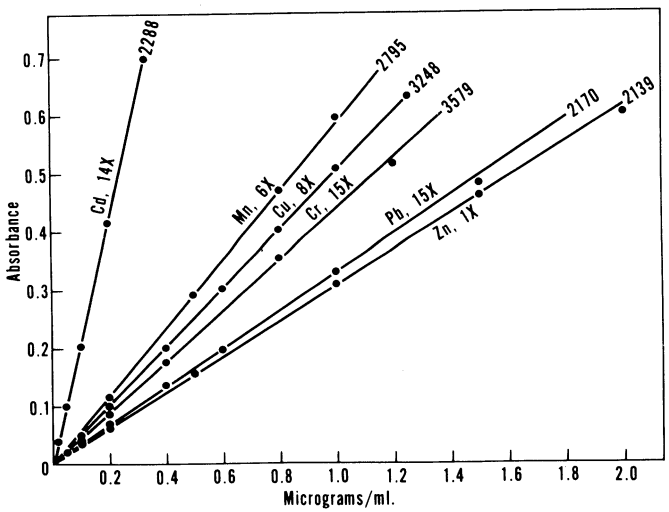

Fig. 1. Atomic absorption calibration curves for individual elements at specified wavelengths and scale expansion.

standardization by diluting metal standard stock solutions (containing 1 mg. metal per ml. in dilute acid medium) to the appropriate concentrations. Concentration ranges covered for the individual elements are indicated by calibration curves (Fig. 1) at specified conditions of wavelength and scale expansion. Atomic absorption data were acquired by aspirating aqueous single-element standard solutions before and after aspirating the ashed corn sample solutions. Both standard and sample solutions were similar in acid concentration.

A hydrogen continuum lamp can be used to identify nonatomic absorption interferences associated with the sample matrix, especially for lead and cadmium analysis. For lead, where the presence of nonatomic interferences are demonstrated in complicated sample matrices, a more reliable reading can be made at the less sensitive 2833-nm. resonance line.

For Mercury by Nonflame Atomic Absorption. To prepare a stock mercury standard solution containing 1 mg. Hg per ml. in 10% aqua regia, 0.1362 g. of mercuric chloride (99.4% pure) was dissolved in 20 ml. 50% aqua regia and finally diluted to 100 ml. with water. Working standards were prepared by successively diluting this stock mercury solution with 10% aqua regia to yield appropriate concentrations of mercury. Increments of a standard solution (containing 0.1 γ Hg per ml.) were transferred to 250-ml. Phillips beakers and all reagents normally added to samples were added simultaneously to the standards. Standards were carried through the entire procedure and they covered the range of 0.1 to 1.6 γ Hg with resultant readings ranging from about 5 to 60% absorption. Absorption values were derived from peak height values.

Although decomposition of corn does not produce a completely clear solution, differences between absorption values for standards and spiked corn samples were quantitatively insignificant. The quantity of antifoam solution added suppressed occasional foaming and yet did not substantially reduce the evolution rate of mercury from the aqueous phase. Concentrated HNO_3 was distilled as previously mentioned, but reagent-grade HCl and stannous chloride were not purified further. Reagent blanks containing these three reagents indicated no measurable quantity of mercury.

Mercury standard solutions in 10% aqua regia medium indicated no mercury adsorption on the walls of Pyrex storage containers, even down to concentrations of 1 ng. Hg per ml. Investigations (17,18) on the stability of dilute mercury

TABLE I. FLAME ATOMIC ABSORPTION OPERATING CONDITIONS

Element	Sample Dilution Factor[1]	Resonance Line λ	Scale Expansion	Sensitivity Attained[2]
Zinc	8×	2139	1×	0.0185
Lead	None	2170	15×	0.0173
Cadmium	None	2288	14×	0.0027
Manganese	8×	2795	6×	0.0097
Copper	None	3248	8×	0.0113
Chromium	None	3579	15×	0.0130

[1]Further dilutions when original sample volume was 25 ml.
[2]Microgram/milliliter/1% absorption.

solutions demonstrated that containers adsorbed extensive amounts of mercury. Coyne and Collins (17) determined that nitric acid at pH 1 was an effective preservative, but that the sample had to be acidified as it entered the container.

Procedures

For Six Metals by Flame Atomic Absorption. To measure effectively the content of six metals (Zn, Mn, Cu, Pb, Cd, and Cr) in corn, at least 10 g. is required. The sample was weighed in a 250-ml. Phillips beaker and 40 ml. distilled concentrated HNO_3 was added to begin a 2-hr. decomposition at room temperature. The Phillips beaker with a glass cover still intact was then placed on a warm hot plate to commence wet-ashing. With a minimum application of heat, an exothermic reaction occurs which is characterized by the presence of oxides of nitrogen; but the heat so generated can be moderated effectively by the quantity of acid present. About 35 min. later the reaction subsides and heat is increased so that the hot plate maintains concentrated HNO_3 (a reagent blank) at approximately 70° C. Heating was continued at this temperature overnight, then 2 ml. 70% $HClO_4$ was added to the cooled solution, and heating was resumed until a clear solution appeared. As all HNO_3 eventually evaporated, fumes of $HClO_4$ appeared; heating was maintained until ashing was complete. The $HClO_4$ was then removed by evaporation.

The residue was treated with 5 ml. concentrated HCl and the acid was refluxed in the beaker; an equal volume of water was then added with subsequent evaporation to dryness. This refluxing process with concentrated HCl followed by evaporation to dryness was repeated. Finally, 1.0 ml. concentrated HCl was added and the mixture was warmed briefly; then 15 ml. water was added and the solution was heated for about 15 min. The cooled solution was transferred to a 25-ml. volumetric flask, made up to volume at room temperature, and transferred to a 60-ml. polyethylene storage bottle. Atomic absorption measurements were then made for each individual element along with the appropriate standard solutions (Table I).

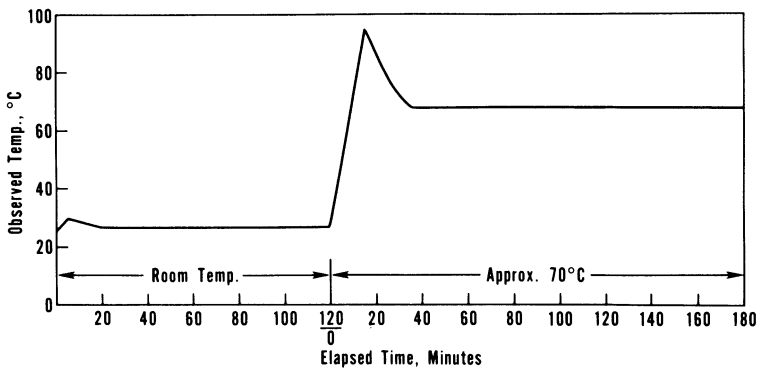

Fig. 2. Temperatures of corn samples (10 g.) in a 250-ml. Phillips beaker treated with concentrated nitric acid (20 ml.) at room temperature and at 70°C. Exothermic reaction subsides after 35 min. of heating.

For Mercury by Nonflame Atomic Absorption. Approximately 10 g. of whole kernel corn was transferred to a 250-ml. Phillips beaker and 20 ml. distilled concentrated HNO_3 was added to start a 2-hr. decomposition at room temperature. The Phillips beaker with a glass cover, still intact, was placed on a hot plate as previously. After the characteristic exothermic reaction (depicted in Fig. 2) subsided, heating was continued at 70° C. for 3 hr. more to oxidize the sample with the nitric acid. The beaker was allowed to cool before 20 ml. concentrated HCl was added. This aqua regia mixture was heated overnight at 70° C. Water (20 ml.) was added and the mixture heated again at 70° C. for 8 hr. The cooled sample was transferred quantitatively with water to a previously weighed 300-ml. BOD bottle. Water was then added to the bottle until the combined weight of the bottle and sample was 90 g. more than the empty BOD bottle. The bottle was stoppered and stored. Mercury was retained quantitatively in solution in this condition for extended periods with no adsorption losses.

The stopper was removed and 2 drops of the specially prepared antifoam solution was added and mixed by swirling the sample solution. Stannous chloride solution (10 ml.) was rapidly added with a pipet; the stream was directed at the side of the bottle above the liquid level to prevent premature mixing. The aerator bubbler on the mercury analyzer, continually circulating air, was immediately transferred from another BOD bottle, containing only double-distilled water, and submerged in the sample solution. The elemental mercury was swept from the solution (approximately 100 ml.) into the absorption cell and the resultant absorption was recorded as a peak on the recorder. More than 50 previously decomposed samples, plus mercury standards, were measured daily.

RESULTS AND DISCUSSION

In the analysis of heavy metals, extraneous metals can be introduced from two general sources: a) from a contaminated environment or apparatus, and b) from reagents. To offset these external sources of metals, the number of procedural operations must be reduced. Few reagents should be used and glass apparatus in contact with the sample should be kept to a minimum. Sample preparation steps should be eliminated or at least reduced. Also, the reagents must be purified as required so as to lower the quantity of metals so introduced.

Proper selection of decomposition procedures precludes losses of metals through volatilization and by retention of the metal on the surface of any container used for ashing. In our work with whole kernel samples, decomposition was by wet-oxidation at temperatures about 70° C. to eliminate losses by volatilization. During the exothermic reaction previously described, a maximum temperature of 95° C. was attained (Fig. 2). With a single sample of 10 g., a quantity not too unwieldy for acid-digestion techniques, six of the metals were determined by flame atomic absorption using scale expansion techniques as needed. For mercury, a separate 10-g. corn sample was required to attain the sensitivity needed to measure the very small concentrations of mercury. For the metals in corn studied here, the end result of all this work has been a definitely recognizable and reproducible difference in magnitude between sample and blank.

TABLE II. HEAVY METAL CONTENT OF WHOLE KERNEL CORN SAMPLES[1] OF KNOWN GEOGRAPHIC ORIGIN (γ per g., d.b.)

Sample Type and Origin	Zinc	Manganese	Copper	Lead	Chromium	Cadmium	Mercury
White							
Texas	18.7 ± 0.7	7.66 ± 0.96	1.96 ± 0.11	0.299 ± 0.011	0.065 ± 0.017	0.061 ± 0.009	0.0018 ± 0.0008
Illinois	23.6 ± 1.1	4.82 ± 0.79	1.87 ± 0.10	0.340 ± 0.020	0.064 ± 0.006	0.042 ± 0.004	0.0018 ± 0.0003
Indiana	27.2 ± 1.8	7.43 ± 0.99	1.91 ± 0.10	0.287 ± 0.024	0.068 ± 0.001	0.035 ± 0.003	0.0035 ± 0.0028
Yellow							
Iowa	22.3 ± 0.9	7.30 ± 2.52	1.99 ± 0.09	0.267 ± 0.017	0.155 ± 0.030	0.065 ± 0.019	0.0062 ± 0.0002
Kansas and Missouri	24.7 ± 0.8	6.97 ± 0.80	1.74 ± 0.10	0.198 ± 0.029	0.061 ± 0.006	0.148 ± 0.018	0.0027 ± 0.0017
Illinois	21.6 ± 0.6	4.52 ± 0.50	1.81 ± 0.04	0.254 ± 0.030	0.025 ± 0.029	0.039 ± 0.009	0.0020 ± 0.0013
Mean	23.0	6.45	1.88	0.274	0.073	0.065	0.0030

[1]Submitted by the American Corn Millers Federation and representing different geographic locations.
[2]Mean and standard deviation for the six different (10 g.) corn samples.

TABLE III. RECOVERY OF METALS FROM SIX CORN SAMPLES[1] SPIKED WITH SEVEN HEAVY METALS

Metal	Spike Data		Mean and Std. Dev. of Spike Recovery[2] γ/g.
	Metal Added/ 10 g. Corn γ	Equivalent Concentration γ -g.	
Zinc	100.0	10.0	9.8 ± 1.9
Manganese	50.0	5.0	5.0 ± 0.8
Copper	5.0	0.50	0.52 ± 0.17
Lead	5.0	0.50	0.50 ± 0.10
Chromium	5.0	0.50	0.57 ± 0.03
Cadmium	5.0	0.50	0.53 ± 0.06
Mercury	1.0	0.10	0.12 ± 0.01

[1]Submitted by the American Corn Millers Federation and representing different geographic locations.
[2]Mean and standard deviation for the six different (10 g.) corn samples.

Heavy Metal Analysis

Concentrated HNO_3 oxidizes most of the corn, and it also converts organomercurials to an inorganic form. After the addition of concentrated HCl, the oxidative process continues but not so vigorously as with the preliminary treatment with HNO_3 only. This aqua regia solution ensures complete solubilization of the mercury salts. Addition of water (20 ml.) to the aqua regia mixture and subsequent heating eliminates oxides of nitrogen. Their presence in the absorption cell when the mercury vapor is aerated will give erroneous absorption readings. Mercury is retained effectively in this aqua regia solution, even for extended periods, until it is reduced chemically for aeration of the vapor, which is measured by nonflame atomic absorption. Under the specified conditions a 1% absorption reading is equivalent to approximately 1.5 ng. Hg per g. corn.

To 10-g. samples of a previously analyzed whole kernel corn (containing approximately 1 ng. Hg per g.) increments of mercury standard solutions were added so as to represent concentrations in the corn ranging from 20 to 160 ng. Hg per g. These spiked samples were then analyzed by the proposed procedure to verify its reliability. Absorption data collected were then converted to absorbance values (Fig. 3). The linear response indicated that the method was effective in measuring the mercury present.

A series of whole kernel corn samples submitted by the American Corn Millers Federation represented both white and yellow varieties and originated from an area covering six states. Analyses clearly established the concentration levels of seven metals (Table II) with no major variations between different types of corn. The mean concentration values ranged from a high of 23.0 γ per g. for zinc down to a low of 0.0030 γ per g. for mercury.

Each of the submitted whole kernel corn samples was also spiked with aqueous standard solutions of the seven metals studied. The spiked samples represented four levels of concentration with equivalent spike concentrations in the corn of:

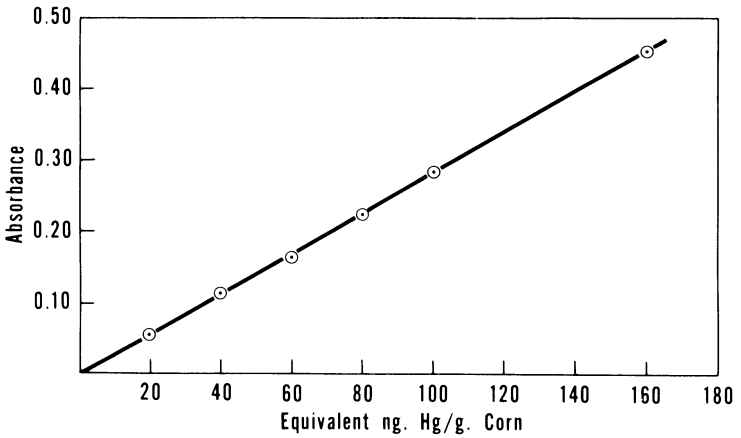

Fig. 3. Absorbance curve for increments of mercury added to 10 g. of corn. Corn sample itself contained about 1 ng. Hg per g. corn.

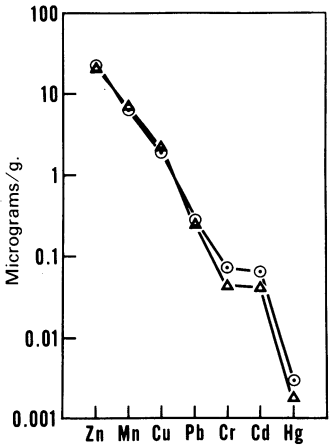

Fig. 4. Comparison of mean heavy metal concentrations of whole kernel corn. Circles indicate six corn samples submitted by American Corn Millers Federation; triangles represent five other varieties.

TABLE IV. MEAN HEAVY METAL CONCENTRATION VALUES FOR 11 WHOLE KERNEL CORN SAMPLES[1]

Element	Concentration γ /g., d.b.	
Zinc	22.9 ± 2.2	(18.7-27.2)
Manganese	6.60 ± 1.10	(4.52-7.78)
Copper	2.08 ± 0.45	(1.65-3.10)
Lead	0.271 ± 0.039	(0.198-0.340)
Chromium	0.060 ± 0.035	(0.025-0.155)
Cadmium	0.055 ± 0.032	(0.035-0.148)
Mercury	0.0024 ± 0.0017	(0.0005-0.0062)

[1]Mean and standard deviation for six samples of known geographic origin plus five other varieties. Concentration ranges are given in parentheses.

10 γ Zn per g.; 5.0 γ Mn per g.; 0.50 γ Cu, Pb, Cd, and Cr per g.; and 0.10 γ Hg per g. All the same analyses and procedures carried out on the other corn samples were carried through on these spiked samples. Recoveries (Table III) show no evidence of losses of these metals. The recovery of chromium was slightly higher than expected.

Metal concentration values for the corn samples reported here were also compared with those from five different whole kernel varieties in another study we conducted. For each of the seven metals determined, the mean concentration values for both groups of samples were plotted on a logarithmic scale so as to compare all values in their respective concentration ranges (Fig. 4). For both groups of samples, the results were generally the same for zinc, manganese, copper, and lead. The values for cadmium, chromium, and mercury were slightly higher for the six corn samples submitted by the American Corn Millers Federation. Table IV lists the mean and standard deviation of the concentration values for the seven metals in the 11 different samples.

CONCLUSION

Seven heavy metals inherent in whole-kernel corn can be effectively determined in the parts per million to parts per billion concentration range, provided appropriate wet-oxidation procedures are used. Flame atomic absorption was satisfactory to measure six metals, but a nonflame technique was required to measure the low concentrations of mercury.

Acknowledgment

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