Determination of Trace Metals in Foods Using Chelating Ion Exchange Concentration

Richard A. Baetz* and Charles T. Kenner

An improved procedure based on the Baetz and Kenner (Baetz, R. A., Kenner, C. T., *J. Agr. Food Chem.* 21, 436 (1973)) multimetal method which eliminates the necessity for the separate analysis of the neutralization precipitate is reported. The sample is digested in HNO_3–H_2SO_4–H_2O_2 catalyzed by V_2O_5 and the lead is coprecipitated with added strontium, filtered, converted to carbonate, dissolved, and determined before neutralization of the digest and column separation of the other metals. Cd, Cu, Co, Mn, Ni, and Zn are eluted from the column with 2 *N* H_2SO_4 and determined by atomic absorption. Sensitivity varies from 10 ppb for Cd to 100 ppb for Co and recoveries of added standards to eight food commodities averaged 97.2%. Cobalt recoveries are lower for certain types of foodstuffs. Values obtained by the proposed method on NBS standards 1571 (orchard leaves) and 1577 (bovine liver) agreed with the accepted correct concentrations. The heavy metal content of eight representative commodities has been determined.

Trace analysis of heavy metals in foods has become increasingly important in medical, ecological and pollution studies due to the toxicity of heavy metals and lack of information as to normal levels in various foodstuffs. Reported average concentrations of many toxic metals are very low (Underwood, 1971) and in many cases have been determined by methods which were not ideally suited for trace quantities. Screening methods which are sensitive, precise, and accurate are needed to determine several metals in a single sample charge of a wide variety of foods.

Previously published methods (Langmyhr *et al.*, 1974; Childs and Gaffke, 1974; Baetz and Kenner, 1973; Schramel, 1973; Gajan *et al.*, 1973; Holak, 1973; Gish and Christensen, 1973; Marks *et al.*, 1972) for more than one metal in various biological materials have used a variety of ashing, extraction, and concentration techniques together with photometric, atomic absorption, polarographic, and other methods of determination.

Both wet oxidation (digestion) and dry ashing techniques suffer from disadvantages (Gorsuch, 1970) for the determination of metals in foods. Losses during dry ashing may be caused by volatilization, adsorption on unburned carbon, and insoluble silicate formation (Thiers, 1957). The use of H_2SO_4 in the wet digestion of foods containing lead causes low results in the presence of large amounts of calcium due to coprecipitation. Hoover *et al.* (1969, 1972) overcame this problem by adding strontium to the digest solution to cause coprecipitation of the lead with strontium sulfate.

Separation and concentration techniques which have been used for trace metals include volatilization, electrodeposition, liquid–liquid extraction, precipitation, and ion exchange. The imino diacetate chelating resin (Chelex 100, Dowex A-1) has been used to separate and concentrate metals from solutions of high ionic strength such as sea water (Imoto, 1961), oil field brines (Collins *et al.*, 1962; Galle, 1971), industrial waste waters (Biechler, 1965), geological samples (Blount *et al.*, 1973), basic fusions of ore samples (Freudiger and Kenner, 1972), and digested food samples (Baetz and Kenner, 1973, 1974).

This paper is concerned with the improvement of the Baetz and Kenner (1973) method for trace amounts of Cd, Co, Cu, Mn, Ni, Pb, and Zn in various foods. The sample is digested with HNO_3, H_2SO_4, and H_2O_2 catalyzed by V_2O_5. Strontium is added to the sample before digestion to aid in the separation of lead. The sulfate precipitate resulting from dilution of the cooled digest is filtered, converted to carbonates, and dissolved in HNO_3, and the lead determined by atomic absorption. The filtrate from the lead separation and conversion is neutralized to pH 6.5 ± 0.5 and passed through a column of Chelex 100. The column is washed with $(NH_4)_2SO_4$ and eluted with 2 *N* H_2SO_4, and the eluted metals determined by atomic absorption. The proposed method is simpler, requires less time, and is more sensitive, precise, and accurate than the original method.

Food and Drug Administration, Dallas District, Dallas, Texas 75204 (R.B.), and the Department of Chemistry, Southern Methodist University, Dallas, Texas 75275 (C.T.K.).

EXPERIMENTAL SECTION

Reagents. Standards used included: stock, 10,000 ppm from the pure metals (Ventron Corp., Alfa Products, Beverly, Mass.) by dissolving in HCl, HNO_3, or H_2SO_4; intermediate, 1000 ppm (stock standard diluted with 1.6 *N* H_2SO_4 for all metals except lead; 2 *N* HNO_3 used for lead; stability approximately 3 months); working, 0–10 ppm (same acids specified for intermediate standards were used; prepared by dilution of intermediate standards at time of use and discarded after use).

Dowex A-1 resin (Chelex 100, 100–200 mesh, Na form) was from Bio-Rad Laboratories. Clark and Lubs pH 6.5 buffer was prepared by adding 140 ml of 0.100 *M* NaOH to 500 ml of 0.100 *M* KH_2PO_4 and diluting to 1 l. (Bower and Bates, 1955). The strontium solution (2% w/v) consisted of 6.00 g of $SrCl_2 \cdot 6H_2O$/100 ml. Ammonium carbonate solution (5% w/v) was prepared by adding 50.0 g of $(NH_4)_2CO_3$ to approximately 600 ml of H_2O in a 1-l. volumetric flask, mixing until dissolved, warming to room temperature, and diluting to volume. The ammonium sulfate solution (5% w/v) consisted of HNO_3 (J. T. Baker No. 5-9603; suitable for mercury determination) and H_2SO_4 (J. T. Baker No. 5-9685; suitable for mercury determination). Purified deionized water was prepared by passing ordinary deionized water through two I. W. T. Research Model I demineralizer cartridges (Arthur H. Thomas Co., No. 3923-D25) and then through a Puritan Model I cartridge (Arthur H. Thomas, No. 3923-D30). Necessary connections were made with Tygon tubing. This water was used for all procedures requiring water. All other chemicals were ACS reagent grade quality.

Glassware. All glassware used must be washed in 10% v/v HNO_3 and rinsed with deionized water. The same glassware was used for all analyses and was not cleaned by any other method or mixed with other equipment.

Apparatus. The Fisher filtrator (Fisher Scientific Co., No. 9-788) used must be free of corrosion. A Büchner filter funnel with fritted disk was used (Kimble No. 28400-30M). The ends were drawn out to permit entry into a 10-ml volumetric flask. The resin tube was prepared by fusing a 24/40 TS female glass joint to the upper end of a

2.5-cm i.d. Allihn filter tube with a 145–175-μm glass frit (Ace Glass Co., No. 7195-02). The eluent reservoir consisted of a 500-ml graduated cylindrical separatory funnel, supplied with Teflon stopcock plug and 24/40 TS male glass joint (Ace Glass Co., No. 7267-T). The flow rate was controlled with the stopcock. Alternatively, the apparatus described by Baetz and Kenner (1973) may be used in place of the resin tube and eluent reservoir. Absorption measurements were made with Perkin-Elmer Models 303 and 503 atomic absorption spectrophotometers equipped with deuterium corrector, Model 56 recorder, and Perkin-Elmer Intensitron Hollow Cathode lamps. Operating parameters were similar to those stated in the standard conditions section of the "Analytical Methods for Atomic Absorption Spectrophotometry" (1973).

Pretreatment of Resin. The Chelex-100 resin was pretreated as described previously (Baetz and Kenner, 1973).

Preparation of Column. The regenerated resin was placed in the filter tube to a depth of 1.0–1.2 cm (approximately 5-ml wet volume) and the separator attached. The column was equilibrated to pH 6.5 ± 0.5 by the addition of 1.0 ml of 1 *N* H_2SO_4 and 100 ml of pH 6.5 buffer. The sample was decanted into the separator and the flow rate controlled at 9 ± 2 ml/min. When the level of the liquid was just above the top of the resin bed, 200–300 ml of water and then 15 ml of 5% $(NH_4)_2SO_4$ were added. The effluent was discarded.

Procedure. *Caution.* Because of trace amounts of some metals of interest in the reagents used, equal amounts should be added to duplicates and reagent blank in all cases. Samples should be prepared in equipment which is free of contamination by the metals of interest.

Sample Digestion. A 50.0-g sample was weighed into a 1500-ml beaker together with 20–30 mg of vanadium pentoxide, 5 or 6 glass boiling beads, and 5.0 ml of strontium solution. Concentrated HNO_3 (100 ml) was carefully added to each and beakers were covered with watch glasses. Mixtures were heated on a hot plate with a gradual increase in temperature until the solutions reached a full boil. Any frothing which occurred was controlled by the addition of small amounts of H_2O. Mixtures were heated until oxide of nitrogen fumes were no longer evolved. Solutions were cooled to room temperature and the volume adjusted to about 200 ml with H_2O. To both sample and blank was added 20 ml of H_2SO_4 and this was evaporated until charring began. H_2O_2 (50%) was cautiously added dropwise to each mixture until the sample solution was clear and the same green color as the reagent blank. Since 50% H_2O_2 is a very strong oxidant, it was added slowly in small amounts to prevent frothing and splattering. The use of rubber gloves is recommended. For some commodities such as wheat, milk, and fish, it is advantageous to add additional HNO_3 just after charring begins and then continue digestion.

Milk. Since the relatively large amounts of calcium in milk interfere with the coprecipitation of lead with strontium sulfate, 10.0 ml of strontium solution was added instead of 5.0 ml, and then the regular procedure was continued.

Removal of Precipitate and Determination of Lead. Cooled digests were placed in an ice bath. After several minutes the sides were rinsed down with three 15-ml portions of H_2O (total 45 ml). The beakers were kept in the ice bath for at least 45 min and swirled occasionally. The Büchner filter funnel was fitted with a rubber stopper and attached to the Fisher Filtrator. Using a 400-ml beaker as a receiver, the solution and precipitate were transferred to the funnel. Vacuum was applied carefully and the solution allowed to filter slowly. The vacuum was turned off and the digest beaker rinsed into the funnel with 15 ml of chilled 1 *N* H_2SO_4. The vacuum was reapplied. The rinse procedure was repeated twice being sure the precipitate was transferred completely to the funnel. $(NH_4)_2CO_3$ solution (5%, 10 ml) was used to rinse down the sides of the funnel. Gentle vacuum was applied and the solution filtered slowly. $(NH_4)_2CO_3$ solution (30 ml) was added to the funnel and 25 min elapsed before applying vacuum. Addition and filtration of ammonium carbonate solution were repeated.

The beaker was removed from the Fisher Filtrator and saved for the determination of Cd, Cu, Mn, Ni, and Zn.

A 10-ml volumetric flask was placed in the filtrator under the tip of the funnel. The precipitate was dissolved with four separate 2.5-ml portions of warm 2 *N* HNO_3. The mixture was cooled to room temperature and diluted to volume with 2 *N* HNO_3.

Lead was determined at 283.3 or 217.0 nm against lead standards (0, 2.5, 5.0, 7.5, and 10.0 μg/ml) which contained 5.0 ml of 2% strontium solution/10.0 ml. Sample absorbances were corrected for the absorbance of the reagent blank.

Separation, Concentration, and Determination of Cd, Cu, Mn, Ni, and Zn. Three drops of 0.05% Methyl Red was added to the filtrate from the lead determination. The mixture was agitated carefully to offset frothing and spattering caused by neutralization of the carbonate and placed in an ice bath, and the pH was adjusted to yellow by careful addition of 50% NaOH. The mixture was cooled to room temperature and a pH meter was used to complete adjustment to pH 6.5 ± 0.5 with 1 *N* NaOH and/or 1 *N* H_2SO_4.

The filtrate was poured through the column and washed with 5% $(NH_4)_2SO_4$ as described under the section on column preparation. The filtrate was eluted with 2 *N* H_2SO_4, collecting the first 23–24 ml of eluent in a 25-ml volumetric flask, and was diluted to volume with 2 *N* H_2SO_4. The resulting solution was approximately 1.6 *N* in H_2SO_4 due to dilution by one bed volume of $(NH_4)_2SO_4$ solution. The absorbance for each metal *vs.* freshly prepared standards was determined and the concentration read from a standard curve or calculated from the absorbance of the nearest standard. Each standard and sample reading was corrected for the standard blank (zero concentration) and for the reagent blank. For metals such as zinc which are often present in high concentrations, serial dilutions of an aliquot of the sample with 1.6 *N* H_2SO_4 were prepared to bring the concentration into the measurable range.

METHOD DEVELOPMENT

In all elution studies the resin was equilibrated to the proper pH by passage of the buffer before addition of the sample, and the sample solution was adjusted to the same pH. The retention and elution of Cd, Co, Cr, Cu, Mn, Ni, and Zn were investigated at pH 6.5 ± 0.5 utilizing the proposed elution procedure. The retention of chromium was investigated further at various pH values using the proposed elution procedure with the exception of the pH of the column and the sample.

The capacity of the resin to retain divalent ions was investigated using the proposed procedure and standard solutions of Cu, Mn, and Zn which were adjusted to the proper pH and approximate ionic strength. The concentration of the metals was increased until the recoveries were significantly less than 100%.

The effect of extended use and regeneration of the resin by the proposed procedure on the capacity and elution characteristics was investigated. A resin which had been used for approximately 6 weeks and which had been regenerated at least 12 times by the proposed method was regenerated for 24 hr at 60° in 30% NaOH (Bio-Rad, 1974). Duplicate determinations of a solution containing a total of 7000 μg of Cu, Mn, and Zn before and after the elevated temperature regeneration were compared.

The recoveries of Cd, Co, Cu, Mn, Ni, and Zn at four different concentration levels of standards were determined utilizing the proposed procedure except for the ad-

dition of strontium and the removal of the $SrSO_4$–$PbSO_4$ precipitate.

The recoveries to be expected from the proposed method were investigated using standards containing Cd, Co, Cu, Mn, Ni, Pb, and Zn in the approximate average ranges found generally in foods. The recovery study was extended by addition of a similar standard to eight different food products.

Recovery of Pb by the procedure of Hoover *et al.* (1969) with minor modifications was investigated.

The effect of time on the completeness of precipitation of Pb and Sr sulfates was investigated during the determination of a sample of shredded wheat to which 2.0 ppm of Pb had been added. Three recoveries were run using the 45-min time in the proposed method and one determination was allowed to stand 48 hr before filtration of the precipitate.

The effect of varying amounts of strontium on the recovery of lead in milk by the proposed procedure was investigated using 3.0 ppm of added Pb.

Duplicate determinations were made for the seven metals using the proposed method on eight different food commodities and two NBS standards.

RESULTS AND DISCUSSION

All standard deviations listed in this paper were calculated from the range by the method of Dean and Dixon (1951), unless otherwise noted.

The investigation of the retention and elution of Cd, Co, Cr, Cu, Mn, Ni, and Zn from the smaller column used in the proposed procedure indicated quantitative recovery of all metals except Cr. The average recovery for the six satisfactory metals ranged from 93.2% for Cu to 102.1% for Cd with an overall average of 98.1% and an overall standard deviation of 0.84%. Only 38% of the Cr was recovered. This low recovery led to the investigation of the effect of pH upon the retention of Cr by the column. The recovery was 60.5% at pH 3.8 and 4.7, 54.9% at pH 5.8, and 7.7% at pH 7.0. In each case, the remainder of the Cr was found in the column effluent from the retention and wash steps. These values substantiate the work of Freudiger and Kenner (1972) who found similar retention and recovery of Cr using a chloride system in place of the sulfate system used in the proposed procedure.

The capacity of the amount of resin used in the proposed procedure was investigated using standard solutions containing Cu, Mn, and Zn at various levels. The average recovery was 97.6% for 73 mmol, 97.9% for 102 mmol, and 93.0% for 224 mmol. These values represent a total of 2300, 3200, and 6000 μg, respectively. Even though there is less recovery above 102 mmol the recovery is still satisfactory for 224 mmol or 6000 μg of total metal ions. This is approximately twice as great as the highest total metal content in any of the samples analyzed.

Since the resin may be used and regenerated at room temperature several times before being discarded or regenerated at elevated temperatures, it was deemed advisable to test a sample of resin which had undergone several regenerations before and after the elevated temperature regeneration recommended by the supplier. Average recoveries of a total of 7000 μg of Cu, Mn, and Zn were the same before and after the elevated temperature regeneration.

The effectiveness of the proposed procedure for different concentration levels was studied by running samples of standards through the procedure except for the addition of strontium and lead and the removal of the $SrSO_4$–$PbSO_4$ precipitate. The overall average recovery for all four levels for the six metals tested was 96.2%. The levels utilized were (in ppm): Cd (0.05, 0.06, 0.12, 0.24); Co (0.20, 0.60, 1.20, 2.40); Cu (0.25, 1.0, 2.0, 4.0); Mn (0.20, 1.0, 2.0, 4.0); Ni (0.20, 0.80, 1.60, 3.20); Zn (0.05, 5.0, 10.0, 20.0). These results are tabulated and will appear in the microfilm edition (see paragraph at end of paper regarding supplementary material).

Table I. Effect of Added Strontium on Lead Recovery in Milk Analysis

Sr added, mg	Pb recovered,[a] %
10	0.0
17	27.9
33	41.7
60	88.3
100	92.7
200[b]	95.5

[a] 2.0 ppm added to each sample. [b] Equivalent to 10 ml of 2% strontium solution.

Table II. Analysis of NBS Standard Reference Materials

Element	Amount found, μg/g	Av., μg/g	NBS value, μg/g
	Orchard Leaves NBS 1571		
Cu	11.8, 11.9, 12.1, 12.1	12.0	12 ± 1
Mn	88.9, 87.6, 90.1, 89.0	88.9	91 ± 4
Pb	45.4, 44.4, 43.5, 44.2	44.4	45 ± 3
Zn	24.2, 24.9, 24.5, 24.8	24.6	25 ± 3
	Bovine Liver NBS 1577		
Cd	0.30, 0.30	0.30	0.27 ± 0.04
Cu	188, 185	187	193 ± 10
Mn	10.3, 10.2	10.3	10.3 ± 1.0
Zn	119, 123	121	130 ± 10

Recoveries of standards containing those levels of concentration which represent the average range of the seven metals (0.02–5.0 ppm) found generally in foods averaged 93.9% with an average standard deviation of 4.72%. Recoveries of a similar standard added to eight food commodities averaged 97.2% (range 84.5–108.4%) for all metals except cobalt, with an average standard deviation of 4.03% (range 2.53–4.56%). The recovery of added cobalt was not satisfactory for the bonita fish, cod fillets, and turnip greens. In each of these commodities, neutralization of the digest with NaOH produced a greenish-brown solution probably due to complex formation (Callahan *et al.*, 1966; Baetz and Kenner, 1973). The use of the proposed method in the determination of Co in these and similar products is not recommended. Even though satisfactory recoveries of cobalt (average 101.8%) were obtained in the other commodities, cobalt results are not reported since no cobalt was found in any of the products studied. All of these recovery results are tabulated and will appear in the microfilm edition.

The inclusion of lead in the precipitate caused by the neutralization step in the original Baetz and Kenner (1973) procedure necessitated the determination of the metals both in this precipitate and in the eluate from the column. To offset this double determination, separation of the lead by dilution, partial neutralization, chilling, and filtering was investigated, but recoveries were too low. The coprecipitation of lead with added strontium suggested by Hoover *et al.* (1969, 1972) appeared promising and, with minor modifications, was adopted. The recovery of lead in the proposed procedure averaged 92.9% with a standard deviation of 5.2%. To test the completeness of the coprecipitation, a 3.0-ppm standard was treated in the

Table III. Determination of Six Metals in Various Food Commodities

Commodity[a]	Amounts found, ppm					
	Cd	Cu	Mn	Ni	Pb	Zn
Tomato catsup	0.06–0.06	3.61–3.38	1.88–1.90	0.98–0.98	0.05–0.06	1.88–1.73
Fresh spinach	0.11–0.11	0.69–0.68	7.31–7.10	0.09–0.11	0.15–0.12	8.89–9.58
Canned Bonita fish in oil	0.03–0.03	0.86–0.84	0.27–0.26	BDL[b] BDL	1.58–1.51	28.50–28.97
Apples	0.03–0.03	0.35–0.30	0.48–0.62	0.08–0.08	BDL BDL	0.75–0.71
Shredded wheat	0.09–0.10	2.10–2.10	23.33–23.86	0.26–0.27	0.07–0.07	39.14–39.83
Homogenized milk	BDL BDL	0.05–0.04	0.08–0.05	BDL BDL	BDL BDL	3.97–3.56
Canned turnip greens	0.04–0.04	0.28–0.28	2.09–2.31	BDL BDL	0.51–0.59	1.29–1.43
Frozen cod fillets	0.02–0.02	0.61–0.60	0.15–0.14	BDL BDL	BDL BDL	3.11–2.94

[a] All commodities reported on "as is" basis. [b] BDL, below detectable level.

usual way and the filtrate from the washing and carbonate conversion steps was analyzed for lead before neutralization. Less than 2% of the lead was found in the filtrate.

The study of the effect that the time the digest is chilled before transfer and filtration showed 45 min to be optimum. The recovery of 2.0 ppm of Pb was 87.0% after 20 min, 97.0% after 30 min, and 98.5% after 45 min. One sample of shredded wheat was allowed to sit for 48 hr after dilution before transfer and filtration. Since the recovery for this sample was 80.2%, it is recommended that the sample be filtered within 30 min after being chilled for 45 min.

During the early work on the determination of the metals in milk, low recoveries were obtained for lead. Since the probable cause was believed to be interference due to the large amounts of calcium in milk, the effect of varying amounts of added strontium was investigated with the results in Table I. While the 5.0 ml used in the proposed method is satisfactory for most commodities, milk and similar products which contain large amounts of calcium require more added strontium to ensure complete coprecipitation of the lead.

The results of the determination of metals by the proposed method on the National Bureau of Standards reference materials No. 1571 (orchard leaves) and No. 1577 (bovine liver) are shown in Table II. In each case the average values obtained agree with the accepted correct concentration and the average standard deviation is 1.42%.

The results of duplicate determinations on eight different types of foodstuffs are shown in Table III. The standard deviation and the sensitivity in all cases are significantly smaller than in the previous Baetz and Kenner (1973) method.

Different batches and different quality reagents were used at various times during the investigation and this caused blanks which differed significantly. The use of the reagent quality specified and the purified deionized water produced the lowest metal blanks. As an example, the blank was reduced from 0.06 to 0.03 ppm for lead and from 1.2 to 0.46 ppm for zinc. The primary source of Ni in the blank is the sodium hydroxide. The Ni blanks varied from 0.12 to 0.29 with different batches of sodium hydroxide but were not affected by different batches of the other reagents. The usual blanks obtained using the high purity acids and purified deionized water were: Pb, 0.03 ppm; Cd, 0.01 ppm; Ni, 0.12 ppm; Mn, 0.06 ppm; Cu, 0.05 ppm; Co, 0.00 ppm; and Zn, 0.46 ppm. The sensitivities (defined as 1% absorption above the blank) for the metals (in ppm) are: Pb, 0.05; Cd, 0.01; Ni, 0.08; Mn, 0.02; Cu, 0.04; Zn, 0.05; and Co, 0.10.

In samples which contain relatively large amounts of iron and produce relatively large amounts of precipitate upon neutralization, the time required for transfer to the column may be lengthened due to clogging of the column by the precipitate. This occurred in the samples of catsup and shredded wheat but did not affect the sensitivity or the precision of the results. Citric acid was used by Baetz and Kenner (1974) in their cadmium procedure to offset this interference of large concentrations of iron. Similar addition of citric acid was tried in the proposed procedure, but interfered with the determination of Mn, Ni, and Cu causing low results. Citric acid should not be used with samples which are expected to contain these three metals. Also, samples which contain citric acid should not be analyzed for these three metals by the proposed procedure.

Excessive delay between steps should be avoided due to the probability of loss by adsorption on the walls of the container. A student who was using the method on some wheat samples for a separate project had to leave the neutralized digest for 2 weeks before transfer to the column and the recoveries were 50% or less. Recoveries for a repeat determination avoiding excessive delay were in the normal range of 90–100%.

The proposed method is simpler, requires less time, and is more sensitive and precise than the original method. Further work is underway to incorporate other important metals such as Hg, As, and Se into the procedure and to improve the sensitivity by use of graphite furnace atomic absorption and/or anodic stripping voltammetry.

Supplementary Material Available. A study of column elution at different concentration levels, recovery studies of standards in the usual ranges found, and recovery studies of added standards to food commodities will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JAFC-75-41.

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A Thermogravimetric Study of the Stability under Heat of Iron–Protein Complexes

Shela Gorinstein

Iron–protein complexes were investigated by methods of differential gravimetric and differential thermal analyses. Studying the thermal stability has shown that, under heating, these complexes undergo two basic stages of thermal dissociation: dehydration (180–240°) and decomposition of the dehydrated complex (300–700°). Differences in heat dissociation of metal–protein complexes show the varying stabilities of their chemical bonds. It was also found that an additional introduction of ferric ion (Fe^{3+}) decreases the stability of iron–protein complexes.

One of the prime indicators of beer quality is its colloid-protein stability (Badgley, 1972; Stage, 1972; Steiner, 1972; Schildbach, 1971; Narziss and Roettger, 1973). This depends on the amount of metal–protein complexes in aqueous ethanol medium. Information on the concentration of metal-protein complexes in ethanol media is scarce in the scientific literature (Clapperton, 1971; Stone, 1972; Lundin, 1963; Djurtoft, 1962). In previous works (Fertman and Gorinstein, 1970; Gorinstein, 1973a, b), it was proven that the strength of the bonds between microelements and proteins is measured by the ratio of their quantity in protein fractions to their total content in beer. The data have shown that the most common complexing agents are elements of the eighth group (iron) of the Periodic Table of the Elements (Bagger, 1969; Davies *et al.*, 1969; Gorinstein, 1973a).

In order to study the differences in composition between complexes with and without Fe^{3+}, we added Fe^{3+} at a concentration of 3.5×10^{-3} mg/l. The deposition limit of beer (*i.e.*, its stability) sharply decreases as the Fe^{3+} concentration increases (Gorinstein, 1973b). By using the above concentration, a sediment was formed in the beer.

In this study, we have undertaken to isolate the iron-protein complexes, establish their change in composition by heat dissociation, and study their thermal stabilities.

MATERIALS AND METHODS

The investigation was carried out on "Zhiguli" nonfiltered beer, produced at Lvov Brewery Firm "Kolos," from 60% light malt and 40% nonmalted adjuncts. Standards of comparison for beer were the brews clarified by cotton filtering masses "Kineshma" (control) and "Evlakh" (test). (Kineshma and Evlakh are the Russian names of samples of cotton fibers. The Kineshma mass is of 34 nephelos units, and the Evlakh of 55 nephelos units. The two are distinguished by their filtering abilities.)

The stability of iron-protein complexes was determined thermochemically (Paulik *et al.*, 1958; Belcher *et al.*, 1960; Keattch, 1967; Gorinstein, 1974). Proteins were concentrated by tannin–caffeine and ammonium sulfate (for details see Fertman and Gorinstein, 1968). The sediment was dried at 30°. Their thermal stability was studied by the thermogravimetric method using the Paulik-Paulik-Erdey derivatograph (Paulik *et al.*, 1958). Four curves were recorded simultaneously on the derivatograph and are presented in Figures 1-4. Curve 1 on all the figures in positions A and C is the curve of differential thermogravimetric analysis, DTG; curve 2 is the curve of differential thermal analysis, DTA; curve 3 is the curve of temperature, *T*; curve 4 on positions B and D is the curve of integral thermogravimetric analysis, TG. Points a-h are the sites of the endothermic effects of substance weight loss at varying temperatures. The investigated substance and the standard—aluminum oxide repeatedly heated—were heated in a platinum crucible. The conditions of the experiment are as follows: weight of substance, 100 mg; thermopair, Pt-Pt/RH; resistance of electric circuit, DTA, 0.1; DTG, 0.1 megohm; rate of heating, 10°/min; range of error of temperature, ±5°. Twelve samples of iron–protein complexes in beer were investigated.

RESULTS AND DISCUSSION

Investigation of iron–protein complexes by the thermogravimetric method has shown that in the temperature interval of 180–240°, one or two endothermic effects of dehydration take place on the DTG and TG curves of all the samples of beer (see Figures 1-3). The nature of the complex is dependent on the radius and the electrical charge of the heavy metals (Fe, Cu, etc.), and dependent on them, in turn, is the temperature of hydration (Kapitonova *et al.*, 1971; Caldin, 1972; Krestov and Kurakina, 1973).

The first endo effect exists in each of the derivatograms presented in Figures 1-3. In the test beer, however, a higher temperature is found than in the other samples. A quantitative interpretation of the different thermoanalysis curves is presented in Table I.

Department of Pharmaceutical Chemistry, The Hebrew University School of Pharmacy, Jerusalem, Israel.