Table III. Total Recovery of 10 ppb of *N*-Nitrosodimethylamine (DMN), *N*-Nitrosodiethylamine (DEN), *N*-Nitrosodi-*n*-butylamine (DBN), *N*-Nitrosopyrrolidine (PYR), and *N*-Nitrosopiperidine (PIP) from Various Samples of Meat Products

Sample	Recovery, %				
	DMN	DEN	DBN	PYR	PIP
Minced meat dough I	92	97	82	48	103
Minced meat dough II	91	78	86	45	84
Fried minced meat	95	88	88	53	89
Smoked Guelder sausage I	70				
Smoked Guelder sausage II	98	83	68	40	78
Luncheon meat II	83				
Fried meat balls, "Dutch frikadel" type	96	89	98	49	87
Frying fat	101	99	100	62	99

are given in Tables II and III.

DISCUSSION

The recovery of *N*-dimethylnitrosamine was good within the range studied (10–50 ppb). At the level of 10 ppb the recovery for *N*-diethylnitrosamine, *N*-di-*n*-butylnitrosamine, and *N*-nitrosopiperidine was also determined and found to be equally as good as the recovery of *N*-dimethylnitrosamine. At the level of 10 ppb the recovery of *N*-nitrosopyrrolidine was in the range of 40–62%. A similar relatively lower recovery for *N*-nitrosopyrrolidine has been observed by others (Crosby et al., 1972; Bryce and Telling, 1972; Telling et al., 1975). In the blank samples none of the *N*-nitrosamines was detected indicating either their absence in the samples or that their concentration was lower than 0.2–0.1 ppb depending on the type of *N*-nitrosamine searched for.

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Quantitative Determination of Zinc, Iron, Calcium, and Phosphorus in the Total Diet Market Basket by Atomic Absorption and Colorimetric Spectrophotometry

Eddie D. McGary* and Barbara E. Young

A simple method has been developed to determine zinc, iron, calcium, and phosphorus using the dry ash procedure of the AOAC (Association of Official Analytical Chemists, "Official Methods of Analysis", 12 ed, Washington, D.C., 1975) with a few minor modifications for 12 total diet market basket composites. The ash is diluted with 0.1 N HCl, made to a known volume, and analyzed for zinc and iron by atomic absorption spectrophotometry (AAS). A second series of dilutions is made from the original dilutions; then lanthanum is added so that each composite contains 1% lanthanum in 0.1 N HCl. These dilutions are then analyzed for calcium by AAS. A third series of dilutions is made from the initial dilutions and phosphorus is then determined by the molybdophosphoric acid colorimetric method (Halmann, M., *Anal. Chem. Phosphorus Compd.* **37** (1972)). The proposed method was applied in part to 45 total diet market baskets. Recoveries averaged between 94 and 99% for the four metals analyzed. Methods for the analysis of zinc, iron, calcium, and phosphorus appear in various scientific literature but no provisions are made for the analysis of these metals in combination in the total diet of man.

This paper describes a procedure for the analysis of zinc, iron, calcium, and phosphorus and may be applied to other metals as well, if the vapor pressure of the metal is not appreciable at the temperature given in this method for the dry ashing step. The total diet market basket composition is given in Table I. A total diet market basket represents the recommended 2-week diet of a 15–20 year old male or female for the region of the country in which it is collected.

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EXPERIMENTAL SECTION

Reagents and apparatus used included the following.

Table I. Market Basket Composition

Composite[a]	Products	Composite[a]	Products
1	Dairy products	7	Root vegetables
2	Meats, fish, and poultry	8	Garden Fruits
3	Grains, cereal products	9	Fruits
4	Potatoes	10	Oils, fats, and shortening
5	Leafy vegetables	11	Sugar and adjuncts
6	Legume	12	Beverages

[a] Preparation of composites is outlined by The Bureau of Foods, Food and Drug Administration, Washington, D.C.

(a) **0.1 N Hydrochloric.** Dilute 8.9 ml of analytical reagent grade (Mallinckrodt) HCl to 1 l. with distilled water.

(b) Hydrochloric acid (1 + 1) was used.

(c) **5% Lanthanum.** Weigh 58.64 g of reagent grade La_2O_3 (Matheson Coleman and Bell) and transfer to a 1500-ml beaker. Add about 90 ml of distilled water and stir with a glass rod. Slowly add with stirring 250 ml of reagent grade HCl until the reaction ceases. Let the solution cool and transfer to a 1-l. volumetric flask and dilute to volume with distilled water.

(d) **2.5 N Nitric Acid.** Dilute 162.5 ml of analytical reagent grade (Mallinckrodt) HNO_3 to 1 l. with distilled water.

(e) **10% Sodium Molybdate.** Weigh 100 g of reagent grade (Merck) sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$), dissolve, and dilute to 1 l. with distilled water.

(f) **Zinc Standard Solution.** Prepare a stock standard solution of zinc by weighing 0.500 g of granular zinc metal (20 mesh from Fisher Scientific Co.), dissolve in 10 ml of HCl, and dilute to 1 l. with distilled water (zinc concentration is 500 μg/ml). Dilute 20 ml of stock standard zinc solution to 1 l. with 0.1 N HCl (zinc concentration is 10 μg/ml).

(g) **Iron Standard Solution.** Prepare a stock standard solution of iron by weighing 1 g of iron wire (Baker Analyzed Reagent, J. T. Baker Chemical Co.) in 1:2 HCl and dilute to 1 l. with distilled water (iron concentration is 1 mg/ml or 1000 μg/ml). Dilute 5 ml of stock standard iron solution to 500 ml with 0.1 N HCl (iron concentration is 10 μg/ml).

(h) **Calcium Standard Solution.** Prepare a stock standard solution of calcium by weighing 1.249 g of $CaCO_3$ (Fisher certified reagent grade ACS), dissolve in 10 ml of HCl, and dilute to 1 l. with distilled water (calcium concentration is 500 μg/ml). Dilute 50 ml of stock standard solution to 250 ml with 0.1 N HCl (calcium concentration is 100 μg/ml).

(i) **Phosphorus Standard Solution.** Weigh 0.2197 g of potassium phosphate monobasic (certified reagent grade, Fisher Scientific) and dissolve with distilled water. Transfer to a 500-ml volumetric flask and dilute to volume with distilled water (phosphorus concentration is 100 μg/ml).

(j) A Jarrel Ash 810 atomic absorption spectrophotometer, division of Fisher Scientific Co., or equivalent was used.

(k) A Beckman DU spectrophotometer or equivalent was used.

(l) **Platinum Crucibles (100 ml Capacity).** Wash with (1 + 1) HNO_3 before and after use and then rinse with distilled water.

Procedure. Weigh 5.00 g of each composite (except use 10.0 g for composite 12) (see Table I) into clean platinum crucibles. Place the crucibles containing the composites into a drying oven at 105 °C for about 2 h. Char under infrared lamps until smoke ceases and ash at about 525 °C in a muffle furnace (raise temperature slowly to 525 °C to avoid ignition) until carbon free (a small amount of carbon usually remains in composites 4 and 11 but does not seem to interfere in the analysis). Dissolve the ash under a watch glass with 5 ml of HCl (1 + 1) (b). Add 20 ml of distilled H_2O and evaporate to near dryness on a steam bath. Add 20 ml of 0.1 N HCl and continue heating for a few minutes. Carefully rinse the watch glass into the crucible and quantitatively transfer the contents through a Whatman filter paper no. 41 or equivalent into a 100-ml volumetric flask, except use a 200-ml volumetric flask for composite 2. Wash the crucible with several portions of 0.1 N HCl, transferring each to the volumetric flask, and dilute to volume with 0.1 N HCl. Duplicate 5-ml portions of 0.1 N HCl are carried throughout the procedure for blanks. One is diluted to 100 ml and the other is diluted to 200 ml with 0.1 N HCl. Use the 200-ml blank for composite 2 and the 100-ml blank for all other composites.

Zinc and iron are quantitatively determined from these solutions using AAS. See Jarrell-Ash (1972) for instrument parameters for zinc and iron analysis.

(1) *Preparation of Zinc Standard Curve.* Dilute the zinc standard solution (f) with 0.1 N HCl to prepare standards containing 0–1 μg/ml of zinc.

(2) *Preparation of Iron Standard Curve.* Dilute the iron standard solutions (g) with 0.1 N HCl to prepare standards containing 0–4 μg/ml of iron.

Calcium Analysis. A second series of dilutions is made from the initial dilutions to analyze for calcium. To these dilutions 20 ml of 5% lanthanum (c) is added to each composite dilution (so that the final lanthanum concentration is 1%) and then diluted to volume with 0.1 N HCl. These dilutions are analyzed by AAS. See Perkin-Elmer (1968) for standard conditions for calcium.

Preparation of Calcium Standard Curve. Dilute the calcium standard solution (h) with lanthanum solution (c) to prepare standards containing from 0 to 12 μg/ml of calcium in 1% lanthanum and dilute to volume with 0.1 N HCl.

Phosphorus Analysis. A third series of dilutions is made from the initial acid dilutions to analyze for phosphorus. Transfer an aliquot to a 50-ml volumetric flask. Add distilled H_2O to about 40 ml volume and quantitatively add 5.0 ml of the sodium molybdate reagent (e) to each flask to develop the yellow color. Dilute each flask to volume with distilled H_2O and shake thoroughly. Read absorbance of yellow solution using a spectrophotometer with 1-cm cells against distilled H_2O at 380 nm.

Preparation of Phosphorus Standard Curve. Transfer the phosphorus standard solution (i) to 50-ml volumetric flasks to prepare standards containing 0–10 μg/ml of phosphorus and proceed as under phosphorus analysis beginning with "add 5 ml of 2.5 N HNO_3. . .".

Calculations. Determine zinc, iron, calcium, and phosphorus from standard curves obtained by plotting absorbance against micrograms per milliliter of standard solutions: ppm of Zn, Fe, Ca, and P = [(μg/ml from standard curve)(dilution factor, ml)]/g of sample.

RESULTS AND DISCUSSION

During method development, ash-aid solution, AOAC (1975), was added to selected composites for zinc analysis of market baskets (2, 3, 4, and 5) before ashing and the results obtained compared to those without the addition

Table II. Comparison of Zinc Found in Four Total Diet Market Baskets Representing 11 Composites, with and without the Use of an Ash-Aid Solution

Market Basket no.	Composite no.	ppm Zn found	
		Ash-Aid Solution	Ash-Aid solution[a] excluded
2	8	2.72	3.00
	9	0.60	0.80
	10	6.20	6.92
3	2	30.00	28.00
	6	6.00	6.80
	7	2.10	2.40
4	2	30.80	27.40
	4	5.00	4.80
	5	1.48	1.40
5	1	4.12	4.08
	3	9.00	8.80

[a] Average of three determinations.

Table III. Zinc, Iron, Calcium, and Phosphorus Found in Total Diet Market Basket Composites

Composite	Zn found, ppm[a]	Fe found, ppm[b]	Ca found, ppm[c]	P found, ppm[d]
1	5.2	2.1	1293	1131
2	26.8	16.8	298	2000
3	7.9	32.7	645	1478
4	4.0	8.1	106	686
5	2.3	8.1	366	297
6	8.0	13.6	371	836
7	2.5	5.1	249	281
8	3.1	5.3	135	266
9	0.9	3.6	101	159
10	5.6	5.9	115	660
11	2.8	12.7	715	707
12	0.8	1.4	51	89

[a] Average of 45 market baskets. [b] Average of 35 market baskets. [c] Average of 35 market baskets. [d] Average of 20 market baskets.

Table IV. Recovery of Zinc Added to Total Diet Market Basket Composites

Composite	Determinations	Added ppm	Found ppm	% recovery	SD
1	9	5.00	5.01	100.1	9.7
2	11	25.00	22.00	88.1	5.1
3	4	5.00	4.81	96.5	11.1
4	9	5.00	5.00	100.0	9.6
5	7	5.00	4.55	91.0	6.9
6	7	5.00	4.81	96.0	11.3
7	6	5.00	4.86	97.2	10.2
8	5	5.00	4.50	90.0	3.1
9	7	5.00	4.91	98.1	14.4
10	13	5.00	4.63	92.6	10.8
11	6	5.00	4.86	97.5	8.1
12	8	5.00	4.68	93.5	8.6

of the ash-aid solution. The results are tabulated in Table II. The results compare favorably; therefore, the ash-aid solution was excluded from the method due to the added step it would have incorporated into the procedure.

The linear working range for zinc, iron, calcium, and phosphorus will depend on the instrument used in the analysis. In our work we found the linear working range for zinc (0.1–1 μg/ml) and iron (0.1–4 μg/ml) in 0.1 N HCl using a Jarrell-Ash 810 (AAS). Calcium (1–12 μg/ml) was determined in 1% La in 0.1 N HCl using a PE 303 (AAS) and phosphorus (0–10 μg/ml) was determined using a Beckman DU spectrophotometer.

Table V. Recovery of Iron Added to Total Diet Market Basket Composites

Composite	No. of Determinations	Added ppm	Found ppm	% recovery	SD
1	4	20.00	21.00	105.5	10.4
2	6	20.00	20.40	102.0	12.8
3	4	20.00	19.80	99.0	5.8
4	7	20.00	21.30	106.6	11.6
5	6	20.00	20.90	104.7	8.2
6	6	20.00	20.90	104.7	4.0
7	3	20.00	21.70	108.7	4.1
8	5	20.00	20.80	104.0	9.4
9	5	20.00	20.40	101.8	4.4
10	7	20.00	20.60	102.9	6.9
11	5	20.00	19.50	97.6	11.6
12	5	20.00	19.60	97.6	6.7

Table VI. Recovery of Calcium Added to Total Diet Market Basket Composites

Composite	No. of Determinations	Added ppm	Found ppm	% recovery	SD
1	4	800	758	94.8	4.6
2	6	800	810	101.3	4.6
3	4	800	774	96.5	3.7
4	6	800	770	96.2	1.7
5	5	800	780	97.4	3.8
6	5	800	793	99.2	5.4
7	5	800	771	96.4	4.0
8	3	800	772	96.7	6.7
9	3	800	799	100.0	4.4
10	5	800	792	99.0	5.8
11	3	800	836	104.7	4.7
12	3	800	795	99.3	2.3

Table VII. Recovery of Phosphorus Added to Total Diet Market Basket Composites

Composite	No. of Determinations	Added ppm	Found ppm	% recovery	SD
1	3	800	783	98.0	3.6
2	5	800	792	99.0	4.7
3	3	800	772	96.7	7.5
4	6	800	796	99.3	3.3
5	4	800	771	96.5	2.4
6	4	800	773	96.8	1.5
7	2	800	767	96.0	0.0
8	2	800	788	98.5	2.1
9	2	800	786	98.0	0.0
10	8	800	800	100.1	6.3
11	4	800	786	98.2	9.3
12	2	800	781	97.5	2.1

It is of interest to note that the standard solutions of zinc (10 μg/ml), iron (10 μg/ml), calcium (100 μg/ml), and phosphorus (100 μg/ml) have been used up to 6 months, when kept at room temperature, to produce standard curves with no significant changes.

To control phosphate interference in the calcium analysis, the samples were prepared to contain about 1% lanthanum in 0.1 N HCl.

The results from the analysis of zinc (45 market baskets), iron (35 market baskets), calcium (35 market baskets), and phosphorus (20 market baskets) are found in Table III. The figures represent the mean values of the total diet market basket composites.

The efficiency of the method was determined by adding known amounts of zinc, iron, calcium, and phosphorus to the composites representing the total diet market baskets and then calculating the percent recoveries. The recoveries obtained indicate that there is good precision in determining these trace elements by the proposed methodology in food products. These results are found in Tables IV–VII.

It has not been our objective to determine the nutritional significance of the data we have obtained from the market baskets analyzed and given in this report. Our objectives have been to develop an analytical method that would give good precision and to give us an idea of how much zinc, iron, calcium, and phosphorus one would expect to find in the total diet market basket.

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Dinitrophenylation of the Compounded Chick Starter Rations for the Estimation of Available Lysine

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Available lysine of compounded starter rations of poultry was estimated by treating the rations with 2,4-dinitrofluorobenzene followed by acid hydrolysis. The hydrolysate was purified by passing through a Sephadex G-50 column and the color was read at 390 mμ using a DBG Beckman spectrophotometer. As the chemically estimated values conformed with the biological results, the present method will be useful for the assessment of compounded poultry rations.

It is not possible to estimate the nutritional availability of lysine units whose ε-amino groups are chemically bound in the heat-processed high carbohydrate rich feeds simply by acid hydrolysis. The process no doubt overvalues the feeds with respect to this particular amino acid content and thus the assessment of compounded poultry rations by chemical methods becomes erroneous. Carpenter (1960) estimated available lysine of foods with the help of 2,4-dinitrofluorobenzene which underwent Sanger reaction (1945) with ε-amino groups. The interfering components were separated by Rao et al. (1963) who used a column chromatographic technique for the estimation of ε-dinitrophenyllysine in oilseed meals, but no attempt was made by them to determine the available lysine content in a compounded ration. This paper describes a simple method for the estimation of available lysine in chick starter rations by applying Sanger reaction and then purifying the acid hydrolysate of the reacted product with the help of a Sephadex G-50 column.

EXPERIMENTAL PROCEDURE

Formulation of the Compounded Starter Rations. First, four starter rations designated as A, A_1, B, and B_1 were prepared by mixing the processed ingredients in requisite amounts. The protein content of the rations in each group was kept more or less constant but the amount of lysine-enriched materials was changed to differentiate the rations in lysine content.

Another set of experiments was conducted by adding known amounts of synthetic L-lysine hydrochloride to the starter ration C which was deficient in lysine content. Thus, four more rations designated as C_1, C_2, C_3, and C_4 which were different in added amounts of lysine were prepared. All other ingredients of these rations were the same as in ration C.

Reaction with 2,4-Dinitrofluorobenzene. To 2 to 3 g of finely ground fat-free material, 30 ml of 50% sodium bicarbonate solution was added with gentle stirring. The mixture was left undisturbed for 15 to 20 min and 1.5 ml of 2,4-dinitrofluorobenzene in 40 ml of ethanol was added. After preliminary stirring the contents were shaken in the dark for 2 h on a mechanical shaker with slow motion. An air stream was passed to remove alcohol and a major portion of water and the residue was taken up for acid hydrolysis.

Preparation of the Acid Hydrolysate. To the residue 200–250 ml of 6 N HCl was added and the mixture was refluxed for 24 h. When hydrolysis was over, the mixture was cooled and filtered cautiously through Whatman filter paper (No. 41). The residue was washed repeatedly with cold distilled water. The filtrate and the washings were mixed together and evaporated to dryness under reduced pressure. The dried residue was again dissolved in cold distilled water and made up to a definite volume.

Purification of the Hydrolysate. A 4-ml aliquot of the prepared acid hydrolysate was extracted 5 times with 25-ml portions of peroxide-free ether and the aqueous phase of the hydrolysate was evaporated to dryness under reduced pressure. The residue was dissolved in 4 ml of 0.3 N hydrochloric acid and introduced into a Sephadex G-50 column, 90 × 1.5 cm, previously equilibrated with 0.3 N hydrochloric acid. The yellow band was collected in one portion by eluting with 0.3 N hydrochloric acid at a flow rate of 35 ml/h. The collected fraction was again evap-

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