DETERMINATION OF CALCIUM, MAGNESIUM, IRON AND ZINC IN PROTEIN CONCENTRATES BY ATOMIC ABSORPTION SPECTROMETRY

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Several researchers have applied atomic absorption method for the trace analysis of vegetal samples and fodder. In some of these works [1—6] the ashing of the sample at 420—550 °C is recommended. The ignition residue is dissolved then in a small amount of hydrochloric acid or a mixture of hydrochloric and nitric acid, and analysis is carried out after dilution. Another method of preparation often used is digestion. Various acid mixtures are used for digestion, thus e.g. nitric acid—perchloric acid—sulfuric acid mixture [7—9] or nitric acid—perchloric acid mixture [10—13]. Several authors[14—17] dissolve without digestion the elements to be determined with hydrochloric acid or with nitric acid [3].

Out of the works dealing with interference problems in flame-photometric atomic absorption, the following should be mentioned: David [8] studied the interfering effect of anions in the determination of calcium in vegetal samples, Willis [18, 19] tried several methods for the elimination of phosphate interference, for which Trent and Slavin [20] recommended as general method the use of lanthane chloride. Willis [21, 22] found that no phosphate interference was met in a nitrous oxide—acetylene flame, while Manning and Capacho-Delgado [23] showed experimentally and explained theoretically the role of alkali metals in the determination of calcium in a dinitrogen oxide—acetylene flame.

Our measurements involved the determination of calcium, magnesium, iron and zinc in protein concentrates prepared from fodder-crops, by means of atomic absorption spectrophotometry. The mineralization of the samples was made by digestion with nitric acid—perchloric acid mixture or with sulfuric acid—hydrogen peroxide mixture and by dry ashing at 500 °C. These methods were compared from the point of view of the accuracy of the determination, and the single methods have been evaluated with respect to time and work requirement. Phosphates present in samples of vegetal and animal origin interfere in the flame photometric and atomic absorption spectrophotometric determination of alkali earth metals. The measure of this interference was studied, and methods proposed for its elimination were critically compared.

Chemicals, devices

For digestion and for the preparation of the standard solutions, chemicals of analytical purity, manufactured by Merck, have been used. Measurements were done with an Unicam SP 90A atomic-absorption spectrophotometer, in acetylene—air and acetylene—nitrous oxide flame, respectively, using Unicam or Original Hanau hollow-cathode lamps. Optimal measuring conditions for the single elements are contained in Table 1. (Burner head

Table 1

Conditions of the determination of the element measured

Element	Wavelength nm	Current of lamp mA	Height of burner mm	Slit mm	Scale expansion	Acetylene l/min.	Air l/min.	Range of measurement μg/ml
Zn	213.8	8	14	0.1	4	1.5	5	0.5— 5
Fe	248.3	7	7	0.1	6	1.5	5	5—20
Mg	285.2	3	11	0.1	3	1.5	5	0.1— 1
Ca	422.7	6	12	0.1	4	1.5	5	1—10

height: distance of the center of the cathode spot, formed in the middle of the flame, from the level of the burner head.) When using a nitrous oxide—acetylene flame, the flow rate of acetylene was 4 lit./min, and that of nitrous oxide 4.8 lit./min.

Preparation of the sample

Three kinds of methods of preparation were used in our measurements; the sample in which the calcium, magnesium, iron and zinc content has been determined, was in each case 0.5 g.

The brief description of the methods of preparation is as follows:

a) *Digestion with nitric acid—perchloric acid*

Digestion was done with a mixture of 10 ml of concentrated nitric acid and 1.5 ml of perchloric acid in an Erdey—Jankovits apparatus [24]. This mixture is not explosive even in the presence of organic substances, provided that nitric acid is in excess as long as organic matter is present [25]. After 40 minutes of intensive digestion, the solution was evaporated to dryness, the residue was dissolved in water, the volume of the solution was made up to 25 ml, and the solution was filtered.

b) *Digestion with sulfuric acid—hydrogen peroxide*

The digestion with sulfuric acid and hydrogen peroxide mixture was carried out in a 50 ml Kjeldahl flask. 3 ml of concentrated sulfuric acid was added to the sample, then in small portions hydrogen peroxide, until a crystal clear solution was obtained after the cooling of the solution [26]. The solution was evaporated to dryness, and the residue dissolved in water. The time required for this digestion was about the same as that needed for the digestion with sulfuric acid—perchloric acid mixture. In the case of a high calcium content, calcium sulfate precipitated was alkalified with 6 n ammonium hydroxide and dissolved with 0.1 m (pH = 9) EDTA solution (35 ml). The volume of the solution was made up to 50 ml.

c) *Dry ashing*

The samples were placed into a porcelain crucible, and ashed in a muffle furnace according to the following schedule: 20 minutes at 100 °C, 20 minutes at 200 °C, 30 minutes at 300 °C, 4 hours at 500 °C. The ash was dissolved in 2 ml of 0.5 n hydrochloric acid solution, the volume made up to 25 ml, and the solution was filtered.

Investigation of the interference of phosphate ions in calcium determination

Samples of vegetal origin always contain phosphate compounds. Phosphate ions interfere in the determination of calcium ions by atomic absorption. The phosphate content of the protein concentrates investigated was determined in form of phosphor molybdene vanadate. In the different samples, phosphorus content varied between 1.5 and 7.5 per cent.

For the investigation of phosphate interference, solutions containing 3, 5, 7, and 10 μg/ml calcium have been prepared, in which the phosphate concentration varied in the range from 0 to 50 μg/ml. The change in calcium extinction was measured as a function of the phosphate content. Measuring results are shown in Fig. 1. The extinction of calcium is strongly decreased by phosphate ions, the decrease in extinction may be as high as 40 percent.

The interference by phosphate can be eliminated by the addition of lanthane salts in excess. Solutions containing 1 percent of lanthane were used for our investigations. Lanthane chloride contains small amounts of calcium, and must be taken into consideration in the measurements.

The interfering effect of the phosphate ions is not manifested in high temperature nitrous oxide—acetylene flames of reducing character.

According to our experiments too, the interference of phosphate can be eliminated by this method. At higher phosphate concentrations (2—3 percent), a slight increase in absorption was observed. In the nitrous—acetylene flame, the sensitivity of calcium determination increases to about threefold, as compared to the air—acetylene flame. The analytical curves obtained in the two kinds of flames are shown in Fig. 2.

Table 2

Determination of the calcium content in different flames and under different additions

Sample	Method of measurement C₂H₂—air Ca+1%La μg/ml	C₂H₂—air Ca μg/ml	Δ%	C₂H₂—N₂O Ca μg/ml	Δ%	C₂H₂—N₂O Ca+500 μgK/ml μg/ml	Δ%
I	8.10	6.41	—21	9.65	+19	8.12	+0.3
II	6.95	5.09	—27	8.45	+22	7.00	+0.5

Since the samples contain in general higher quantities of alkali metals, sodium and potassium, besides calcium, the effect of these elements on the determination of calcium by atomic absorption has been investigated. Alkali metals increase substantially the extinction of calcium in nitrous oxide — acetylene flame. Fig. 3 shows the effect of sodium and potassium. The percentage increase in extinction, referred to the extinction of the solution containing only calcium, is plotted on the ordinate as a function of sodium and potassium concentration. In the high temperature nitrous oxide—acetylene flame, the ionization of calcium is considerable. Alkali metals, ionizing easily, decrease the ionization of calcium, which results in an increase of the extinction of calcium. This effect must be taken into account in the development of the method of analysis, and sodium or potassium must be added in a concentration of 500 μg/ml to the test and standard solutions.

The calcium content of the two samples was determined by different methods: in air—acetylene flame in the presence of 1 percent of lanthane and without this addition, in nitrous oxide—acetylene flame without any additive, and in the presence of 500 μg/ml of potassium. Measuring results are shown in Table 2, following the concentrations. It is seen from the table, that the same results are obtained in air—acetylene flame in the presence of lanthane and in nitrous oxide—acetylene flame in the presence of potassium. When measuring calcium in an air—acetylene flame without additive, concentrations by 20 to 27 percent lower, and in a nitrous oxide —acetylene flame, similarly without additive, by 19 to 22 percent higher than the actual concentration are obtained.
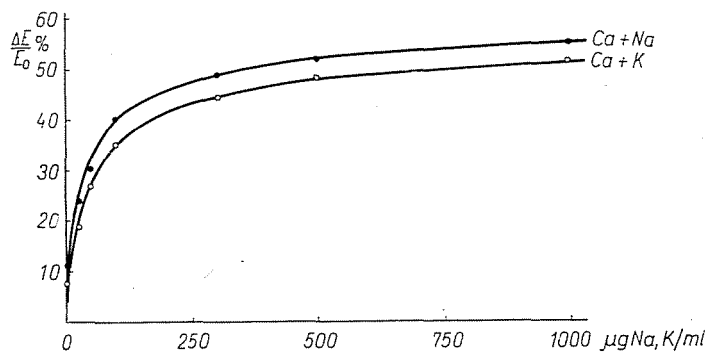
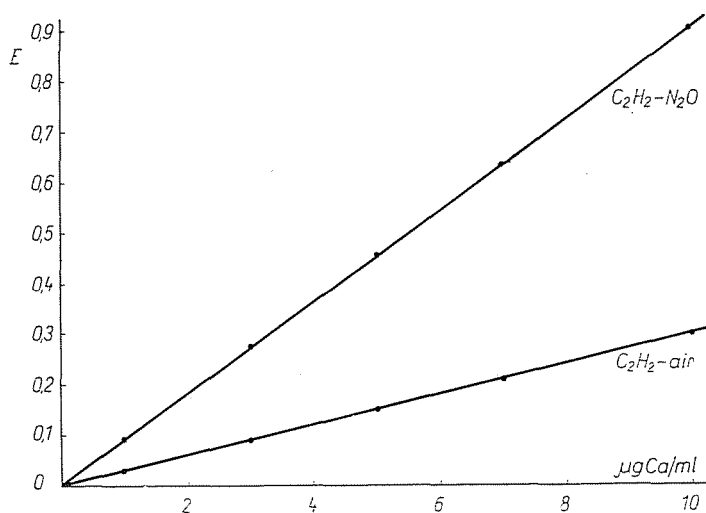*Fig. 1.* Effect of the increase in phosphate content on the extinction of calcium

Fig. 2. Sensitivity of calcium determination in acetylene—air and in dinitrogen oxide—acetylene flames

Determination of iron by various methods

Measuring results obtained by atomic absorption were checked in the case of iron by spectrophotometry. Sulfosalicylic acid and α,α'-dipyridyl were used for the measurements.

Preparation of the sample for spectrophotometric determination

In the case of sulfosalicylic acid, the solution was prepared as follows: 10 ml of the neutral solution to be investigated was introduced with a pipette into a 20 ml normal flask, 2 ml of a 10 percent sulfosalicylic acid reagent
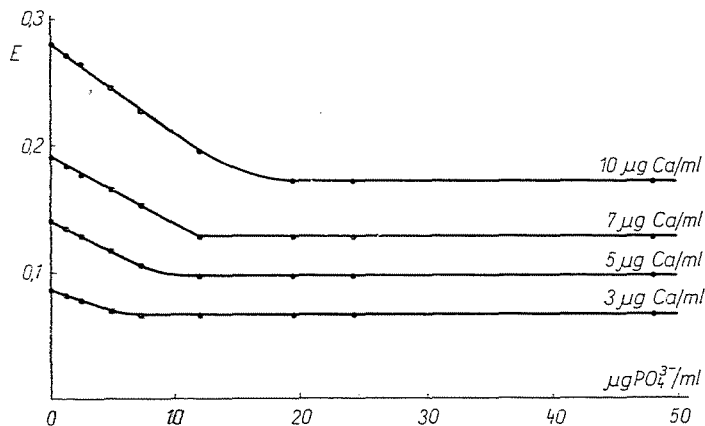
Fig. 3. Effect of sodium and potassium on calcium determination in nitrous oxide—acety-
lene flame

and 2 ml of concentrated ammonium hydroxide was added, and the flask
was filled up with water to the mark. Measurement was made at 420 nm, in
a cell of 1 cm, with a Hilger Uvispek spectrophotometer, with water as blank
solution [27].

When using α,α'-dipyridyl, the solution was prepared as follows: 20 ml
of the solution to be measured was introduced with a pipette into a 100 ml
normal flask, 2 ml of ascorbic acid, 5 ml of acetate buffer and 1 ml of a 2 percent
α,α'-dipyridyl solution were added, and the flask was filled up with water
to the mark. The solution was left to stand for 30 minutes, and measured at
525 nm in a cell of 1 cm with a Spekol spectrophotometer, with water as the
blank solution [27].

Evaluation of the measuring results

With each of the three methods of preparation, 11 parallel preparations
were done, and the calcium, magnesium, iron and zinc content of the samples
was measured. Evaluation was made on the basis of an analytical curve
plotted for standard solutions, and by addition evaluation. In the measure-
ment of calcium and magnesium, sample solutions had to be diluted to hundred-
fold, to permit a measurement within the straight section of the calibration
curve. Results obtained by the different methods of preparation are compiled
in Table 3. Deviations from the results of digestion with nitric acid—per-
chloric acid are given, as well as the standard deviations of the results.

Standard deviations characteristic of the accuracy of the methods
indicate that digestion with nitric acid—perchloric acid is the best for a
reproducibility. As concerns the time requirement of the methods, the nitric

Table 3

Comparison of different methods of preparation

(μg/ml — concentration of the samples; $\sigma\%$ — relative error of the results; $\Delta\%$ — percentage deviations referred to the results of the digestion with nitric acid—perchloric acid)

Element	Method of preparing sample								
	Destruction						Dry combustion		
	HNO$_3$—HClO$_4$		H$_2$SO$_4$—H$_2$O$_2$						
	μg/g	$\sigma\%$	μg/g	$\sigma\%$	$\Delta\%$	μg/g	$\sigma\%$	$\Delta\%$	
Zn	89.5	±1.8	86.3	±4.0	—3.5	82.2	±4.7	—8.2	
Fe	794	±3.5	783	±5.0	—1.4	784	±3.0	—1.3	
Mg	3720	±0.6	3560	±4.5	—4.3	3690	±3.0	—0.8	
Ca	29700	±1.3	28000	±5.7	—5.7	28100	±4.6	—5.4	

acid—perchloric acid method is superior to the sulfuric acid—peroxide method, because the former does not require further chemical operations. The dry ashing method required the shortest time, but from other aspects, it is inferior to the nitric acid—perchloric acid method.

Summary

In the measurement of the calcium content of fodder concentrates by atomic absorption, the same results were obtained for the elimination of the interfering effect of phosphate, when lanthane chloride was added in excess in the case of air—acetylene flame, or when alkali buffers were added in the case of nitrous oxide—acetylene flame. For the determination of trace elements in protein concentrates by atomic absorption, the nitric acid—perchloric acid method is recommended for accuracy, while dry ashing is the quickest method when a great many samples have to be analysed.

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