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Determination of calcium intake by help of atomic absorption spectrophotometry

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Calcium is the main nutritional element forming bones and teeth. It thus comprises the most abundant mineral in the human body (1). An infant is born with a store of calcium of about 30 g and has to build up approximately 1200 g until he becomes an adult (2). With the dynamic nature of calcium turnover, it is estimated that man needs approximately 800 mg of calcium daily. Higher amounts are recommended during adolescence (3, 4). However, much lower amounts may be considered adequate (5, 6). In besides to skeleton formation, calcium is indispensable for many other vital biological processes (7). Determination of calcium intake of a population or population group(s) is therefore important. It helps to assess the nutritional status of the concerned population, to determine adequacy of their diet, to lay down sound feeding programs, to provide information for more realistic overview of calcium requirements, and to serve in other purposes. The picture obtained in a developed healthy community would serve as a guide for less developed areas.

In order to determine intake of a nutrient rather accurately, a dietary survey need be carried out, and samples of food consumed, invariably numerous, are analyzed for that nutrient. Although calcium is present in foods in different forms, and reaches food from different sources, fortunately this will not be of concern, and only the total calcium content is the desirable estimate.

Calcium has been determined in foods by several methods, e.g. precipitation as oxalate, chelation as the ethylene diamine tetraacetate salt, and emission spectroscopy. When large numbers of samples need to be analyzed, as in case of dietary surveys, a method is required, that is likewise simple, rapid, accurate, needs minimum sample preparation and analyst expertise, and can be automated as well. The atomic absorption technique suggests itself.

Literature on atomic absorption spectrophotometry (AAS), its application to food analysis and calcium determination by this technique has become extensive (8–10). The present work investigates calcium determination in different food items by AAS, effect of salts expected in the samples, and control of their interference. Samples of single food items and of food mixtures are analyzed, and results are compared to those in Dutch food composition table (DFCT). Calcium intake of different population groups in the Netherlands is thus determined.

Materials and methods

Sampling and sample preparation

Samples were obtained as random purchases from retail shops in the city of Utrecht, a central city in the Netherlands where most railways of the Kingdom meet. This was considered reasonably representative of goods on sale in the country since foodstuffs there were usually manufactured and distributed by large firms, thus reaching every part of the country. Collection of samples from a large number of cities would be too costly and would result in respectively too high a number of samples.

In order to further reduce the number of samples analyzed, foodstuffs were grouped in composite samples, each formed of items similar in nature, and in quantities proportional to the habitual consumption of the Dutch. As an example, the bread sample was composed as follows:

White bread, forming 62 %, prepared from 2 sliced and 1 unsliced loaves plus 1 loaf milk bread;

Brown bread, forming 30 %, prepared from 2 sliced loaves, only one end included (3 ends excluded);

Light rye bread, forming 2.5 %, prepared from 3 sliced loaves;

Dark rye bread, forming 2.5 %, prepared from 3 sliced and 1 unsliced loaves;

Currant bread, forming 3.0 %, prepared from 1 loaf and 3 buns.

Wherever necessary, samples were prepared by peeling, trimming, or deboning, so as only the edible part will be used for analysis. They were comminuted by a suitable manner, thoroughly mixed, reduced, and preserved by drying, freeze-drying, freezing or cooling. Remixing before weighing a sample for analysis was a common practice.

Ashing: The sample (containing 2–8 mg phosphorus)[1]) was dry ashed using magnesium acetate as ashing aid. Thus the effect of mineral acids used in wet ashing on suppressing calcium atomic absorption was avoided. The ash solution in hydrochloric acid was evaporated on water bath, residue redissolved in the acid and filtered. This treatment was found sufficient to eliminate any silicates (11), presence of which would suppress absorption signals.

Ash was made up to 100 ml by water. A volume containing 0.1–0.25 mg Ca was transferred to a 25 ml volumetric flask for calcium determinations.

Detailed description of sampling, preparation of samples and sample mixtures, ashing procedures and reagents used throughout, had been given elsewhere (11).

Instrumental: Most of the work was carried out using a "Perkin-Elmer 290 B" instrument with a "Hitachi 159" recorder. A "Varian Techtron AA-5" with a "Kipp & Zonen" recorder has been employed for the study of air/hydrogen flame, phosphate interference, and analysis of some samples. Parameters were adjusted for highest sensitivity as summarized hereunder:

	Perkin Elmer 290 B	Varian Techtron AA-5
Wave length nm	422.7	422.7
Slit width nm	0.7	0.2
Lamp current ma	5.0	3.0
Air pressure kg/c^2	2.4	2.4
Acetylene pressure kg/c^2	0.56	0.7
Hydrogen pressure Lb/in^2	unemployed	10.0
Acetylene flow rate l/min	not measured	2.0
Hydrogen flow rate l/min	unemployed	2.5
Burner height mm	unmeasurable	4.0

Sample flow rate in the PE instrument was found to be 3.2 ml/min.

Method of determination: Based on the undergoing study, a method for calcium determination was worked out. Standard solutions were prepared to contain

[1]) Phosphorus was likewise determined in the same samples.

0–10 mg Ca %. Lanthanum was added both to standard and sample solutions to inhibit ionic interference, particularly from phosphate. Air acetylene flame was used throughout. Calcium content of the sample was computed from the formula:

$$\text{mg Ca \%} = 125A/S.V.W.$$

where A stood for absorption signal of the ash solution, S stood for slope of the standard graph, V stood for volume taken of the 100 ml ash solution and further diluted to 25 ml before spraying, and W was sample weight in grams.

Results and discussion

Sensitivity: Calcium absorption showed good linearity with its concentration above the 2 ppm level (fig. 1). The slight curvature at the lower concentrations might be due to a matrix effect, since it was not encountered with solutions of higher matrices, e.g. containing sodium chloride. Sensitivity in air/acetylene (A/A) flame was found to be 0.1 ppm ca/1 % absorption, in good agreement with earlier reports (13, 14). Sensitivity in air/hydrogen (A/H) flame was found much higher (fig. 2). However, A/A flame was used throughout due to weaker interferences than in A/H flame as shown below.

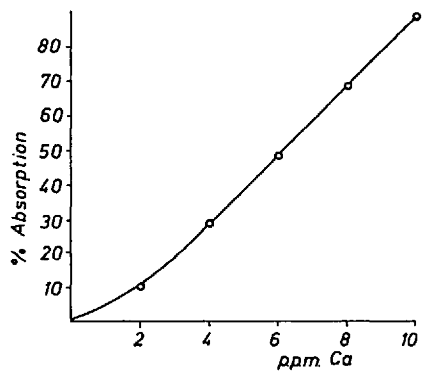

Fig. 1. Relation between calcium content and its atomic absorption.

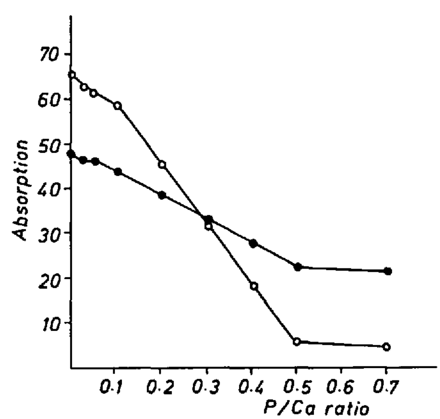

Fig. 2. Calcium atomic absorption in
●——● air/acetylene flame,
○——○ air/hydrogen flame

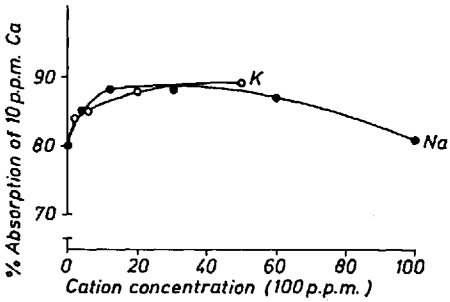

Fig. 3. Effect of sodium and potassium on absorption of 10 ppm calcium.

Interferences and their control

Cationic interference: Low concentrations of sodium or potassium (as chlorides) slightly enhanced absorption signals, whereas higher concentrations tended to lower them (fig. 3). The enhancing effect would not be attributed to radiation scattering (14) since at these very low concentrations the salt will easily be dissociated, and no refractive crystals will be present. It would be rather due to effect of the easily ionizable monovalent cations on saving more calcium atoms from ionization into the flame. The decreased absorption at the high cationic concentrations might be explained by a matrix effect (15).

Contrary to findings by Willis (14) magnesium showed an effect similar to that of sodium, in accordance with earlier results (10).

Lanthanum, in a 1 % concentration, was found sufficient to control interference of 1000 ppm of the studied cation (table 1).

Table 1. Control of cationic interferenc by lanthanum.

p.p.m. Calcium	% absorption in absence and presence of 1000 p.p.m. of the cation			
	No cation	Sodium	Potassium	Magnesium
0.0	00	00	00	00
3.2	20	20	20	20
8.0	50	49	50	49
11.2	68	67	67	68
16.0	92	92	91	92

Table 2. Effect of sulphate on absorption of 10 p.p.m. calcium.

p.p.m. SO^4	% absorption
0	80
4	78
10	75
20	68
40	68
80	67
120	66
200	65

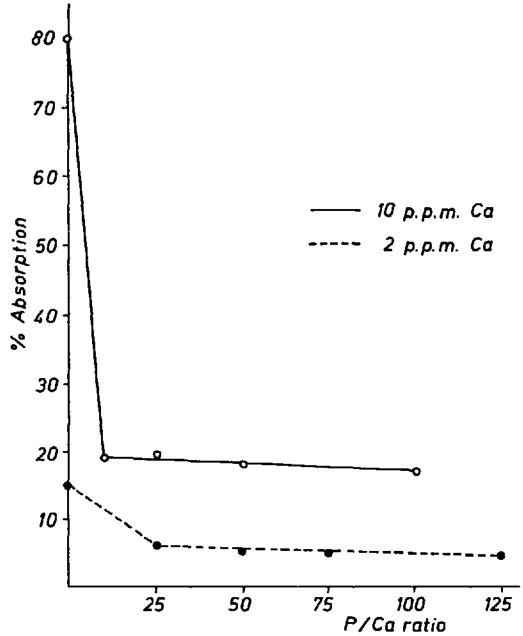

Fig. 4. Effect of high phosphate content on absorption of calcium
●- - -● 2 ppm Ca,
○——○ 10 ppm Ca

Anionic interference: Anions suppress calcium absorption (14). No serious concentrations of the vanadates or aluminates were to be expected in foods. Silicates were eliminated during ash preparation (see Materials and methods). At least in sulfated foods (e.g. dehydrated fruits), sulfates would be present in high concentrations. Most of the suppressing effect on absorption of 10 ppm Ca was found to be brought about by the first 20 ppm of sulfate. Higher concentrations of this anion showed a decreased effect (table 2).

High concentrations of phosphate showed strong suppressing effect on absorption of calcium (fig. 4). Interference in A/H flame exceeded that in A/A flame (fig. 2) due to lower temperature of the former one and its insufficiency to dissociate the formed calcium salt(s) (15). Extent of phosphate interference not only depended on phosphorus concentration, but

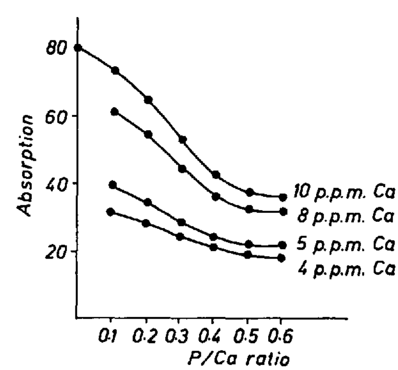

Fig. 5. Effect of P/Ca ratio on suppression of calcium atomic absorption.

Table 3. Effect of lanthanum and strontium on controlling phosphate interference (500 p.p.m.) with absorption of calcium (5 p.p.m.).

p.p.m. La or Sr	% absorption in presence of La	Sr
4,000	60	44
6,000	79	61
8,000	83	72
10,000	85	80
12,000	85	80

Table 4. Effect of lanthanum on anionic interference.

p.p.m. anion	% absorption of 5 p.p.m. Ca	10 p.p.m. Ca
Sulphate		
0	54	
700	54	
1750	54	
3500	48	
Nitrate		
0	54	
4000	54	
Phosphate (P)		
0		80
250		80
500		80
750		74
1000		67
% hydrochloric acid		
0	48	
0.6	48	
1.8	47	
3.0	46	

Table 5. Control of combined interferences by lanthanum.

Ion concentration (p.p.m.) PO_4	H_2SO_4	Mg	HNO_3	Absorption of 5 p.p.m. Ca
0	0	0	0	54
0	0	1000	1350	54
0	1750	1000	0	53
100	1750	0	0	54
100	1750	1000	0	53
200	0	0	0	54
200	0	1000	0	54

Table 6. Recovery of calcium added to ash solutions.

Sample	P/Ca ratio	% recovery of added p.p.m. calcium 0	2	4	6	10
Sugar	–	0	103	–	101	98
7-µp	–	0	100	110	–	–
Paprika	0.2	0	103	103	–	–
Cheese	0.6	0	106	103	–	–
Milk	0.75	0	100	100	–	–
Veal	25.00	00	106	103	–	–

Table 7. Reproducibility of calcium determination.

Sample	mg Ca % of 4 determinations 1	2	3	4
Apple	2.6	2.6	2.8	2.9
Cod fish	17.5	17.7	18.4	19.2
Apricot jam	12.8	12.8	13.2	13.2
"Gouda" cheese	748	452	755	762
Peanuts	58.2	59.8	60.0	60.4
Cola drink	1.6	1.7	1.8	–

Table 8a. Calcium content of some Dutch foodstuffs.

Foodstuff	mg Ca/100 g	
	by AAS[1)]	Calculated from DFCT[2)]
Sugar	3	–
Apple treacle	29	11
Strawberry jam	11	20
Apricot jam	13	20
Milk	119	120
Evaporated milk	260, 275, 290[3)]	300
Gouda cheese, young	754	560
Cod fish	18	20
Green paprika	12	25
Peanuts	60	60
7µp	6	6
Orange juice	11, 16, 33	10

1) Atomic absorption spectrometry.
2) Dutch food composition tables.
3) More than one figure indicates analysis of more than one trade mark.
4) Range of more than three different trademarks.
– analysis not shown in DFCT.
S. From: Nährwert-Tabellen, Souci, S. W., et al. Die Zusammensetzung der Lebensmittel, Stuttgart 1969.
V. From: Weinchemie und Weinanalyse, in: Handbuch der Kellerwirtschaft by Vogt, E., ed. Eugen Ulmer, Stuttgart 1953.

Table 8b. Calcium content of some Dutch foodstuffs often treated with phosphates.

Foodstuff	mg Ca/100 g	
	By AAS	From DFCT
Ginger bread	11, 12, 16	65
Knäckerbrod	45, 142	50
Rusks "bischuit"	28	–
Small cakes	22, 36	10
Pan cakes	83, 232	–
Pan cake flour	90	–
Cream crackers	16	–
Salted crackers	12, 36, 38	15
Cheese crackers	270	–
Self-raising flour	10, 12	–
Baking powder	1	–
Cake mix	4, 5	–
Pudding powder	140–308[4])	–
Infant food	14, 22	–
Child food	183	
Ice cream	62–172	160
Molten cheese	449–635[4])	450, 675
one sample	1274	
Ham	5, 7, 9	10
Sausage, fumed	10, 12, 22	10
Sausage, frankfurter	9, 10	(8)[S]
Fishsticks	11, 13, 14	(11)[S]
Tuna fish, canned	14	(7)[S]raw
Herring fillets in tomato sauce	32	150 raw
Mackerel, canned	168	175
Sardines, canned	216	20
Mayonnaise	12	10
Salad sauce	5	4
Chocolate, bitter	10, 41	50
Milk chocolate	202	200
Orange juice	11, 16	10
Orange juice, concentrated	33	–
Cola drinks	2–6	5
Condensed milk	260–290	300
Instant cocoa powder	15	140

also on P/Ca, i.e., at a constant calcium level, extent of interference decreased with increasing P/Ca ratio (fig. 5).

Lanthanum showed better capacity in controlling ionic interference than did strontium (table 3). It successfully controlled, in a 1 % concentration, interferences of one kind or combinations of different ions (tables 4, 5).

Accuracy and reproducibility: Using the standard addition method (16), calcium determination by AAS showed reasonable accuracy when applied to foods of variable P/Ca ratios (table 6). Reproducibility was also acceptable (table 7).

Table 8c. Calcium content of some Dutch food mixtures.

Foodstuff	mg Ca/100 g	
	By AAS	From DFCT
Bread mixture	29	15
Grains	20	18
Potatoes: new	11	–
old	9	10
Glucose and sugar treacle	9	50
Apple treacle	29	11
Whole milk	111	120
Standardized milk	106	120
Cheese	833	610
Meat	7	10
Offals, edible	15	10
Hen eggs	54	60
Herring	35	–
Plaice	33	–
Haddock	54	–
Pulses	99	80
Fats	5	10
Cream	73	70
Vegetables	38	50
Fruits	19	23
Fruits, subtropical	29	30
Fruits, dried	119	50
Nuts	110	130
Coffee, whole	120	(146)^{S}
Coffee, extr.	45	–
Tea, whole	377	(302)^{S}
Tea, extr.	15	–
Wine	6	(2)^{V}
Beer	4	2
Soft drinks	4	6

Calcium content of some Dutch foodstuffs

Table 8 summarizes results of calcium content of some foods as obtained by analysis, and from DFCT (4).

On the over all, values obtained by analysis did not differ much from those given in the DFCT. However, significant differences did exist, in e.g. ginger bread, ice cream, canned sardines and instant cocoa. These differences would be due to differences in the commercial brand of the food analyzed (see e.g. ice cream). Thus DFCT could be satisfactorily used for general purposes, and whenever samples of food consumed in a dietary survey were not available. However, for more accurate results, samples of the diet consumed must be analyzed.

The per caput calcium intake of the Dutch population

The per caput calcium intake of the Dutch population (table 9) determined from analysis of the food mixtures and food consumption in the

Table 9. Calcium intake of differnet population groups.

Group	Per caput mg calcium per day of food groups																						
	(1)			(2)			(3)			(4)			(5)			(6)			(7)			Total	
	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b
Whole population	59	76	7.9	755	691	71.9	35	38	4.0	14	8	0.9	118	116	12.1	18	14	1.4	41	17	18	1040*)	960
Men in a village	-	129	8.2	-	1264	80.5	-	54	3.4	-	17	1.1	-	105	6.6	-	31	0.2	-	1	0.0	-	1572
Men in town	-	98	8.8	-	828	74.0	-	47	4.2	-	12	1.1	-	130	11.7		1	0.1	-	1	0.1	-	1117
Town men in summer	-	75	6.5		948	81.9		37	3.2		15	1.3		82	7.1								1156
Town men in winter	-	69	7.1		778	79.7		35	3.5		12	1.3		109	8.4								976
Girl students		57	3.3		1372	78.8		38	2.2		34	2.0		153	8.9					66	3.8		1720
Boy students		80	7.3		840	77.5		24	2.3		-	-		104	9.6					37	3.4		108
Lactating mothers		51.8			1168.5			8.0			-			31.6			160						1420
Nursing mothers		44.7			630.3			7.1			-			25.6			160						-
Children 1 year		30			695			16			7			47			-			-			796
Children 2 years		34			613			20			10			52			-			-			795
Children 3 years		41			642			18			11			56			-						768

*) Calculated by Mulder (DFCT):980
a = computed from food tables
b = by analysis
c = % of total intake

(1) Carbohydrate-rich foods
(2) Dairy products
(3) Protein-rich foods
(4) Fat-rich foods
(5) Fruits and vegetables
(6) Nuts
(7) Drinks

Netherlands as calculated by Mulder (18) did not differ much from that published by the last author, being 980 mg, nor from the Dutch recommendations (0.8 g). It is higher than recommendations of many other countries (e.g. 0.5 for Canada and France, 0.6 for Japan, 0.7 g for South Africa, 0.8 g for Germany, Norway and USA) (19). The fact that dairy products shared with most (≈ 72 %) of the total calcium intake should be a healthy one, since milk calcium is known to be more easily absorbed than e.g. bread calcium (17).

It is needless to say that the per caput intake of any nutrient does not give a detailed picture of intake of this nutrient. Some individuals might consume much higher or much lower amounts than the calculated average. Intake could be affected by factors such as age, sex, social standing, economic status or job. This called for study of intakes of different population groups. Nevertheless, it must be borne in mind that individual variation within one group could still be very large as was shown by den Hartog (20) and Wigbout (21).

Calcium intake of some Dutch population groups

Calcium intakes of the Dutch population groups were computed from previously published data on food intake of some groups (11, 22, 23, 24, 25). In general, calcium intakes of these groups were found at least close to or higher than the recommendations (table 9).

It was noted that village men enjoyed highest calcium intake within the groups studied, not only due to high food intake, but particularly to high consumption of milk and dairy products sharing with 80 % versus 74 % in the case of town men. Town men exceeded villagers only in fruit and vegetable consumption.

Climate showed strong effect on calcium intake. Food intake, except for fruits and vegetables, was higher in summer than in winter. However, share of each food group in the total intake of calcium remained almost constant.

The case of the girl student with an intake two times the recommendations for her age, could be a good example of individual variations.

Compared to the boy student, or to the average Dutch, her consumption of bread was very low, but her consumption of fruits and vegetables, of fat-rich foods, and particularly of milk and dairy products was especially high, even exceeding that of lactating mothers.

Lactating mothers enjoyed an intake of calcium, on the average, close to Dutch recommendations (1.5 g). The worry would be for mothers with too low intake due to individual variations. To be noted was that milk and dairy products shared with more than 80 % of mothers' intake of calcium versus 3.6 % for the bread group. High dietary fiber (e.g. in bread) was shown to inhibit absorption of calcium (26).

Unfortunately, there were no dietary surveys for adolescents available so that to compute their calcium intake and compare it to their high recommended allowances (1.2 g according to Dutch recommendations). Daily calcium intake of children was found more or less lower than the Dutch recommendations of 800 mg. The case would be more serious when individual variations in the intake could be taken into account. The case was more evident with the 2-year-old child, where the infant might no more be breast- or bottle-fed with milk and became more interested in playing than in being fed. As a matter of fact, irregular consumption habits had been reported for this age (25).

It is concluded that local reliable and updated food composition tables could be safe enough for use in computing nutrient intake for general purposes, saving lots of time, effort and costs consumed in food collection and analysis. For more specific purposes where large number of samples will be available for analysis, preparation of well designed food mixtures can lower this number appreciably. The atomic absorption spectrometry as a simple and rapid technique provides a suitable method for metals' determination in food, particularly when such large number of samples in a dietary survey must be analyzed.

Study of calcium intake in the Netherlands showed that recommendations of most population groups were covered, that well diversified diets were consumed, and that milk and dairy products presented the main source for dietary calcium in this country. This would reflect a status of a well-informed population in a developed country, and fruitful efforts of concerned organizations such as «het voorlichtingsbureau voor de Voeding» (The information office for Nutrition). It is believed that foundation of such an organization in developing countries would have very remarkable results.

Summary

In a dietary survey, large numbers of a wide variety of food samples have to be analyzed. The atomic absorption spectroscopy (AAS), with its simplicity, accuracy and rapidity, suggests itself for determination of many elements in such a case.

In this investigation, calcium determination by AAS is reviewed. Analytical parameters, interferences and their control are studied. Food samples are dry ashed and treated so as to eliminate silicates. Most serious interference comes from phosphates and is successfully controlled by addition of lanthanum. Calcium is determined in the range 2–10 ppm with a sensitivity of 0.1 ppm. Single food samples and samples representing food mixtures are analyzed, and calcium intake of different population groups is computed. In most cases, results compare favourably well with those obtained from food composition tables.

Calcium intake in the Netherlands both of the average per caput and of chosen population groups is found to cover the recommendations except for infants. Differences do exist between intakes of different groups and in different seasons.

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