

Rapid Determination of Calcium, Magnesium, Sodium and Potassium in Milk by Flame Atomic Spectrometry After Microwave Oven Digestion

Miguel Angel de la Fuente and Manuela Juárez*

Instituto del Frío (CSIC), Ciudad Universitaria s/n, 28040 Madrid, Spain

A rapid method for determining Ca, Mg, Na and K in skim, semi-skim and whole milk was studied. These elements can be accurately determined in milk by atomic absorption (Ca and Mg) and atomic emission (Na and K) spectrometry after digestion of the sample with HNO₃ in domestic and analytical microwave ovens in sealed poly(tetrafluoroethylene) reactors. Microwave-oven mineralization allowed accurate determination as tested against certified milks. The results were also in good agreement with those obtained after precipitation with trichloroacetic acid in certified and real liquid milk samples. Low recoveries for Ca in whole milk were found, owing to matrix effects, and the method of standard additions was necessary to avoid them. Mean recoveries determined for one of the milk reference materials studied were 99.3, 100, 100.5 and 100.3% for Ca, Mg, Na and K, respectively. The method gave relative standard deviations of 1–2% for the four elements. The limits of detection were 63, 15, 18 and 72 µg l⁻¹ for Ca, Mg, Na and K, respectively. The method is very practical for routine laboratory analyses of relatively large numbers of samples.

Keywords: Calcium, magnesium, sodium and Potassium determination; flame atomic spectrometry; milk; microwave-oven digestion

Introduction

Although a variety of instrumental methods (fluorimetry, X-ray fluorescence, neutron activation, inductively coupled plasma atomic emission spectrometry) have been used in recent years to determine the different mineral elements in milk, flame atomic spectrometry is perhaps the most prevalent because it is sufficiently sensitive for the macro-elements and easy to operate, having successfully replaced the conventional spectrophotometric methods.

Sample preparation represents an important stage in analysis and some publications have been devoted to this subject.^{1,2} For milk, preparation of the solution for spectrophotometric measurement is of paramount importance, and this varies depending on the authors: some simply dilute the milk with de-ionized water;³ another method involves drying the sample⁴ preceded by a preliminary heating step to evaporate the milk to dryness;^{5,6} and one of the most common procedures is that involving precipitation of the milk proteins with trichloroacetic acid (TCA).^{7–10} Both TCA precipitation and dry incineration methods have the drawback of requiring different reagents and/or long operating times.

Two developments are apparent in the sample preparation procedures used or recommended over many years: first, the use of sealed pressure vessels to accelerate sample digestion and minimize contamination and loss of volatile elements; and second, the use of microwave radiation to assist in digestion.

Recently, rapid, safe and efficient acid decomposition methods based on the use of microwave ovens have been proposed for the determination of different elements in a variety of matrices and materials including foods.^{11,12} In milk and dairy products, only a few reports have been published involving microwave digestion, more specifically trace elements in infant formula powdered milk¹³ and cheese,¹⁴ and none of them studied the major elements contained in milk.

The purpose of this work was to establish operating conditions for the determination of Ca and Mg by atomic absorption spectrometry (AAS) and Na and K by atomic emission spectrometry (AES) in milk, using samples prepared by digestion in domestic and analytical microwave ovens. The sample preparation procedure was compared with that of precipitation with TCA.

Experimental

Apparatus

The determination of K and Na was carried out by AES using a Model 5100 PC atomic absorption spectrometer (Perkin-Elmer, Norwalk, CT, USA). Ca and Mg were determined by AAS using the same instrument with a multi-element (Ca, Mg, Zn) hollow-cathode lamp. The elements under study were determined using an air-acetylene flame and the recommended values for the instrumental parameters are given in Table 1.

Reagents

High-purity water with a metered resistivity of 18 MΩ cm was used to prepare all samples and standards. All reagents used were of the highest purity available and at least of analytical-reagent grade.

HNO₃, 65% m/v. Suprapur grade (Merck).

H₂O₂, Perhydrol. Suprapur grade (Merck).

Ca, K, Na and Mg standard solutions for spectrophotometry, 1 ± 0.002 g l⁻¹ (Panreac).

Ca, Mg, Na and K standard Titrisol solutions, 1 ± 0.002 g l⁻¹ (Merck).

LaCl₃ solution, 5% m/v. Prepared by mixing lanthanum oxide (Phaxe) (5.86 g), 5 ml of distilled water, 25 ml of concentrated HCl (Merck) and diluting to 100 ml with distilled water.

Trichloroacetic acid, 24% m/v aqueous solution.

Standard Solutions

Two groups of standard solutions were prepared. First, to measure the elements by the TCA deproteinization procedure, the composition described by Juárez and Martínez-Castro⁹ was used. Second, to determine major cations by the

* To whom correspondence should be addressed.

microwave-oven digestion method, two standard solutions were used with Ca, Mg, Na and K contents of 2.0, 0.2, 3.0 and 1.0 and 5.0, 0.5, 7.0 and 2.5 mg l⁻¹, respectively. Lanthanum was added to all standards at 500 µg ml⁻¹.

Samples

Milk samples utilized for repeatability experiments were purchased in the market and corresponded to three types of UHT milk with different fat contents: 3.6% for whole milk, 1.8% for semi-skim milk and 0.1% for skim milk. During the course of the assays, samples were stored in refrigerated conditions.

Reference Material

Validation of the method presented in this study was performed by using two reference materials: one from the National Institute of Standards and Technology (NIST), Standard Reference Material (SRM) of 1549 Non Fat Milk Powder, and the other from the Community Bureau of Reference, BCR-63 Skim Milk Powder. Amounts of 5 g of these powders were dissolved in water and diluted to 50 ml in order to reconstitute the liquid milk for subsequent use in the different assays.

Procedures

Sample preparation by de-proteinization with the TCA method was described in previous papers.^{8,9}

For the microwave-oven digestion method, different reagent volumes and conditions (times and power settings of the microwave oven) were tested in order to establish the experimental parameters of the recommended procedures as described below.

For sample preparation using a domestic microwave oven, six reactors were taken and filled with 1 ml of milk sample and 2 ml of concentrated HNO₃. The reactors were sealed and placed at outer positions inside a lidded plastic container, which was placed on the revolving plate. The alternative digestion mixture consisted of 2 ml of HNO₃ and 2 ml of H₂O₂ for the same amount of milk sample (1 ml). The six samples were then irradiated according to the following programme: 4 min at 583 W followed immediately by 8 min at 247 W. After irradiation, the reactors were set in an ice-bath to cool before opening. Appropriate dilutions must be carried out for sample measurements in the linear range of each of the elements to be determined.

The procedure using the analytical microwave oven was similar to that described above for the domestic microwave. The six reactors were placed in the carousel at alternate locations, leaving a gap between every two reactors so that the radiation would be evenly distributed among them. Lanthanum was added to the solution measured at 500 µg ml⁻¹ as a releasing agent to break up the Ca₃(PO₄)₂ in all the samples.

Table 1 Instrumental conditions for measurement of Ca, Mg, K and Na by flame atomic spectrometry

Element	Wave-length/nm	Slit width/nm	Lamp intensity*/mA	Flame characteristics/ l min ⁻¹	
				Air	Acetylene
Ca	422.7	0.7	15	10	2.0
Mg	285.2	0.7	15	10	3.8
K	766.5	0.4	—	10	2.0
Na	589.0	0.4	—	10	2.0

* Multi-element (Ca, Mg, Zn) Intensitron (Perkin-Elmer).

Microwave Ovens and Reactors

Two types of microwave oven were used for the assays. The domestic microwave oven was a Texet Model-112, programmable for time and microwave power in three discrete steps with nine power settings (ranging from 112 to 650 W) and equipped with a revolving plate. For safety reasons the domestic microwave was placed in an isolated area of the laboratory. The magnetron frequency was 2450 MHz. The poly-(tetrafluoroethylene) (PTFE) vessels used for the solutions were laboratory-made and had a volume of approximately 100 ml with a 10 mm wall thickness and tight-fitting screw-cap lids.

A commercially available analytical microwave oven, Model MDS-2000 (CEM, Indian Trail, NC, USA) with a magnetron frequency of 2455 MHz and 650 W output power, was also used. This apparatus was equipped with pressure and temperature control systems and a carousel for 12 vessels. Lined digestion vessels (100 ml) made from perfluoroalkoxy (PFA)-Teflon were obtained from CEM. The vessels can be used at temperatures up to 250 °C and a maximum pressure of 1400 kPa. Venting of the digestion or reaction products is controlled with a proprietary sealing and vent stem (a thin fluoroplastic rupture foil is inserted between the body and the cap to act as a seal) and the vessel can easily be tightened or opened by hand.

Results and Discussion

Irradiation Efficiency

For domestic ovens that are not specifically designed for analytical purposes it is important to note that different positions in the oven cavity are not identical in terms of microwave irradiation.¹⁵ Thus a prior study of the irradiation efficiency at different positions on the revolving plate was necessary. In order to carry out this study, 61 beakers (28 mm in diameter), each containing 25 ml of distilled water, were placed inside the cavity of the microwave oven on the revolving plate, covering its entire surface in nine energetically different positions. The oven was operated at maximum power for 30 min, then the loss of water from each beaker was measured and related to the level of microwave power absorbed, taking the maximum loss of water as equivalent to a power absorption level of 100%. Fig. 1 shows the microwave distribution on the plate, from which it was concluded that the central position is the least energetic and outer positions received more microwave power. During the assays, the reactors were placed at outer positions on the revolving plate to ensure maximum irradiation of the solutions.

The power distribution depends on the amounts of materials (samples and reagents) employed, because the PTFE vessels are transparent to microwave radiation.¹⁶ In the assays six vessels were used simultaneously.

Digestion Conditions

As the basic aim of this study was to arrive at the mildest possible conditions of mineralization with the smallest possible number and lowest concentrations of reagents and with safety margins sufficient to prevent deterioration of both the microwave oven and the reactors, the experiments were designed as described below. At the same time, these conditions had to be adequate for the preparation of a solution that would meet analytical requirements for subsequent measurement by atomic spectrometry.

The temperature and especially the pressure inside the reactor are limiting factors in the digestion of different matrices in closed vessels.¹⁷ In order to ascertain the pressure and temperature conditions inside the reactors in the domestic microwave oven, the digestion conditions described under

Procedures for the domestic microwave oven were reproduced in the analytical microwave oven. The evolution of both parameters is illustrated in Fig. 2. The temperature increased exponentially during the first moments of digestion up to 100 °C, thereafter rising more slowly up to 140 °C for the final part of the first stage (4 min) of high-power irradiation (583 W). During the second mineralization stage, the temperature dropped to 125 °C, then remained constant. During the first stage, the pressure increased gradually up to 235 kPa (4 min), settling down to 150–160 kPa for the remaining 8 min of the programme. The mineralization temperature and pressure ensure oxidation of the sample because the microwave energy

penetrates the sample in depth and so causes heating throughout the liquid rather than at the surface only. When microwaves are absorbed by an acid, they cause heating by two mechanisms: dipole rotation and ionic conduction, which can occur simultaneously.¹⁶

The acid used in digestion was HNO₃ in view of its high oxidizing capacity and the solubility of nitrates in aqueous media. It was decided not to use HCl in view of its weaker oxidizing capacity and the high pressure that it produces, and perchloric acid was rejected in order to obviate problems with corrosive fumes and its explosive potential,^{15,18} which could damage the reactors. As an alternative to HClO₄, H₂O₂ was used in combination with HNO₃ in order to improve the digestion efficiency and achieve a clearer post-digestion solution, which would make for more accurate atomic spectrometric measurement. The samples mineralized without H₂O₂ were yellowish, whereas those mineralized with H₂O₂ provided a clear solution. Table 2 gives the results for the elements studied for mineralization of samples under identical conditions with and without added H₂O₂ for the remainder of the study.

Matrix Effects

At the outset of the study, the final atomic spectrometric determination of the four elements was carried out by external calibration through extrapolation from the straight lines determined using solutions of known concentration. In this way the results given in Tables 2 and 3 were arrived at. However, on attempting to determine Ca in whole milk samples in order to establish the precision of the procedure (as has been noted, the certified milks assayed were reconstituted powdered skim milks), the concentrations of this element were found systematically to be lower than those determined by TCA de-proteinization. The decrease with respect to the values as measured by the method of Brooks *et al.*⁸ were in excess of 15%. This loss did not appear to affect the other three elements, however. Longer microwave digestion times (20–25 min) produced no improvement. It was thought that addition of H₂O₂ combined with HNO₃ to the digestion mixture might enhance the destruction of organic matter and hence the release of Ca; however, as the results noted previously (Table 2) showed, the presence of H₂O₂ did not alter the Ca concentration as determined in whole milk.

To remove the matrix effects, in the determination of Ca in whole milk, it was decided to perform the final atomic spectrometric measurement by standard additions because the conditions could not be optimized for accurate measurement by straight calibration. The values found for Ca by the standard additions, method are close to those determined by TCA de-proteinization (Table 4), and therefore this procedure was adopted to determine Ca in repeatability tests using whole milk.

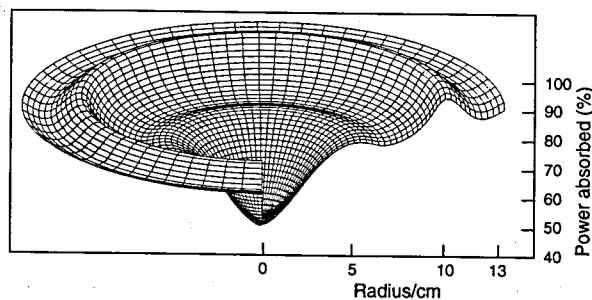


Fig. 1 Distribution of microwave radiation within the oven cavity on the revolving plate.

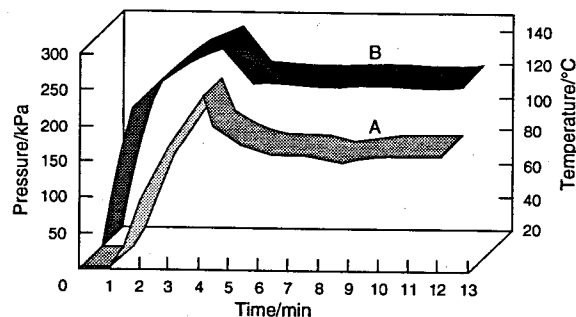


Fig. 2 Pressure (A) and temperature (B) evolution during the programmed digestion in the microwave oven (4 min at 583 W and 8 min at 235 W).

Table 2 Mean values and standard deviations for Ca, Mg, Na and K content (mg l⁻¹) in whole milk as determined by flame atomic spectrometry following mineralization in a domestic microwave oven in the presence (A) and absence (B) of H₂O₂. Each result is the mean of eight determinations

Digestion	Ca	Mg	K	Na
A	1008 ± 18	101 ± 1	1628 ± 19	650 ± 9
B	1008 ± 20	100 ± 1	1622 ± 13	643 ± 7

Table 3 Certified and experimental mean (± standard deviation) values for Ca, Mg, Na and K (mg l⁻¹) in reconstituted certified milks BCR-63 and SRM-1549. Measurement performed by flame atomic spectrometry following precipitation with trichloroacetic acid (TCA) or digestion with domestic (A) or analytical (B) microwave oven. The values shown are the means of five determinations

Sample	Method	Calcium	Magnesium	Potassium	Sodium
BCR-63	Certified values*	1260 ± 30	112 ± 3	1780 ± 60	457 ± 16
	TCA	1292 ± 18	110 ± 3	1736 ± 22	460 ± 14
	Microwave A	1261 ± 22	112 ± 2	1780 ± 10	446 ± 14
	Microwave B	1261 ± 32	113 ± 2	1782 ± 13	449 ± 3
SRM-1549	Certified values*	1300 ± 50	120 ± 3	1690 ± 30	497 ± 10
	TCA	1298 ± 16	119 ± 4	1683 ± 28	496 ± 13
	Microwave A	1306 ± 16	120 ± 1	1692 ± 17	500 ± 6
	Microwave B	1308 ± 12	123 ± 1	1690 ± 19	499 ± 4

* With 95% confidence limits.

In order to confirm the extent to which fat content influences Ca determination, microwave digestion was performed on commercial milks with different fat contents (whole, semi-skim and skim milk); Ca was determined by external calibration and the results were compared with those obtained by precipitation with TCA. The results are given in Table 5. In skim milk as in the certified milks, the Ca levels as determined by external calibration were similar for both procedures. In semi-skim milk, the Ca concentration was found to be slightly lower after mineralization in the microwave oven when determined by external calibration, whereas in whole milk this difference was marked. These results confirm the noticeable influence of the matrix on the analysis of whole milk. Association of two thirds of the Ca with the casein micelles in the presence of fat¹⁹ may be the reason behind this marked matrix effect on the determination of Ca, because organic materials are not totally decomposed to CO₂ and water. This effect was not apparent (Table 5) with Mg (only one third of which is bound to the caseins and whose over-all concentration is of a lower order of magnitude than that of Ca) or Na or K, both of which are present almost entirely in the soluble phase.²⁰

Precision of the Method

The repeatability of the procedure was studied by carrying out eight replicate assays on a single sample of whole milk prepared by mineralization in analytical and domestic microwave ovens and also by precipitation with TCA. The eight assays were performed in two lots in each microwave oven. The mean values and standard deviations are given in Table 4.

Table 4 Precision in determination of Ca, Mg, Na and K (mg l⁻¹) in whole milk (mean values \pm standard deviations) mineralized by domestic (A) and analytical microwave (B) oven digestion and by precipitation with trichloroacetic acid (TCA). Each result is the mean of eight determinations

Method	Calcium	Magnesium	Potassium	Sodium
TCA	1198 \pm 23	98 \pm 2	1535 \pm 21	573 \pm 21
Microwave A	1205 \pm 15	97 \pm 1	1518 \pm 16	578 \pm 7
Microwave B	1206 \pm 19	98 \pm 1	1531 \pm 16	573 \pm 11

Table 5 Determination of Ca and Mg (mg l⁻¹) by atomic absorption spectrometry (quantified by external calibration) in whole, semi-skim and skim milk (mean value \pm standard deviation) following mineralization by domestic microwave oven or precipitation with trichloroacetic acid (TCA). Each result is the mean of eight determinations

Milk	Calcium		Magnesium	
	TCA	Microwave	TCA	Microwave
Whole	1198 \pm 23	982 \pm 14	98 \pm 2	97 \pm 1
Semi-skim	1115 \pm 14	1105 \pm 18	106 \pm 2	109 \pm 1
Skim	1264 \pm 6	1261 \pm 23	112 \pm 2	112 \pm 1

The variability of the TCA and microwave methods are not statistically different ($P \leq 0.05$). The relative standard deviations (s_r) for the four elements were between 1 and 2% (Ca 1.2 and 1.6%; Mg 1%; K 1.1%; Na 1.2 and 1.9%). In all instances, the values found for samples prepared by digestion in the two ovens were lower than for those prepared by TCA precipitation (Ca 1.9%; Mg 2%; K 1.4%; Na 3.7). These results were comparable to those reported by Gaines *et al.*⁴ for Ca (1.1%) in a dry-ashing procedure.

Accuracy

In order to assess the accuracy of the procedure, in addition to examining BCR-63 and SRM-1549 certified samples, percentage recoveries were determined after addition of known amounts of Ca, Mg, Na and K to the latter.

Table 3 gives the contents of the elements studied in the certified samples prepared both by digestion in the two experimental microwave ovens and by precipitation with TCA.

For SRM-1549, the results following the two mineralization procedures were within the range of certified values, those of samples prepared by microwave being slightly higher. The results after mineralization in the two different types of microwave oven were comparable. For BCR-63 the results were within the certified range, although the results for Na were closer to the certified value in samples prepared by precipitation with TCA. However, the concentration of Ca as determined by the latter procedure was slightly above the certified value. The spread of the results, with the exception of Ca in BCR-63, was found to be smaller when samples were mineralized with either of the microwave ovens.

Table 6 shows the recovery study in which 1 ml of SRM-1549 reconstituted with 1 ml of standard solution was spiked with 1.0, 0.1, 0.5 and 1.5 g l⁻¹ of Ca, Mg, Na and K, respectively. The mean recoveries after digestion in the domestic microwave oven ranged from 99.3% for Ca to 100.5% for Na. These results are closer to 100% than those reported by Juárez and Martínez-Castro⁹ using the TCA de-proteinization method for Na (101.9%) and Mg (101.4%) and comparable to those for Ca and K (99.4% and 99.9%, respectively). Using dry mineralization in a furnace at 550 °C, Gaines *et al.*⁴ achieved Ca recoveries between 101% and 102%.

It may be concluded that milk digestion with HNO₃ (and H₂O₂) in PTFE reactors using microwave heating for AAS and AES provides an efficient alternative to dry mineralization or de-proteinization methods. The limits of detection were 63 (Ca), 15 (Mg), 18 (Na) and 72 (K) μ g l⁻¹. The precision and accuracy of the proposed method are similar to those of the techniques cited and are appropriate for the determination of Na, Ca, Mg and K in real samples. It also has the added advantages of simplicity in the digestion procedure and above all rapidity of execution: six milk samples can be digested in only 12 min. The possibility, here confirmed, of performing digestion in such affordable apparatus as a

Table 6 Recovery of Ca, Mg, Na and K added to reconstituted SRM-1549. The sample was digested with the domestic microwave oven and measured by flame atomic spectrometry. Each result is the mean of five determinations

Element	Certified values* mg l ⁻¹	Amount added/ mg l ⁻¹	Total calculated mg l ⁻¹	Amount found†/ mg l ⁻¹	Recovery (%)
Calcium	1300 \pm 50	1000	2300 \pm 50	2284 \pm 32	99.3
Magnesium	120 \pm 3	100	220 \pm 3	220 \pm 1	100.0
Potassium	1690 \pm 30	1500	3190 \pm 30	3200 \pm 37	100.3
Sodium	497 \pm 10	500	997 \pm 10	1002 \pm 19	100.5

* With 95% confidence limits.

† Mean value \pm standard deviation.

domestic microwave oven means that this procedure can easily be applied to routine determinations.

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