Optimization of Analytical Procedures Applied to Skimmed and Pasteurized Goat's and Cow's Milk for the Total Determination of Ca, Cu, Fe, Mg, K, Na, and Zn by Flame Atomic Spectrometry

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ABSTRACT

Optimization of the analytical procedures applied to skimmed and pasteurized goat's and cow's milk samples for the determination of Ca, Cu, Fe, Mg, K, Na, and Zn using flame atomic absorption spectrometry (FAAS for Ca, Cu, Fe, Mg, and Zn) and flame atomic emission spectrometry (FAES for K and Na) is presented. For this purpose, 40 random samples (20 each of goat's and cow's milk) of different brands were purchased locally in the city of Maracaibo, Venezuela. The analytical procedures applied to the milk samples included: (i) direct dilution with deionized water; (ii) wet digestion at atmospheric pressure with HNO₃; and (iii) wet digestion at atmospheric pressure with HNO₃ and H₂O₂. Spectrometric determination of the metals was performed using calibration curves, for non-spectral interferences using the standard addition method, yielding average errors between the slopes of <5% for Ca, Cu, Fe, Mg, and Zn. For FAES analysis, the K and Na concentrations were established by the quantification factors.

The accuracy was assessed by recovery studies and the results were between 95 and 105%. The analytical precision was established at an average RSD of 1.37% (ca. <5%). The limits of detection (3σ) and quantification (10σ) were 0.027 and 0.091 mg/L for Ca, 0.024 and 0.081 mg/L for Cu, 0.042 and 0.139 mg/L for Fe, 0.005 and 0.018 mg/L for Mg, 0.002 and 0.007 mg/L for K, 0.002 and 0.007 mg/L for Na, and 0.008 and 0.025 mg/L for Zn, respectively. The characteristic concentrations (CO) were: Ca 0.080 mg/L, Cu 0.080 mg/L, Fe 0.120 mg/L, Mg 0.010 mg/L, and Zn 0.010 mg/L. Skimmed and pasteurized goat's and cow's milk had similar concentrations of Ca, Fe, Mg, and Zn, with significant differences for Cu, K, and Na ($p < 0.05$).

The FAAS- and FAES-based methods described for Ca, Cu, Fe, Mg, K, Na, and Zn represent viable analytical tools for the evaluation of these different types of milk samples, and permitted the accurate, precise, and interference-free determination of Ca, Cu, Fe, Mg, K, Na, and Zn in these important food samples.

breed, milking frequency and interval, season of the year, gestational age, heredity, and other environmental variables (3).

The essential metals such as calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), potassium (K), sodium (Na), and zinc (Zn) are among the most important inorganic compounds present in milk (4) and have essential biochemical functions. Because of these benefits and its low cost, cow's milk is one of the main foods in Venezuela and other countries; however, it can cause allergic reactions and gastrointestinal problems (5). Goat's milk is an alternative in cases of intolerance to cow's milk and provides many benefits such as easy digestibility in patients who cannot tolerate cow's milk due to allergies to its proteins or the globule size of fat. For these reasons, the production of milk and milk products from other mammals such as goats and sheep has increased (6).

Since the levels of macro- and micronutrients such as Ca, Cu, Fe, Mg, K, Na, and Zn, among others, are critical in cow's and goat's milk, they must be evaluated in order to ensure product quality and to preserve the milk's nutritional composition (7). It is, therefore, very important to use reliable and reproducible analytical techniques that allow the determination and quantification of these metals in a short time and with simple and minimal sample handling.

INTRODUCTION

Milk is an important food source in the human diet and is consumed by children and adults of all ages (1). It can be defined as a complex

biological fluid secreted by the mammary glands of female cattle, goats, buffalo, sheep, and other mammals. Milk is made up of fats, proteins, vitamins, enzymes, sugars, and essential inorganic compounds for the proper function of the vital processes of the human body (1,2). The composition of milk varies considerably with the animal's diet and

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The analytical techniques widely used for the determination of trace metals in milk samples are inductively coupled plasma mass spectrometry (ICP-MS), electrothermal atomization atomic absorption spectrometry (ETA-AAS), flame atomic absorption spectrometry (FAAS), and flame atomic emission spectrometry (FAES) (8, 9, 10). FAAS and FAES are two of the most widely used techniques due to adequate precision and accuracy, simplicity, speed, and low cost of analysis (11-13). However, the direct determination of trace metals in milk by FAAS and FAES is difficult due to matrix interferences and low sensitivity because some of the metals present in milk such as copper, iron, and zinc are present at very low concentrations with respect to the organic matter (matrix) (11-13). Therefore, the determination of these metals by FAAS and FAES should be preceded by a pre-treatment such as digestion or mineralization (11-13) in order to eliminate the associated matrix without loss of analyte and for improving detectability (2, 14, 15). In this study, the concentrations of Ca, Cu, Fe, Mg, K, Na, and Zn in goat's and cow's milk were determined by FAAS- and FAES-based analytical methodologies previously optimized and validated.

EXPERIMENTAL

Instrumentation

The determination of Ca, Cu, Fe, Mg, and Zn was performed using a PerkinElmer® Model 2380 atomic absorption spectrophotometer (PerkinElmer Inc., Shelton, CT, USA) in the FAAS mode, and for K and Na determination in the FAES mode. Hollow cathode lamps specific to Ca, Cu, Fe, Mg, and Zn analysis were used as the primary radiation sources. The operating parameters used for the determination of each metal are listed in Table I (16). A Denver model 200-

DS analytical balance (ca. sensitivity ± 0.0001 g) (Denver Instrument Company, Bohemia, NY, USA) was used to weigh the metal salts. The volumes were measured with a Gilson micropipette Pipetman (models P-100, P-200, P-1000, and P-5000) using premium grade polypropylene tips (Gilson France SA, Villiers Le Bel, France). The digestion of cow's and goat's milk was performed using a Thermolyne model 2200 hot plate (Barnstead Thermolyne Corporation, Dubuque, IA, USA).

Reagents and Standard Solutions

All chemical reagents used were of analytical grade. The stock solutions of Ca, Cu, Fe, Mg, K, Na, and Zn (ca. 1000 mg/L) were prepared gravimetrically from salts of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (Riedel de Haën, Hannover, Germany), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (Fisher Scientific Co., Pittsburgh, PA, USA), $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (JT Baker, Phillipsburg, NJ, USA), $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (Merck, Darmstadt, Germany), KCl (Fisher Scientific Co.), NaCl (Fisher Scientific Co.), and concentrated Titrisol® solution of ZnCl_2 (Merck), all prepared in 1.0 mol/L nitric acid (Merck), respectively. These stock solutions were prepared in 1.0 mol/L nitric acid (Merck). All solutions were prepared with grade I triply distilled deionized water as estab-

lished by the American Society for Testing and Materials (electrical resistivity >16.6 MQ/cm at 25 °C) (17), in polypropylene-calibrated flasks (Nalgene Labware, Nalge, Rochester, NY, USA). A concentrated solution of lanthanum was prepared from lanthanum chloride ($\text{LaCl}_3 \cdot 7\text{H}_2\text{O}$, Merck) and used in the preparation of the aqueous standards and samples for the determination of Ca and Mg in order to avoid chemical interferences caused by phosphates (16). The calibration curves for every standard solution were obtained daily by direct dilution of the stock solution of each metal in 0.01 mol/L nitric acid (i.e., 0.5, 1.0, 2.0, 3.5, 5.0, and 6.0 mg Ca/L; 0.05, 0.10, 0.50, 1.00, 2.00, 3.00, 4.00, 5.00, 6.00, and 7.00 mg Cu/L; 0.10, 0.50, 1.00, 3.00, 5.00, 6.00 and 8.00 mg Fe/L; 0.10, 0.20, 0.30, 0.40, 0.50 and 0.60 mg Mg/L; and 0.05, 0.10, 0.20, 0.30, 0.40, 0.60, 0.80, and 1.00 mg Zn/L). Digestion of the samples was performed using concentrated nitric acid (ca. 65% v/v, Merck), and different combinations of hydrogen peroxide (ca. 30% v/v, Merck) as a co-oxidant.

Sampling

A random sampling was carried out by using 40 samples of skimmed and pasteurized liquid milk (20 samples each of goat's and cow's milk). The most consumed brands

TABLE I
Operational Parameters for the Determination of Metals
by Flame Atomic Spectrometry (16)

Element	Wavelength (nm)	Spectral Bandwidth (nm)	Gases in the Flame	Instrumental Mode
Ca	422.7	0.7	Air-Acetylene	AA
Cu	324.7	0.7	Air-Acetylene	AA
Fe	248.3	0.2	Air-Acetylene	AA
K	766.5	4.0	Air-Acetylene	AE
Mg	285.2	0.7	Air-Acetylene	AA
Na	589.0	0.14	Air-Acetylene	AE
Zn	213.9	0.7	Air-Acetylene	AA

AA: Atomic absorption; AE: Atomic emission.

of milk were obtained directly from supermarkets and bakeries located in Maracaibo city, commercially packaged in bottles of 500 mL and 1000 mL. The milk samples were transferred into polyethylene containers (125 mL Nalgene®), properly identified with the brand name, expiration date, and type of milk, and kept refrigerated at 4 °C before analysis to prevent decomposition and microbial growth.

Sample Pretreatment

Prior to the analytical determination of Ca, Cu, Fe, Mg, and Zn by FAAS, and K and Na by FAES, three different treatments were applied to the milk samples with the purpose of selecting the analytical method and sample treatment most suitable for the quantification of all metals.

(a) Treatment A (TA) involved dilution of the milk samples with deionized water, using the appropriate dilution factor for the analytical determination of each metal (ca. 2-fold for Cu, Fe, and Zn; 250-fold for Ca and Mg; and 100-fold for K and Na).

(b) In Treatment B (TB), an acid digestion was conducted at atmospheric pressure by mixing 5.0 mL of milk and 2.5 mL of concentrated nitric acid (HNO₃ 65%), then heating on a hot plate at 70–80 °C. The resulting solutions were brought to volume with deionized water in 10-mL flasks. A gravity filtration stage (Whatman® grade 1, Whatman International Ltd, Maidstone, Kent, England) was used in this last stage due to the presence of fat particles that were not removed during the digestion step.

(c) Treatment C (TC) was a digestion process by mixing the milk samples with HNO₃ and H₂O₂: 5.0 mL of milk, 2.5 mL of concentrated nitric acid (HNO₃ 65%), and a heating step at 70–80 °C in order to reduce the volume to about 2.0 mL. Subsequently, 2.5 mL of hydro-

gen peroxide (H₂O₂ 30% v/v) was added, and the solution heated again to reduce the volume to about 0.5 mL, cooled to room temperature, and the remaining solution transferred to a 10-mL flask and brought to volume with deionized water (18). Then, the samples were filtered by gravity to separate the fat that was not removed during the digestion process, and finally stored under refrigeration at 8 °C until spectrometric analysis. A dilution factor of 250-fold was necessary prior to the analytical quantification of Ca, Mg, K, and Na, while the determination of Cu, Fe, and Zn was performed directly due to their low concentrations in the digested solutions.

Volume Optimization of H₂O₂

With the aim of optimizing treatment C, different experimental assays were conducted by varying the amount of hydrogen peroxide (in order to evaluate the detectability of the metals), reagents consumption and digestion time. Different experiments were performed varying the volume of hydrogen peroxide. Thus, 1.0, 2.5, and 4.0 mL of 30% (v/v) H₂O₂ were added to three aliquots of the same milk sample digested with 2.5 mL of concentrated nitric acid. The concentrations of Ca, Cu, Fe, Mg, K, Na, and Zn were measured and compared to obtain the optimum amount of hydrogen peroxide for digestion of the milk samples.

Study of Filter Paper

The filtration step used in treatments B and C was required to remove the fat particles that were not removed at the end of both digestion processes. A study was conducted to determine the effect of the filter paper (Whatman®, grade 1) variation in the analytical signals, and thus the concentration for certain metals. In this study, the same digested blank was equally divided (ca. 25 mL for each one),

and then only a portion was filtered partially, and the filtered and unfiltered blanks were read spectrophotometrically. Blank preparation was performed by using 25.0 mL of deionized water, 12.5 mL of HNO₃, and 5.0 mL of 30% H₂O₂, then bringing the resulting solution to 50 mL volume (following the same proportion of reagents as used in the acid digestion of the milk samples). Five blank aliquots were filtered and the rest of the solution was measured unfiltered. The absorbances were averaged and compared for both filtered and unfiltered blanks.

Analytical Procedures

After the study of non-spectral interferences (also known as chemical or matrix interferences), calibration curves were used for the quantification of the metals. The standard addition method was used by varying the concentrations of the studied metals and adding 1.0, 2.0, and 3.0 mg Ca/L; 0.5, 1.0, and 2.0 mg Cu/L; 1.0, 1.5, and 2.0 mg Fe/L; 0.05, 0.10, and 0.20 mg Mg/L; and 0.10, 0.20, and 0.30 mg Zn/L to the milk samples. The slopes obtained by the standard addition method were compared with those obtained for the calibration curves prepared with aqueous solutions of the analytes studied in a 0.01 mol/L nitric acid matrix in order to confirm the use of the calibration curve for quantification of these metals.

Quantification of K and Na by FAES was performed by preparing aqueous standards of the evaluated analytes with concentrations immediately higher than those in the samples under study, resulting in a variable mathematical factor to obtain the true concentration in the analyzed test portion using the linear relationship calculated (17). For both analytes, the aqueous standard used was 15 mg/L, with readings of 99 E.a.u. (emission arbitrary units) for each one. Therefore, the quantification factor was 0.152 mg /

E.a.u. L for both K and Na (17). An optimization of the analytical parameters of merit was performed, such as accuracy, precision, detection and quantification limits, instrumental sensitivity, and the applicability of the methods to the real samples was studied. The accuracy of the analytical method was checked by recovery studies, selecting three levels of fortification or enrichment (i.e., low, medium, and high) for each analyte in each sample fortified or enriched, performing these experiments in pentaplicates and the signal readings were taken in pentaplicates (19,20). To assess the precision [repeatability (within-run) and reproducibility (between-runs)] of the analytical methods, each sample was prepared in triplicate and absorbance or E.a.u. readings collected in pentaplicates, obtaining for each test portion the arithmetic mean ($\bar{X}$), the standard deviation (SD), and the relative standard deviation [$RSD = (SD/\bar{X}) \times 100$].

Statistical Analysis

Statistical analysis was carried out by conventional methods (i.e., linear regression analysis, *t*-test, Mann-Whitney two-sample test,

analysis of variance, etc.), with the help of commercial statistical programs (i.e., Excel® 2000, Microsoft Corporation, USA). Differences and correlations were considered statistically significant when *p* was <0.05.

RESULTS AND DISCUSSION

Sample Treatment Selection

The three treatments applied to the milk samples were evaluated with the aim of selecting the most suitable procedure for a reliable and reproducible quantification of the essential metals Ca, Cu, Fe, Mg, K, Na, and Zn. In TA, a direct measurement of the milk samples was performed using two-fold deionized water dilutions for Cu and Fe, 10-fold for Zn, 250-fold for Ca and Mg, and 100-fold for K and Na, and compared with TB (digested with HNO₃) and TC (digested with HNO₃ and H₂O₂), both based on wet digestions of the sample with heating at atmospheric pressure to remove the organic matter present.

Table II shows the comparison of the metal concentrations obtained from the milk samples after application of the three differ-

ent treatments. TA was less effective in increasing the detectability of certain metals. However, TB and TC allowed an increase in the detectability of all metals determined. TC was more effective in increasing the detectability for most metals (i.e., Fe, Mg, Na, and Zn), especially metals that are found in smaller proportion in the sample, except for Cu which was not detectable in the samples analyzed during the methodological development. In the case of Ca and K, higher concentrations were obtained using TB; however, the differences were not statistically significant (*p* < 0.05) in comparison to those obtained with the TC.

Table III presents the statistical analysis comparing the concentrations (in mg/L) of each metal obtained with treatments A, B, and C. The concentrations obtained with each treatment were statistically significant with a confidence level of 95% (*p* < 0.05). Additionally, comparison tests (Tukey HSD) and the Student's *t*-test analysis were performed comparing each pair of treatment (i.e., A:B, A:C, and B:C). TC yielded a higher concentration of Fe, Mg, Na, and Zn in most of

TABLE II
Comparison of Metal Concentrations Obtained with Three Different Treatments Applied to the Milk Samples

Sample	Treatment	Analyte Concentration (mean $\pm$ SD, in mg/L)						
		Ca	Cu	Fe	Mg	K	Na	Zn
1	A	601.80 $\pm$ 24.90	ND	ND	94.05 $\pm$ 0.25	1375.10 $\pm$ 25.15	329.20 $\pm$ 19.10	1.12 $\pm$ 0.01
	B	827.90 $\pm$ 14.20	ND	0.25 $\pm$ 0.01	98.33 $\pm$ 2.34	1575.10 $\pm$ 47.25	416.70 $\pm$ 19.10	1.28 $\pm$ 0.03
	C	820.90 $\pm$ 9.80	ND	0.38 $\pm$ 0.01	99.96 $\pm$ 0.80	1525.10 $\pm$ 63.39	462.50 $\pm$ 21.70	1.41 $\pm$ 0.07
2	A	924.90 $\pm$ 24.30	ND	0.30 $\pm$ 0.01	130.30 $\pm$ 2.49	2370.90 $\pm$ 24.10	554.80 $\pm$ 2.80	1.91 $\pm$ 0.01
	B	1004.86 $\pm$ 30.06	ND	0.22 $\pm$ 0.01	136.10 $\pm$ 1.83	2419.40 $\pm$ 21.15	574.20 $\pm$ 2.80	2.12 $\pm$ 0.03
	C	988.49 $\pm$ 8.34	ND	0.34 $\pm$ 0.01	138.60 $\pm$ 3.05	2432.80 $\pm$ 20.12	577.40 $\pm$ 2.80	2.18 $\pm$ 0.04
3	A	713.30 $\pm$ 5.50	ND	ND	97.90 $\pm$ 0.10	3065.50 $\pm$ 72.13	1005.40 $\pm$ 4.70	1.82 $\pm$ 0.02
	B	744.60 $\pm$ 5.10	ND	0.21 $\pm$ 0.01	99.79 $\pm$ 0.56	3226.20 $\pm$ 21.13	1018.80 $\pm$ 4.70	1.86 $\pm$ 0.01
	C	775.10 $\pm$ 1.90	ND	0.26 $\pm$ 0.05	100.50 $\pm$ 0.66	3214.29 $\pm$ 0.10	930.10 $\pm$ 4.70	1.95 $\pm$ 0.02

Samples 1 and 2: Diluted skimmed and pasteurized cow's milk.

Sample 3: Diluted skimmed and pasteurized goat's milk.

A: Dilution with H₂O, B: HNO₃ Digestion, C: HNO₃-H₂O₂ Digestion.

ND: Not detectable.

the analyzed samples, with a statistically significant 95% confidence interval ($p < 0.05$). In the specific case of Ca and K, their detectabilities were increased using TB (samples 1 and 2 for Ca, samples 1 and 3 for K). But comparing the concentrations obtained from TB and TC, no significant differences ($p > 0.05$) were found in the analyzed samples. Thus, the most suitable method for the determination of Ca, Fe, Mg, K, Na, and Zn in the milk samples was found to be TC.

HNO₃/H₂O₂ Digestion

In treatment C, the concentrated nitric acid oxidized most of the organic matter at the digestion temperature used (ca. 60–70 °C) without loss of analyte (considering that the metals under study were not volatile under the conditions employed), observing the precipitation of proteins (especially casein) and separation of the butter fat. Lowering the pH of milk below 4.6 by the addition of HNO₃ produced denaturation of casein. At the same time, the acidity of the medium increased the solubility of the minerals, so that the calcium and phosphorus contents in the organic micelle gradually became soluble in the aqueous phase (21). Other factors favoring the destabilization of casein are the sudden change in temperature, molecular agitation, and impaired concentration, among others. This process freed up the different metals present in the milk as ions. When much of the evaporation volume of the digested sample was reduced, hydrogen peroxide (30%) was added to complete the digestion. Thus, H₂O₂ acted as a co-oxidizing agent that allowed to intensify and accelerate the oxidation reaction of the organic matter in the sample resulting in a homogeneous solution with light coloration (7, 21, 22).

Optimizing the Volume of Hydrogen Peroxide

After selecting the TC using hydrogen peroxide as the digestion treatment, the H₂O₂ volume was optimized. Tests were conducted on samples of milk previously digested with 2.5 mL concentrated HNO₃ and adding 1.0 mL, 2.5 mL, and 4.0 mL of 30% hydrogen peroxide. The results showed that there was no significant variation (95% confidence) in the metal concentrations when using any of the listed amounts of H₂O₂. For this reason, the lowest volume (ca. 1.0 mL) was selected as the most suitable for the treatment, which also is lowest in cost and results in the complete digestion of the samples.

Study of Filter Paper

In 2000, Silvestre et al. (23) reported the effect of filter paper in the variation of the metal concentrations of the obtained filtrate, especially if used for strongly acidic solutions. For this reason, a study was conducted to determine the effect of the filtration step required to separate the fat that was not removed during the digestion of the milk samples in TC. The absorbance and E.a.u. values obtained for digestion of the blanks in TC for the determination of Ca, Cu, Fe, Mg, K, Na, and Zn are listed in Table IV. It can be seen that there is no difference in the absorbance values for Cu, Mg, and Zn, whereas Ca and Fe varied

TABLE III
Statistical Comparison Between Metal Concentrations Obtained With Treatment A, B, and C Using One-way ANOVA and *t*-Student

Sample	Treatment	ANOVA / <i>t</i> -Student						
			Ca	Fe	Mg	K	Na	Zn
1	A, B, C	A:B	X	X	X	X	X	X
		A:C	X X	X X	X X	X X	X X	X X
		B:C	*	X	X	*	X	X
2	A, B, C	A:B	X	X	X	X	X	X
		A:C	X X	X X	X X	X X	X X	X X
		B:C	*	X	X	*	*	X
3	A, B, C	A:B	X	X	X	X	X	*
		A:C	X X	X X	X X	X X	X X	X X
		B:C	X	X	X	*	X	X

A: Dilution with H₂O, B: HNO₃ Digestion, C: HNO₃-H₂O₂ Digestion.

X: Significant differences ($p < 0.05$);

*: No significant differences ($p > 0.05$).

Concentration of Cu was not detectable.

TABLE IV
Comparisons Between Mean Signals for Filtered and Unfiltered Blanks for the Spectrometric Determination of Ca, Cu, Fe, Mg, K, Na, and Zn

	Mean Absorbance						
	Ca	Cu	Fe	Mg	K ^a	Na ^a	Zn
^a Unfiltered Blank	0.001	0.001	0.002	0.002	00	01	0.003
^b Filtered Blank	0.002	0.001	0.003	0.002	01	01	0.003

^a In E.u.a.: Emission arbitrary units.

^b As mean of five test portions prepared of the Blank solution for each analyte, and five replicate absorbance or E.a.u. readings.

slightly ($p < 0.05$). For K and Na, no statistical differences between the E.a.u. values were observed. Moreover, the absorbance and E.a.u. values obtained were reproducible when comparing the different aliquots of the filtered blanks. Due to the filtration of the blanks from the digested milk samples, any minimal metallic input from the filter paper was eliminated when the value was subtracted from the absorbance of the blank of the digested samples, ensuring the reliability of filter paper for the determination of Ca, Cu, Fe, Mg, and Zn in milk samples with the selected digestion treatment. For K and Na, the blanks showed no different analytical signals during the spectrometric measurements.

Optimization of Analytical Parameters

Quantification of the metal concentrations using flame atomic absorption spectrometry (for Ca, Cu, Fe, Mg, and Zn) and flame atomic emission spectrometry (for K and Na) and applying the optimized digestion treatment to the milk samples requires evaluation of the analytical parameters of merit such as mathematical-statistical processing of the calibration curve, accuracy, precision, spectral and non-spectral interferences, detection and quantification limits, instrumental sensitivity and applicability.

Calibration Curve

In order to find the optimal linear working range for each metal studied, the calibration curves for Ca, Cu, Fe, Mg, and Zn were optimized. The optimal linear range for Ca was from 0.0 to 8.0 mg/L; for Cu, from 0.0 to 6.0 mg/L; for Fe, from 0.0 to 7.0 mg/L; for Mg, from 0.0 to 0.6 mg/L; and for Zn, from 0.0 to 0.8 mg/L. These results are listed in Table V as well as the curve equations and the Pearson linear correlation coefficients.

Highly significant values can mathematically transform the absorbances obtained for the test portions of the actual milk samples to the concentrations of the analytes studied. The quantification of K and Na was performed using aqueous standards of 15 mg/L for each, applying a quantification factor of 0.152 mg/E.a.u. L (Table V) instead of calibration curves because the linear range for both metals was very limited. Considering the high concentrations of K and Na in real milk samples, overdilution to quantify these metals in the samples would have maximized the random experimental error.

Accuracy

The accuracy of the spectrometric methods for the analytical determination of metals considered in this study was verified by recovery studies of each metal added in the milk samples. When performing this study, the average recoveries obtained for Ca, Cu, Fe, Mg, K, Na, and Zn with TC were 96.34 ± 1.14 ; 98.22 ± 3.06 ; 102.23 ± 2.21 ; 99.90 ± 1.20 ; 100.13 ± 0.34 ; 99.52 ± 1.86 , and $97.61 \pm 0.68\%$, respectively (Table VI). These results demonstrated adequate accuracy of the proposed methods for the determination of Ca, Cu, Fe, Mg, and Zn by FAAS, and K and Na by FAES, and

are within the theoretical range of 95–105%, which is internationally accepted as optimum (24).

Precision

The results obtained in the precision studies [within run (analytical repeatability) and between-runs (analytical reproducibility)] of the proposed methods for the spectrophotometric determination of Ca, Cu, Fe, Mg, K, Na, and Zn are listed in Table VII, and are expressed as standard deviation (SD, in mg/L) and relative standard deviation (RSD, in %) (20). In this study, the mean RSD for the within-run and between-runs was 1.37%, which is below the reported international maximum value of 5% as the optimum (24). This confirms adequate precision of the analytical methods used for the FAAS and FAES quantification of the metals in the milk samples analyzed.

Study of Spectral and Non-spectral Interferences

For all metals, the presence of spectral interferences was not taken into account due to the simplification of the matrix through appropriate processes in the mineralization of the real samples, evidenced by adequate recovery levels obtained from the samples mineralized.

TABLE V
Calibration Curves and Correlation Coefficients for the Determination of Ca, Cu, Fe, Mg, and Zn by FAAS, and Quantification Factors for K and Na by FAES

Element	Equation of the Calibration Curve	Correlation Coefficient	Linear Range (mg/L)
Ca	$A = 0.0598 c + 0.0044$	$r = 0.9994$	8.0
Cu	$A = 0.0517 c + 0.0013$	$r = 0.9998$	6.0
Fe	$A = 0.0337 c + 0.0032$	$r = 0.9998$	7.0
Mg	$A = 0.5251 c + 0.0025$	$r = 0.9998$	0.6
K	0.152 ^a	-	15.0
Na	0.152 ^a	-	15.0
Zn	$A = 0.3180 c + 0.0042$	$r = 0.9995$	0.8

^a Quantification factors in mg / E.a.u. L units when using FAES.

The study of non-spectral interferences was carried out by comparing the slopes of the working curves with those obtained by the method of standard addition for each metal. The slopes and relative errors between slopes (listed in parentheses, in %) obtained for the calibration and standard addition curves were for Ca, 0.0598 ($r = 0.9994$, $p < 0.001$) and 0.0568 ($r = 0.9995$, $p < 0.001$) (5.02 %); for Cu, 0.0517 ($r = 0.9998$, $p < 0.001$) and 0.0509 ($r = 0.9997$, $p < 0.001$) (1.55 %); for Fe, 0.0337 ($r = 0.9998$, $p < 0.001$) and 0.0350 ($r = 0.9996$, $p < 0.001$) (3.86 %); for Mg, 0.5251 ($r = 0.9998$, $p < 0.001$) and 0.5151 ($r = 0.9999$, $p < 0.001$) (1.90 %); and for Zn, 0.3180 ($r = 0.9995$, $p < 0.001$) and 0.3094 ($r = 0.9994$, $p < 0.001$) (2.70 %). Relative error between slopes for each metal indicates an adequate parallelism, implying non-significant matrix effects in the analytical determination of these metals. Experimental relative errors were in all cases $\leq 5\%$, a value considered appropriate according to international criteria (19). These results implied the absence of non-spectral interferences in the FAAS analyses for the developed Ca, Cu, Fe, Mg, and Zn methods and permitted the use of aqueous calibration graphs for metal quantification in the mineralized milk samples. For K and Na, the study of non-spectral interferences was not carried out due to the simplification of the matrix through suitable mineralization processes and high dilution performed (ca. 100-fold) of the real milk samples, which minimized the interfering effects of the matrix under study. Furthermore, in the analytical determination of K and Na, calibration curves were not employed, instead quantification factors were used as indicated above.

Methodological Detectability and Instrumental Sensitivity

The methodological detectability expressed across the limits of

detection (LOD), which is defined for each analyte as three times the SD of the blank solution, and the limits of quantification (LOQ), defined as 10 times the SD of the blanks, both expressed in mg/L. The instrumental sensitivity, calculated as characteristic concentration (CO, in mg/L), for all of the metals determined by FAAS; for K and Na, determined by FAES, only LOD and LOQ values are presented) is listed in Table VIII. The experimental values for the CO were near to the theoretical values reported

for the instrument (16). These values allowed adequate detectability, quantifiability, and analytical sensitivity for the spectrometric determination of Ca, Fe, Mg, K, Na, and Zn in the mineralized milk samples as described above.

Concentrations of Ca, Cu, Fe, Mg, K, Na, and Zn in Milk Samples

After optimizing the analytical parameters of merit, the proposed methodologies were applied to a representative number of samples

TABLE VI
Accuracy Study for Determination of Ca, Cu, Fe, Mg, and Zn by FAAS, and K and Na by FAES in Milk Samples Digested with $\text{HNO}_3\text{-H}_2\text{O}_2$

Element	Added (mg/L)	Expected (mg/L)	Found (mg/L)	Recovery (%)
Ca	1.00	3.31	3.15	95.16
	2.00	4.31	4.20	97.44
	3.00	5.31	5.12	96.42
				$\bar{X} \pm \text{SD } 96.34 \pm 1.14$
Cu	0.500	0.545	0.519	95.23
	1.000	1.045	1.059	101.34
	2.000	2.045	2.006	98.09
				$\bar{X} \pm \text{SD } 98.22 \pm 3.06$
Fe	1.000	1.187	1.184	99.74
	1.500	1.687	1.754	103.97
	2.000	2.187	2.252	102.97
				$\bar{X} \pm \text{SD } 102.23 \pm 2.21$
Mg	0.050	0.240	0.243	101.25
	0.100	0.290	0.287	98.96
	0.200	0.390	0.388	99.48
				$\bar{X} \pm \text{SD } 99.90 \pm 1.20$
K	0.50	13.98	14.00	100.14
	1.00	14.48	14.45	99.79
	1.50	14.98	15.05	100.47
				$\bar{X} \pm \text{SD } 100.13 \pm 0.34$
Na	1.00	10.54	10.27	97.44
	2.00	11.54	11.66	101.04
	3.00	12.54	12.55	100.08
				$\bar{X} \pm \text{SD } 99.52 \pm 1.86$
Zn	0.100	0.222	0.215	96.84
	0.200	0.322	0.316	98.13
	0.300	0.422	0.413	97.86
				$\bar{X} \pm \text{SD } 97.61 \pm 0.68$

TABLE VII
Within-Run and Between-Runs Precision Study for the Determination of Ca, Cu, Fe, Mg, K, Na, and Zn in Milk Samples by FAAS and FAES

Sample ^a	Mean (mg/L)	Within Run (Analytical Repeatability)		Between-Runs (Analytical Reproducibility)	
		SD (mg/L)	RSD (%)	SD (mg/L)	RSD (%)
Ca	4.88	0.0143	0.29	0.0136	0.28
Cu ^b	0.046	0.0018	3.91	0.0021	4.57
Fe	0.136	0.0037	2.73	0.0024	1.79
Mg	0.412	0.0019	0.45	0.0017	0.40
K	6.75	0.1254	1.86	0.0282	0.42
Na	4.33	0.0351	0.81	0.0968	2.23
Zn	0.100	0.0021	2.07	0.0031	3.08

^a Cow's milk sample, prepared in triplicate and read in pentaplicates.

^b Cu was not detectable in cow's milk sample; therefore, skimmed and pasteurized goat's milk was used for this study.

TABLE VIII
Methodological Detectability and Instrumental Sensibility for the Determination of Ca, Cu, Fe, Mg, and Zn by FAAS, and K and Na by FAES in Milk Samples (mg/L)

	Ca	Cu	Fe	Mg	K	Na	Zn
LOD	0.027	0.024	0.042	0.005	0.002	0.002	0.008
LOQ	0.091	0.081	0.139	0.018	0.007	0.007	0.025
CO	0.080	0.080	0.120	0.010	-	-	0.010

LOD: Limit of detection.

LOQ: Limit of quantification.

CO: Characteristic concentration.

of skimmed and pasteurized goat's and cow's milk (n = 15 each one) of different commercial brands purchased and consumed in the city of Maracaibo, Venezuela. Table IX shows the comparison among the spectrometric concentrations of the essential metals Ca, Cu, Fe, Mg, K, Na, and Zn obtained for the milk samples. Skimmed and pasteurized goat's and cow's milk had similar concentrations of Ca, Fe, Mg, and Zn, with significant differences for Cu, K, and Na ($p < 0.05$). The metals Cu and Na were present at high concentrations in the goat's milk samples. It should be mentioned that copper was quantified in 10 of the 15 goat's milk samples, since the Cu concentrations were below the detection limit of the metal (ca. 0.024 mg Cu/L) giving an average concentration of 0.092 mg Cu/L. For potassium, the concentration was 1596.6 ± 110.4 mg/L in skimmed and pasteurized cow's milk, and thus was higher than the values in the goat's milk samples. In the literature, several authors reported higher levels of Ca, Cu, Fe, Mg, K, and Na, among others, in goat's milk than in cow's milk (3, 25-27). However, in this research goat's milk only had higher concentrations of copper (ca. 0.092 ± 0.005 mg/L) and sodium (ca. $1296, 61 \pm 41.44$ mg/L), and lower values for potassium. Finally, it should be pointed out that the high concentrations of Na in goat's and cow's

TABLE IX
Concentrations (mean $\pm$ SD, in mg/L) of Ca, Cu, Fe, Mg, K, Na, and Zn in Skimmed and Pasteurized Liquid Goat's and Cow's Milk Samples

	Ca	Cu *	Fe	Mg	K *	Na *	Zn
Goat's Milk (n=15)	961.2 ± 64.2	0.092 ± 0.005	0.197 ± 0.090	98.84 ± 0.12	1416.5 ± 23.1	1296.6 ± 41.4	1.905 ± 0.2
Cow's Milk n=15)	1120.3 ± 157.3	ND ^a	0.279 ± 0.099	93.28 ± 12.13	1596.6 ± 110.4	1060.2 ± 106.0	1.949 ± 0.6

* Significant differences ($p < 0.05$).

^a Not detectable (LOD = 0.024 mg Cu/L).

milk could be detrimental for people with high blood pressure and fluid retention (27, 28).

CONCLUSION

In this study, the FAAS- and FAES-based methods applied to goat's and cow's milk samples were reliable, reproducible, and free from interferences for the determination of Ca, Cu, Fe, Mg, K, Na, and Zn. The wet digestion with heating at atmospheric pressure, and using nitric acid and hydrogen peroxide, was effective for simplifying the milk matrix, increasing the detectability of the metals, particularly those found in smaller concentrations in the sample (i.e., Cu, Fe, and Zn). Skimmed and pasteurized goat's and cow's milk presented levels in most of the studied metals, except Cu, K, and Na. A high concentration of sodium was found for both types of milks studied, which could be harmful for people with high blood pressure and coronary diseases.

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