Chemometric Studies of Several Minerals in Milks

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A statistical study of correlation, factorial, and discriminant analysis on the metal composition (Se, Fe, Cu, Zn, Na, K, Ca, Mg) of different types of milks (human, cow, goat, pasteurized, and powdered infant formula) was carried out to establish the relationships between the metal concentrations and, therefore, differentiate the samples according to the type of milk. A large number of significant intermetallic correlations were found in all samples, which could be due to biological relationships between the metals studied. After the factorial analysis, the dimension space was reduced from eight variables to two factors, accounting for ∼71.4% of the total variance. After an orthogonal rotation, the first factor was positively correlated with Ca and the second factor with Fe. The representation of the *scores* makes it possible to separate not only human milk from powdered infant formula but also to separate both of these from the other milks. In the discriminant analysis, four discriminant functions were obtained, which are linear combinations of the quantitative variables that best explain the differences among the different milks analyzed. These functions make it possible to classify 98% of the samples analyzed within each type of milk correctly. Therefore, discriminant functions obtained here can be used to identify the origin of any milk sample.

Keywords: *Se; Fe; Cu; Zn; Na; K; Ca; Mg; milk; multivariate analysis*

INTRODUCTION

The metal concentrations in milks vary as a function of two kinds of factors related to its secretion from the mammary gland, such as the animal species, the time of year, and the breed of the animal, and factors related to handling by humans. Among these factors are the technological processes required to prolong shelf life, because pasteurization and sterilization processes significantly influence the metal concentrations (Moreno-Rojas et al., 1991, 1993; Zurera-Cosano et al., 1994).

Multivariate data analysis and, in particular, principal factor analysis can be used to obtain more information on major, minor, and trace components in foods. Holt (1993) has studied the interrelations of the concentrations of some metals of human milk by comparison with cow and goat milks. Martín Hernández et al. (1992) have applied stepwise discriminant analysis of the milk samples to yield the mineral contents and ratio among them and to differentiate the cheeses made from different milks. Several studies (Favretto et al., 1987, 1994; Gabrielli Favretto et al., 1989; Rincón et al., 1994; Zurera-Cosano et al., 1994) have applied these statistical tools to minor and trace elements present in milks. In general, a reduction of the number of variables and the differentiation of some types of milks analyzed were deduced.

In this paper the Se, Fe, Cu, Zn, Na, K, Ca, and Mg concentrations of several milks (from cow, goat, human, powdered infant formula, and pasteurized) have been determined by atomic absorption spectrometry and spectrofluorometry (Se). A statistical study of correlation, factor analysis, and discriminant analysis was carried out to differentiate the analyzed milks (human, cow, goat, pasteurized, and powdered infant formula) on the basis of the mineral composition (Se, Fe, Cu, Zn, Na, K, Ca, and Mg).

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MATERIALS AND METHODS

Apparatus. A Perkin-Elmer model MFP-44A spectrofluorometer with automatic recorder, arc of xenon Osram XBO, differential corrected spectra unit DCSU-1, digital lector VDR-3, and heating water bath Selecta Frigitherm S-382 were used. The emission intensity measuring system of the spectrofluorometer was calibrated daily, using the Perkin-Elmer set of fluorescent polymer blocks.

Atomic absorption spectrometry was performed with a Varian Spectr AA-10 Plus equipped with a D_2 lamp background correction system.

Reagents and Solutions. Se, Fe, Cu, Zn, Na, K, Ca, and Mg standard solutions (1 g/L) from Fisher were used for atomic absorption spectrometry. Working standards were prepared for dilution of this solution with Milli-Q water, DAN solution (0.04%, m/v), or 0.04 M EDTA-Na_2.

Trichloroacetic acid (TCA) (15%), HNO_3, $HClO_4$, and HCl used were of analytical quality.

Distilled and deionized water from Millipore-Milli-Q was used.

Samples. *(1) Human Breast Milk*. Fifty-eight samples of 12 healthy volunteer women (21−35 years old) were obtained during 1994−1996. Samples were collected from 2 weeks to 5 months postpartum (mature milk). The sampling was carried out in accordance with the standard procedures described in the IAEA/WHO document (Parr, 1978). Breast milk was directly sampled into precleaned polyethylene tubes using a conventional mechanical breast pump.

(2) Cow Milk. A single bulk tank sample of fresh, mature Holstein cow's milk was collected from each of six farms every 15 days during 1995. The 151 total samples were collected under normal working conditions.

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Table 1. Average Metal Concentrations (Milligrams per Liter) for the Different Types of Milks Studied[a]

metal	human	powdered infant formula[b]	cow	pasteurized	goat
Se[c]	15.69 ± 4.07[b]	7.47 ± 3.69 (57.5)[c d]	16.44 ± 4.41[b]	14.85 ± 2.85[b]	19.98 ± 5.18[a]
Fe	0.465 ± 0.191[b]	6.587 ± 0.901 (50.6)[a]	0.515 ± 10.178[b]	0.170 ± 0.026[c]	0.520 ± 0.213[b]
Cu	0.329 ± 0.140[b]	0.529 ± 0.135 (4.07)[a]	0.076 ± 0.032[e]	0.110 ± 0.039[d]	0.173 ± 0.053[c]
Zn	2.10 ± 1.10[d]	4.34 ± 1.05 (33.4)[abc]	4.41 ± 0.67[a]	3.06 ± 0.14[c]	3.31 ± 0.60[b]
Na	162.8 ± 90.1[b]	223.2 ± 60.9 (1717)[b]	534.1 ± 109[a]	549.2 ± 119[a]	513.7 ± 145[a]
K	602.7 ± 119[d]	767.2 ± 73.9 (5902)[c]	1424 ± 200[b]	1419 ± 55.4[b]	1585 ± 184[a]
Ca	368.6 ± 62.4[e]	786.2 ± 205 (6048)[d]	1945 ± 243[a]	1540 ± 72.6[c]	1804 ± 200[b]
Mg	40.11 ± 9.75[c]	41.16 ± 22.2 (317)[c]	113.9 ± 18.7[b]	121.1 ± 14.1[b]	156.8 ± 26.7[a]

[a] Results in the same horizontal line with the same superscript were not significantly different ($p < 0.05$). [b] In parentheses, concentration expressed as mg/kg. [c] Micrograms per liter. [d] Micrograms per kilogram.

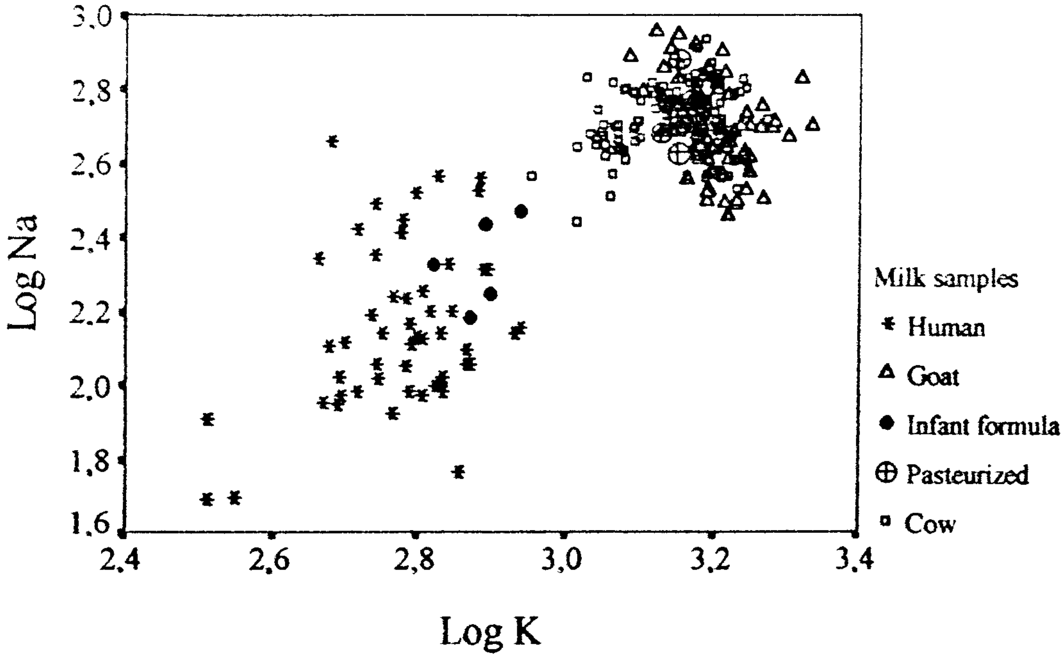

Figure 1. Plots of the double-logarithmical correlation between K and Na.

(3) Goat Milk. Seventy-eight samples of milk from 20 goats were collected during the March−August 1995 period. Sampling was carried out similarly to that for the cow's milk. Fresh samples of milk from local goats were supplied by a local farm.

(4) Commercial Milk. Three samples of five different kinds of powdered infant formulas for children <6 months and six different kinds of pasteurized whole milk marketed in Spain were taken from supermarkets in Tenerife.

All samples were stored in acid-washed polypropylene containers without preservatives, and they were frozen at −20 °C until analysis.

Sample Preparation. *Determination of Fe, Cu, Zn, Na, K, Ca, and Mg.* Three milliliters of milk (or 0.39 g of powdered infant formula) and 10 mL of HNO_3/$HClO_4$ (9:1) acid mixture were placed in a beaker, which was closed and left overnight. Next morning, the temperature of this acid mixture was slowly increased, using a hot-plate, to 160−170 °C until fumes of $HClO_4$ appeared. This solution was quantitatively transferred and adjusted to a 10 mL flask with Milli-Q water. These metals were determined by atomic absorption spectrometry.

Determination of Se. Fluorometric determination of the Se in milks was carried out according to methods published previously (Rodríguez Rodríguez et al., 1994, 1997).

Statistics. All of the statistical analyses have been performed by means of the SPSS release 6.0 for Windows (Ferrán, 1996). Analysis of variance was applied for all variables studied to compare the mean values obtained among the different groups of the population studied. The mean values obtained in the different groups were compared by one-way ANOVA and *t* test, assuming that there were significant differences between mean values when statistical comparison gave $p < 0.05$. Simple linear and logarithmical correlation analysis was used to obtain a measure of the correlation strength of the relationship between two variables. Factor analysis (utilizing principal components as the method from extraction of factors) was used to summarize the information in a reduced number of factors, and discriminant analysis was used to select the most useful variables in the differentiation among aggregations.

RESULTS AND DISCUSSION

Metal concentrations of all the milks analyzed can be arranged in the sequence Ca > K > Na > Mg > Zn > Fe > Cu > Se with two exceptions: (1) for human milk, the K concentration is higher than the Ca concentration, and (2) for powdered infant formula, the Fe concentration is much higher than the Zn concentration, which is a consequence of supplementation.

Table 1 shows the results for the metal concentrations (Se, Fe, Cu, Zn, Na, K, Ca, Mg) in the samples corresponding to the five types of milks studied. The results of the one-way ANOVA (least significant difference) for comparison of the mean values are also included in Table 1. The Se mean concentration of goat milk and powdered infant formula were significantly ($p < 0.05$) higher and lower, respectively, than the Se mean concentrations obtained in the other milks. No significant differences were observed between Se concentrations of cow and human milks or between Se concentrations of natural and pasteurized cow milks.

Table 2. Results of the Factor Analysis of the Matrix of Data

variable	communality	factor	eigenvalue	% variance	cumulative variance
Se	0.408	1	4.391	54.9	54.9
Na	0.754	2	1.307	16.3	71.2
K	0.792	3	0.870	10.9	82.1
Ca	0.930	4	0.573	7.2	89.3
Mg	0.728	5	0.365	4.6	93.8
Fe	0.740	6	0.268	3.4	97.2
Cu	0.642	7	0.153	1.9	99.1
Zn	0.704	8	0.073	0.9	100.0

The Fe and Cu mean concentrations of powdered infant formulas (and the Cu mean concentration of human milk) were significantly higher than those of the other milks, whereas the contents of Zn in human milk and of Fe in pasteurized milk were significantly lower than those in the other milks. The cow and goat milks presented significant differences in their levels of Cu and Zn and similar values of Fe. The cow milk also showed higher concentrations of Fe and Zn and lower concentrations of Cu than were observed in pasteurized cow milk. The differences were significant in the three cases.

The human milk presented mean concentrations of K and Ca significantly lower than those found for powdered infant formulas, and both types of milks presented mean concentrations of Na, K, Ca, and Mg that were significantly lower than those in the rest of milks analyzed. The mean concentrations of K and Mg in goat milk were higher than the mean concentrations of cow milk. However, the mean concentrations of Na and Ca were higher than those concentrations observed in cow milk. For the Ca concentrations, cow milk showed higher levels than the goat milk and the goat milk showed higher levels than pasteurized milk.

A correlation study between the variables after logarithmical transformation was carried out. It is interesting to point out the relationships between Na and K, present in the milk as free ions, and between Ca and Mg, mainly associated with the casein fraction, which have already been reported by Holt (1993). These correlations define the following regression equations:

Table 3. Factor Matrix Obtained after a Varimax Rotation

variable	factor 1	factor 2
Se	0.153	−0.620
Na	0.862	−0.107
K	0.870	−0.189
Ca	**0.954**	−0.142
Mg	0.790	−0.323
Fe	−0.030	**0.859**
Cu	−0.704	0.382
Zn	0.784	0.300

$$\log \text{Na} = 1.228 \times \log \text{K} - 1.192 \quad r = 0.812$$

$$\log \text{Ca} = 1.077 \times \log \text{Mg} + 0.981 \quad r = 0.873$$

Also, Na and K are correlated ($p < 0.05$) positively with Ca and Mg, and the correlation coefficient varies between 0.767 for Na−Mg (log Na = 0.826 × log Mg + 0.954) and 0.911 for K−Ca (log K = 0.541 × log Ca + 1.395). When the plots of these four correlations are represented, it is possible to differentiate between the samples of human milk and powdered infant formulas with respect to the rest of milk samples. Figure 1 presents the plot of the correlation between Na and K. Two groups can be differentiated; the samples of human milk and powdered infant formulas are grouped in one group, and the other group includes the samples of cow, goat, and pasteurized milk. All of the alkaline and alkaline-earth metals analyzed here present significant and positive correlations with Zn and negative correlations with Cu; however, these metals (Na, K, Ca, and Mg) do not show significant correlations with Fe. The correlation coefficients of Zn with Na and K are higher than for Cu. Copper and Zn are significantly and negatively ($r = -0.377$) correlated and Fe is positively correlated ($p < 0.05$) with Cu and Zn. However, the correlation coefficients observed are rather low. When the correlation study was carried out on the samples

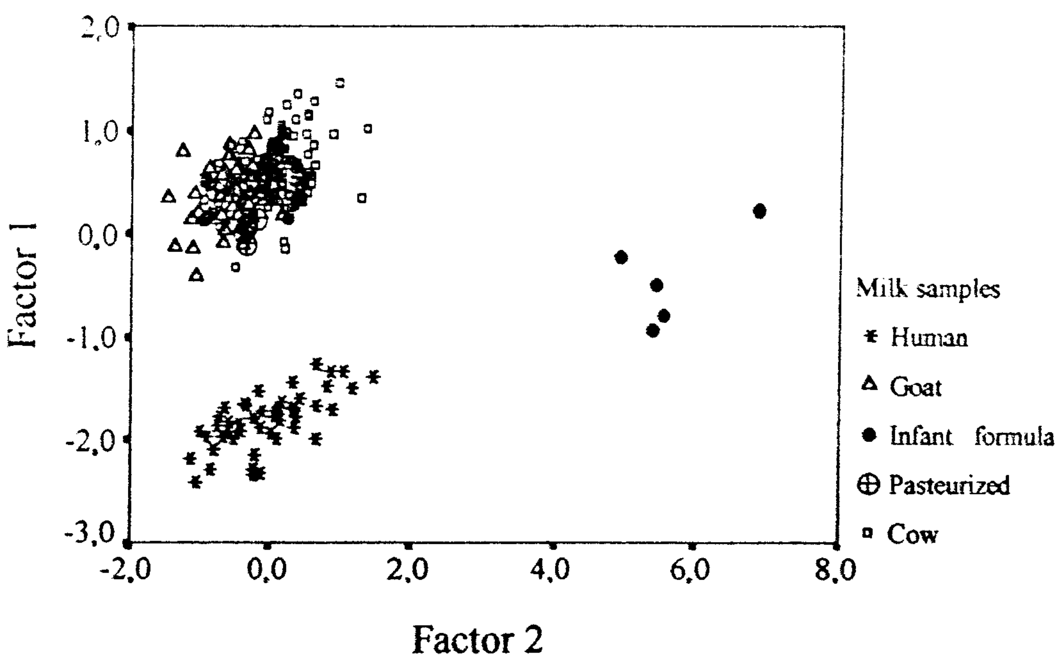

Figure 2. Scores of the milk samples on axes representing the two factors.

Table 4. Stepwise Discriminant Analysis

step	variable	Wilks's lambda	sig
1	Fe	0.0565	0.000
2	Ca	0.0053	0.000
3	Mg	0.0021	0.000
4	Cu	0.0015	0.000
5	Zn	0.0011	0.000
6	K	0.0008	0.000
7	Na	0.0007	0.000

within each type of milk, a lower number of significant correlations was observed. The correlation coefficients were lower, too. This is a consequence of the relatively narrow range of metal concentrations found within each type of milk.

Multivariate factor analysis was applied to the 298 samples of milk studied to obtain a more simplified view of the relationship between quantitative variables (Se, Na, K, Ca, Mg, Fe, Cu, and Zn). The first two factors were chosen (71.2% of the total variance) because their eigenvalues were >1, and therefore they explain more variance than an original variable (Table 2). All variables, except the Se, present communality >0.6, and therefore they are well represented by these two factors. An orthogonal rotation (Varimax rotation) was carried out to minimize the number of variables that influence each factor and then to facilitate the interpretation of the results (Table 3). The first factor that explains the higher percentage of variance (54.9%) is mainly related with Ca and to a lesser degree with K and Na. This agrees with the correlation study below. On the other hand, the second factor is highly correlated with Fe. Consequently, one can deduce that Ca and Fe are the variables that make it possible to characterize the system without losing very much information.

The scores for the two factors are plotted as a scatter diagram in Figure 2, concluding that the samples are differentiated by the type of milk. However, one can observe that the samples are distributed in three groups clearly differentiated. The samples of cow, goat, and pasteurized milks were in one group, and powdered infant formulas and human milks were in the other. In the latter group the two milks were also separated. In this sense, the powdered infant formulas can be differentiated from the remaining milks by means of the variable correlated with the second factor, Fe. This is explained because the high content of this metal in the powdered infant formulas is due to supplementation. On the other hand, the Ca variable associated with the first factor allows one to differentiate the human milk and powdered infant formulas with respect to cow, goat, and pasteurized milks.

Table 5. Discriminant Functions

function	eigenvalue	% variance	cumulative variance	canonical correlation
1	20.463	53.27	53.3	0.976
2	15.233	36.65	92.9	0.969
3	2.670	6.95	99.9	0.853
4	0.049	0.13	100.0	0.216

Discriminant analysis for the quantitative variables (Se, Fe, Cu, Zn, Na, K, Ca, and Mg) was applied to classify all of the milk samples by taking the type of milk as the classification variable. All of the variables, except the Se (because it does not satisfy one of the criterions of the discriminant analysis), were selected using the stepwise method. The process of variable selection for each step is represented in Table 4. Depending on the method, the values of Wilk's lambda decreased (~7 times) from 0.056 to 0.0007. Thus, if the milk samples are represented in the space generated by seven quantitative variables, the milk samples of the different types of milk are well separated. Consequently, the discriminant functions obtained must provide a high percentage of the samples correctly classified within the different types of milk considered.

Four discriminant functions were obtained, which are linear combinations of the quantitative variables that best reveal the differences between the types of milk studied (Ferran, 1996; SAS, 1989). The total variance attributed to the two first functions is 92.9% (Table 5). In addition, these two functions present high eigenvalues and canonic correlations near to unity. Thus, it can be concluded that the scatter of the data is due to differences between the types of milk. Table 6 shows the coefficients corresponding to the discriminant func-

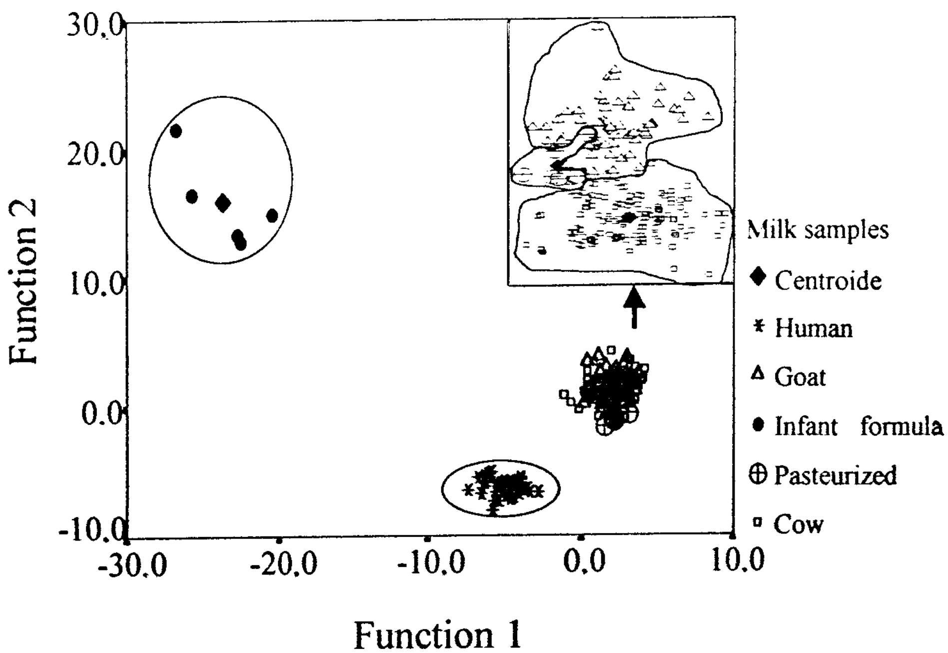

Figure 3. Scatter diagram of the milk samples on the axes representing the two-function discriminant.

Table 6. Coefficients of Discriminant Function

variable	function 1	function 2	function 3	function 4
Na	0.262	0.183	−0.028	−0.853
K	0.333	0.344	0.302	−0.321
Ca	0.264	0.246	0.795	0.286
Mg	0.212	0.536	−0.196	0.427
Fe	−0.685	0.777	0.0196	−0.103
Cu	−0.369	−0.299	0.617	0.413
Zn	0.146	−0.059	−0.614	0.502

Table 7. Results of Discriminant Analysis

		predicted group				
actual group	N	cow	pasteurized	powdered infant formula	goat	human
cow	115	113 (98.2%)	1 (0.9%)	0 (0.0%)	1 (0.9%)	0 (0.0%)
pasteurized	6	0 (0.0%)	6 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
powdered infant formula	5	0 (0.0%)	0 (0.0%)	5 (100.0%)	0 (0.0%)	0 (0.0%)
goat	62	3 (4.8%)	0 (0.0%)	0 (0.0%)	59 (95.2%)	0 (0.0%)
human	48	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	48 (100.0%)

tions obtained, which define the following equation:

$$D_s = B_{s1} \times \mathrm{Na} + B_{s2} \times \mathrm{K} + B_{s3} \times \mathrm{Ca} + B_{s4} \times \mathrm{Mg} + B_{s5} \times \mathrm{Fe} + B_{s6} \times \mathrm{Cu} + B_{s7} \times \mathrm{Zn}$$

where D_s is the discriminant function, B_{sn} is the coefficient obtained for each metal, and $s = 1-4$ represents the four discriminant functions.

The classification of the samples in the five types of milk analyzed is shown in Table 7. After the discriminant analyses were carried out, 97.9% of the samples were correctly classified. The samples of powdered infant formulas and human and pasteurized milks were perfectly (100%) classified. For the samples of cow milk, 98.2% were correctly classified. Only two samples were associated with pasteurized and goat milks, whereas three samples of goat milk were incorrectly classified as cow milk. Similar results were obtained by Rincón et al. (1994), which could be distinguished by previous application of discriminant analysis on eight quantitative variables (Fe, Cu, Zn, Mn, Na, K, Ca, and Mg contents) between cow milk on the one hand and ewe and goat milk on the other.

In Figure 3, a scatter diagram of two first-discriminant functions derived from the seven selected variables is represented. Similar to the results of factorial analysis, three clearly differentiated groups are observed. One corresponds to the samples of human milk, the second is associated with powdered infant formula, and the samples of cow, goat, and pasteurized milks are included in the third group. However, a zoom of the scatter diagram shows the almost complete separation of all the types of milk studied.

Discriminant functions may be used as a complementary tool to identify the type of milk. Therefore, if the concentrations of Fe, Cu, Zn, Na, K, Ca, and Mg are known, it is possible to differentiate the milk samples within the five milk types considered in this paper, with a 2% incorrect classification. Although the concentration of a mineral may vary for many reasons, the use of combinations such as discriminant functions reduces the importance of the variation of any single element (Rincón et al., 1994).

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