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Chemometric application in foodomics: Nutritional quality parameters evaluation in milk-based infant formula

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A method to verify the differentiating characteristics of milk-based infant formula is proposed in this work. In order to evaluate a classification of the milks according to their nutritional profile, the concentration of 24 elements were determinated. Supervised methods PCA-LDA, PLS-DA, and SIMCA were contrasted. PCA-LDA and SIMCA provided significantly better results for milk classification of the two studied classes (infant formula and infant formula fortified). As an alternative approach SIMCA was capable to discriminate an overlapped group consisting of baby milks administrated during first 6 months of life. Chemometric methods employed highlight four metal concentrations (Zn, Mn, Cu, and S) which could be associated to relevant nutritional parameters in baby growth. Thus, proposed methodology provides a simpler, faster and more affordable classification for simple study on Foodomics in milk-based infant formula.

1. Introduction

Ensuring food safety and quality is one of the most challenging tasks for food researchers, government, and food industry. *Foodomics* has grown as a discipline that studies food along nutrition through the application and integration of advanced '*omics*' analytical techniques with multivariate data analysis (chemometrics), to reach the main objective: improve human health, well-being, and knowledge; connecting food components with health [1–3].

Milk-based infant formula has been recognized as a very important dairy product regarding food quality and safety [4,5]. There are a large variety of formulas available for children under 1 year, which vary in nutrients, calorie count, taste, ability to be digested and cost. However, as fast as the production and demand for milk have grown international authorities and regulators are challenged to incessantly improve both compliance and safety regulations [6,7]. Thus, is necessary to consider robust and specific methods for nutritional elements determination in milk-based infant formulas to ensure rigorous control of their composition, including elements added routinely in order to satisfy mineral content requirements [8–12].

The promising research on foodomics area requires advanced mass spectrometry technologies, such as inductively coupled plasma mass spectrometry (ICPMS). Advantages include the ability to determine multielemental composition simultaneously with low detection limits, gaining widespread interest for nutritional studies where the identification of compounds containing metals and metalloids that are toxic to human or other organism is of great importance [12]. Therefore, with multielemental determination of milk-based infant formulas by ICPMS a large number of chemical variables data is reached to be used for multivariate statistical analysis.

Foodomics studies normally needs chemometrics tools to handle such large data sets of multivariate analysis and assist to extract qualitative and quantitative relevant chemical information, aiming to improve quality control of food products [13–15]. Chemometric techniques allow construction of models to characterize target samples within previously defined and validated groups. There are three major pattern recognition methods: unsupervised principal component analysis (PCA), hierarchical cluster analysis (HCA), and supervised discriminant analysis (DA) and these provide either cluster plots or dendrogram structures for segregation and discrimination. Recently, soft independent modeling of class analog (SIMCA) has also been extensively employed for different classification purposes [16–20]. SIMCA could

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be used in various foodomics studies, such as food adulteration, food authenticity, food traceability, and food effects on human health.

Regarding foodomics, chemometric models to identify milk-based infant formula were proposed in this work, considering child's nutritional requirements in different stages of growth. A method to discriminate different milk-based infant formula according to their elemental composition is developed employing three supervised techniques: linear discriminant analysis (LDA), partial least square discriminant analysis (PLS-DA), and soft independent modeling of class analogy (SIMCA). Discriminant capacity of each obtained model was evaluated.

2. Materials and methods

2.1. Reagents

Throughout the work water was distilled and de-ionized, with a resistivity of 18.2 MΩ cm, produced by an Easy pure RF system from Barnstead (Dubuque, IA, USA). Concentrated nitric acid (65%v/v) from Sigma-Aldrich (Germany), Dimethylformamide from Acroorganics (New Jersey, USA), were used. Certified multielement standard solutions 2, 3 and 5, and Rh mono-elemental standard solution from Perkin Elmer Pure Plus-Atomic Spectroscopy Standards, (Norwalk, USA) were used for calibration and recovery studies.

2.2. Instrumentation

For multielemental determination, an inductively coupled plasma mass spectrometer from Perkin-Elmer SCIEX, ELAN DRC-e (Thornhill, Canada) was used. The Ar gas with a minimum purity of 99.996% was supplied by Air Liquide (Río IV°-Córdoba, Argentina). An HF-resistant and high performance perfluoracetate (PFA) nebulizer model PFA-ST, coupled to a quartz cyclonic spray chamber with internal baffle and drain lines, cooled with PC^3 Peltier inlet system from ESI (Omaha - NE, USA) were used. Tygon black/black 0.76 mm i.d. and 40 cm length peristaltic pump tubing were used. The instrument conditions were: auto lens mode on, peak hopping measurement mode, dwell time of 15 ms, 30 sweeps per reading, 1 reading per replicate and 3 replicates. Nickel sampler and skimmer cones were used. A water bath model HH-S from Arcano (Argentina) was also used for sample pre-treatment with DMF.

2.3. Analytical procedure

Sample introduction was carried out using a PFA high-efficiency microconcentric nebulizer coupled to a baffled cyclonic spray chamber, at −5 °C as desolvating temperature. An optimization for maximum analyte intensity and minimum back-ground was performed in a previous work [21], the conditions adopted were the following: solutions were introduced into the plasma at 700 μL min^{-1}, applying 1100 W of radio frequency power and using 0.7 L min^{-1} nebulizer of argon gas flow rate; samples were triplicate analyzed.

Twenty four analytes were evaluated in this study. For element quantification, each sample was independently treated with DMF as recommended in a previous work [21]: 250 μL of milk was accurately weighed directly in a 15-mL polypropylene flask and then, 2 mL of DMF was added and shaken vigorously. The volume was completed to 10 mL with HNO_3 (1.0%). Matrix matching calibration (added with 20%v/v DMF and 1% nitric acid) was used as calibration method. The analytes concentrations were 1.0; 5.0; 10.0; 20.0; 40.0 and 80.0 μg L^{-1}. Rhodium (10 μg L^{-1}) was added to all solutions as internal standard.

2.4. Samples

Forty five commercial infant formulas products (15 milk formula × 3 box of each one) available in Argentinean local markets were purchased for analysis: 27 baby milk (1–12 life months), and 18 fortified baby milk (0–12 months old). The samples were stored at 5 °C and immediately prepared and analyzed after being opened. The accessible milks are bestowed in Table 1. A Standard Reference Material (SRM) – skim milk powder BCR 063R – from Community Bureau of reference, Geel, Belgium was used for optimization and to assess the trueness and precision of the analytical methods.

2.5. Data analysis

A multivariate analysis to evaluate the ratio between elemental concentration and different milk samples was evaluated. Principal component analysis (PCA) was used as descriptive tool for the data visualization. In order to analyze several supervised pattern recognitions, Linear Discriminant Analysis (LDA), Partial Least Squares Discriminant Analysis (PLS-DA), and Soft Independent Modelling Analysis (SIMCA) were performed using the software Unscrambler × 10.3 (CAMO-ASA, Trondheim, Noruega). The validation step for each of the algorithms was performed using full cross-validation [22].

3. Results and discussion

3.1. Analytical validation and elemental concentrations for milk-based infant formula

Table 2 presents the figures of merit for trace elements determinated in milk-based infant formula: quantification limits ranged between 1,9 (Gd) and 92 (Zn) μg L^{-1}, respectively. Precision calculated as relative standard deviation (RSD%) was better than 7.2. Analytical method was validated through accuracy by a certified reference material: skim milk powder (BCR 063R) from Community Bureau of reference. The certified values and the found values for the analytes Cu, Pb and Zn that are present in the reference material are shown in Table 3. The results were compared through a paired *t*-test and no significant differences were observed ($n = 3$, $p = 0.05$).

The presence of Co, Cu, Mn, Mo, Ni, Rb, S, Sr, V and Zn was evidenced in milk-based infant formula. The remaining analytes were lower than quantification limits. Average concentrations of the analytes found in milk samples and comparison of the obtained results between baby infant formulas and baby fortified infant formulas for the different growth stages - 0 to 6 months (1), 6 to 12 months (2), after 12 months (3) - are shown in Table 2.

3.2. Classification models development

In the development of milk-based infant formula classification models according to nutritional requirements in different growth

Table 1
Milk-based infant formula commercialized in Argentina.

Milk-based infant formula		Growth stages
A1	Baby 1	0–6 months
A2	Baby 2	6–12 months
A3	Baby 3	1–3 years
B1	Baby 1	0–6 months
B2	Baby 2	6–12 months
B3	Baby 3	1–3 years
C1	Baby 1	0–6 months
C2	Baby 2	6–12 months
C3	Baby 3	1–3 years
AP1	Baby Premium 1	0–6 months
AP2	Baby Premium 2	6–12 months
AP3	Baby Premium 3	1–3 years
BP1	Baby Premium 1	0–6 months
BP2	Baby Premium 2	6–12 months
BP3	Baby Premium 3	1–3 years