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Chemometric authentication of the organic status of milk on the basis of trace element content

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ABSTRACT

The objective of this study was to develop a method for authenticating organic milk samples in North Spain on the basis of its trace mineral composition. Fourteen elements in 98 samples were determined by inductively coupled plasma mass spectrometry. Although concentrations of Co, Cr, Cu, I, Se and Zn where statistically higher in conventional milk and As in organic, none of these elements by itself was able to discriminate between organic and conventional milk. The chemical data was examined by principal component analysis and cluster analysis, revealing a natural separation between organic and conventional milk. In a second step, several supervised pattern recognition techniques were used to construct mathematical models for predicting the type of milk (organic or conventional) based on the metal content. The results proved that the model constructed using the artificial neural network is capable of correctly identifying the type of milk in almost 95% of cases.

1. Introduction

Since the 1980s the authenticity of food products has been recognized as an important quality criterion by consumers, producers and regulatory bodies. In this context, the European Union has developed various laws and regulations in order to protect traditional foods in different regions, encourage rural life and agricultural activity, add value to authentic products and prevent consumers being defrauded and misled by the sale of unauthentic goods. Rapid, specific methods of detecting adulterations, verifying the quality and guaranteeing the geographical origin or type of production of different food products are nowadays widely in demand in food control laboratories (Ashurst & Dennis, 1996).

Organic farming is also regulated in the European Union (European Parliament & of the Council, 2007; European Commission, 2008), and organic products are strictly controlled as regards the use of additives, processing aids, chemically synthesized ingredients and genetically modified organisms. Organic products are well-received by consumers and are generally sold for much higher prices than conventional products. The use of objective methods of distinguishing organic products from intensively produced goods is of special interest as regards preserving the image of organic producers and protecting the economic interests of consumers.

Many food authentication systems involved the use of chemometric and mathematical pattern recognition models based on the information obtained from chemical analysis of authentic and unauthentic food products. Different food components (such as trace elements, stable isotope ratios, flavor volatile compounds, vitamins, proteins and amino acids, etc.) have been used for this task. Successful authentication models have been published for diverse food products including wine, beer, cider and other alcoholic beverages, coffee, tea, fruits and vegetables, cheese, milk and other dairy products, cereals, honey, oil and meat (Dong, Xiao, & Luo, 2017; Dong et al., 2016; Karoui & Baerdemaeker, 2007; Luo et al., 2015; Luykx & van Ruth, 2008).

In view of the large increase in the consumption of organic milk, the ease with which it can be replaced or adulterated with conventional milk is of great concern (Zain, Behkami, Bakirdere, & Koki, 2016). The chemical composition of organic milk has been widely studied in recent years (Schwendel et al., 2015; Średnicka-Tober et al., 2016). Studies have focused particularly on the fatty acid profile, as organic milk contains a healthier balance of omega-6 and omega-3 fatty acids than

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conventionally produced milk (Butler, Stergiadis, Seal, Eyre, & Leifert, 2011; Ellis et al., 2006). However, diet-related differences in the fatty acid profile of milk (particularly the amount of fresh/preserved forage and concentrate feed) have prevented development of a method for testing the authenticity of organic milk products (Schwendel et al., 2015). Milk represents an essential source of the trace elements I and Se (particularly in some countries such as the UK; Bath & Rayman, 2016), and various studies have demonstrated that organic milk generally contains lower concentrations of these elements than conventional milk (Schwendel et al., 2015; Średnicka-Tober et al., 2016). Significant differences in the contents of other trace and toxic metals in organic and conventional milk have also recently been reported and attributed to the different patterns of trace element supplementation in the concentrate feed and consumption of soil by cows during grazing (Rey-Crespo, Miranda, & López-Alonso, 2013).

Mineral and trace elements provide potentially useful information for developing chemometric procedures for authentication studies, for the following reasons: i) the mineral content of foodstuffs is related to the soil and the environment in which the product has been produced; ii) the mineral content of foodstuffs generally remains stable after sampling; iii) pretreatment of samples is simple (usually only involving an acid wet digestion), and iv) accurate and precise multielemental determination can be obtained by atomic spectrometric techniques such as inductively coupled plasma atomic emission spectroscopy (ICP-AES) and inductively coupled plasma-mass spectrometry (ICP-MS). On the basis of these advantages, trace element composition has been widely and successfully used for the chemometric discrimination of foods (Gonzalvez, Armenta, & de la Guardia, 2009). In Spain, particularly in northern Spain, a wide variety of food products of different quality and/or geographical origins have been satisfactorily classified on the basis of their metal contents: wine (Rebolo et al., 2000), alcoholic distillates (Iglesias-Rodríguez et al., 2010), potatoes (Herrero-Latorre, Barciela-García, García-Martín & Peña-Crecente, 2013), and cow's milk (Sola-Larrañaga & Navarro-Blasco, 2009), among others.

The objective of this study was to develop rapid method for authenticating genuine organic milk samples on the basis of the concentrations of selected mineral elements determined by ICP-MS in northern Spain. The authentication procedure was based on chemical analysis and subsequent data treatment by chemometric techniques to develop classification models for the milk samples on the basis of the type of production: organic or conventional. The method could be used to help prevent other types of milk of lower quality and price being falsely sold as organic milk.

2. Material and methods

2.1. Milk samples: collection and pretreatment

Milk samples in which this study is based were collected in northern Spain (latitude 43°65 – 41°75, longitude – 9°29 – −4°51) from organic (n = 39, accounting for 80% of the dairy organic production of the region) and conventional (n = 59) representative farms. Briefly, in organic farms the cows were grazed and received a low proportion of the dry matter (DM) intake as concentrate feed (16%). On the contrary, conventionally managed cows received more intensive diets (35% of concentrate feed) and were either grazed or kept indoors and fed total mixed rations (TMR). The herd size was similar in both types of farms (mean milking cows per herd ranging between 47 and 52), although milk production was higher on the conventional farms (on average a 305-d-corrected milk yield of 9040 kg, relative to the corresponding value of 7122 kg on the organic farms).

Soils in the sampled area in northern Spain are composed mainly of palaeozoic materials (granite) with metamorphic rocks such as gneiss, slate and schist in the center and west, which were subject to Alpine folding (Gibbons & Moreno, 2002). Information on toxic and trace element concentrations in soils (n = 33) and diets (n = 99) of a subgroup of farms participating in the present study has been published elsewhere (López-Alonso, Rey-Crespo, Herrero-Latorre, & Miranda, 2017).

In each farm representative milk samples (0.1 L) were obtained directly from the bulk tank during the daily milk collection in November 2015. Samples were immediately refrigerated and transported to the laboratory, where 3 aliquots (each 20 mL) were frozen at −20 °C for mineral analysis.

Milk samples were acid digested before ICP-MS analysis. One mL of milk was digested in 8 mL of 69% concentrated nitric acid and 2 mL of 33% w/v hydrogen peroxide, in a microwave-assisted digestion system. The microwave oven temperature program consisted of two steps: (i) increase from room temperature to 200 °C, ramp time 10 min, and (ii) 200 °C for 10 min. Once cooled, the digested samples were transferred to polypropylene sample tubes and diluted with Milli-Q ultrapure water. Samples were prepared for iodine determination by a high temperature alkaline extraction procedure (EN., 2007).

2.2. Apparatus

Essential trace and toxic element concentrations were determined by ICP-MS (VG Elemental PlasmaQuad S-Option equipped with a Micromist low-flow nebulizer for a Peltier device, quartz double pass spray chamber and a quartz torch: Thermo Fischer Scientific). A microwave digestion system (Ethos Plus, Milestone, Sorisole, Italy) was used for acid digestion of milk samples.

2.3. Reagents, standard solutions and certified reference materials

All solutions were prepared from ultrapure water of resistance 18 MΩ cm^{-1} produced with a Milli-Q purification system (Millipore Corp., Bedford, MA). Stock ultrapure grade standard solutions (1000 mg/L) of the elements determined were purchased from Merck (Poole, U.K.): ICP Multi element standard solution IV certiPUR® in the case of Co, Cr, Cu, Fe, Mn, Ni and Zn; and ICP standard certiPUR® for Se; stock standard solutions for As and Mo were obtained respectively from SCP Science (Montreal, Canada) and from Panreac (Barcelona, Spain). A stock solution of tetramethylammonium hydroxide (TMAH) (Panreac) was prepared in water (25% w/v) for use in the alkaline extraction. A standard stock solution of iodide (1000 mg L^{-1}) was prepared from potassium iodide (Panreac). Ultrapure nitric acid (69%) was obtained from Merck and hydrogen peroxide (33% w/v) from Panreac.

Validation of the analytical methods was performed using certified reference material: NIST SRM-1549 (non-fat milk powder) from the National Institute for Standards and Technology (NIST) (Gaithersburg, MD, USA).

2.4. Analytical methods

The concentrations of 14 mineral elements (As, Cd, Co, Cr, Cu, I, Fe, Pb, Mn, Hg, Mo, Ni, Se and Zn) were determined in triplicate in all milk samples. The experimental conditions for the ICP-MS method used are described in detail in a previously published paper (Rey-Crespo et al., 2013).

Analytical quality control was applied throughout the study (Table 1). Blank samples were processed with test samples, and the values used in further data processing were calculated by subtracting the blank from the sample readings. Detection limits were calculated as three times the standard deviation of the reagent blanks. Analytical recovery, determined from the certified reference material (CRM) NIST SRM-1549 analyzed together with the samples, show acceptable agreement between the measured and certified values. The CRM was not certified for Ni, so analytical recovery (91%) was determined using spiked samples (10 µg/L).