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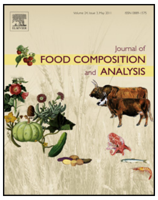

Original Research Article

Non-toxic and potentially toxic elements in Italian donkey milk by ICP-MS and multivariate analysis

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ABSTRACT

Concentrations of 6 non-toxic and 6 potentially toxic elements in donkey milk, forage and feed samples from 3 Italian farms were determined by ICP-MS. Zn (mean value range: 1.403–4.567 mg kg^{-1}) and Fe (0.733–2.220 mg kg^{-1}) concentrations were the highest in all analyzed milk samples, followed by Cu (0.125–0.199 mg kg^{-1}), Mn (0.024–0.046 mg kg^{-1}) and Se (0.030–0.040 mg kg^{-1}). In consideration of the foregoing and of the RDA values, donkey milk appears a good source of selenium for both adults (3.64–23.27%RDA) and children (20–128%RDA). Furthermore, the majority of samples did not reveal residues dangerous for human health. Indeed, Hg (0.96–1.22 μg kg^{-1}) and As (42.84–78.05 μg kg^{-1}) are within the prescribed range, while Cd (3.96–11.19 μg kg^{-1}) and Pb (9.77–18.11 μg kg^{-1}) were, at times and only for children, above the respective benchmark. In 11% of total milk samples Pb levels exceed the European limit (0.02 mg kg^{-1}), while Sb (28.77–38.04 μg kg^{-1}) and Ni (30.00–36.00 μg kg^{-1}) levels could not be compared due to the lack of available parameters. Data were analyzed by chemometric assessment to elicit the correlation within the different samples and to evaluate statistically significant differences among samples from the farms and seasonal variations during the sampling periods. Findings have established the geographical origin of milk on the basis of the mineral profile and can be used as a tool to elicit the authenticity of donkey milk.

1. Introduction

Recent clinical studies have demonstrated that donkey milk provides nutritional adequacy and good palatability and is a safe and alternative food in both IgE- and non-IgE-mediated cow's milk protein allergies (CMPA). CMPA incidence is attested at 3% in children under one year of age, whereas in Italy 10,000–15,000 newborns show intolerance to cow's milk proteins (Carroccio et al., 2000; Monti et al., 2007).

Donkey milk has unique nutritional features such as low casein content, high essential amino-acid content (Guo et al., 2007), high and balanced essential fatty acid content, such as linoleic and linolenic acid (Salimei et al., 2004; Salimei and Chiofalo, 2006), high lactose content (Schaafsma, 2003) and an interesting amino profile (La Torre et al., 2010).

Although in trace and ultratrace metal levels are important to evaluate safeness and quality in milk, data are scanty. Mineral content determination of milk may represent a valid instrument to evaluate both the nutritional status in milch animals and the nutritional value of their milk. Additionally, the presence of heavy metal residues may be indicative of the sanitary status in milk and an indirect indicator of pollution in the environment where food is produced (Licata et al., 2004). Mineral presence in milk can be linked to the forage, feed, water and to the drugs administered. The literature on the levels of heavy metals and on metal residues in milk from different Italian regions, either in cow and in human milk, is consistent (Prohaska et al., 2000; Gundacker et al., 2002; Honda et al., 2003; Muñoz and Palmero, 2004; Yamawaki et al., 2005; Naccari et al., 2006; Suárez-Luque et al., 2007; Benincasa et al., 2008; Birghila et al., 2008; Patra et al., 2008; Tajkarimi et al., 2008; Qin et al., 2009; Rahimi et al., 2009; Nascimento et al., 2010; Soares et al., 2010; Bilandžić et al., 2011; Herwig et al., 2011). Results vary according to the sampling zones and to the analytical methods employed. At present there are few data on essential macro minerals and trace elements in donkey milk (Fantuz et al., 2012; Salimei and Fantuz, 2012).

Stripping voltammetry (Muñoz and Palmero, 2004), capillary zone electrophoresis (Suárez-Luque et al., 2007), atomic absorption spectrometry (Gundacker et al., 2002; Honda et al., 2003; Licata et al., 2004; Patra et al., 2008; Tajkarimi et al., 2008; Qin

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et al., 2009; Rahimi et al., 2009; Soares et al., 2010; Bilandžić et al., 2011) were employed to determine metals in milk.

At present, Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Prohaska et al., 2000; Benincasa et al., 2008; Herwig et al., 2011) and Inductively Coupled Plasma Atomic Emission (ICP-AES) (Yamawaki et al., 2005; Birghila et al., 2008; Nascimento et al., 2010) allow the simultaneous determination of several trace elements.

In this work the determination of concentration of six non-toxic elements (Fe, Zn, Cr, Cu, Se and Mn) and of six potentially toxic elements (As, Pb, Cd, Hg, Ni and Sb) by ICP-MS in donkey milk, forage and feed samples from three Italian farms is described. The first aim of the research was to assess quality and safety in the analyzed samples and to survey the correlation between milk, forage and feed concentrations. The second aim was to evaluate the statistically significant differences among samples from different farms and the seasonal variations during the sampling periods using chemometric analyses. Furthermore, Principal Component Analysis (PCA) was employed to correlate milk samples to the geographical areas. This approach can be considered useful to classify and to identify the sources that influence mineral profiles in this type of milk and the assessment of the geographic origin of donkey milk (Di Bella et al., 2012). This last serves to gain the Protected Designation of Origin (PDO) or Protected Geographical Indication (PGI) labels.

2. Material and methods

2.1. Instrumentation

Milk, forage and feed samples were digested by means of a closed-vessel microwave digestion system (Ethos 1, Milestone, Bergamo, Italy) equipped with sensors for temperature and pressure control. The equipment is provided with PTFE vessels capable of withstanding pressures of up to 110 bar.

The determination of non-toxic and potentially toxic elements in digested samples has been carried out by Agilent 7500cx (Agilent Technologies, Santa Clara, CA) ICP-MS spectrometer powered by a 27.12 MHz radiofrequency solid-state generator at 1600 W. This instrument was equipped with a MicroMist glass concentric pneumatic nebulizer coupling with a cooled Scott double pass type spray chamber made of quartz. The ICP torch was a classic Fassel-type torch with wide diameter (2.5 mm) fitted with a shield torch system. Ni sampler and skimmer cones of 1.0 mm and 0.4 mm were used. An octopole collision/reaction system with helium gas to minimize polyatomic interferences resulting from plasma and matrix was used. The equipment is provided with off-axis ion lens, a quadrupole mass analyzer and an electron multiplier detector. This instrument also includes an autosampler ASX520 (Cetac Technologies Inc., Omaha, NE, USA) and an integrated sample introduction system.

2.2. Chemicals and standard solutions

The gases 99.9990% argon and 99.9995% helium were supplied by Rivoira gases (Rivoira S.p.A., Milan, Italy). Concentrated (65%, v/v) nitric acid trace metal analysis grade (J.T. Backer, Mallinckrodt Backer, Milan, Italy) and concentrated (30%, v/v) hydrogen peroxide (J.T. Backer, Mallinckrodt Backer, Milan, Italy) were used to digest samples. High purity water with resistivity of 10 MΩ cm (J.T. Backer, Mallinckrodt Backer, Milan, Italy), was used throughout.

Stock standard solutions (1000 mg L^{-1} in 2% nitric acid) of each element under investigation were purchased from Fluka, Milan, Italy (Fe, Zn, Cr, Ni, Cu, Se, Pb, As and Sb) and from Merck, Darmstadt, Germany (Hg, Mn and Cd). Also, solution of Re at 1000 mg L^{-1} in 2% nitric acid was acquired by Fluka (Milan, Italy) and was used, as preparation standard, to verify the digestion of sample and to correct the volumetric changes.

Stock standard solutions of Sc, Rh and Bi (1000 mg L^{-1} in 2% nitric acid) were purchased from Fluka (Milan, Italy) and were used as on-line internal standards (at level of 1 mg L^{-1}) to correct instrumental drift and variations due to the matrix.

To tune the instrument, an ICP-MS tuning solution containing 1 μg L^{-1} of ^{7}Li, ^{59}Co, ^{80}Y and ^{205}Tl in 2% HNO_3 was obtained from Agilent (Santa Clara, CA). Mixed working calibration standards were prepared at concentration ranges suitable for the analytes being investigated (range from 0.05 to 10 μg L^{-1} for the elements Cr, Mn, Ni, Se, Sb and Pb, from 0.10 to 20 μg L^{-1} for As, from 0.05 to 3 μg L^{-1} for Cd, from 0.05 to 1.5 μg L^{-1} for Hg, from 1 to 100 μg L^{-1} for Cu, from 0.01 to 1 mg L^{-1} for Fe, and from 0.05 to 1.5 mg L^{-1} for Zn) from multi-element standard solutions of 1000 μg L^{-1} prepared from single element standard solution of 1000 mg L^{-1}. The calibration curves were obtained by 5 standard solutions.

The internal Re standard was prepared at 0.8 mg L^{-1}. Before use, glassware was washed with 5% HNO_3 for at least 12 h, rinsed with ultra-pure water and then dried.

The certified reference materials used for the validation were: whole milk powder NIST RM 8435 (National Institute of Standard and Technology, NIST, Gaithersburg, MD, USA); non fat milk powder NIST SRM 1549, (National Institute of Standard and Technology, NIST, Gaithersburg, MD, USA); hay powder IAEA-V-10 (International Atomic Energy Agency, IAEA, Vienna, Austria); rice flour NIES CRM 10 (National Institute for Environmental Studies, NIES, Onogawa, Tsukuba, Japan).

2.3. Samples

A total of 45 donkey milk samples were obtained from conventional Italian donkey farms, in Sicily (Farm A), Calabria (Farm B) and Emilia-Romagna (Farm C). Three bulk milk samples were collected from each farm in the months of September and November 2009, and in January, March and May 2010. All donkeys (mean age 3–8 years) were healthy and no illness during pregnancy was reported. Milk samples were collected using hand milking by standardized procedures at the same time of day (in the morning between 08.00 and 10.00 a.m.), and were preserved in PET container; immediately refrigerated on ice, then frozen at −20 °C, and once in the laboratory, they were stored until analysis.

One forage sample and one feed sample from each farm was collected in each month (a total of $5 \times 3 = 15$ forage samples and $5 \times 3 = 15$ feed samples were analyzed). Forage samples were polyphyte grass silage, while feed formulation were based on barleycorn, wild oat, fava bean, locust bean, and they did not contain added elements. Forage samples were first oven-dried at 65 °C for 72 h, and then ground and homogenized; feed samples were only ground and homogenized.

2.4. Sample preparation

For the digestion of the samples, approximately 0.5 g of milk, forage or feed were first added with 1 mL of internal Re standard at 0.8 mg L^{-1}, and then were digested with 7 mL of HNO_3 (65%, v/v) and 1 mL of H_2O_2 (30%, v/v) in acid-prewashed PTFE vessels.

The mineralization was carried out at 200 °C, at 1000 W, for 20 min. After cooling down to room temperature, the digested samples were weighted, quantitatively transferred into pre-cleaned 10 mL volumetric flasks, diluted to mark using deionized water, and stored at 4 °C.

The certified reference materials were digested under the same conditions of the samples. Prior to mineralization, the certified