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Determination of the concentrations of essential and toxic metals in UHT milk produced in Mato Grosso State, Brazil

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Abstract

The aim of this study was to determine the Na, K, Ca, Mg, Cu, Cr, Zn, Fe, Mn, Pb, and Cd in UHT milk produced in Mato Grosso State, Brazil. Three lots of whole, skimmed, and semi-skimmed UHT milk from four different producers were collected in the Cuiabá city. The samples were prepared by wet decomposition and quantification was performed using FAAS. The linear correlation coefficients were greater than 0.99 and the LOD and LOQ ranged from 1.8 x 10^{-3} to 2.4 x 10^{-1} mg/100 g, and from 5.4 x 10^{-3} to 7.2 x 10^{-1} mg/100 g, respectively. The precision and accuracy of the method were evaluated in addition and recovery tests. For the fortification levels evaluated, recoveries were in the range 71-112%, with RSD values lower than 12%. The concentrations of Na, K, Ca, Mg, Cu, and Zn varied according to the type of milk, and in some cases did not comply with the recommended values given in the Table of Food Composition. In the case of the toxic metals, some samples showed concentrations of Pb and Cr that exceeded the maximum values allowed by Legislation. The results indicated a need for improved quality control in milk production.

Introduction

Mato Grosso State is the eighth largest Brazilian milk producer, with an annual output of approximately 680 million liters and an estimated 139% increase in production over the last 10 years (IMEA, 2012). However, the dairy sector mainly consists of family-run enterprises that have generally not benefitted from investments in technical expertise and the technologies required for improvement of milk quality (BNDES, 2010). As a result, there is concern about the health risks and nutritional properties of milk produced in Mato Grosso.

Milk plays an important role as the only source of nutrients for young mammals, providing a complex mixture of lipids, proteins, carbohydrates, vitamins, and minerals (Swaisgood, 2010). Due to its widespread consumption, milk must be of satisfactory quality in order to protect the health of the community. It should be obtained from healthy animals, with collection and storage under hygienic conditions that avoid contamination (Okada *et al*., 1997).

Mineral salts comprise a small fraction (8-9 g L^{-1}) of the total composition of milk, with calcium, magnesium, sodium, and potassium as the main cations, and phosphate, citrate, and chloride as the main anions. These ions may be partially associated with casein micelles (Gaucheron, 2005).

Essential minerals are vital for the functioning of the human organism, since they participate in many metabolic processes, and regulate biochemical reactions. In addition, Fe, Cu, Zn, and Mn play crucial roles in protecting the body against the negative effects of toxic free radicals (Król *et al*., 2012). Dobrzanski *et al.* (2005) reported that the concentrations of the main milk minerals are fairly constant, but may be influenced by stage of lactation, quality of nutrition, use of chemical additives, and chemical pollutants present in the environment.

In addition to the essential minerals, milk can contain potentially toxic metals, notably Cd and Pb, due to environmental contamination and the use of low quality mineral supplements in cattle feed (Okada *et al*., 1997; Marçal, 2005; Antunovic *et al*., 2005; Rodrigues *et al*., 2011; Abdulkahaliq *et al*., 2012). Lukáčová *et al.* (2012) determined concentrations of potentially toxic metals in samples of raw and UHT milk and detected the presence of cadmium. Dawd *et al*. (2012) determined the concentrations of metals in whole milk of the Addis Ababa region in Ethiopia, and found that the milk represented a risk to public

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health due to metal contamination that exceeded acceptable limits. Gonçalves *et al*. (2008) determined the levels of Pb, Cd, Cu, Fe, and Zn in pasteurized cow's milk produced in Goiás State, Brazil, and detected contamination by Pb as well as mineral deficiency in the samples.

The aim of this study was to determine the concentrations of essential minerals (Ca, Na, K, Zn, Fe, Cr, Cu, Mn, and Mg) and potentially toxic metals (Pb and Cd) in whole, semi-skimmed, and skimmed UHT milk produced in Mato Grosso State, and compare the results with the limit values established in current legislation.

Material and Methods

Instrumentation

Flame atomic absorption spectroscopy (FAAS) analyses were performed using a SpectrAA 220 instrument (Varian) fitted with hollow cathode lamps. Acetylene and nitrous oxide (both 99.5%, Linde Gás, São Paulo, Brazil) were used as the fuel and oxidant gases, respectively, for the determination of Ca. For the measurements of Na, K, Mg, Fe, Cu, Zn, Mn, Cr, Cd, and Pb, acetylene and compressed air were used as the fuel and oxidant gases. The aspiration rate of the working standard solutions or samples was adjusted to 2.00 ± 0.2 mL min^{-1}. The measurements were carried out according to the manufacturer's recommendations for maximum sensitivity. Table 1 shows the instrumental operating conditions used for each analyte.

An analytical balance with an accuracy of ±0.0001 g (Model AW 220, Marte, São Paulo, Brazil) was used for weighing the samples and reagents. Sample decomposition was performed using a hot plate (Quimis, São Paulo, Brazil). Micropipettes (Boeco, Germany) with adjustable volumes of 5-50, 50-200, and 100-1000 µL were used for the preparation of the standard solutions and samples.

Reagents, solutions, and samples

High purity deionized water (resistivity 18.2 MΩ cm, Milli-Q system, Millipore, Bedford, MA, USA) was used for the preparation of the standard solutions and samples. The standard solutions were prepared by sequential dilution of 1000 mg L^{-1} aqueous stock solutions (Carlo Erba, Italy). All flasks and glassware were washed with tap water, immersed in 7.0% (v/v) HNO3 solution for at least 24 h, and rinsed thoroughly with deionized water. The sample preparation procedure employed analytical grade HCl (38% w/w, Quimis, São Paulo, Brazil).

Twenty-four whole, semi-skimmed, and skimmed UHT milk samples from four producers in Mato Grosso State were obtained locally in the city of Cuiabá and identified by numbering them 1, 2, 3, and 4. For the first producer, three samples from different lots of whole, semi-skimmed, and skimmed milk were collected (9 samples). The second producer only offered whole milk, and three samples from different lots were collected (3 samples). The other producers offered whole and skimmed milk, and three samples from different lots were collected (6 samples). All samples were maintained under refrigeration after opening.

Instrumental parameters

Determination of the limits of detection (LOD), limits of quantification (LOQ), and linear correlation coefficients (r) employed analytical curves constructed using the external standardization method (Skoog, 2006). The following concentration ranges (in mg L^{-1}) were used: 0.0-100.0 (Na); 0.0-6.0 (K); 0.0-100 (Ca); 0.0-25.0 (Mg); 0.0-10.0 (Fe); 0.0-8.0 (Pb); 0.0-5.0 (Cu); 0.0-3.0 (Zn); 0.0-0.5 (Mn); 0.0-3.0 (Cd); 0.0-1.0 (Cr). The LOD and LOQ values were determined according to the method described

Table 1. Instrumental operating conditions

Analyte	λ (nm)	Current (mA)	Slit width (nm)	Fuel/oxidant gases	Flame stoichiometry
Na	330.0	5.0	0.5	Acetylene/air	Oxidizing
K	769.9	6.0	1.0	Acetylene/air	Oxidizing
Ca	422.7	10.0	0.5	Acetylene/nitrous oxide	Reducing
Mg	202.6	5.0	1.0	Acetylene/air	Oxidizing
Fe	272.0	5.0	1.0	Acetylene/air	Oxidizing
Mn	279.5	5.0	0.2	Acetylene/air	Oxidizing
Zn	213.9	5.0	1.0	Acetylene/air	Oxidizing
Cu	327.4	10.0	0.2	Acetylene/air	Oxidizing
Cr	357.9	7.0	0.2	Acetylene/air	Oxidizing
Cd	228.8	10.0	0.5	Acetylene/air	Oxidizing
Pb	324.7	10.0	0.5	Acetylene/air	Oxidizing

by Currie (1999). All measurements were made in triplicate (n=3) and included an analytical blank.

Sample preparation

Sample preparation consisted of transferring 10 g of milk to a 50 mL Erlenmeyer flask, followed by addition of 30 mL of a solution of HCl (1:1, v/v). The top of the flask was partially covered with a plastic wrapper to allow reflux, and the mixture was heated at 100°C for 2 h on a hot plate. At the end of the heating period, the digested sample was cooled to room temperature and filtered through a quantitative filter paper into a 25 mL volumetric flask, and the volume was made up to the mark using deionized water.

Addition and recovery tests

The precision and accuracy of the proposed method were evaluated by means of addition and recovery tests. These tests were performed at three concentration levels for each analyte (Table 2) and were conducted by adding different volumes of 1000 mg L^{-1} aqueous spectroscopic standards to the three batches of whole UHT milk samples. After the addition, the samples were allowed to remain at rest for 24 h to permit sample/metal interaction. Subsequently, wet decomposition was performed as described above (Section 2.4). The addition and recovery tests were performed in triplicate, followed by an analytical blank. The fortification levels used for each analyte were selected considering the sensitivity of the instrument, the LOQ, and the LOD.

Table 2. Fortification levels used in the addition and recovery tests

Analyte	Amount added (mg g^{-1})		
	Level 1	Level 2	Level 3
Na	0.05	0.1	0.2
K	0.3	0.6	0.8
Ca	0.009	0.018	0.13
Fe	0.02	0.04	0.06
Cu	0.00005	0.00007	0.00011
Zn	0.04	0.05	0.08
Mg	0.02	0.03	0.05
Mn	0.02	0.04	0.06
Cr	0.0005	0.0008	0.0012
Cd	0.001	0.002	0.008
Pb	0.2	0.4	0.5

Table 3. Instrumental parameters

Analyte	r	LOD (mg/100 g)*	LOQ (mg/100 g)*
Na	0.999	2.4×10^{-1}	7.2×10^{-1}
K	0.998	3.9×10^{-3}	1.2×10^{-2}
Ca	0.997	5.8×10^{-3}	1.8×10^{-2}
Fe	0.997	1.8×10^{-3}	5.4×10^{-3}
Cu	0.999	8.8×10^{-3}	2.7×10^{-2}
Zn	0.999	2.9×10^{-1}	8.8×10^{-1}
Mg	0.999	1.4×10^{-1}	4.2×10^{-1}
Mn	0.998	1.9×10^{-3}	5.8×10^{-3}
Cr	0.996	6.8×10^{-3}	2.1×10^{-2}
Cd	0.994	1.9×10^{-3}	5.8×10^{-3}
Pb	0.997	2.1×10^{-2}	6.4×10^{-2}

*Analyte mass/milk mass

Determination of minerals and potentially toxic metals in milk samples

All samples were submitted to wet decomposition, as described previously, and the analytes were quantified by FAAS. The results were compared with the values given in the Brazilian Table of Food Composition (TACO) (NEPA, 2011), Decree No. 55.871 (1965) of the Brazilian National Health Surveillance Agency (ANVISA), which stipulates the levels of metals in foods (Brazil, 1965), and ANVISA Resolution No. 42 (2013), which implements the Mercosul Technical Regulation concerning maximum levels of inorganic contaminants in foods (Mercosul, 2013). Analysis of variance (ANOVA) with the post-hoc Tukey test ($p<0.05$) was used to identify any significant differences among the samples in terms of metal concentrations. These statistical procedures were performed using Assistat software.

Results and Discussion

The analytical curves showed r values higher than 0.99 for all analytes (Table 3), indicating a good linear correlation between the analytical signals and the analyte concentrations. In the addition and recovery tests, the recoveries varied in the range 71-112%, with RSD values lower than 12% (considering all concentration levels) (Table 4). According to the Association of Official Analytical Chemists (AOAC), recoveries of between 80 and 110%, with RSD values up to 15%, are acceptable for concentration levels in the mg g-1 range. It should be noted that a wider spread of recovery and RSD values may be acceptable, depending on the purpose of the analysis, the complexity of the matrix, and the analytical method employed. Depending on the complexity of

Table 4. Percentage recoveries of analytes (n=3) from spiked whole UHT milk samples (mean ± RSD)

Analyte	Recovery ± RSD (%)		
	Level 1	Level 2	Level 3
Na	103 ± 10	97 ± 12	77 ± 12
K	109 ± 5	94 ± 6	97 ± 8
Ca	98 ± 2	88 ± 9	112 ± 11
Fe	108 ± 4	97 ± 8	96 ± 10
Cu	88 ± 11	91 ± 0	88 ± 5
Zn	104 ± 11	93 ± 6	99 ± 6
Mg	102 ± 8	94 ± 3	80 ± 5
Mn	76 ± 8	71 ± 2	102 ± 10
Cr	109 ±4	108 ± 2	106 ± 5
Cd	105 ± 4	103 ± 8	100 ± 6
Pb	93 ± 3	81 ± 1	81 ± 6

the matrix, the recovery interval can be extended to between 50 and 120%, with precision of ±16% (AOAC, 1998; Ribani *et al.*, 1994; Taverniers *et al.*, 2004). The proposed method was subsequently used to determine the concentrations of Na, K, Ca, Fe, Cu, Zn, Mg, Mn, Cr, Cd, and Pb in the different whole, semi-skimmed, and skimmed UHT milk samples produced in Mato Grosso State (Table 5).

The levels of calcium measured in the whole milk samples complied with the TACO values, but the levels in the semi-skimmed and skimmed milk were lower than recommended. The sodium concentrations in all the samples of whole milk were lower than the recommended values, but were higher than the TACO values for all the semi-skimmed and skimmed milk samples. In the case of magnesium, only one sample of whole milk showed a concentration below the TACO value. The variability in the concentrations of these minerals can be attributed to the stage of lactation, type of cattle feed, use of chemical additives during processing, and veterinary drugs, amongst other factors (Dobrzanski *et al.*, 2011).

The concentrations of Cu and Zn in all the samples were below the TACO values. Low concentrations of Cu and Zn in milk have also been observed in previous studies (Licata *et al.*, 2004; Birghila *et al.*, 2008), and have been attributed to interference from other milk components (Cozzolino, 1997). It is noteworthy that depending on the concentrations present in food, Cu and Zn can be considered as potentially toxic metals. The current Brazilian legislation that defines the criteria for these elements is Decree No. 55,871, of March 26, 1965 (Brazil, 1965), and the samples analyzed in this work showed values below the maximum permitted limits.

For potassium, all the milk samples showed concentrations higher than the TACO values. This could be explained by the use of chemical additives such as stabilizers (potassium citrate), or supplementation of cattle diets with potassium salts. These salts show high bioavailability in the metabolism of the animal, where they act in regulating osmotic balance and body water content, participate in protein and carbohydrate metabolism, and are important in muscle and nerve activity (EMBRAPA, 2005).

The samples of semi-skimmed and skimmed milk showed chromium concentrations above the current maximum permissible levels (Brazil, 1965). Although chromium can be considered an essential element in food, it may also be toxic, depending on its concentration and oxidation state (Ambushe *et al.*, 2009). Chromium contamination in milk can occur due to pollution from mining and the use of agrochemicals, as well as during milk processing (Beltrame *et al.*, 2005). Another possible source of contamination with Cr is the supplementation of dairy cattle diets with chromium salts such as chromium chelate in order to compensate mineral deficiencies in grazing systems of Mato Grosso (Tronco, 2003; Branco, 2008; Moreira, 2012).

One sample of whole milk (A2) and two samples of skimmed milk (A7 and A8) showed lead concentrations above the permitted limits. Possible sources of lead include industrial and agro-industrial activities, the use of contaminated feed, and mineral supplements (Okada *et al.*, 1997; Marçal, 2005; Antunovic *et al.*, 2005; Rodrigues *et al.*, 2011; Abdulkahaliq *et al.*, 2012). The vast extent of Brazilian agriculture means that the country is a major consumer of mineral supplements for animal use (Marcal *et al.*, 2003). In order to reduce costs, and following government authorization (Brazil, 1997), the agricultural sector began to use alternative sources of phosphorus from phosphate rock in the production of supplements, which could contribute to contamination with toxic metals and radioactive elements.

Lead contamination was also reported by Licata *et al.* (2004) for bovine milk samples collected in the city of Calabri, Italy, where the highest measured concentration was around 0.0009 mg/100 g. Abolfazi *et al.* (2012) found concentrations of between 0.0182 and 0.001 mg/100 g in milk produced in the eastern region of Azerbaijan. Gonçalves *et al.* (2008) reported concentrations varying from 0.025 to 0.020 mg/100 g in pasteurized bovine milk collected in five regions of Goiás State, Brazil.

Analysis of variance revealed significant differences ($p<0.05$) between the samples in terms of the concentrations of Ca, Mg, Mn, Cr, and Pb. The results highlight the heterogeneity of milk composition, which can be caused by multiple factors including the stage of lactation, diet of the herd, animal breed, use of agrochemicals, chemical additives,

Table 5. Analyte concentrations in samples of UHT milk produced in Mato Grosso State, Brazil (mean value of the three lots ± RSD%, n=3)

Sample	Concentration (mg/100 g) ± RSD%										
	Na	K	Ca	Fe	Cu	Zn	Mg	Mn	Cr	Cd	Pb
A1(W)	55 ± 5a[*]	6312 ± 6a	123 ± 3ab	≤LOQc	≤LOQ a	0.2± 1a	9 ± 2 b	0.002 ± 9c	≤LOQc	≤LOQ a	≤ LOQ d
A2(W)	55 ± 1a	5813 ± 8a	126 ± 6a	≤LOQc	≤LOQa	0.3 ± 2a	11 ± 5ab	0.002 ± 1c	≤LOQc	≤LOQ a	0.07 ± 12a
A3(W)	56 ± 2a	5004 ± 8a	126 ± 3a	≤LOQc	≤LOQa	0.2 ± 8a	12 ± 7a	0.004 ± 1bc	≤LOQc	≤LOQ a	≤LOQ bc
A4(W)	50 ± 8a	5531 ± 5a	128 ± 4a	≤LOQc	≤LOQa	0.2 ± 11a	12 ± 4ab	0.003± 2c	≤LOQc	≤LOQ a	≤LOQ d
A5(SS)	57 ± 6a	5435 ± 10a	116 ± 6 abc	0.1 ± 8b	≤LOQa	0.3 ±1.5a	11 ± 7ab	0.007±11ab	0.05.±.6b	≤LOQ a	≤LOQ d
A6(S)	57 ± 4a	6275 ± 8a	117 ± 4abc	0.1 ± 1a	≤LOQa	0.3 ±1.7a	12 ± 1ab	0.008±12a	0.05.± 6ab	≤LOQ a	≤LOQ d
A7(S)	53 ± 3a	5243 ± 3a	105 ± 2bc	0.1 ± 8b	≤LOQa	0.3 ± 5a	12 ± 2ab	0.008± 7ab	0.05 ± 6ab	≤LOQ a	0.03 ± 2b
A8(S)	51 ± 2a	4962 ± 4a	104 ±2c	0.1 ± 4a	≤LOQa	0.3 ± 7a	10 ± 1ab	0.01± 1a	0.06 ± 1a	≤LOQ a	0.02 ± 2cd
TACO[1] (W)	64	133	123	Tr	0.02	0.4	10	Tr	-	-	-
TACO[1] (S)	51	140	134	Tr	0.02	0.4	10	Tr	-	-	-
ANVISA[2]	-	-	-	-	3.0	5.0	-	-	0.01	0.1	0.005
MERCOSUL[3]	-	-	-	-	-	-	-	-	-	0.005	0.002

[*] Values followed by the same letter (a, b, c, or d) in the same column indicate no significant difference between the samples at the 5% confidence level; (W) whole milk; (SS) semi-skimmed milk; (S) skimmed milk;1 Brazilian Table of Food Composition; 2 Maximum permitted values according to Decree No. 55,871, of March 26, 1965; 3 Maximum permitted values according to Resolution No. 42, of August 29, 2013; Tr: trace

veterinary drugs, and environmental pollution.

Conclusions

Concentrations of Na, K, Ca, Mg, Cu, and Zn varied according to the type of UHT milk and did not always comply with the TACO values. This could be attributed to factors related to the management of the dairy cattle, milk processing, and the interference of other milk components. Three UHT milk samples showed lead concentrations above the maximum allowed value, and all the samples of semi-skimmed and skimmed UHT milk showed Cr concentrations above the maximum permissible value. Further work will be needed to determine the underlying causes of the transfer of these two toxic metals to milk in this Brazilian region. Improvements are also needed in terms of the quality control of milk production and the management of dairy cattle.

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