Determination of Heavy Metal Contents in Human Colostrum Samples by Electrothermal Atomic Absorption Spectrophotometry

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Summary

Determination of lead, cadmium, nickel, chromium, manganese and copper levels in colostrum samples (n = 30), collected from middle-class mothers, was carried out by electrothermal atomic absorption spectrophotometer (ETAAS) with Zeeman effect background correction using a tungsten–palladium–citric acid chemical modifier mixture. A wet-ashing procedure was applied to dissolve the samples and to remove the fat. The graphite furnace temperature programme for analytes determined by ETAAS was studied, and the optimum pyrolysis temperatures of Pb, Cd, Ni, Cr, Mn and Cu were determined in the presence of the modifier mixture. Detection limits of Pb, Cd, Cu, Mn and Cr were determined using a modifier mixture solution. Zinc and iron levels were determined by Zeeman flame atomic absorption spectrometer (F-AAS). Heavy metal levels (mean values) found in colostrum samples were 14.6 µg/l of Pb, 2.8 µg/l of Cd, 27.8 µg/l of Ni, 8.6 µg/l of Cr, 43.2 µg/l of Mn, 278 µg/l of Cu, 12.9 mg/l of Zn, and 3.5 mg/l of Fe. These metal levels were compared with results from other studies in different countries.

Introduction

Milk is a fundamental food for infants. The most natural and best source is breastfeeding and this is greatly encouraged for the first 6 months of life and should be continued for as long as 2 years.[1] The beneficial effects of breastfeeding are many. Human milk is usually considered to contain the right balance of fully usable nutrients required for a growing, full-term infant. The existence of host-resistant factors in human milk gives the infant immunologic protection, and the presence of food antibodies in human milk may also prevent food allergies.[2,3] Milk synthesis is promoted by increased prolactin serum levels shortly after delivery. The initial low secretion of the mammary glands, which lasts from 1 to 5 days post-partum, is called colostrum. The secretion during the following 5 or more days is called 'transitional milk', and from 2 to 6 weeks after delivery until the termination of lactation is called 'mature milk'.[2] Colostrum has higher contents of proteins, minerals and fat-soluble vitamins than mature milk.[4,5] Lactation is influenced by nutrients, drugs, stress, and chemicals which are able to modify hormone status.[6,7]

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Maternal milk may sometimes contain chemical contaminants which could have adverse effects on nursing infants. Occupational chemicals or drugs, and hazardous persistent environmental chemicals are the major sources which are the crucial factors limiting breastfeeding in some cases.[8]

Toxic heavy metals (lead and cadmium), toxic and essential metals (nickel and chromium), and also essential elements (iron, copper, manganese, and zinc), have special importance among breastmilk contaminants for pregnant and lactating women. With increasing environmental pollution, a heavy metal exposure assessment study is necessary.[9] Heavy metals enter the woman's body through inhalation and ingestion. Exposure of the newborn through milk to the toxic heavy metal may constitute a hazard, especially when the initial body burden is already raised due to placental transfer.[2] In breastfeeding cases, the constituents of human milk also have special importance. Inadequate essential mineral levels may lead to growth retardation since the newborn infant gets them only via mother's milk.[6] Therefore, balanced food consumption is also essential during gestation and the lactation periods for the mother, the fetus and the suckling infant. The colostrum sample has also higher transitional, toxic and essential metal contents than mature milk and it is necessary to analyse these elements in this milk fraction.

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The concentrations of trace elements in human milk are usually 1/10 the levels encountered in most biological materials.[6] It is also important that sample collection and pretreatment prior to analysis is controlled carefully to avoid the risk of contamination from sample handling and the reagents employed.[7,10]

During the direct determination of volatile elements, such as cadmium (Cd) and lead (Pb) in colostrum samples by electrothermal atomic absorption spectrophotometer (ETAAS), chemical interferences occur in a graphite furnace because of covolatilization of the analyte with the matrix.[11] Presence of residue in the colostrum/milk sample may cause difficulty in accurate determination since these residue materials are volatilized together with analyte metals in a graphite furnace. This is called background or matrix effect. To overcome such background effect matrix modifiers are required.[11–13]

Metal salts continue to be the most popular modifiers in ETAAS.[12–14] Palladium is the most efficient and universal modifier that has been shown in many studies, but there is a limited number of publications regarding tungsten.[11,12,14,15] Mixed modifiers, such as tungsten and palladium, often exhibit more versatile effects and higher efficiency than single modifiers.[13–16]

The aim of this study was to determine the concentrations of toxic and essential elements in colostrum samples, to evaluate their environmental toxic metals exposure and nutritional status, and to compare the results with those obtained in other studies in different countries. In order to obtain an accurate determination of the heavy metals in the colostrum samples, it was necessary for maximum pyrolysis temperatures for each heavy metal to be optimized by using a suitable modifier mixture.

Materials and Methods

Apparatus

A Hitachi Model 180/80 flame and graphite furnace (Hitachi 180/078) atomic absorption spectrophotometer, equipped with Zeeman effect background corrector, an autosampler (P/N-170/0126), and an automatic data processor were used for all measurements. All the operating parameters were set as recommended by the manufacturer. The injected sample volume was adjusted to 20 µl.

Reagents and standards

All reagents, the stock standard solutions of palladium, tungsten and citric acid from Merck, and the metal standards from BDH Chemicals, were of analytical grade and further dilutions were prepared in distilled-deionized water before use. Milk powder and Whey powder standard reference materials were obtained from the International Atomic Energy Agency to ensure reliability of the method proposed to estimate the levels of lead, cadmium, copper, chromium, manganese, nickel, iron and zinc in the samples. Milk powder was industrially prepared skimmed cow's milk. Whey powder was also cow's milk collected from a processing plant in Europe.

Sample collection and processing

Thirty colostrum samples were collected from middle-class mothers residing in Ankara (one of the most over-populated cities in Turkey) during the period from December 1995 to February 1996 with the help of the Paediatric Department of the Social Insurances Hospital. The education level of the mothers was primary school. Their ages ranged from 18 to 33 years. Six donors were smokers. Iron supplementation had been given to 18 women during their pregnancy. Approximately 8 ml of colostrum samples were taken using a breast pump, placed in silicone-coated glass vacutainer tubes 48 h postpartum, and kept in a fridge until analysis.

After mixing by manual shaking, the sample tubes were put into a cold sandy bath. The temperature was gradually increased up to 80ºC and 4 ml of a mixed acid solution (nitric acid–perchloric acid) was added to each sample and reheated to 80ºC.[17] Following the wet-ashing procedure, the sample was cooled, 2–3 ml of 0.2 per cent nitric acid was added, and the volume of the sample was increased to its original volume by adding deionized water. Milk powder and Whey powder were dry-ashed at a temperature of 400ºC and then treated with the mixed acid solution.[1] Blank solutions were also prepared in order to check possible contamination during the preparation of the sample solutions, by using the same reagents under the same conditions.

Procedure

For the determination of lead, cadmium, copper, chromium, manganese and nickel contents in colostrum, 1 ml of the colostrum sample solution was added to 1 ml of modifier mixture solution. A volume, 20 µl, of the prepared sample solution was injected into the graphite tube. The best conditions for the determination of analytes in colostrum samples, using the graphite furnace with a mixed modifier solution, were obtained by changing the temperarture and time. Iron and zinc were determined differently by using the flame atomic absorption spectrometer (F-AAS) technique.

Results

The optimized temperature programme and maximum pyrolysis temperatures for metals determined in colostrum samples by ETAAS, using a mixed modifier solution, are given in Table 1.

Lead, cadmium, copper, chromium, manganese and nickel were analysed by graphite furnace, and iron and zinc were determined by the F-AAS

TABLE 1
Optimized graphite furnace temperature programme

Element	Drying temperature (ºC)	Times(s) Ramp	Times(s) Hold	Pyrolyis temperature (ºC)[a]	Atomization temperature (ºC)[b]	Cleaning temperature (ºC)[c]
Cu	50–120	20	20	120–1300	2700	2800
Cd	50–120	20	30	120–900	1600	1800
Ni	50–120	20	20	120–1300	2700	2800
Cr	50–120	20	30	120–1350	2700	2800
Pb	50–120	20	30	120–1250	2100	2400
Mn	50–120	20	20	120–1350	2500	2800

[a] Ramp time = 30 s, hold time = 30 s; [b] atomization time = 7 s; [c] cleaning time = 5 s.

TABLE 2
Concentrations of Pb, Cd, Cu, Ni, Mn, Fe and Zn in standard reference materials from IAEA

Reference material	Element	Unit	Concentrations Certified value	Concentrations Found[a]
Milk powder (A-11)	Pb	ng/g	54 (29–79)	50 ± 3
	Cd	ng/g	1.7 (1.2–2.2)	1.8 ± 0.2
	Cu	ng/g	378 (354–402)	374 ± 16
	Cr	ng/g	17.7 (13.7–21.7)	15.8 ± 1.3
	Mn	ng/g	257 (248–266)	259 ± 11
	Fe	µg/g	3.65 (2.89–4.41)	3.61 ± 0.22
	Zn	µg/g	38.9 (36.6–41.2)	39.4 ± 1.8
Whey powder (155)	Pb	ng/g	104 (71–136)	101 ± 5
	Cd	ng/g	16 (12.5–19.6)	15.6 ± 1.3
	Cu	mg/g	0.57 (0.46–0.68)	0.54 ± 0.04
	Cr	µg/g	0.59 (0.51–0.66)	0.55 ± 0.05
	Mn	µg/g	9.30 (8.78–9.82)	9.18 ± 0.36
	Ni	µg/g	0.54 (0.44–0.64)	0.52 ± 0.04
	Fe	µg/g	62 (50–74)	60 ± 5
	Zn	µg/g	34.3 (32.8–35.7)	33.8 ± 1.9

[a] The mean of eight replicate measurements with 95% confidence level, $\overline{X} \pm$ t.s/$\sqrt{n}$.

technique for all the Milk powder, Whey powder and colostrum samples. As shown in Table 2, the obtained metal levels from the standard reference materials agreed with the certified values to within about ± 6 per cent. Mean values and ranges of the eight heavy metal concentrations obtained from 30 colostrum samples are given in Table 3. Some of the metal contents in mature milk and colostrum which were reported in previously published studies are given in Table 4.

TABLE 3
Mean values and ranges of heavy metal concentrations in colostrum samples

Element	Mean value[a] (µg/l)	Ranges (µg/l)
Lead	14.6 ± 5.5	8.8–35.4
Cadmium	2.8 ± 1.7	1.2–9.0
Nickel	27.8 ± 8.0	14.9–47.9
Chromium	8.6 ± 6.0	2.3–23.9
Manganese	43.2 ± 14.4	12.5–70.7
Copper	278 ± 58	179–454
Zinc[b]	12.9 ± 7.3	4.5–27.1
Iron[b]	3.5 ± 1.8	2.1–11.0

[a] $\overline{X} \pm$ standard deviation, n = 30 samples; [b] in mg/l.

Discussion

The proposed tungsten–palladium–citric acid modifier mixture was found to be very efficient for

TABLE 4
Ranges of metal concentrations in human milk obtained by other studies

Metal	Ranges (µg/l)		References	
	Mature milk	Colostrum	Mature milk	Colostrum
Lead	2–277	35.8	2, 3, 7, 19	18
Cadmium	0.1–43.0		2, 3	
Nickel	10–20		2, 3, 7	
Chromium	0.18–11.2	0.2–2.4	2, 3, 22–25	23
Manganese	2.0–30.0	4.0–32.0	10, 23, 27	23, 27, 28
Copper[a]	0.1–3.6	0.4–1.1	2, 3, 10, 23, 28	23, 27
Zinc[a]	0.6–8.7	8.0–16.0	10, 23, 24, 28	23
Iron[a]	0.2–4.0	0.9–1.1	2, 3, 10, 23, 24	23

[a] In mg/l.

the thermal stabilization of cadmium, copper, nickel, chromium, lead and manganese in the colostrum samples, and in reducing interferences and removing volatile matrix components from the samples. This modifier mixture was capable of determining the relatively volatile elements.[13,15] Detection limits of lead, copper, manganese, chromium and cadmium were found to be 0.92, 0.71, 0.11, 0.26 and 0.62 µg/l by using the proposed modifier mixture.

Intake of lead and cadmium have no beneficial importance and their exposure to humans should be prevented.[18] Their exposure, via lead-based paint, industrial emissions and auto exhausts, can cause potential health hazards to humans.[2,3] Organic lead compounds, i.e., tetraethyl and tetramethyl lead, are extensively used as gasoline additives in Turkey. Due to the environmental contamination, bioaccumulation of lead increases in the mother's body mass, which causes increased lead levels in colostrum and mature milk. Ankara, where the present study was carried out, has minimal to low industrial activities, thus traffic is the predominant environmental pollution source of lead. Measured lead levels in the colostrum sample (Table 3) were considered to be quite safe when comparing mature milk and colostrum with reported studies from other countries (Table 4).[3,7,18,19] Another toxic metal, cadmium, is also found as an environmental pollutant in air, food and water.[3,20] A major non-occupational source of cadmium is cigarettes. Smoking one or more packs of cigarettes/day may double the body burden of cadmium. With continuous exposure, these toxic metals progressively accumulate in various tissues, and manifest their toxic effects. Chronic exposure to a low dose of cadmium may also cause hypertension and infertility in male rats.[21] Exposure to cadmium from any source, including cigarette smoking, during the gestation period may lead to increased cadmium secretion in the colostrum. This high ratio of cadmium secretion decreases gradually to 50 per cent of its previous value.[2] In our study, six out of 30 donors were smokers (5 cigarettes/day or more). However, there was no significant difference between the colostrum cadmium levels for the smoker and non-smoker groups, probably indicating that our donors were not heavy smokers. The cadmium levels measured in our 30 samples might not be potentially hazardous for infant development.

Trace quantities of trivalent chromium are essential for carbohydrate metabolism, and deficiency of chromium may cause growth retardation, insufficiency in protein, and lipid metabolism.[6,20,22] Similar effects have been observed in nickel deficiency, causing body growth retardation, anemia and significant hypoglycemia in rats.[20] Excess intakes of these metals (chromium and nickel) may exert toxic effects especially in children and elderly people. The nickel and chromium levels of our colostrum samples were quite high compared to previously reported results.[7,23–25] This might be due to the composition of food consumed by our subjects or the kitchenware used, which is made from chromium and nickel alloys. Further detailed investigation may explain the higher nickel and chromium levels in the colostrum samples and the possible effects on the suckling infants, both for nutritional and toxicological interpretations.

Iron, copper, manganese and zinc are essential elements found in milk secretions, and they are of nutritional importance in infant development.[6,23,26–28] Higher metal levels have been found in the colostrum fraction of a mother's milk than in mature milk. Initial levels of these elements decline gradually during the lactation period.[29] From the nutritional and toxicologic perspective, monitoring of a mother's milk samples from the early stage through to established breastfeeding, has obvious importance for both the mother and the infant. Measured essential element concentrations in our colostrum samples (Table 3) are quite similar to previously reported results (Table 4).[23,27,28]

It can be concluded that collected colostrum

samples from middle-class mothers resident in Ankara contain non-toxic levels of cadmium, lead, nickel and chromium, and sufficient levels of essential trace elements. In addition to this, we stress the importance of periodic analysis by using reliable, simple and economical methods proposed for both toxic and essential metals in colostrum and other biological fluids, which reflect the degree of environmental pollutions, exposure levels, and the nutritional status of pregnant or lactating women.

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