The Minerals and Heavy Metals in Cow's Milk from China and Japan

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(Received November 10, 2008; Accepted December 25, 2008)

Cow's milk is an important foodstuff and beneficial to human health. In the present study, commercial milks from China and Japan and raw milk from Inner Mongolia of China were collected. The contents of 18 elements were determined using inductively coupled plasma-optical emission spectrometry (ICP-OES), atomic absorption spectrometry (AAS) and atomic fluorescence spectrometry (AFS). Our analysis showed both Chinese and Japanese milks are rich in macroelements, such as calcium, potassium. However, the milk contents of chromium, manganese and zinc, which belong to microelements, were higher in Chinese commercial milks than in Japanese commercial milks. Heavy metals in food pose potential healthy risk. Although lead and cadmium contents in Chinese milk did not exceed the tolerance limits of Chinese National Standards, they were higher than those in Japanese commercial milks. Based on the high contents of some microelements and heavy metals in Chinese milk, we should innovate the technology and improve quality control for milk process and decrease environmental pollution.

Key words —— milk, mineral, heavy metal, lead, cadmium

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INTRODUCTION

Milk is a complex, bioactive substance to promote growth and development of the infant mammals. Cow's milk is widely consumed by human children and adults after the age of weaning. Among the nutrients of milk, calcium (Ca) has been well recognized by researchers and the public because of greater stature in children and less osteoporosis in old people. In fact, milk is an ideal source of macroelements, such as Ca, potassium (K), phosphorus (P). Moreover, microelements and even heavy metals can be found in milk.[1] Microelements, also called trace elements such as copper (Cu), iron (Fe), selenium (Se) and zinc (Zn) are known to be essential for normal growth. However, heavy metals such as arsenic (As), cadmium (Cd), mercury (Hg) and lead (Pb) have no beneficial effects on human.

Historically, cow's milk was seldom consumed by populations in China (except some minorities) and Japan. After World War II, liquid milk consumption rapidly increased in Japan, with 36.7 liters *per capita* in 2005. Although *per capita* consumption of milk in China is as low as 8.8 liters, milk consumption increased 20% every year.[2] Thus, milk has become and will become important foodstuff consumed in Japan and China. Considering the healthy problem of infant, the minerals, especially heavy metals in breast milk have been intensively researched.[3–6] Some studies monitored microelements or heavy metals in cow's milk around polluting areas.[7–10] However, the data on minerals in commercial milk are sparse and the comparisons of minerals in cow's milk from different countries are missing. In China and Japan, minerals in commercial milk were found to be published as abstract in local journal.[11, 12] Since the methods used were not sensitive enough, the re-evaluation of minerals in commercial milk is necessary.

In the present study, we collected commercial milks from China and Japan to determine the mineral contents. Because pasteurization and sterilization processes may influence their contents,[13] raw milks collected from Inner Mongolia of China were also analyzed in this study.

MATERIALS AND METHODS

Instrumentation and Reagents —— Vista MPX type inductively coupled plasma-optical emission spectrometry (ICP-OES) and Spectr AA 110/220 type atomic absorption spectrometry (AAS) was supplied by Varian Inc. (Palo Alto, CA, U.S.A.). 230 E type atomic fluorescence spectrometry (AFS, Beijing Kechuang Haiguang Instrument Co., Ltd, Chao Yang District, Beijing, China) was used. And microwave Lab Stations (Milestone Inc., Sorisole, Italy) was used in this research.

Unless indicated otherwise, all the reagents used were at least of analytical reagent grade obtained from Sinopharm Chemical Reagent Co. (Shanghai, China). Milk powder GBW10017 was purchased from Institute of Geophysical and Geochemical Exploration, Chinese Academy of Geological Sciences (Langfang, Hebei, China). 1.0% (m/v) KBH_4 solution was prepared by dissolving KBH_4 in a 1.0% (m/v) KOH solution. Deionized water of 18 MΩ·cm was used for preparing the solutions.

Sample Collection —— Two brands of nationally available commercial milk were purchased from the local supermarkets in Suzhou city, China and Yamanashi prefecture, Japan, respectively. Milks were collected every one and half months from October 2006 to October 2007. Thus, we got 8 samples each milk. These milks were designated as China 1, China 2, Japan 1 and Japan 2. All of milks were produced from 100% raw milk and were classified as whole milk. Japan 2 was the milk from Jersey cows and other three were not indicated. Raw milk was collected from Inner Mongolia Prairie of China at May 2007. It was milked by nomads in the morning and stored in 50 ml centrifuge tube. The milks collected from Japan and Inner Mongolia were transferred at 4°C by airplane. All samples were stored at −20°C in our laboratory.

Sample Digestion —— For the digestion of samples, approximately 10 g of milk was digested with 10 ml of HNO_3 (65%) and 2 ml of H_2O_2 (30%) in acid-prewashed Teflon vessels. After standing overnight, samples were digested using Microwave Lab Stations with the following program: 250 W, 1 min; 0 W, 1 min; 250 W, 6 min; 400 W, 5 min; 600 W, 5 min. After sufficient cooling, samples were moved to Teflon vessels and diluted to 35 ml with distilled-deionized water. Milk powder GBW10017 was used as reference material and treated as described above. Analytical blanks were prepared with each batch of digestion set. All samples were prepared in triplicate run.

Milk treatment for As and Se determination referred to the Chinese National Standard (GB/T 5009.11-2003; GB/T 5009.93-2003). In brief, 10 ml of 6.0 mol/l HCl was added to the digested samples mentioned above. They were slowly heated until color fading. After cooling, 5 ml of 50 g/l thiourea and 50 g/l ascorbic acid was added for As determination. 2.5 ml of 10 g/l $K_3[Fe(CN)_6]$ was added for Se determination. Finally, all samples were diluted to 35 ml with distilled-deionized water.

Table 1. Instrumental Conditions for ICP-OES

Parameters	Values	
RF power	1.20	kW
Argon cooling	15.0	l/min
Argon auxiliary	1.5	l/min
Nebuliser pressure	240	kPa
Read delay	5	sec
Source equilibration time	15	sec
Rinse delay	30	sec
Pump rotation rate	15	rpm
Washing time	10	sec
Number of replicates	5	

Element Determination —— Ca, K, magnesium (Mg), P, sodium (Na) in samples were determined using ICP-OES. Table 1 shows the parameters optimized for ICP-OES. The emission lines were Ca 393.336 nm, K 766.491 nm, Mg 280.270 nm, P 213.618 nm, Na 589.592 nm. Fe, Mn (manganese) and Zn determined by flame-AAS at spectral lines λ = 248.3 nm for Fe, λ = 279.57 nm for Mn, λ = 213.9 nm for Zn. Cd, chromium (Cr), Cu, molybdenum (Mo), Pb by graphite-furnace-AAS at spectral lines λ = 228.8 nm for Cd, λ = 357.9 nm for Cr, λ = 324.8 nm for Cu, λ = 313.3 nm for Mo and λ = 283.3 nm for Pb. As, Hg and selenium (Se) were determined using AFS at spectral lines λ = 193.7 nm for As, λ = 253.7 nm for Hg, λ = 196.0 nm for Se. KBH_4 was used to react with As(III) and Se(IV) to produce AsH3 and H2Se for their determinations in AFS. The instrumental parameters for AAS and AFS come from the softwares present in the machine.

Statistics —— Values are expressed as mean ± S.D. Analysis of variance (ANOVA) was performed to compare the differences among the groups using SPSS 12.0 programme (SPSS Inc., Chicago, IL, U.S.A.). $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

To evaluate the accuracy and the precision of our analytical method, we analyzed a standard reference material of milk powder. Unfortunately, the certified value of Cd is not given, and only the recommended value of Hg is given. The determined values and the certified values of the rest 16 elements were very close, suggesting the practicability of our methods (Table 2). In fact, we have successfully analyzed the contents of twenty elements in garlic using ICP-OES, AAS and AFS.[14)]

Table 2. Comparison between the Determined Values and the Certified Values of the Elements in Standard Reference Material of Milk Powder (Mean ± S.D., Standard Materials were Determined in Triplication)

Element	Determined value	Certified value
As (μg/kg)	26 ± 10	31 ± 7
Ca (g/kg)	9.1 ± 0.3	9.4 ± 0.3
Cd (μg/kg)	1.5 ± 0.4	
Cr (mg/kg)	0.28 ± 0.09	0.39 ± 0.04
Cu (mg/kg)	0.42 ± 0.13	0.51 ± 0.13
Fe (mg/kg)	8.1 ± 0.4	7.8 ± 1.3
Hg (μg/kg)	ND	(2.2)
K (g/kg)	11.3 ± 0.4	12.5 ± 0.5
Mg (g/kg)	1.03 ± 0.03	0.96 ± 0.07
Mn (mg/kg)	0.60 ± 0.15	0.51 ± 0.17
Mo (mg/kg)	0.27 ± 0.08	0.28 ± 0.03
Na (g/kg)	4.3 ± 0.1	4.7 ± 0.3
P (g/kg)	7.0 ± 0.1	7.6 ± 0.8
Pb (mg/kg)	0.07 ± 0.01	0.07 ± 0.02
Rb (mg/kg)	12.9 ± 0.4	11.6 ± 0.7
Se (μg/kg)	88 ± 11	110 ± 30
Sr (mg/kg)	4.5 ± 1.9	5.3 ± 0.6
Zn (mg/kg)	30.4 ± 2.6	34 ± 2

We determined five macroelements in commercial milks and raw milk (Table 3). The contents of Ca and K reached 1000 mg/kg, followed by P, Na and Mg. K contents we determined were significantly higher in raw milk than in commercial milks. Because raw milk was collected at one time point, this difference should be interpreted with caution. Na contents were significantly higher in China 1 than in other three commercial milks and raw milk. However, their contents were in the normal range of the food composition issued by China and Japan (Table 4).[15,16)] China food composition provides 10 kinds of whole milk for their nutrients and food composition in Japan only shows an average value. It is a common knowledge that milk and milk products are rich in Ca and other macroelements and its consumption is encouraged. Chinese Food Guide recommends a daily intake of 300 g of milk and milk products.[17)] The recommendation in Japanese Dietary Guidelines is 2 servings of milk products and alternatives (1 serving = 100 ml of milk or yogurt).[18)]

Nine microelements were determined in the present study. In generally, the microelements in milk decreased in the order of Zn > Fe > Mn, Cu, Mo, Cr > Se > Sr > Rb (Table 3). Zn contents

Table 3. The Determined Values of the Element Contents in Different Kinds of Milks (Mean ± S.D., $n = 8$ in Triplication for Each Sample)

Element	China 1	China 2	Japan 1	Japan 2	Raw milk
Macroelement					
Ca (mg/kg)	1006 ± 47	980 ± 29	1011 ± 16	1053 ± 72	1041 ± 53
K (mg/kg)	998 ± 43$^{a)}$	1003 ± 33$^{a)}$	995 ± 10$^{a)}$	908 ± 46$^{a)}$	1117 ± 56
Mg (mg/kg)	125 ± 8	117 ± 4	119 ± 5	122 ± 9	123 ± 16
Na (mg/kg)	574 ± 83	377 ± 22$^{b)}$	362 ± 24$^{b)}$	361 ± 31$^{b)}$	384 ± 96$^{b)}$
P (mg/kg)	873 ± 59	838 ± 35	883 ± 18	910 ± 80	861 ± 61
Microelement					
Cr (mg/kg)	0.17 ± 0.05	0.16 ± 0.04	0.09 ± 0.03$^{b,c)}$	0.06 ± 0.02$^{b,c)}$	0.14 ± 0.03
Cu (mg/kg)	0.17 ± 0.06	0.28 ± 0.06$^{a)}$	0.23 ± 0.02	0.33 ± 0.05$^{a)}$	0.17 ± 0.08
Fe (mg/kg)	2.21 ± 0.56	2.44 ± 0.59	1.51 ± 0.12	1.63 ± 0.16	1.93 ± 0.96
Mn (mg/kg)	0.38 ± 0.07	0.36 ± 0.05	0.18 ± 0.06$^{b,c)}$	0.19 ± 0.02$^{b,c)}$	0.20 ± 0.01$^{b,c)}$
Mo (mg/kg)	0.18 ± 0.07	0.19 ± 0.06	0.19 ± 0.04	0.13 ± 0.03	0.13 ± 0.05
Rb (μg/kg)	1.48 ± 0.16	1.84 ± 0.12	1.70 ± 0.32	1.29 ± 0.11	1.43 ± 0.39
Se (μg/kg)	45.77 ± 18.79	67.15 ± 23.07	51.91 ± 9.33	66.44 ± 18.59	42.14 ± 18.50
Sr (μg/kg)	5.66 ± 0.23	5.45 ± 0.25	4.94 ± 0.33	4.63 ± 0.29	5.06 ± 1.12
Zn (mg/kg)	3.65 ± 1.79	3.62 ± 1.66	2.72 ± 0.61	3.04 ± 0.46	2.38 ± 0.50
Heavy metal (Toxic metal)					
As (μg/kg)	ND	ND	ND	ND	ND
Cd (μg/kg)	4.53 ± 3.01	4.25 ± 3.03	1.13 ± 0.64	2.01 ± 1.06	4.19 ± 3.80
Hg (μg/kg)	ND	ND	ND	ND	ND
Pb (μg/kg)	35.01 ± 8.63	32.97 ± 11.24	11.98 ± 3.27$^{a,b,c)}$	12.95 ± 2.94$^{a,b,c)}$	28.15 ± 11.23

a) Significantly different from Raw milk. *b*) Significantly different from China 1. *c*) Significantly different from China 2.

Table 4. Mineral Contents in Liquid Milk

	From The Composition of Chinese foods	From Standard Tables of Food Composition in Japan
Ca (mg/kg)	820–1130	1100
Cu (mg/kg)	Tr-0.2	0.1
Fe (mg/kg)	1–6	0.2
K (mg/kg)	1320–3480	1500
Mg (mg/kg)	80–120	100
Mn (mg/kg)	Tr-0.2	0.2
Na (mg/kg)	248–2921	410
P (mg/kg)	590–1030	930
Zn (mg/kg)	2.5–6.7	4.0

in two Chinese commercial milks varied between 1.34–5.22 mg/kg (3.65 ± 1.79 mg/kg) and 1.38–4.96 mg/kg (3.62 ± 1.66 mg/kg); however, these ranges were 2.23–3.56 mg/kg (2.72 ± 0.61 mg/kg) and 2.85–3.73 mg/kg (3.04 ± 0.46 mg/kg) in two Japanese commercial milks. Relatively high content in Chinese commercial milks with a large variation was also observed in Fe contents. Zn and Fe can transfer to food from the tools and machines used in the milk collection and production. We speculated that it was caused by the differences of technology and quality control between two countries. Mn contents were significantly higher in Chinese commercial milks (0.38 ± 0.07 mg/kg, 0.36 ± 0.05 mg/kg) than in Japanese commercial milks (0.18 ± 0.06 mg/kg, 0.19 ± 0.02 mg/kg). Since Mn content in raw milk was 0.20 ± 0.01 mg/kg, the process of milk production seems to contribute to the high Mn contents in Chinese commercial milks. Cu contents were slightly higher in the four determined commercial milk when comparing with the food composition (Table 4); however, their contents were obviously lower than the milk sample from special area. Simsek *et al.* found Cu in milk was as high as 0.96 (0.77–1.20) mg/kg in Industrial region.[7] Thus, Cu content in milk is likely to be strongly influenced by environment. Cr contents were significantly higher in Chinese commercial milks (0.17 ± 0.05 mg/kg, 0.16 ± 0.04 mg/kg) than in Japanese commercial milks (0.09 ± 0.03 mg/kg, 0.06 ± 0.02 mg/kg). Although Cr and other microelments are essential to maintain the metabolic systems of human body, they can lead to poisoning at higher level. Chinese commercial milks we determined are safe for drinker in this issue because the tolerance limit of Cr in milk is 0.3 mg/kg (GB/T 1461-94). We also determined Mo, Rb, Se and Sr in milks, whose contents are in μg/kg level (Table 3). These elements have a great impact on human health. For example, Se has a relatively narrow range of safety, and large amounts can lead to hair lose, brittle nails and other side effects.[19] However, there are no reports about these elements in milk. The contents of these elements also have not included in the food composition table. Thus, our analysis provided useful basic data for the further research.

As, Cd, Hg and Pb in milk have drawn much public attention due to the food safety issues and potential healthy risk. No As and Hg were detected in the collected milk samples. The detection limits of As and Hg in our study were 0.06 μg/kg and 0.015 μg/kg. Our results are in agreement with other reports. For example, As and Hg contents in milk were less than 10 μg/l in Spain.[20] Hg even cannot be determined in the milk from heavy traffic region and industrial region.[7] Cd is considered to be the most important contaminant in modern times. Cd contents in Chinese commercial milks (4.53 ± 3.01 μg/kg, 4.25 ± 3.03 μg/kg) are 2–3 times more than Japanese commercial milk (1.13 ± 0.64 μg/kg, 2.01 ± 1.06 μg/kg). Since Cd content in raw milk from Inner Mongolia (4.19 ± 3.80 μg/kg) was similar with Chinese commercial milks, the high content of Cd is not likely from the process of milk production. Through 3 years' monitor, Vidovic found a decrease of Cd (93%) in atmospheric deposits, followed by a decrease in soil (30%), in cattle feeds (17%) and in milk (13%),[8] suggesting an atmospheric deposits-soil-cattle feed-milk chain. Pb is a heavy metal of wide occupational and environmental concern. Pb contents in Chinese commercial milks (35.01 ± 8.63 μg/kg, 32.97 ± 11.24 μg/kg) and raw milk (28.15 ± 11.23 μg/kg) were significantly higher than in Japanese commercial milks (11.98 ± 3.27 μg/kg, 12.95 ± 2.94 μg/kg). The tolerance limit of Pb in milk is 50 μg/kg according to GB/T 14935-94. One of samples in China 1 and China 2 exceed this limit. In Japan, Pb concentration in atmospheric air was 15–81 ng/m^3,[21] however, it was as high as 90–2800 ng/m^3 in China.[22] Therefore, environmental pollution leads to high Pb content in milk through atmospheric deposits-soil-cattle feed-milk chain. On the other hand, the pollution from milk process and milk container should not be ignored. Food Safety Committee of Japan has set 1 ppm as the limit of heavy metal in the solution when milk container is extracted by 4% acetic acid.[23)]

In conclusion, milk contains considerable amounts of minerals, which are beneficial to human health. Milk is not the main source of heavy metal intake from foodstuffs[8)] and these commercial milks are safe for drinker according to the current standards. However, some microelements and heavy metals in Chinese commercial milk are high when compared with Japanese commercial milk. It suggests that the technology and quality control for milk process should be improved and environmental pollution should be controlled.

Acknowledgements This work was supported in part by grants from National Natural Science Foundation of China (30771808), and from Medical Development Foundation of Soochow University (EE126712). Li-Qiang Qin and Xiao-Ping Wang contributed equally to the work. Authors are grateful to the contributions of Xiang-Liu Su, Yi-Ming Wu and Xiao-Dong Pan.

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