Original Article

Arsenic and Lead Contaminations in Commercial Fruit Juices of Markets in Mashhad, Iran

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ABSTRACT

Background: Contamination of fruit juices with heavy metals presents a risk for human health. Concentrations of lead, and arsenic in fruit juices of market in Mashhad, Iran, were measured using atomic absorption spectrometry (AAS).

Methods: Fifty varieties of commercial fruit juices (grape, apple, orange, pomegranate, and multi-fruit) from 5 popular brands (A, B, C, D and E) were purchased from local markets of Mashhad during spring and winter 2016. Five samples of each brand were analyzed by AAS.

Results: The mean concentrations of lead and arsenic were 39.4 and 1.9 ng/mL, with a range of 18.5–54.6 and 1.52-2.35 ng/mL, respectively. The orange A and multi-fruit A samples had a higher lead level than the others, whilst multi-fruit E contained the lowest. The lowest arsenic content was found in grape B, while the highest was found in multi-fruit C.

Conclusion: Eighty-three percent of the samples had lead levels exceeding the maximum level (30 ng/mL) accepted by the Codex Alimentarius Commission, while arsenic levels were below the maximal permissible value (10 ng/mL). Due to the fact that fruit juices are used by all age groups including infants and children, it is necessary to minimize the health risk from heavy metal contamination in fruit juices. For this reason, the amount of heavy metals contaminants in fruit juice samples should be regularly checked and controlled by the local health authorities.

Keywords: Arsenic, Atomic Absorption Spectrometry, Fruit Juice, Iran, Lead.

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INTRODUCTION

Fruit juices are widely consumed by all age groups in the world and their intake over the past two decades has rapidly increased because they can reduce the risks of many chronic and degenerative diseases [1–3]. However, heavy metal contaminants might accumulate during fruit growth, transportation, processing, handling, and packaging [4]. The concentration of heavy metals in fruit juices depends on many factors such as the nature of the fruit, the mineral composition of the soil and the irrigation water, the climatic conditions, as well as the agricultural practices such as the types and amounts of fertilizers [5,6]. Heavy metals are natural constituents of the earth's crust and are found throughout the environment in the air, water, and soil. As results of natural processes or human activity, they can be released and accumulate into the food or water [7]. Lead (Pb) and arsenic (As), for instance, are two of major contaminants of the food supply that can accumulate in the body and cause harmful effects [8].

There is a relationship between As exposure and increased risks of both carcinogenicity and systemic health effects [9]. Cardiovascular disease, neurotoxicity, diabetes, hearing loss, and hematologic disorders (anemia, leucopenia, and eosinophilia) are the main toxic effects of long-term exposure to As [10]. Pb is identified as a cumulative toxicant that is distributed to the brain, liver, kidneys, and bones. It can cause several unwanted effects such as disruption in biosynthesis of hemoglobin resulting in anemia, nervous system damage, increased blood pressure, kidney

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dysfunction, and miscarriages [11–13]. This metal is particularly harmful to infants and young children because of ongoing development of central and peripheral nervous system. A decreased intelligence quotient and behavioral deficits are the most common side effects of Pb poisoning in children [14,15]. Because low levels of blood Pb can cause damages during childhood, no safe blood Pb level in children has been identified [16,17]. Hence, different international organizations, such as WHO and Food and Agriculture Organization of the United Nations (FAO) have established maximum levels of heavy metals in food and drink. Codex Alimentarius Commission (CAC) that is a joint inter-governmental body of the FAO and WHO has set the amounts of Pb and As permitted in individual foods and foodstuff. The maximum acceptable level of inorganic As in drinking water is 10 ng/mL and for Pb in fruit juices and nectars is 30 ng/mL [18,19]. Although, many reports [4–6,20–27] have been published concerning Pb and As levels in fruit juices in many parts of the world and also in Iran, there is still considerable concern regarding levels of these contaminants.

This study focused on monitoring contamination of Pb, and As in different fruit juices (grape, apple, orange, pomegranate, and multi-fruit) available in the Mashhad market and to compare them with reference levels established by the WHO and CAC, as well as with similar published data.

MATERIALS AND METHODS

As and Pb standard solutions, 1000 mg/L (SpectrosoL) was obtained from BDH (BDH Chemicals Ltd, Poole, England). HNO_3 65%, H_2O_2 30%, HCl 32%, NaOH, $NaBH_4$, and potassium iodide (KI) were purchased from Merck Company (Darmstadt, Germany). Deionized water (DI) was used for preparing all solutions. All laboratory glassware and plasticware were soaked in HNO_3 solution (20% v/v) for two days. Before using, all of them were rinsed four times with DI water and dried. Standard working solutions at various concentrations were prepared daily by appropriate dilutions of the stock solution with DI water. A Perkin-Elmer model 3030 atomic absorption spectrometer (PerkinElmer, USA) equipped with graphite furnace, flame, and MHS-10 mercury/hydride system was used for absorbance measurements. The instrumental parameters were listed in Table 1 and 2. Fifty varieties of commercial fruit juices (grape, apple, orange pomegranate, and multi-fruit) from 5 popular brands (A, B, C, D and E) were purchased from local markets of Mashhad during spring and winter 2016. The total number of samples was 25 for the winter sampling and 25 for the spring sampling. Five samples of each brand were analyzed for the presence of heavy metals.

To determine the Pb levels in fruit juices by graphite furnace atomic absorption spectroscopy (GFAAS), 5 mL of each sample was transferred into a Teflon digestion vessel with a cover. Then, 6 mL of the acid mixture (HNO_3/ H_2O_2 2:1) along with 2 mL DI water were added and the mixture left overnight at room temperature. In the following day, some organic contents of the matrix were decomposed by heating the vessel at 100 °C for about 40 min on a hot-plate. After cooling, 2 mL of H_2O_2 was added to the solution and then further decomposed by microwave oven digestion with the following microwave programming: the heating from room temperature to 140 °C for 20 min, holding at 140 °C for 20 min (up to 800 W) and turning off the microwave and waiting for 20 min. When the vessel cooled, the contents were gently heated at 100 °C to evaporate the sample nearly to 4 mL. Next, 3 mL of the acid mixture HNO_3/ H_2O_2 was added again and the above mentioned procedure was repeated. After cooling, the contents of the vessel were transferred to a 25.0-mL volumetric flask by washing the interior surface of the vessel with 0.05 mol/L HNO_3 for three times. Afterward, the solution was diluted to the mark with DI water. Blank solutions were also prepared to document contamination resulting from the analytical process. For determination of As in fruit juice by hydride generation-flame atomic-absorption spectroscopy (HG-FAAS), 10 mL of standard or juice sample was transferred into a 15 mL glass tube. Then 3 mL of HCL 32% and 1 mL of KI 10% (w/v) were added and incubated for 60 min at room temperature thereafter. Finally, a portion of the mixture was injected into the MHS 10 system for the FAAS determination of As III hydrides.

Data were statistically analyzed using Student *t*-test to determine significant differences in the data of two seasons. Statistical tests were performed using INSTAT software (GraphPad, San Diego, CA). *P*-values less than 0.05 were considered significant. The values are expressed as mean ± standard error of the mean (SEM).

RESULTS

The obtained concentrations of As and Pb for 50 commercial fruit juices were shown in Table 3. The plots of the metal concentrations in these samples were presented in Fig. 1. The contents of Pb were 19.9-43.7 ng/mL in grape, 25.5-52.2 ng/mL in apple, 22.9-54.6 ng/mL in orange, 25.4-50.6 ng/mL in pomegranate, and 18.5-54.6 ng/mL in multi-fruit. Orange A and multi-fruit A samples had a higher Pb level than the others, whilst multi-fruit E contained the lowest (Fig. 1a). The Pb levels in fruit juices ranged from18.5 to 54.6 ng/mL, and 83% of these values were above the maximum values stated by the CAC (30 ng/mL).

These values for As were 1.52-2.15 ng/mL in grape, 1.82-2.21 ng/mL in apple, 1.68-2.21 ng/mL in orange, 1.62-2.15 ng/mL in pomegranate, and 1.55-2.35 ng/mL in multi-fruit (Fig. 1b). The lowest As content was found in grape B, while the highest was found in multi-fruit C. The contents of As ranged from 1.52 ng/mL to 2.35 ng/mL and were all below the maximum value recommended by WHO (10 ng/mL).

Table 1. GFAAS operating condition.

Wavelength (nm)	**282.3**	**Lamp current (mA)**		**10**
Slit width (nm)	**0.7**	**Sample Injection (µL)**		**50**
Step	**Temperature (°C)**	**Ramp time(s)**	**Hold time(s)**	**Ar flow rate(mL/min)**
Dry 1	130	1	30	250
Dry 2	300	5	30	250
Pyrolysis	800	15	10	250
Atomize	2000	1	3	0
Clean	2500	1	2	250

Table 2. HG-FAAS operating condition.

Flame type	**Air-acetylene**
Wavelength (nm)	193.7
Slit width (nm)	0.7
Lamp current (mA)	6
Ar (purge gas) flow rate (mL/min)	1.5
Pre-reductant solution	KI 10% (w/v)
Reductant concentration (NaBH4)	3% (w/v) of NaBH4 in 1% (w/v) of NaoH

Table 3. The concentrations of As and Pb, in analyzed commercial packaged-fruit juices.

Type	**Spring**		**Winter**	
	Pb (ng/mL)	**As (ng/mL)**	**Pb (ng/mL)**	**As (ng/mL)**
Grape A	41.5	1.82	43.7	1.92
Grape B	29.5	1.58	38.1	1.52
Grape C	42.5	1.79	41.1	1.93
Grape D	42.0	2.15	43.5	2.08
Grape E	19.9	1.75	ND[a]	ND
Mean ± SEM	35.1± 4.50	1.82± 0.09	41.6± 1.31	1.86± 0.12
Minimum value	19.9	1.58	38.1	1.52
Maximum value	42.5	2.15	43.7	2.08
Apple A	40.8	1.94	44.2	2.21
Apple B	36.8	1.82	35.2	1.96
Apple C	38.8	2.06	36.5	2.15
Apple D	46.4	1.88	52.2	2.05
Apple E	25.5	2.02	ND	ND
Mean ± SEM	37.7 ± 3.43	1.94 ± 0.04	42.0 ± 3.93	2.09 ± 0.05
Minimum value	25.5	1.82	35.2	1.96
Maximum value	46.4	2.06	52.2	2.21
Orange A	51.1	1.68	54.6	1.83
Orange B	36.6	1.74	38.5	2.01
Orange C	45.6	1.85	50.2	1.92
Orange D	34.8	1.84	33.5	1.78
Orange E	22.9	2.04	28.5	2.21
Mean ± SEM	38.2 ± 4.84	1.83 ± 0.06	41.1 ± 4.94	1.95 ± 0.07
Minimum value	22.9	1.68	28.5	1.78
Maximum value	51.1	2.04	54.6	2.21

[a]ND means not detected

Table 3. *(continued).*

Type	Spring		Winter	
	Pb (ng/mL)	As (ng/mL)	Pb (ng/mL)	As (ng/mL)
Pomegranate A	33.4	1.70	32.3	1.88
Pomegranate B	40.1	1.62	44.2	1.75
Pomegranate C	38.9	1.74	40.1	2.15
Pomegranate D	43.8	1.78	50.6	1.86
Pomegranate E	25.4	1.67	ND[a]	ND
Mean ± SEM	36.3 ± 3.20	1.70 ± 0.03	41.8 ± 3.83	1.91 ± 0.08
Minimum value	25.4	1.62	32.3	1.75
Maximum value	43.8	1.78	50.6	2.15
Multi-fruit A	48.4	1.72	54.6	1.85
Multi-fruit B	30.6	1.55	34.8	1.70
Multi-fruit C	46.9	2.05	45.8	2.35
Multi-fruit D	48.4	1.82	52.1	1.98
Multi-fruit E	18.5	2.12	20.8	2.19
Mean ± SEM	38.6 ± 6.03	1.85 ± 0.10	41.6 ± 6.22	2.01 ± 0.11
Minimum value	18.5	1.55	20.8	1.70
Maximum value	48.4	2.12	54.6	2.35

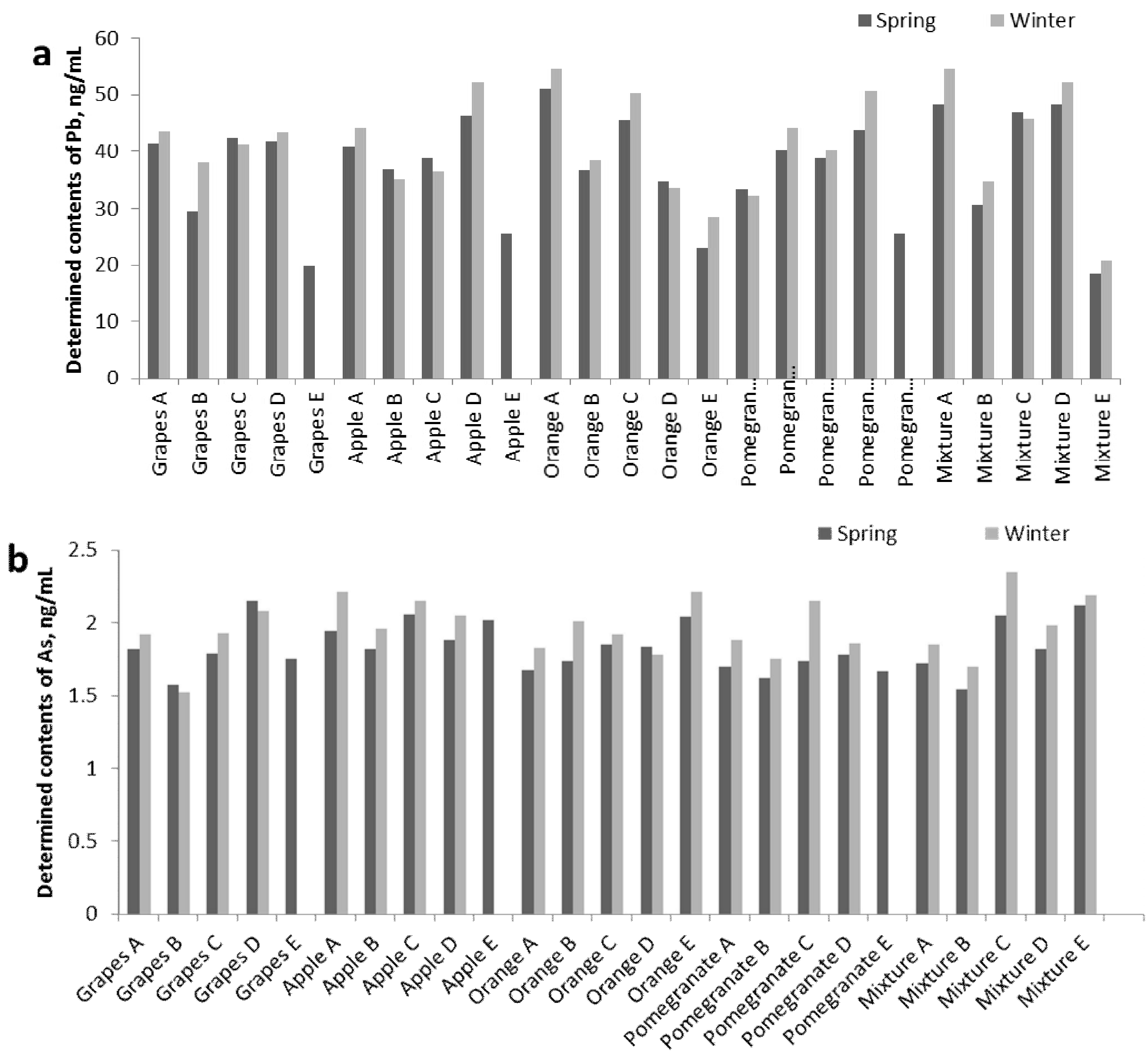

Figure 1. Pb (a) and As (b) concentrations in commercial packaged-fruit juices collected during spring and winter 2016. On the whole, 50 samples were analyzed (five samples for each brand): spring, 25 samples and winter, 25 samples.

DISCUSSION

The results were compared to those of other published studies (Table 4). The reported levels of Pb in some of mentioned studies [4,20–22] were higher than those of the present study. Moreover, Pb levels in the rest of mentioned studies were lower than or nearly equal to our results [5,6,23–26]. The contents of As in the present study were similar or lower than those of all mentioned studies. The contents of selected heavy metals in commercial fruit juice vary in a wide range.

The metal variability comes from the raw materials used in the fruit juices production and used processing technologies. The soil composition, ground-water chemistry, the external conditions during fruit growing and fruit harvesting are factors that may influence the concentration and distribution of metal contaminants in raw material.

Therefore, the raw materials must be monitored to trace the origin of these contaminants. In addition, processing equipment and procedures performed during the fruit processing can also be considered as a possible source of heavy metals contamination. For example, some of the Pb and As present in juices may be due to contamination of the water used in the fruit juices reconstruction or the added sugar or the imported dried fruits.

Hence, in order to reduce the transfer of these heavy metals to juices, processing procedures are carefully checked to reduce the concentration of these contaminants in final products.

Table 4. Pb and as concentration (ng/mL) in fruit juice samples in other published studies as well as in the present study.

Fruit juice	Number of samples	Elements	Country of origin	Concentration (ng/mL)	Ref.
Grape	4	Pb	Iran	96-1521	[4]
Orange	5			28.5-274	
Apple	6	Pb	Romania	4.66-75.68	[5]
		As		<DL-4.36	
Orange	6	Pb		1.02-10.03	
		As		<DL-3.02	
Apple	11	Pb	Poland	51-460	[20]
Orange	10			46-251	
Grape	-	Pb	Nigeria	80	[21]
Apple					
Orange					
Grape	3	Pb	Poland	106	[22]
Apple				670	
Orange				91	
Pomegranate	42	Pb	Turkey	0.0- 20	[23]
Orange	4	Pb	Ghana	ND-50.0	[24]
		As		ND	
Pomegranate	1	Pb	Romania	1.14	[25]
		As		1.66	
Grape	4	Pb	Brazil	<10	[6]
Apple	31	Pb	Romania	0.02-11.02	[26]
		As		0.18-1.14	
Apple	6	As	Spain	2.2-14	[27]
Grape	50	Pb	Iran	19.9-43.7	Current study
		As		1.52-2.15	
Apple				25.5-52.2	
				1.82-2.21	
Orange				22.9-54.6	
				1.68-2.21	
Pomegranate				25.4-50.6	
				1.62-2.15	
Multi-fruit				18.5-54.6	
				1.55-2.35	

CONCLUSION

All samples had As levels that did not exceed the maximum acceptable levels recommended by CAC. However, in 83 percent of those samples, Pb levels were higher than related maximum permissible levels (>30 ng/mL). Therefore, materials that provide significant exposure to heavy metals such as fruit juices must be closely and regularly monitored.

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