

## Determination of Heavy Metals through Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) in Iranian Cheese and Their Potential Health Risks to the Adult Consumers

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### ABSTRACT

In Iran, cheese is one of the dairy products that widely consumed as a main diet for breakfast. Moreover, trace metals in dairy products have recently gained considerable attention.

Iranian cheese samples were collected from Tehran, Iran (February to May 2013). Trace metals including Pb, Cd, Ni, Fe, Sn, Zn, Cr, and Cu were analyzed using inductively coupled plasma optical emission spectrometry (ICP-OES) after dry ashing.

All the tested metals were detected in the cheese samples. The mean concentration of metals in cheese showed the following decreasing order Zn > Fe > Cu > Ni > Sn > Cr > Pb > Cd, with values of 12.98, 7.95, 1.96, 0.83, 0.46, 0.37, 0.34, and 0.01 mg/kg, respectively.

There were no significant differences between types of cheese samples in terms of content of trace metals ( $p > 0.05$ ). All the samples had Pb contents of greater than Codex limit (0.02 mg/kg). According to the measured values of the metals in this study, the intake of all the studied elements through the common consumption of cheese in Iran was below the dangerous level according to permissible intake value for each metal. Also, levels of correlations between the element pairs were analyzed.

**Key words:** Trace Metal; Cheese; Inductively Coupled Plasma Optical Emission Spectrometry; Intake of Element.

### INTRODUCTION

Dairy products are important components of the human diet. One of the basic dairy products is cheese that is rich in fat, protein, calcium, riboflavin, and some other vitamins [1]. During the last 25 years, consumption of fermented dairy products such as cheese has been continuously growing [2].

In Iran, white cheese is widely consumed as a main diet for breakfast. Texture of Iranian white cheese is close to brined cheese made from cow's milk, sheep's milk, or mixtures of both. It is like Beyaz peynir (Turkish white cheese) and Feta but differs from Feta in its composition method. It is, for example, manufactured without dry salting of curd and slime formation on the curd surface before brining [3].

Lighvan cheese is a kind of traditional semi-hard cheese which is produced from ewe's raw milk using lamb rennet paste. No selected or natural starter cultures are added to it and this cheese is originally manufactured in Lighvan village, Tabriz province, Iran [4].

Cream cheese is a product with diacetyl flavors, slightly acidic taste, and creamy white texture. This product is soft, un-ripened, and mild. Cream cheese is produced via mixing milk and cream or coagulation cream using starter culture [5].

Trace metals in dairy products have recently gained considerable attention and many researchers have determined the concentration of heavy metals and trace elements in cheese [6, 7]. Milk and dairy products may be contaminated with heavy metals through a number of sources including: food stuff and water or through manufacturing and packaging processes [8, 9]. It is important to note that existence of metals in dairy products can reveal some points about them. For example, they can indicate the condition of manufacturing practices, possible contamination related to equipment in process, environmental pollution, and quality of animal feeding due to its effect on milk characteristics and cheese properties [5]. Metals in the composition of milk products can be divided into two groups:

essential metals (Fe, Mn, Cu, Zn, Co, Cr, etc.) and non-essential metals (represented mainly by Hg, Cd, Pb, etc.). The second group, even at low concentrations, leads to metabolic disorders with extremely serious consequences. Increasing concentration of both heavy metal groups above the optimal limits has toxic effects on the consumers of milk and milk products [10]. Exposure to trace metals in food products has numerous disadvantages for humans. Symptoms of trace metal toxicity include: acute and chronic symptoms, dizziness, nausea, vomiting, diarrhea, sleeping disorders, loss of appetite, and reduced conception rate. Trace metals have been also related to cardiovascular diseases, depressed growth, impaired fertility, nervous and immune system disorders, increased spontaneous abortions, and elevated death rate among infants [5]. In addition to what was mentioned, several parameters such as pH, characteristics of raw materials in containers, and equipment cause oxidation of containers and equipment. If oxidation increases, metal contents of food will be increased [11]. For example, iron and copper have an important role in the final quality of cheese. Increased Fe and Cu concentrations from external contamination damage cheese quality, because they catalyze oxidation reactions of lipids via the development of unusual flavors due to ionized form of these metals [12]. Therefore, from technological and nutritional points of view, it is very important to know the levels of trace metals in foods such as cheese.

In different studies for investigating metals in milk and dairy products, different techniques have been applied. One of these techniques is inductively coupled plasma optical emission spectrometry (ICP-OES), which determines elements in environmental and biological samples both simultaneously and sequentially. Furthermore, it has some advantageous such as short-time and low-limit of detection [13]. Hence, the mentioned method was used for measuring heavy metals in this study.

In Iran, although cheese plays an important role in people's diet, there are few studies on the levels of heavy metal in dairy products, such as cheese. Therefore, the aim of this study was to determine the levels of some heavy metals in some cheese collected from Iran by inductively coupled plasma optical emission spectrometry (ICP-OES).

## MATERIALS AND METHODS

### *Collection of cheese samples*

In total, 153 samples and three types of industrial cheese (white, Lighvan, and cream) were collected from Tehran, Iran (February to May 2013). Fat in cheese samples was 16-26% and humidity was 54-

65%. Cheese samples were purchased in their original packages and then taken to the lab in an ice box. Before analysis, the cheese samples were dried at 105°C for 24h. Then, the dried samples were homogenized by a stainless steel blender and stored in polyethylene bottles at -18°C until analysis.

### *Reagents and chemicals*

For preparing all the solutions, analytical reagents-grade chemicals were applied. All the plastic and glassware containers were washed with detergent and cleaned by soaking in dilute nitric acid (1 + 9). Then, they were rinsed several times with distilled water prior to use. Nitric acid 65% (from Merck Company, Darmstadt, Germany) and double-deionized water was used for the dilutions.

### *Digestion procedures and analysis*

Dry cheese samples (1.0g) were weighted by a digital analytical balance and then dry ashed in an electric furnace (Gallenkamp, England) at 450–500°C for 16h. In order to obtain white ash, 1 mL of concentrated nitric acid was added and this mixture was ashed again for 6 h. The ash was dissolved with 1–2 mL of concentrated nitric acid and filtered through Whatman filter paper No. 41. Then, the sample was transferred to a 10ml volumetric flask and made up to volume with distilled water [5]. Finally, the metal contents were determined in this solution by inductively coupled plasma optical emission spectrometry (ICP-OES) (Spectro Arcos, Germany). A blank sample was performed in the same way. The ICP-OES operating conditions are listed in Table 1a,b. Before quantitative analysis of the samples, calibration curves of the desired metals were prepared using a series of diluted standard solutions. Correlation coefficient  $r^2$  obtained for all the cases was 0.999 and detection limits were 0.049, 2, 0.29, 0.096, 0.3, 0.161, 0.16, and 0.27ppb for Cd, Pb, Ni, Cr, Cu, Sn, Fe, and Zn, respectively. The recovery percentage was 90%–95%. The recovery percentage was 90%–95%. The operating parameters of determination of elements by ICP-OES are given in Table 1 (a, b).

### *Validity and reliability of the digestion procedure*

In order to verify the validity and reliability of the digestion procedure, a certain amount of each investigated metal (0, 10µg/g and 20µg/g) was added to cheese samples. Then the amount of each metal was measured after digestion (dry ashing) cheese samples. And the obtained values were compared with the added value (0, 10µg/g and 20µg/g) and the percent recovery were obtained. Results showed recovery 77-101.7 %. The relative standard deviation for three digestion replicates of each sample was in the range from 2% to 12 %.

### Statistical analysis

Tukey's test was used to compare the means obtained for the trace metals determined in cheese samples. Correlations between the metals were tested by Pearson correlation test and graphical display was performed using R software.

**Table 1a:** The operating parameters of determination of elements by ICP-OES.

Method	Parameters
RF power (W)	1400
Plasma generator	Argon
Plasma gas flow rate (L/min)	14.5
Auxiliary gas flow rate (L/min)	0.9
Nebulizer gas flow rate (L/min)	0.85
Sample uptake time(s)	240 total
Rinse time of (s)	45
Initial stabilization time (s)	Preflush:45
Pump rate (rpm)	15
Measurement Replicates	3
Frequency of RF generator (MHz)	resonance frequency: 27.12
Type of detector	Solid state CCD
Type of spray chamber	Cyclonic Modified Lichte

**Table 1b:** The operating parameters of determination of elements by ICP-OES.

Element	R <sup>2</sup>	line (nm)
Cd	0.99943	214.438
Cr	0.99987	205.618
Fe	0.99990	259.941
Cu	0.99992	324.754
Zn	0.99950	213.856
Pb	0.99964	220.353
Sn	0.99979	189.991
Ni	0.99989	221.648

## RESULTS AND DISCUSSION

This study was carried out to evaluate the trace metal contents of Iranian cheese. Metal contents (Pb, Cd, Ni, Fe, Sn, Zn, Cr, and Cu) (dry weight) of the samples determined by ICP-OES are reported in Table 2. Concentration of these metals was in the order of Zn > Fe > Cu > Ni > Sn > Cr > Pb > Cd. No significant differences were observed among different types of cheese in terms of metal content ( $p > 0.05$ ).

There was no Maximum Residue Level (MRL) for the elements studied in cheese, except in the case of Pb. The limit value was established as 0.02mg/kg w/w in secondary milk products [14]. Thus, results were compared with those of similar studies, which had used dry ashing digestion method for preparation of the samples (Table 3). Also, because there were no significant differences between three kinds of cheese in terms of metal content, the average value of this study was compared.

### Heavy metal contents

#### Lead content

All cheese samples had measurable Pb contents. Pb contents of all the samples (after calculating wet weight) exceeded the Codex limit (0.02 mg/kg w/w). Average Pb concentration was 0.34 mg/kg in the samples (Table 2). It was almost similar to the value obtained by Orak *et al.* and Coni *et al.*; but, Ereifej and Gharaibeh reported a higher value (Table 3). High levels of Pb in cheese samples were associated with Pb bonding characteristic of casein and fat [18]. Also, it may be due to the materials used for production objectives [9]. Maas *et al.* (2011) stated that the origin of Pb content in cheese cannot be only explained by a concentration effect from milk to cheese; but, it may be derived from the external source of contamination during the manufacturing process. Also, Coni *et al.* suggested that leaching of heavy metals from the equipment to the cheese might happen during processing at a relatively high temperature. It was suggested that, in the phase of cheese ripening, levels of lead contents may be increased through local environmental contamination [8]. In addition, Pb contamination in milk and cheese may be due to feeding cows with fodder collected from road sides [9].

#### Cadmium content

Average Cd content was 0.01mg/kg (Table 2). The concentration detected by Orak *et al.* was almost similar to this value; but, it was slightly greater than the value of the present study. Ereifej and Gharaibeh also reported a higher value. Lower results were found earlier by Coni *et al.* (Table 3). High Cd content of cheese samples may be attributed to uncontrolled production and use of contaminated water in the production process [9]. Moreover, [19] believed cheese manufacturing could cause a significant increase in Cd concentration.

#### Tin content

Average Sn content was 0.43mg/kg in the samples (Table 2). This value was less than the value reported by Ereifej and Gharaibeh (Table 3). They suggested that Sn content in cheese was probably originated from the tin container and/or the salt. In Iran, the value reported as the maximum permitted levels of Sn in brined cheese is 200mg/kg [20]. Present values were largely less than this level. It should be also noted that tinplate is used in food and beverage packaging, which may result in dissolving tin in the food content, especially when plain internal surfaces are used without any cover [21].

#### Iron content

Average Fe concentration was 7.86mg/kg in the samples (Table 2), which was less than the value reported by Ereifej and Gharaibeh and Kira and Maihara. The present value was higher than the one reported in the literature as 3.610mg/kg [15] (Table 3). Ereifej and Gharaibeh suggested that Fe levels in

their samples might be due to tin containers which were manufactured in poor conditions. García *et al.* stated the existence of some probably uncontrolled factors associated with goats' diet, which influenced the Fe concentration of dairy products.

#### Copper content

Average Cu concentration was 2mg/kg in the samples (Table 2), which was less than the reported value in the literature [17]. Lower Cu levels have been found by Coni *et al.*, Orak *et al.*, and Kira and Maihara (Table 3). Coni *et al.* demonstrated that the effect of the manufacturing process on concentrations of Cu. Al, Cd, Cu, and Fe may be released from alloys of materials and tools in the productions of dairy utilized materials and tools [18], [16]. Maas *et al.* stated that the Cu levels may be due to use of large copper vats exclusively in the Comté PDO cheese manufacturing.

#### Zinc content

Mean Zn concentration was 13.37mg/kg in the samples (Table 2), which was almost similar to the value reported by Orak *et al.*. However, Kira and Maihara reported a lower value. Higher values were recorded by Ereifej and Gharaibeh and Coni *et al.* (Table 3), who suggested that Zn levels in brined cheese preserved in tin containers may be originated from milk or tin. Coni *et al.* stated that curdling in cheese-making may increase concentration of elements such Zn. They also suggested that this increase might be ascribed to bonding caseins and fat.

#### Chromium content

Average Cr content was 0.37mg/kg in the samples (Table2), which were higher than the average value reported by Coni *et al.*, Orak *et al.*, and Kira and Maihara (Table 3). High levels of chromium in cheese may be attributed to the point that this chromium is preferentially bound to milk components such as caseins and moves mainly in curd during the curdling step [8]. Some diets contain chromium and natural levels of Cr can increase in raw products owing to technology used in food and beverage processing. Application of this metal in the food industry, especially in stainless steels, has been widespread [24].

#### Nickel content

Average Ni concentration was 0.84mg/kg in the samples (Table2), which were slightly higher than the value reported by Orak *et al.*. Ereifej and Gharaibeh also found a higher value, while Coni *et al.* detected a lower value (Table 3). Nickel contents in foods may be due to environmental contamination, production, or storage of foods, for example drying, cooking, and packaging materials [5]. Also, Smart and Sherlock stated that one of the sources of nickel in foods which can cause food contamination is use of nickel in utensils, food-processing equipment, and catalysts.

#### Correlation analysis

Correlation analysis between metals in cheese samples showed statistically significant positive correlations between Pb-Sn ( $p < 0.001$ ), Zn-Cr ( $p < 0.01$ ), Zn-Ni ( $p < 0.01$ ), and Zn-Fe ( $p < 0.05$ ) (Fig. 1). Thus, according to the found correlations, it can be deduced that the sources of correlated metals were likely the same.

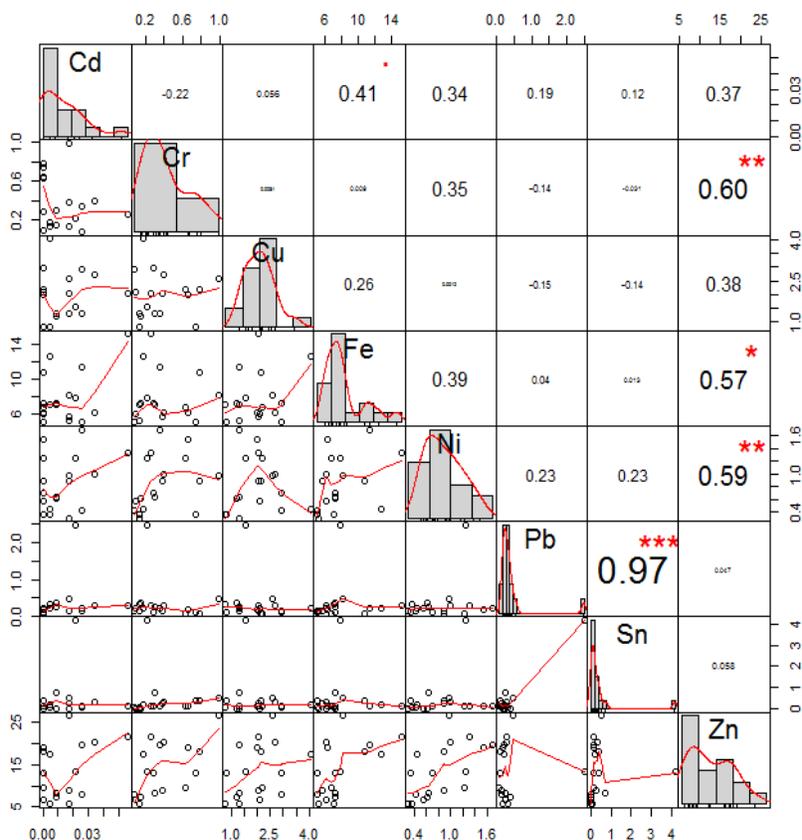
**Table 2:** The concentration levels of Cd, Cr, Cu, Fe, Ni, Pb, Sn and Zn (mg/kg) in cheese species, n=153.

Cheese species	Cd	Cr	Cu	Fe	Ni	Pb	Sn	Zn
White cheese	0.02 ± 0.02	0.53 ± 0.27	1.81 ± 0.55	8.32 ± 3.42	1.16 ± 0.42	0.50 ± 0.81	0.72 ± 1.40	16.32 ± 6.16
Lighvan	NDa	0.41 ± 0.33	3.05 ± 0.94	10.26 ± 2.82	0.68 ± 0.22	0.13 ± 0.06	0.23 ± 0.12	16.07 ± 2.77
Cream	0.01 ± 0.01	0.14 ± 0.08	1.61 ± 0.77	6.31 ± 1.01	0.48 ± 0.14	0.25 ± 0.09	0.21 ± 0.29	6.98 ± 1.12
Mean ± sd	0.01 ± 0.01	0.37 ± 0.27	1.96 ± 0.82	7.95 ± 2.83	0.83 ± 0.43	0.34 ± 0.55	0.46 ± 0.95	12.98 ± 6.04

a: Not Detected in Cd (<0.000049 mg/kg)

**Table 3:** The trace metal concentration (mg/kg) in cheese samples from different countries.

Authors	Country	Specie of cheese	Cd	Cr	Cu	Fe	Ni	Pb	Sn	Zn
Ereifej and Gharaibeh 1993. [17]	Jordan	White brined cheese	0.4	-	4.6	20.8	3.1	7.8	6.90	29.6
Coni <i>et al.</i> 1995. [16]	Italy	Cheese	0.0017	0.055	0.995	-	0.15	0.105	-	19.5
Orak <i>et al.</i> 2005. [15]	Turkey	White cheese	0.127	0.131	0.629	3.610	1.057	0.415	-	15.576
Kira and Maihara 2007. [22]	Brazil	Petit Suisse cheese	-	0.07	0.19	30.1	-	-	-	3.16



**Fig. 1:** Correlations chart between element pairs in cheese samples (according to mean value for each metal)

\* Correlation is significant at the 0.05 level, \*\* Correlation is significant at the 0.01 level, \*\*\* Correlation is significant at the 0.001 level

#### Implications for consumers based on calculation of metal intake

Metals enter our body via different sources, not only through eating cheese; however, in this study, share of cheese in the intake rate of studied metals was calculated. If the weekly ration consumption of cheese in Iran is considered as almost 91g/ week wet weight (per capita consumption of cheese in Iran is 4.7 kg) [26, 27], the intake of  $0.0045\mu\text{g}/\text{week}$  of Cd (corresponding to mean concentration) can be represented. Thus, the contribution is below the Tolerable Weekly Intake (TWI) for a 70kg average adult (TWI =  $490\mu\text{g}$ ). Intake of other metals in this study was calculated in this way and represented in Table 4. FAO/WHO has not yet recommended Provisional Maximum Tolerable Daily Intake (PMTDI) or Provisionally Tolerable Weekly Intake

(PTWI) for chromium; thus, percentage of these values for this metal was not estimated. Finally, results were stated as percentages of PTWI (Table 4). As can be seen in Table 4, contributions of the tested metals to weekly dietary intake by cheese were less than 1% (except for Ni). Nickel, had maximum percentages of the allowed value among the studied metals (1.15%). Therefore, cheese was suggested as a non-significant contributor to the intake of the investigated heavy metals.

Although the intake of trace metals from cheese was below the permissible intake for each metal and there were no toxicological problems from the consumption of cheese, it should be considered that cheese is not the only source of metal in our diet and MRL for Pb in present study is greater than Codex limit.

**Table 4:** Mean dietary intake of Cd, Cr, Cu, Fe, Ni, Pb, Sn and Zn through eating cheese and mean intake % of PTWI.

Metal	PTWI <sup>a</sup>	PTWI <sup>b</sup>	EWI <sup>c,d</sup>	Mean intake % PTWI	Reference
Cd	7	490	0.41	0.08	FAO/WHO 2004. [28]
Pb	25	1750	10.92	0.62	FAO/WHO 2004. [28]
Cr <sup>e</sup>	-	-	12.74	-	-
Fe	5600	392000	273	0.07	FAO/WHO 2004. [28]
Cu	3500	245000	67.34	0.03	FAO/WHO 2004. [28]
Zn	7000	490000	448.63	0.09	FAO/WHO 2004. [28]
Sn	14000	980000	14.56	0.002	EU 2006. [29]
Ni	35 <sup>f</sup>	2450	28.21	1.15	WHO 1993. [30]

a provisional Permissible Tolerable Weekly Intake (PTWI) in  $\mu\text{g}/\text{week}/\text{kg}$  body weight.

b PTWI for 70 kg adult person ( $\mu\text{g}/\text{week}/70$  kg body weight).

c Estimated weekly intake (EWI) ( $\mu\text{g}$ ) through eating cheese (mean trace metal levels in cheese samples after calculation in wet weight).d Based on mean weekly (91g/ person/week) consumption of cheese in Iran.

e The JECFA has not recommended PTWI for chromium.

f WHO recommends a TDI (tolerable daily intake) of 5  $\mu\text{g}/\text{day}/\text{kg}$  body weight (WHO, 1993), but Calculated for a week ( $\mu\text{g}/\text{week}/\text{kg}$  body weight).

## CONCLUSION

The present study provided information on heavy metal levels in Iranian cheese. In this regard, levels of Pb were higher than Codex value. Thus, it is recommended to apply strict regular monitoring of heavy metal contamination in dairy products such as cheese and take controlling measures in Iran. Finally, it is necessary to do further investigations to identify the exact sources of metals, especially Pb, in cheese. For example, these investigations can include the assessment of environmental parameters (such as levels of soil, vegetation, grassland, or cow's feed), utensil and equipment in processing and packaging materials. Then, they can notify both farmers and company managers about this issue. It is recommended to establish a maximum limit for Pb in Iran, not only in cheese but also in other dairy products.

## ETHICAL ISSUES

Ethical issues have been completely considered by the authors.

## CONFLICT OF INTEREST

The authors have declared that there are no conflicts of interest

## AUTHORS' CONTRIBUTION

All authors equally participated in drafting, revising and approving of the manuscript.

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