



Prevalence, Risk Assessment, and Leachability of Al in Milk and some Dairy Products

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Abstract | One hundred and twenty random samples of different dairy products were collected to be analyzed for Al (Al) concentration, to estimate the intake of Al via consumption of these products, and to investigate the leachability of Al from Al utensils into milk during boiling, storage, and reconstitution of infant formula milk powder (IFMP). The analysis of samples occurred, using Buck Scientific Atomic Absorption Spectrophotometry (AAS). The obtained results showed highly significant difference among the examined samples in Al levels $P < 0.0001$, where the highest level was recorded in rice pudding samples (5.35 ± 0.34) and the lowest level was reported in infant formula samples that decreased significantly from other groups (0.36 ± 0.05). Also, Al residues were considered lower compared with the provisional tolerable weekly intake (PTWI) established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The results of the experimental study showed that, processing of milk in Al containers raised Al residues in the products and the level of contamination increased by increasing the time of contact either during boiling or storage. On the other hand, Al exposure from tap water used for formula preparation had not a potential risk itself. Nevertheless, by reconstitution, it will elevate the Al content in it than in milk powder.

Keywords | Al, Dairy products, Risk assessment, Leachability, IFMP

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INTRODUCTION

Al (Al) is considered one of the widely spread elements in our environment. It forms about 8.13% of the earth's surface, mainly in soil, and rocks (Ahmed et al., 2016). Although it is widely spread, it does not appear to play any vital role in the human body (Exley, 2013). It causes many diseases including, anemia, osteomalacia, and dialysis encephalopathy particularly for old people and those with renal diseases. Furthermore, scientists proved that Al plays an essential role in the appearance Alzheimer's disease and other severe disorders in the brain (Karbouj et al., 2009). In addition, it can be accumulated finally in many organs, causing severe lesions as liver, lungs, thyroid, kidney, and brain (Ahmed et al., 2016).

Food is considered the main source of Al in the human

body, either in natural or synthetic form. Artificially added Al is the major source of dietary Al, as it includes different food additives which may be added to food during manufacturing (Yokel et al., 2008).

Milk and milk products are considered a good source of high-quality nutrients essential for the development of a healthy body. However, the presence of any chemical contaminants in them may cause health hazards, mainly in infants, children, or even old people with immunodeficiency diseases (Gonzalez-Weller et al., 2010). Al can contaminate milk and dairy products in many ways, dairy animals may ingest Al while grazing or feeding on contaminated concentrate feeds, then it can reach the milk. Al can be also, introduced into the dairy products during handling, manufacturing, packaging, or storing (Soni et al., 2001).

Although the concern of food safety has motivated scientists highlighting the hazards of consumption of foods containing high levels of Al, information about Al residues of Al in dairy products in Egypt, and assessment of public health hazards associated with consuming these contaminated products are scanty. Moreover, an additional vision on the leaching of Al from processing utensils is still needed. For the previous reasons, this work was conducted to evaluate the presence of Al residues naturally present in milk and some dairy products, which do not contain artificial Al salts. Also, this study aimed to assess the potential health risks for adults, and infants through consuming milk and milk products using target health quotients (THQ). Additionally, an experimental study was designed to give information about the leachability of Al from Al cookware into milk during boiling, and storage, and compare it with a stainless steel one. Finally, to determine the influence of Al content in the boiled tap water on the final concentration of Al in reconstituted powder formulas.

MATERIALS AND METHODS

COLLECTION AND PREPARATION OF SAMPLES

A total of 120 samples of milk and dairy products, including 30 samples for each of milk, kariesh cheese, and rice pudding, 15 samples for each of butter and IFMP were randomly collected from different farmers, dairy shops, groceries, and supermarkets in Sharkia Governorate, Egypt. Samples were transferred directly to the laboratory in clean, dry bottles, or in their original package and were stored frozen at -20°C until analysis.

PREPARATION AND ANALYSIS OF AL CONCENTRATION

Each sample was digested by adding 5 ml nitric acid to the digestion vessel and shaken gently, wait about 30 min, then add 2 ml of hydrogen peroxide and microwave assisted digestion carried out according to a previously validated method (Chuchu et al., 2013). Then all filtrated samples were analyzed for the presence of Al on the basis of methods of Food Additives and Contaminants (2003) by using Buck Scientific Atomic Absorption Spectrophotometry (AAS) model 210VGP at wavelengths of 309.3 nm at The Central Laboratory, The Faculty of Veterinary Medicine, Zagazig University. Three replicates were done for more accuracy.

CALCULATIONS

ESTIMATED DAILY INTAKE (EDI)

The daily intake of Al for a person from the consumption of milk and other products was calculated according to Food and Drug Organization (FAO, 2009) and Nutrition Institute, Cairo (2007). Depending on the mean concentration of Al in the examined dairy product samples, the amount of these products consumed per day, and the body weight of the adult human (60 kg BW). Where the

average amount of these products consumed per day was considered to be 200 mL, 45 gm, 106 gm, and 6gm for milk, kariesh cheese, rice pudding, and butter, respectively. The results obtained were compared with PTWI recommended by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2011). Concerning IFMP, the estimated daily intake was calculated for 0- to 12 month old infants of 3.5 to 10 kg in weight, using the feeding tables and dosages recommended by the manufacturers and the daily intakes of the analyzed metals in infant formulas were recorded as mg/baby/day (Table 3).

TARGET HAZARD QUOTIENT (THQ)

The THQ for the consumers via consumption of polluted milk and other examined dairy products was evaluated depending on the determined dose and the reference oral dose (RFDO) for the pollutant according to United States Environmental Protection Agency (USEPA, 2014). The severity of health hazards is enhanced by increasing its value more than one (Zhuang et al., 2009).

It was determined by the following equation.

$$\text{THQ} = \frac{\text{EDI (mg/kg-day)}}{\text{RFDo (mg/kg-day)}}$$

EXPERIMENTAL STUDY

To determine the leaching of Al from different utensils (Al and stainless steel), 3 Liters of raw milk were divided into 11 equal parts. Milk was handled in both Al and stainless steel utensils separately as following:

1. one part was analyzed before processing while it was fresh and raw (control).
 2. Two raw parts without boiling were individually stored in Al and stainless steel utensils at 4°C for 24 h.
 3. Two parts were separately heated to the boiling temperature for 5 min in both utensils.
 4. Two parts were heated to the boiling temperature for 10 min in both utensils.
 5. Four samples were taken from 10 min boiled milk and stored at 4°C for two days in both utensils and examined daily (two samples daily one for each utensil). All milk samples were collected and stored at -20°C until analysis.
- On the other hand, three types of drinking water samples were examined (15 sample for each type), one from the tap water (act as a control) and two from boiled water (one type boiled in Al cookware and the other type boiled in stainless steel cookware).
 - Two batches of reconstituted IFMP were prepared from water boiled in Al and stainless steel cookwares.

Samples were reconstituted by following up the reconstitution instructions on its original package. We also take a sample from the IFMP before reconstitution. All samples were reconstituted using the examined tap water samples to know the influence of Al content in the boiled tap water on the final concentration of Al in the reconstituted powder formulas.

DATA ANALYSIS

All calculations were performed with the SPSS pocket program for windows (version 16, 2007). Data were reported as Mean±SE. Minimum and maximum values were calculated. One-way Welch's ANOVA was applied to test differences between groups of milk products. Games-Howell post hoc test was the follow up test. Results were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

As shown in Table 1, the minimum level of Al in raw milk samples was 2.00 and the maximum level was 5.34 ppm, with a mean value of 3.20 ± 0.15 ppm. Al-Ashmawy (2011) and Hardisson et al. (2017) recorded lower Al values in raw milk. While, Meshref et al. (2015) found higher results than our study.

Table 1: Concentrations of Al (ppm) in milk and milk product samples.

Product	No.	Min.	Max.	Mean ± SE
Farmer raw milk	30	2.00	5.34	3.20 ± 0.15^c
Kariesh cheese	30	0.40	9.12	4.23 ± 0.44^{ab}
Butter	15	2.19	6.13	4.12 ± 0.24^b
Rice pudding	30	3.46	11.55	5.35 ± 0.34^a
Infant formula	15	0.09	0.60	0.36 ± 0.05^d

No.: Number of samples Results are represented as ppm (mg/kg); ^{a,b,c,d} Means with different superscript letters are statistically different $P < 0.05$.

The presence of Al residues in milk can be occurred during collection and transport of milk from the farms to the dairy shops as a result of the environmental contamination (Meshref et al., 2015). This can explain the presence of the relatively low amount of Al in our work compared with other studies as the examined samples were directly collected fresh from farmers so the chance for contamination due to the previous causes is minimized.

Regarding kariesh cheese, the Al contents were in the range of 0.40–9.12 ppm, with a mean value of 4.23 ± 0.44 ppm (Table 1). Lower results were obtained by Gonzalez-Weller et al. (2010). Higher results were detected by Meshref et al. (2015) where the mean value for Al was 52.36 ± 1.7 ppm.

Kariesh cheese is one of the most popular types of soft cheese because of its noticeable low price and high palatability. It is always consumed by diseased persons like those suffering from obesity, cholesterol, or heart diseases because of its relatively low fat percentage (Awad et al., 2015). Al contamination of kariesh cheese can be occurred as a result of environmental, or milk contamination. This may be attributed to the fact that Al is attracted to casein and fat particles which are the main component of cheese curd. Also, a certain accumulation during the production process through Al utensils cannot be excluded as they are accused for chelation of Al in acidic food (Al Zubaidy et al., 2011).

Rice pudding is considered one of the most popular, tasteful, nutritious, and relatively cheap milk based desserts consumed in Egypt. The results of this work revealed that, the mean value of Al content in rice pudding was 5.35 ± 0.34 ppm, which ranged from 3.46 to 11.55 ppm (Table 1).

Higher values were reported by Meshref et al. (2015) while Burrell and Exley (2010) reported lower content. The presence of Al residues in rice pudding can be ascribed to milk contamination, or to the residues that may be present in rice, sugar, starch and processing equipment.

On the other side, the Al content of butter in the present study ranged from 2.19 to 6.13 ppm, with a mean value of 4.12 ± 0.24 ppm (Table 1). Cooking butter is a traditional product consumed widely in Egypt, where it can be eaten as butter, used for cooking, or as bakery ingredients as it has the ability to produce more palatable food (Ghasemloy et al., 2017). This element can contaminate butter at various stages of its manufacture and storage. Unfortunately, no available data have been described regarding butter as Al residues in butter are neglected at all in Egypt and other countries. However, detection of heavy metal residues in butter is very rare, it was only assessed in few works (Meshref et al., 2014; Ghafari and Sobhanardakani, 2017; Hashemi et al., 2017). Butter need more investigation to highlight the metal content, even it was low, as it used daily in many types of food and desserts and so, the daily diets may contain other sources of metals which may have a cumulative health risk to human.

In this work, Al content had been reported also, in different types of imported infant formula samples, which are widely available in Egypt. The concentration of Al in these formulas ranged from 0.09 to 0.60 ppm (Table 1). The present results were less than others determined by Burrell and Exley (2010) and Chuchu et al. (2013) and higher than the values detected by Sipahi et al. (2014), and Ahmed et al. (2016).

Table 2: Comparison of the provisional tolerable weekly intake (PTWI) value of Al with the calculated weekly intake from consumption of milk and dairy products.

Products	Mean concentration of metals (ppm)	Average daily intake of metals (mg/day/person)	PTWI (mg/day/60 kg person)	Average weekly intake (mg/60 kg person/week) from consumption of milk and dairy products/day/60kg person ^b	
				(mg/person/week)	Contribution to PTWI (%)
Milk	3.20	0.64	120 ^a	4.48	3.73
Kariesh cheese	4.23	0.19		1.33	1.11
Butter	4.12	0.02		0.14	0.12
Rice pudding	5.35	0.57		3.99	3.44
Total	16.9	1.42		9.94	8.4

a: JECFA (2011); b: Daily consumption for adult person (60 kg b.w.) acc. to Nutrition Institute, Cairo, 2007 and FAO, 2009

Table 3: Comparison of the provisional tolerable weekly intake (PTWI) value of Al with the calculated weekly intake from consumption of IFMP in different age.

Age	Daily consumed IFMP 'g'	Mean body weight of baby 'kg'	Average daily Intakes of Al from consuming IFMP (baby)	PTWI mg/baby/week	Average weekly intake	
					(mg/baby/week)	Contribution to PTWI (%)
0-2 weeks	75	3.5	0.02	7	0.14	2
2-4 weeks	100	4.2	0.031	8.4	0.22	2.6
2 months	110	4.7	0.034	9.4	0.24	2.6
4 months	145	6.5	0.045	13	0.32	2.5
6 -12 months	135	10	0.042	20	0.30	1.5

Daily consumed powder formula and mean body weight of baby values were obtained from feeding tables and dosages recommended by manufacturers.

It is worth mentioning that, there are many chances for contamination of the stored milk powder by Al in the packaging containers which include a significant amounts of Al in their materials (Shafer and Siefert, 2006). Other sources may be present, as Al can come from the ingredients used in these formulations, or contamination during their manufacturing to produce the final packaged product (Chuchu et al., 2013).

Since infants are likely more prone to heavy metals, IFMP should be checked regularly and examined for the presence of toxic metal contamination as well as levels of essential trace elements (Sipahi et al., 2014). One way Welch's ANOVA result showed highly significant difference among groups of milk and milk products in Al levels ($P < 0.0001$). The highest level was recorded in rice pudding samples (5.35 ± 0.34^a) then kariesh cheese that showed non-significant difference than rice pudding (4.23 ± 0.44^{ab}). The lowest level was reported in infant formula that decreased significantly from other groups (0.36 ± 0.05^d).

In the present work, the Al daily intakes from the consumption of different examined samples were 0.64, 0.19, 0.02 and 0.57 mg day⁻¹ for milk, kariesh cheese, butter, and rice pudding, respectively (Table 2). These values are equivalent to 3.73, 1.11, 0.12, and 3.44% of the Provisional Tolerable Weekly Intake (PTWI) that

was established for the examined products by the JECFA (2011). The estimated total intake of Al via consuming of milk and other dairy products was calculated as 9.94 mg week⁻¹ which corresponds to 8.4% of the PTWI (Table 2). Where, butter contributed the least portion of the Al intake, while milk and rice pudding contributed the largest portion. Lower results were detected by Bratakos et al. (2012), while higher results were recorded by Meshref et al. (2015), as they recorded high results exceeded the recommended PTWI. Concerning the daily Al intakes from consumption of infant formula milk samples, it differs according to age, and weight of the infant and the amount of milk consumed. Table 3 cleared that, the mean values of the daily Al intake in the examined milk powder formulas were under the accepted daily intake (ADI) value (1 mg/kg bwt) set by the World Health Organization (WHO, 2006) for babies from 0- to 12 months old. Where the highest daily Al intake from consuming of infant formulas was 0.045 mg day⁻¹ which is equivalent to 2.5% of the PTWI of the examined samples for 4 months-old babies weighing 6.5 kg. (Table 2). While Sipahi et al. (2014) recorded the highest daily Al intake from consuming infant formula for 2 to 4-week-old babies.

The potential health risk might have been evaluated by the target hazard quotients technique (THQ) (Yu et al., 2015).

That has been distinguished as a helpful parameter for assessment of dangers associated with consuming of metal contaminated food. The THQ is a ratio of the determined dose of a pollutant to a reference oral dose (RFD) for that substance (Zhuang et al., 2009). The RFD for Al is 1 mg/kg bw/day (US EPA, 2014). As stated by the results of the available study, the THQ values of heavy metals for adults via consumption of milk and other examined products, and for infants through consumption of IFMP were found to be less than one (Table 4). These results were similar to those detected by (Odularu et al., 2013). The Al contamination of the examined food items was inadequate to cause a health hazard, suggesting that the local inhabitants in our governorate will not be exposed to a potential health risk from consuming of milk and other milk products, but there are additionally different sources of metal exposures such as dust breathing, dermal contact and ingestion of other types of food and water in the diet, which were not incorporated in this work.

Table 4: Target hazard quotient (THQ) for daily exposure to Al through consumption of milk and dairy products.

Products	EDI (mg /day/ Kg. bwt)	RFD mg/ kg/day	THQ
Milk	0.011	1.0 ^a	0.011
Kariesh cheese	0.003		0.003
Butter	0.0003		0.0003
Rice pudding	0.010		0.010
Infant formula milk powder	0.007		0.007

a: US EPA (2014).

Al is utilized in the manufacture of domestic cooking utensils, because of its formability, and heat conductivity. Also, it has a protective compact Al₂O₃ layer on its surface that does not affected by the aqueous medium, although, the solubility of this protective layer increases in acidic and alkaline medium (Al Juhaiman, 2010). The extent of the increase of Al is dependent on some factors such as temperature, pH value, duration of contact or heating, presence of sugar, organic acids, salt and other ions as discussed by Ranau et al. (2001). Information about the leachability of Al from Al cookwares into milk and other milk products is still scarce and in our work we will try to detect their role in milk.

Concerning the experimental part, that was applied to give informations about the leachability of Al from different cookware into milk during boiling, and storage, the concentrations of Al in milk after boiling in Al and stainless steel utensils are presented in Table 5. Al level in milk boiled for 10 min in Al cookware was approximately twice higher than that of the raw milk (Al level was elevated from 3.56 to 8.11 ppm). Also, Al concentration in boiled

milk for 10 minutes was exceeded the Al concentration in milk, which boiled for 5 minutes only from 5.94 to 8.11 ppm, whereas, leaching of Al during boiling in stainless steel cookware was found to be negligible. Similar information has also been reported by Meshref et al. (2015). While Al-Ashmawy (2011) concluded that the elevation of Al content of raw milk due to the leaching Al ions from cooking pans were relatively low. Odularu et al. (2013) inferred that Al leaching was detected in all forms of new and old cooking utensils. Additionally, Al Juhaiman (2010) showed that significant amounts of Al may migrate from Al cookwares into milk, that can raise the levels of Al to abnormal amounts and may exceed the suggested levels set by WHO/FAO.

Furthermore, the concentrations of Al in milk after storage in different utensils for 48 hours (Table 5), showed that Al content was elevated in milk stored in Al containers and it increased with time as the longer the contact time the higher Al content, as it reached 9.19 ppm and 10.11ppm after 24 and 48 hours respectively. While leaching of Al during storage in stainless steel utensils was nearly not affected.

Our results, cleared that leaching of Al increased during boiling in Al cookware than during storage. These results can be explained by the results of Karbouj (2007) and Turhan (2006) who reported the relation between the cooking temperature and the Al leaching and cleared that leaching is dramatically higher at 100°C than at ambient temperature, regardless of the cooking time, because the corrosion rate is to assume to be depended on the temperature at which the leaching is running. Also, Al Zubaidy et al. (2011) reported that Cooking for additional hours in Al pots, more than two, or storing food in the cooking pot, would elevate the leaching rate. On the contrary, Al-Ashmawy (2011) concluded that, Leaching of Al increased to a significant percent more during storage than during boiling.

The Al concentration in natural waters differs significantly according to numerous physicochemical, mineralogical and geochemical factors. Also, as a result of using Al sulphate to remove organic matter during water treatment in purifying plants, a residual amount is retained and supplied in drinking water, even if good operating conditions were applied (Rondeau et al., 2000). Generally, IFMP products, reconstituted with tap water are exposed widely to a high risk from metals contamination (Soylak et al., 2006). As a consequence, it is important to assess the Al level in tap water and its relative contribution to dietary intake of infants fed on reconstituted powder formulas.

Table 5: Al levels (ppm) in raw milk after processing in Al and stainless steel utensils.

Utensil	Raw milk	Raw milk refrigerated for 24h	Boiled		Refrigerated after boiling for 10 min.	
			for 5min	for 10 min	24h	48h
Al	3.56	3.71	5.94	8.11	9.19	10.11
Stainless steel		3.00	2.94	2.97	3.12	3.53

Table 6: Al levels (ppm) in IFMP after reconstitution in Al and stainless steel utensils.

	Water used for reconstitution						IFMP	Reconstituted formula	
	Tap water		Boiled in Al		Boiled in stainless steel			Mean ± SE of Al in Al utensils	Mean ± SE of Al in Stainless steel utensils
	No. of samples	Mean±SE	No. of samples	Mean±SE	No. of samples	Mean±SE			
Al levels	15	0.0255±0.002	15	0.54±0.07	15	0.0136±0.003	0.45	1.19 ± 0.09	0.530± 0.03

The results in Table 6 revealed that the mean value of Al residues in tap water was 0.0255 ± 0.002 ppm, where water contamination level for Al was less than the maximum contamination level set by US EPA (2013) (0.05-0.2 mg/l). Nearly similar results were detected by Blasco and Galindo (2003), while Hajira and Shaharuddin (2015) detected higher results.

The mean value of Al in boiled tap water samples in both Al and stainless steel cookwares was used to establish the influence of Al content in drinking water on the final concentration of reconstituted infant formulas also, to assess the effect of boiling of water in Al and stainless steel cookwares on leaching of Al into water which used in the reconstitution process of IFMP. The results in Table 6 showed that the mean value of Al in water boiled in Al cookwares and stainless steel were 0.54 ± 0.07, 0.0136 ± 0.003 ppm, which give 1.19 ± 0.09 and 0.530 ± 0.03 ppm of Al in reconstituted formula, respectively. Our results cleared that using of Al cookware in boiling of water increased Al content in water and subsequently increased Al in the reconstituted milk. These results can be explained by the results reported by Al Zubaidy et al. (2011) who concluded that, the amount of Al leaching depends on temperature and time of exposure.

The present study outcomes indicated that Al exposure from tap water used for formula preparation is not clearly a potential risk itself as the recorded residues were less than the maximum contamination level set by US EPA (2013). Nevertheless, by reconstitution of milk powder, it will increase the Al content in it than in milk powder, which will increase daily intake but not more than the accepted levels and it is not considered a potential risk. On the other hand, it should be kept in mind that the amount of Al residues in drinking water can vary according to the geographic area and geochemical medium where the water supply is sited.

Efforts should be made by manufacturers to reduce the Al content of these products to an achievable practical minimum by applying suitable precautions during processing, especially with regard to packaging materials containing Al, since this is a potential source of contamination (Motil, 2000).

CONCLUSIONS AND RECOMMENDATIONS

The present work detected that milk and other dairy products in Sharkia Governorate may be contaminated with different concentrations of Al residues, but these residues are considered low compared with the PTWI established by the JECFA and do not have great hazard effects to consumer's health. However, eating large amounts of them on a daily basis in different types of food, containing Al additives or contaminated with Al residues may expose a person to higher levels of Al, have adverse effects on health. Concerning babies, they are probably going to be exposed to higher metal levels through reconstituted infant formula than through breast milk. Thus, regular monitoring and more concern during the manufacturing of this product should be applied to avoid the contamination with Al and consequently, lower its content. Regarding the leaching of Al, the results cleared that preparing and keeping of milk in Al utensils raise the Al content, so it is recommended to use glass or stainless steel containers for the handling of milk products.

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AUTHOR'S CONTRIBUTION

ENA designed the study, supervised the experiment,

and wrote the manuscript draft. SSA performed sample collection, and data analysis. All the authors have read, revised, and approved the final manuscript.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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