Precipitation of Aluminum in the Digestion Solution of Fried Bread Sticks by Mixed with Drinks: A Quantitative Analysis

Zhong Wensi [1], Ren Ting [2] and Zhao Lijiao [2+]

[1] The Experimental High School Attached to Beijing Normal University, Beijing 100032, China

[2] Beijing Key Laboratory of Environmental and Viral Oncology, College of Life Science and Bioengineering, Beijing University of Technology, Beijing 100124, China

Abstract. Excessive exposure to aluminum (Al) can result in some serious health risks. Diet is considered to be a main source of oral Al intake. Chinese fried bread sticks (FBS), a popular breakfast food in China, usually contain relatively high level of Al because of the use of Al-containing food additives in its processing. In this work, the Al level was determined in the FBS samples purchased from breakfast dealer in China. The precipitation of Al in the digestion solution of FBS mixed with various beverages was also investigated. The determination was performed by high-resolution continuum source atomic absorption spectrometry (HRCS-AAS). The methods used for sample preparation, digestion and quantification were established, generating satisfactory analytical precisions (represented by relative standard deviations ranging from 1.7% to 4.3%) and recoveries (98.7% to 103.0%). Precipitations were observed in the mixtures of the FBS digestion solution with coffee, herbal tea and jasmine tea, in which the amount of precipitation in the mixture with coffee was much more than the others. The results indicated that Al levels were higher than 1000 mg/kg in the FBS digestion solution, while was significantly reduced to 168 mg/kg in the filtrate of the mixture with coffee. It was speculated that coffee reduced the Al contents in the FBS digestion solution due to the effect of precipitation. The quantitative method established in this work lays a foundation for preventing Al toxicity in human from diet and will help establish more healthy style of food combination for the traditional Chinese food.

Keywords: fried bread stick, aluminium, drinks, precipitation, atomic absorption spectrometry

1. Introduction

Aluminum (Al) is a ubiquitous element in the environment. As a non-essential element for human beings, Al is widespread in all the food and commonly used in food technologies [1]. However, the risks of Al are well known that it is neurotoxic and bioaccumulative, producing toxicity to the central nervous, skeletal and hematopoiietic systems [2]-[5]. Al accumulation is related to Alzheimer's disease and dialysis encephaopathy [6]. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has revised the safety of Al and lowered the provisional tolerable weekly intake (PTWI) to 2 mg/kg body weight (bw) in 2011 [7] and the European Food Safety Authority (EFSA) established 1 mg/kg bw as the PTWI for all Al compounds in foods, including additives [8]. High contents of Al have been reported from various food products, for instance, bakery products (muffin, cake and pancake, etc.), fried snacks, jelly and mung bean vermicelli, probably due to the addition of Al-containing food additives [9]-[12]. As a kind of the most widely consumed breakfast foods in China, fried bread sticks (FBS) always deep fried to have delicious flavor and make chewy and crispy taste. One of the consumers' major concerns over FBS with traditional procedures is their high Al levels.

In this study, the contents of Al in FBS were determined using high-resolution continuum source graphite furnace atomic absorption spectrometry (HRCS-GFAAS). The Al concentrations in the digestion

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solution of FBS mixed with various types of drinks were quantitatively analyzed to investigate the precipitation of Al in FBS. The quantitative method established in this work lays a foundation for preventing the toxicity of Al in human from eating FBS and other fried food. Moreover, this work is possibly to provide more healthy style of food combination for the traditional Chinese food.

2. Experimental

2.1. Instruments and Reagents

A ContrAA 700 (Analytik Jena AG, Jena, Germany) high-resolution continuum source atomic absorption spectrometer equipped with a short-arc xenon lamp, a high-resolution double echelle grating monochromator and a charge-coupled device (CCD) array detector was used. A MPE-60 autosampler for liquid samples was installed on the GFAAS. High purity Ar (99.999%) was supplied as the protective and purge gas. The method was optimised based on the proper atomic line for Al (309.2713 nm). A BT-214D electronic balance (Sartorius, Göttingen, Germany) was used to weigh the samples. An ETHOS A Microwave Digestion System (Milestone, Milano, Italy) was used for the samples digestion.

The Al standard solution (100 mg/L) was purchased from the National Research Center for Certified Reference Materials of China. The working standard solutions were prepared daily through a stepwise dilution of the standard stock solutions using 0.5% (v/v) HNO_3. $Mg(NO_3)_2$ (Sigma-Aldrich, St. Louis, MO, USA) was used as chemical modifier for the determination of Al. The reagents were of analytical grade, and all solutions were prepared using deionized water (18.2 MΩ/cm) produced by a PureLab Prime system (PALL, Washington, NY, USA). All containers and glassware were cleaned by soaking in the 5 mol/L HNO_3 for at least 24 h and rinsed three times with deionized water prior to use.

2.2. Sample Preparation

The FBS samples were ground into powder using a ZM 200 ultra centrifugal mill (RETSCH Technology, Haan, Germany) after drying to constant weights. Approximately 0.25 g of dried powder was weighed and added into the polytetrafluoroethylene (PTFE) digestion vessel with the addition of 7 mL of concentrated HNO_3 and 1 mL of hydrogen peroxide (H_2O_2). Subsequently, the samples were digested using a two-step temperature program. The temperature was linearly increased to 190 °C over 10 min followed by staying at 190 °C for 30 min. The maximum power of the rotating magnetron was 1000 W. After digestion and cooling, each solution was evaporated to approximately 2 mL and diluted with deionized water in a 50 mL volumetric flask. Then 5.00 mL of the FBS digestion solution or blank reagent were mixed well with 5.00 mL of 10 types of beverage, including barreled drinking water, soybean milk, milk, orange juice, grape juice, coca cola, sprite, instant coffee (10 g dissolved in 100 mL of boiling water), herbal tea (Wang Laoji), jasmine tea (1 g infused in 100 mL of boiling water), respectively, and the before and after comparisons were shown in Fig. 1. A control sample was prepared by mix 5 mL of the FSB digestion solution with 5 mL of deionized water. The mixtures were vortexed for 1 min followed by standing for 5 min. Then the samples were centrifuged at 5000 r/min for 5 min to separate the aqueous solution and precipitation. The supernatants were filtered through a 0.45 μm micropore membrane and acidified to pH 1~2 by nitric acid. Finally, 20 μL of the aqueous sample solutions or the standard solutions were transferred to GFAAS together with the modifiers for the determination. The results were reported as the average of three repeated measurements, and all digestions were conducted in triplicate.
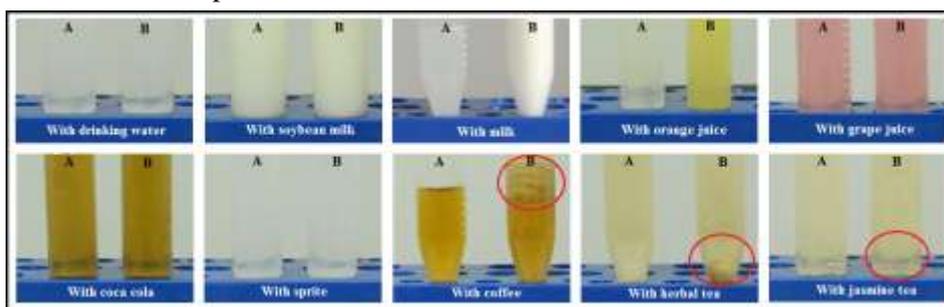

Figure Note: "A"—After the filtration, "B"—Before the filtration

Fig. 1: Comparison of samples before and after the filtrations.

2.3. Analysis Conditions

The analytical working solutions included the following: 0, 50.0, 100.0, 150.0 and 200.0 µg/L for Al. The working solutions were prepared daily by appropriately diluting the stock standard solution of Al. The instrumental condition and temperature program for the determination are listed in Table 1. The contents of Al were calculated as shown in formula (1). In which, X means the content of Al in the samples (mg/kg); c_{mix} stands for the determined Al concentration in the mixture of FBS with different drinks (µg/L); $c_{blank\text{-}drinke}$ refers to the Al concentration in the mixture of blank reagent with different drinks (µg/L); m is the sample mass (g); V stands for the volume of the digested sample solution (50 mL); f_{mix} is the dilution ratio for mixture of samples; $f_{blank\text{-}drink}$ is the dilution ratio for the blank reagent; f refers to the dilution ratio of the digestive solution; n is the ratio of sample mass before to after drying procedure.

Table 1. Graphite furnace program for the determination of Aluminium

	Temperature (℃)	Ramp (℃/s)	Hold (s)
Drying	110	50	10
Pyrolysis	1300	300	10
Atomizing	2400	1500	3
Clean	2500	500	4

$$X = \frac{(f_{mix}c_{mix} - f_{blank-drink}c_{blank\text{-}drink}) \times V \times f}{m \times 1000} \times n \tag{1}$$

3. Results and Discussion

3.1. Conditions of Microwave Digestion

Wet digestion and microwave digestion are commonly used for sample digestion when analysing trace metals with AAS. The latter was chosen for this study because of its advantages including less sample pollution, limited analyte evaporation, lower acid consumption, shorter digestion time and significant blank value reduction. The FBS samples were mixed with the digestion agents (HNO_3 and H_2O_2) and sealed in digestion vessels. In the acidic digestion mixture, H_2O_2 decomposed to form high-energy reactive oxygen, and the HNO_3 degraded to form catalytically active NO_2. Reactive oxygen and NO_2 accelerate the oxidation process and improve the sample digestion. In this work, a mixture of HNO_3-H_2O_2 (volume ratio 7:1) was used to digest the powdered FBS samples to colourless and transparent liquid.

3.2. Optimization of Temperature Program

The temperature program of the GFAAS analysis consisted of four steps, i.e., drying, pyrolysis, atomizing and clean. Using a ramped temperature program including slow heating for drying might prevent the loss of analytes. Pyrolysis removed all or most of the coexisting elements while minimising analyte loss. Excessively high pyrolysis temperature may cause analyte loss, sensitivity reduction and poor repeatability, while low pyrolysis temperature will generate high background absorption values [13]. During atomization, the target elements were turned into atomic vapour at the ground state. Appropriate atomization temperatures completely evaporate the analytes without leaving residue, and prolong the life of the graphite tubes. By achieving the highest absorption intensity, the optimal temperature program was obtained and was indicated in Table 1.

Matrix modifiers are widely employed in heavy metal analysis with GFAAS because they can improve the thermal stability of the analytes and reduce the matrix interference by facilitating the volatilization of the sample matrix in a graphite tube. A suitable matrix modifier is necessary for applying high pyrolysis temperatures to eliminate the matrix effects before atomization, as well as reduce interference and background signal [14]. $Mg(NO_3)_2$ is frequently used as a chemical modifier for determining Al in food, biological and environmental samples using GFAAS. In the research from Ren et al. [13], 1 g/L $Mg(NO_3)_2$ was used as the matrix modifier for the determination of Al in wheat flour food. López et al [15] reported the determination of Al in drinking water, fruit juices and soft drinks by GFAAS using $Mg(NO_3)_2$ as the matrix modifier, giving more reproducible results. A report also showed that $Mg(NO_3)_2$ was used as the efficient matrix modifier while determining Al contents in tea infusion and chocolate using GFAAS [16]. A method

for measuring Al contents in wine, beer and other alcoholic beverages consumed in Spain was developed using GFAAS with 5 g/L $Mg(NO_3)_2$ as modifier [17]. In this work, 5 μL of 0.5 g/L $Mg(NO_3)_2$ was used for the determination of Al in each sample. With the addition of the modifier, the pyrolysis and atomization temperatures were optimized to 1300 and 2400 ℃, respectively. The absorbance values observed after adding the modifier were much higher compared with those without modifiers.

3.3. Method Validation

A series of freshly prepared working solution was diluted with 0.5% (v/v) HNO_3 for plotting the calibration curves: 50, 100, 150 and 200 μg/L. The linearity of the calibration curve was evaluated based on the correlation coefficient (R^2). As shown in Fig. 2, the R^2 value of the non-linear curve is obviously higher than that of the linear curve. Therefore, the non-linear calibration curve with $R^2 > 0.999$ was selected for quantifying the Al levels in the samples.
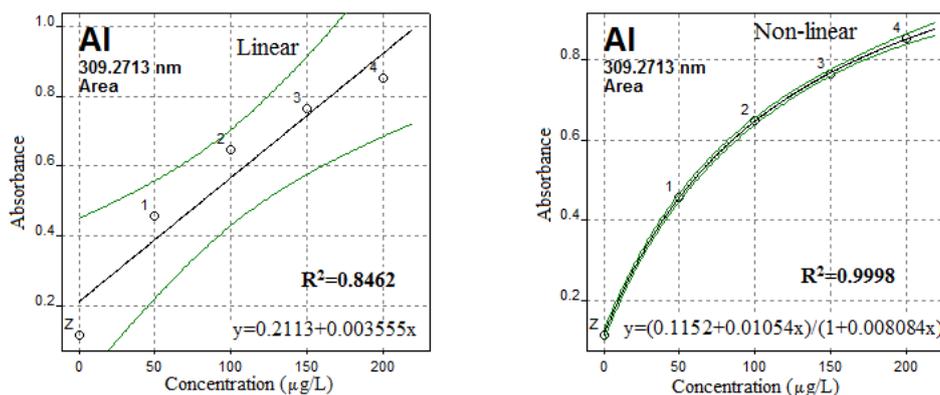

Fig. 2: Comparison of the linear and non-linear standard curves for aluminium.

To evaluate the influence of the entire procedure on the measured results, the recovery of Al was measured. Four quality control (QC) samples were prepared by adding various amounts (0, 10, 30 and 50 μL) of the standard solution mixtures (containing 5 mg/mL Al) to quadruplication of FBS samples (0.25 g for each), respectively. All the mixtures were diluted 40 times and the concentrations of Al in the QC samples were analyzed six times to determine the relative standard deviation (RSD). The recovery was calculated as shown in formula (2). As listed in Table 2, the recoveries ranged from 98.7 to 103.0%, and the precision represented by RSD ranged from 1.7 to 4.3%.

Recovery = (determined concentration with addition of standards − determined concentration without standards) / added concentration $\times 100\%$ (2)

Table 2. Repeatability of the aluminium determinations

Element	Added (μg)	Determined (μg)	Recovery (%)	RSD (%)
	0	121.1	—	4.3
	50	176.3	103.0	3.2
Al	150	274.1	101.1	1.7
	250	366.3	98.7	2.9

To validate the accuracy of this method, the contents of Al in three certified reference materials (CRMs) of food were determined using the same method as that used for the FBS samples. As shown in Table 3, the determined concentrations were in good agreement with the certified values. The recovery, precision and accuracy indicate that the quantitative methods employed in this work are appropriate for measuring Al levels in FBS. The limit of detection (LOD) was calculated as three times the standard deviation (SD) of the reagent blank readings. The SD was obtained from 20 analyses of the blank, which only contained 0.5% (v/v)

HNO$_3$ and modifier, using the same temperature program as that used for the samples. The LOD was 2.96 μg/L.

Table 3. Determined contents of aluminium in three reference materials (n=4)

Reference material	Certified value (g/100g)	Determined (g/100g)
GBW 10011 Wheat	0.0104 ±0.0010	0.0110 ±0.0007
GBW 10012 Maize	0.032 ±0.003	0.031 ±0.003
GBW 10043 Rice	0.045 ±0.007	0.046 ±0.005

3.4. Application of the Proposed Method

A total of 11 mixtures of blank reagent or digested FBS solution with water or 10 types of beverages were tested for Al levels, and the results were summarised in Fig. 3. As it can be seen from Fig. 3(a), all the samples of blank reagent with the beverages were found to contain very low Al contents, ranging from 0.01 to 0.75 mg/kg, with the highest Al level of 0.75 mg/kg from the mixture of blank reagent with jasmine tea. However, as shown in Fig. 3(b), the mixtures of FBS digestion solution with water or various beverages were found to contain much higher Al levels. The mixture of FBS with deionized water has the highest Al level (mean value: 587.10 mg/kg), followed by mixtures of FBS with jasmine tea (529.15 mg/kg), barreled drinking water (519.34 mg/kg), soybean milk (454.11 mg/kg), coca cola (446.10 mg/kg), orange juice (439.05 mg/kg), milk (430.83 mg/kg), herbal tea (422.66 mg/kg), sprite (417.86 mg/kg), grape juice (338.68 mg/kg). The mixture of FBS digestion solution with coffee was found to contain the lowest level of Al (168.46 mg/kg). As shown in Fig. 3, the mixture of FBS with tea was found to contain less amount of Al than digested FBS solution; however, the effect of Al reduction was not obvious. It is worth nothing that the Al content in tea was the highest among all the analyzed 10 types of beverages. There are numbers of studies reported that the tea infusions contained relatively large amounts of Al [18], [19]. As reported in a defined diet study on the estimation of the oral Al intake in Australia, instant tea provided an Al level of 1.8 mg/L, which was >50% of the Al consumed daily (3.2 mg/day) by the American Water Works Association [20]. The amount of Al present in coffee beans and coffee infusions also attracted extensive concern in the past decade [21]-[22]. As reported by Koch et al. [21], the Al level in coffee infusions (0.23-0.34 mg/kg) was lower than that in tea, but this reported Al level is much higher than that determined in this study (0.04 mg/kg). Precipitations were observed in the mixtures of the FBS digestion solution with coffee, herbal tea, and jasmine tea, in which the amount of precipitation in the mixture with coffee was much more than the others. The results indicated that Al levels were higher than 1000 mg/kg in the FBS digestion solution, while was significantly reduced to 168 mg/kg in the filtrate of the mixture with coffee. It was speculated that coffee reduced the Al contents in the FBS digestion solution due to the effect of precipitation.

The high level of Al, which was found in FBS digestion solution, was possibly due to the use of Al-containing food additives in the processing [9]. According to the National Food Safety Standards of China GB 2760-2011-Standards for Uses of Food Additives, FBS is permitted to contain aluminium potassium sulfate or aluminium ammonium sulfate as bulking and stabilizing agents, while the amount of Al residue should be no more than 100 mg/kg [23]. The Al level in the FBS samples analyzed in this study exceeded this limitation. According to EFSA, the PTWI of Al is 1 mg/kg body weight. If a 60 kg adult weekly consumes a piece of FBS (about 50 g) with the Al level of 1170 mg/kg as determined in this study, the level of exposure to Al exceeds the PTWI. Recently, several investigations demonstrated that the processing procedure could be improved without the addition of Al-containing food additives [24], which is a possible approach to reduce the risk of Al to health by eating FBS.
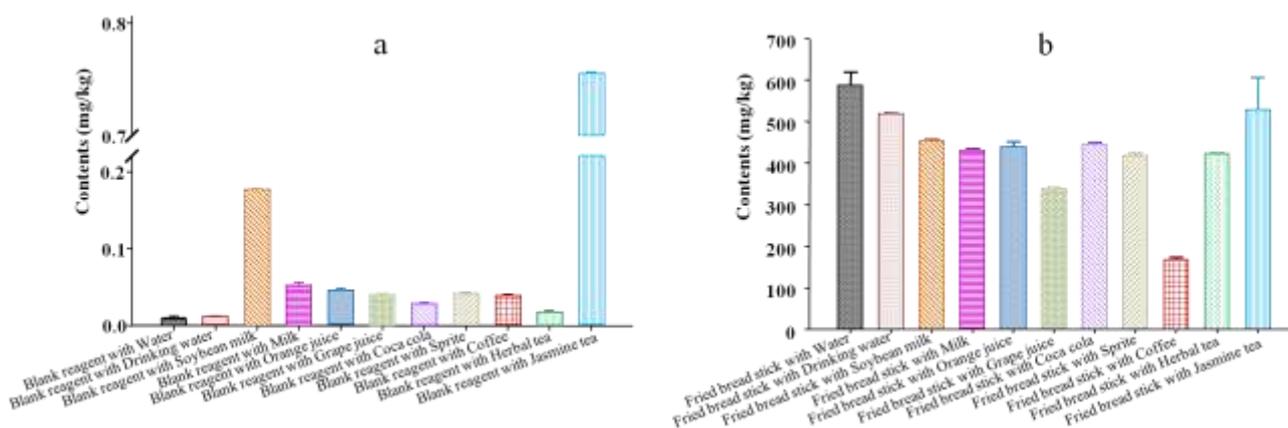
Fig. 3: Aluminium contents in the mixture of blank reagent (a) or digested FBS digestion solution (b) mixed with various beverages determined by HRCS-GFAAS

4. Conclusions

The use of Al-containing food additives is one of the important factors involved in the Al intake via diet. In this study, the amounts of Al in FBS and the mixture of FBS digested solution with 10 types of beverages produced and marketed in China were analyzed using HRCS-GFAAS. The Al level in FBS was about 10 times higher above the limitation stipulated in the related standards (GB 2760-2011). In the mixture of the FBS digestion solution with the beverages, precipitations were observed after the FBS digestion solution mixed with isovolumetric coffee, herbal tea, and jasmine tea; and the amount of precipitation in the mixture with coffee was much more than the others. Especially, the Al levels were significantly reduced from 1170 mg/kg in the FBS digestion solution to 168 mg/kg after mixed with coffee. Therefore, it was speculated that coffee was possibly to reduce the risk of Al to human health due to its precipitation effect. The quantitation method established in this work lays a foundation for strengthening the supervision and decreasing the overconsumption of Al-containing food additives. Meanwhile, this work will assist in establishing more healthy style of food combination to reduce the risk of Al to human health.

5. Acknowledgements

6. References

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