Determination of Aluminum in Edible Jellyfish Using Chrome Azurol S with Spot Test on Filter Paper

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Aluminum (Al) has been well known as an environmental factor that may affect several enzymes and other biomolecules related to Alzheimer's disease. The increasing use of Al in the preparation and storage of food currently represents the main form of Al exposure for the general public. The present study was aimed to develop a household procedure for the rapid test determination of Al in edible jellyfish. The method was developed based on the reaction of Chrome Azurol S with Al in acidic medium, forming a colored compound on the surface of filter paper. Experimental design methodologies were used to optimize the measurement conditions. The proposed method was applied successfully to the analysis of Al in edible jellyfish products in clinical laboratory and household settings.

Keywords Aluminum, diffuse reflectance, filter paper, edible jellyfish, household

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Introduction

Aluminum (Al) biotoxicity was first recognized in patients to whom Al was administered parenterally, *i.e.* through dialysis fluid¹ and intravenous nutrient admixture.² Absorption and accumulation of Al in humans can be caused by the diet, vaccines, drinking water, parenteral fluids, antacids, inhaled fumes and particles from occupational exposures.^{3,4} It has been clearly shown that individuals ingesting large amounts of Al compound do absorb significant amounts and show elevated plasma Al levels.⁵ The plasma Al can alter the blood-brain barrier; as a result of which it enters into the central nervous system under normal physiological conditions easily and accumulates in the brain.^{6,7} Epidemiological investigations have shown that Al can cause memory impairment and cognitive dysfunction, which would lead to neurodegenerative diseases, such as Alzheimer's disease⁸ and Parkinson's disease.⁹

The increasing use of Al in preparation and storage of food, such as in Al vessels, foils and cans, may increase the Al content, particularly in salty, acidic or alkaline food.^{4,10} Orally ingested Al additives currently represent the main form of Al exposure for the general public. However, an international panel of the European Food Safety Agency, on request of the European Commission, has given a scientific opinion on safety of Al from dietary intake, defining as tolerable the weekly intake (TWI) of 1 mg Al/kg of body weight.¹¹ Jellyfish (Cnidaria: Scyphozoa: Rhizostomeae) have always been an important fishery commodity in the world.¹² It has been reported that China produced 62969 tonnes of jellyfish in 2009, creating revenue of about 149 million USD, which was a 33% increase from the year before.³ Jellyfish possess negligible fat content

(0.4 – 1.8 g/100 g D.W.) and low calorific value (1.0 – 4.9 kcal/g D.W.), while minerals (15.9 – 57.2 g/100 g D.W.) and protein (20.0 – 53.9 g/100 g D.W.) are found to be the richest components. It has been considered an appealing source of nutritive ingredients for the development of functional food.¹³ However, as metal pollution is prominent in the coastal marine environment, the accumulation of copper, zinc, silver, iron and Al in jellyfish has been reported.^{14–17} Therefore, in the present study we focus on the determination of Al content in edible jellyfish under household conditions in order to help consumers avoid excessive intake of Al by eating jellyfish.

Numerous techniques have been reported for the determination of Al, including electrothermal atomic absorption, ICP-MS, neutron activation analysis, HPLC, and fluorometry.¹⁸ The simplest and most frequently applied method employs spectrophotometric detection based on the Chrome Azurol S reaction because of its relatively low cost and good sensitivity. Improvement of the reagent with nonionic or cationic surfactants substantially improves the spectral characteristics of the compound and reduces the detection limit of Al.¹⁹ Spot reactions have been successfully used in applications including large-scale clinical, environmental, food and water analyses.²⁰ Paper, composed of cellulose fibers, has served as a medium for chemical analyses for many decades. The filter paper supplies a high quality surface for spot tests, because it is white, ensuring a bright, high contrast background for reflectometric analysis.

The possible health impact of the exposures to Al from food would be inevitable for each family. Hence, there is a great need to develop methods that are more applicable to the household environment and comply with the 12 principles of Green Chemistry. The fact that Al is water-soluble facilitates its extraction with hot and acidic water. To the best of our knowledge, there have been no previous reports on the use of household extraction methods with quantitative spot tests for the determination of Al in food samples. Therefore, the goal of

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this paper was to impregnate chromatographic cellulose paper with Chrome Azurol S, to study the properties of this reagent on the paper surface, and to develop a household procedure for the rapid test determination of Al in edible jellyfish.

Experimental

Materials and reagents

Qualitative filter paper (Whatman No. 1) was used as the solid support for the spot tests. All reagents were analytical grade and the solutions were prepared by ultrapure water. The chromogenic agent solution consisted of Chrome Azurol S (Aldrich) and hexadecyltrimethylammonium bromide (Sigma) at concentrations of 3.2×10^{-3} and 2.2×10^{-3} mol/L, respectively, plus acetate buffer at a concentration of 6×10^{-1} mol/L.

Working standard solutions of Al (0.5, 0.75, 1, 2, 5, 10, and 20.0 mg/L) were prepared by dilution of a certified commercial standard (Beijing Nonferrous Metal Research Institute; 1 g/L Al) daily into deionized water.²¹ Working solutions of Al were prepared daily by appropriate dilution of the stock solution with deionized water.

Samples

Jellyfish (red jellyfish, *R. esculentum*) samples were collected from local markets in the municipality of Binhai New Area (Tianjin, China). Edible jellyfish have two distinct body parts, the bell and the oral arms. Mimicking the common processing practice of jellyfish fishermen, freshly caught jellyfish were cut into bell and oral arms and immediately transported back to the laboratory on ice. In the laboratory, jellyfish samples were cleansed thoroughly with distilled water and then stored at -20°C prior to use.¹³

Procedure

Production of test paper. The test paper was produced by successively treating the Whatman No. 1 qualitative filter paper with aqueous solutions of hexadecyltrimethylammonium bromide, Chrome Azurol S and acetate buffer, respectively. The paper was dried for 120 min in a desiccator at 50°C and cut into rectangles (40×10 mm).

Spot test reaction. The standard aluminum solution (the concentrations of Al were 0.5, 0.75, 1, 2, 5, 10, and 20.0 mg/L, respectively) with a given pH was pipetted onto a ready-to-use paper. After 1-min natural drying, a camera from Fuji-shi (Fine Pix J25) was used to take photographs of the paper. To obtain a visual test scale, chromaticity measurements²¹ were obtained by using a portable colorimeter (CR-10, Minolta, Japan). The range and color characteristics of the scale were calculated on the basis of the measured diffuse reflectance spectra for a light source C in the CIE uniform color system (1976) using converted color coordinates (a and b) and luminosity (L).¹⁹ The color difference in luminosity (ΔL) and the color coordinate b difference (Δb) were calculated by the following equations: $\Delta L = L_0 - L$ and $\Delta b = b_0 - b$ where L , b , L_0 , b_0 are the color coordinates of the sample in the study and a reference sample, respectively. A paper impregnated with Chrome Azurol S was used as a reference sample.

Study of interferences. The possible interference of several ions, Fe(III), Cu(II), Na(I), Zn(II), Mg(II), Ca(II), and K(I) was evaluated. Solutions containing Al (10 mg/L) and each of the ions at concentrations equal to or 10 times greater than that of Al were evaluated under the same conditions described in the recommended procedure.

Sample preparation and test. Jellyfish samples were excised

into approximately $5 \times 5 \times 5$ mm cubes, 5 g of triturated sample was weighed out and Al was extracted using hot and acetic water (50 mL). The extract filtrate was obtained and analyzed by the proposed method. The results obtained were compared with standard a color card for Al. The density of the test paper was analyzed by using the ImageJ software.

Estimation of Al content. Weighed jellyfish samples (0.1 – 0.3 g) or jellyfish extraction (0.1 – 0.3 mL) were digested with nitric and perchloric acids. Residues were taken into 1% v/v nitric acid and the aluminum concentrations determined by inductively coupled plasma mass spectrometry (ICP-MS) (7500cx, Agilent Technologies Corporation, USA).²²

Statistical analysis. The results were expressed as the means $\pm$ standard deviation (SD) of triplicate using SPSS 17.0. The statistical significances of data were determined using one-way analysis of variance (ANOVA) followed by Dunnett's contrast, P value < 0.05 was regarded as significant.

Results and Discussion

Optimization of paper impregnation and aluminum determination

This study describes for the first time the development of a combined spot test for Al determination on ready-to-used filter paper, based on the reaction between Chrome Azurol S and Al in acetic acid medium to produce an intense blue-green color ($\lambda = 620$ nm).²³ Spot tests can be used for quantitative analysis by reflectance measurements with reproducible results if the color of the spot is uniform over the entire surface of the solid support. The factors that contribute to the uniformity of the spot test are: order and rate of reagent addition, quality of filter paper and volume of solution added.²⁴ Studying the order of treating the paper by reagent solutions revealed that the best conditions are as follows: first, the chromatographic paper is immersed into the solution of hexadecyltrimethylammonium bromide for 30 min; next, it is impregnated with the Chrome Azurol S solution for 30 min; and finally, it is treated with the solution of acetate buffer for 30 min in an airtight condition to supply the best pH for the reaction of Al with Chrome Azurol S (3.2×10^{-3} mol/L) (Fig. 1A). The paper was dried for 2 h in a vacuum desiccator at 50°C and cut into rectangles (10×30 mm) (Fig. 1B). No change in the colored spot could be tested when different volumes (0.5, 1, 5 and 10 μL) of the Al solution were pipetted onto the paper. Therefore, it was decided to add 5 μL of the Al solution. This volume made a sufficient spot to be tested by the colorimeter. The average diameter of the spots was about 0.8 cm, and the inner parts were a uniform blueish color. As such, the chromaticity measurements were on the inside of the spots. Ten sample points were tasted in each spots. As shown in Fig. 1C, the relative density gradually decreased with an extension coloration time. This made it the difficult to obtain precise measurements of the resulting color of the spot. The optical stability of the colored spot was found to be stable for 1 min. In conclusion, the recommended procedure is that at first 5 μL of the sample solution be pipetted onto the test paper and after 1 min, the chromaticity measurements carried on the inside of the spots.

To optimize the range of the scale for the visual test determination of Al, we used chromaticity measurements. The color of the complex formed on the paper surface was identified by chromaticity coordinates (a , b , L). The color difference in the square of luminosity (ΔL) and the square of color coordinate b difference (Δb) are directly proportional to the Al^{3+} concentration in solution in the same range. $C_{\text{Al}}(\text{mg/L}) = 0.49645 - 0.03978\Delta L_2 + 0.01224\Delta b^2$ (Fig. 2). The test scale for

Fig. 1 Influence of concentration of Chrome Azurol S (A), drying method (2 h in a vacuum desiccator at 50°C) (B) and coloration time (C) on the spot test of Al. The level of Al (10 mg/L) was determined by the proposed method and quantified by densitometry.

Fig. 2 The standard color card and the linearity range for the ΔL^2 , Δb^2 and C_{Al} (mg/L) using standard Al(III) solutions with concentrations of 0.5, 0.75, 1, 2, 5, 10, and 20.0 mg/L (A). The plot of ΔL^2 against C_{Al} (B) and the plot of Δb^2 against C_{Al} (C).

Table 1 Recoveries of aluminum added to water samples

Sample	Added value/ mg L ⁻¹	Found value/ mg L ⁻¹	Recovery, %
Water	0.501	0.502	104.6
	1.003	1.003	97.43
	5.002	5.302	105.8
			$a = 102.6 \pm 4.5$
Jellyfish	0.502	0.501	94.62
	1.001	1.002	96.73
	5.004	4.906	97.71
			$a = 96.32 \pm 1.51$

a. Average $\pm$ standard deviation (SD), $n = 3$.

the visual determination of Al(III) was constructed within the linearity range for the ΔL^2 , Δb^2 and C_{Al} (mg/L) using standard Al(III) solutions with concentrations of 0.5, 0.75, 1, 2, 5, 10, and 20.0 mg/l. The plot of ΔL^2 against C_{Al} and the plot of Δb^2 against C_{Al} have been presented in separate panels in Fig. 2 (B, C).

Validation of the proposed method

The limit of detection (LOD) and limit of quantification (LOQ) were determined according to IUPAC recommendations²⁵ $LOD = 3 \times (s/S)$ and $LOQ = 10 \times (s/S)$, where “ s ” is the standard deviation of measurements of the blank ($n = 10$) and “ S ” is the slope of the linear dynamic range.²⁶ The LOD and LOQ were 0.05 and 0.5 mg/L, respectively.

A change of the diffuse reflectance exceeding $\pm 5\%$ in the determination of Al, due to the existence of other ions or compounds commonly present (Sect. “Study of interferences”) was considered to be indicative of interference. The results obtained showed that no interferences occurred for the ions and compounds investigated under the experimental conditions used. Therefore, the present method had good selectivity and could be applied to the analysis of Al in edible jellyfish.

Possible matrix interferences were studied using standard additions, and the accuracy of the method was tested using the recovery of Al.²⁶ Recovery tests were performed by water sample and jellyfish sample with known amounts of Al standard, followed by detection using the proposed method. The results obtained for jellyfish and water samples are presented in Table 1. The recoveries ranged from 94.6 to 105.8% in the samples. These data showed that the composition of the jellyfish product and the water sample did not interfere in the analysis of Al significantly. No clean-up steps were necessary for the water samples.

Optimization of the Al extracting conditions in edible jellyfish

The influence of different extracting conditions (volume of the white vinegar, temperature and duration of the extracting procedure) were studied in the general step proposed above, to extract a maximum amount of Al in jellyfish (Fig. 3). The optimum volume of white vinegar needed was found to be 5 mL in the extracting solution. The effect of temperature (range: 25 – 100°C) on the dissolution of Al was investigated. As can be seen, the optimum temperature of the stage is 40°C. In order to evaluate the influence of extraction duration on the dissolution of Al in edible jellyfish, a set of studies was carried out by varying the duration range between 1 to 60 min. Based on the results observed, the optimum extracting duration is 30 min.

Determination of Al in jellyfish samples with the use of the test paper

The amount of Al in the bell and the oral arm of jellyfish

Fig. 3 The influence of volume of white vinegar, temperature and reaction time on the extraction of Al in jellyfish.

water extraction ($n = 3$) were detected both by the proposed method and by ICP-MS. The values, grouped according to the type of jellyfish body parts, tested by the two procedures are shown in Table 2. Comparison between test paper (x) and ICP-MS (y) results indicated good accordance between the Al content obtained from all samples (using t-tests at a 95% confidence level) with those studied by the official method,²⁷ irrespective of different jellyfish body parts, using the two methods; their relationship was demonstrated by a linear regression, shown by the equation $y = 1.0159x - 0.0724$, $R^2 = 0.9624$, $P < 0.001$ (Fig. 4). Compared with the previous visual spot tests for Al,¹⁶ which depend on the operator's visual acuity, the quantitative proposed method has high precision. Additionally, the proposed method was applied to Al determination in food products (jellyfish) as well as in water samples.

Conclusions

The present study demonstrates the feasibility of analysis of Al using a spot test on a filter paper surface. The method proposed here combines simple sample treatment, high sample throughput,

Table 2 Concentrations of aluminum in different jellyfish samples by inductively coupled plasma mass spectrometry (ICP-MS) and test paper

Jellyfish body parts	Sample	Al concentration/mg kg ⁻¹ (mean ± SD, <i>n</i> = 3)	
		Test paper	ICP-MS
Bell	A	108 ± 5	108 ± 3
	B	75 ± 9	76 ± 6
	C	101 ± 7	107 ± 2
Oral arms	A	124 ± 3	123 ± 1
	B	114 ± 3	118 ± 3
	C	95 ± 6	91 ± 4

Fig. 4 Relationship observed among Al concentrations (mg/L) in jellyfish (bell, oral arm) obtained by test paper (x) and ICP-MS (y). Obtained results were plotted all together, irrespective of jellyfish body parts.

and reduced analysis time together with sufficient sensitivity and acceptable reproducibility, similar to those obtainable with the more demanding ICP-MS techniques. It was successfully applied to the determination of Al in edible jellyfish products. Its ease of use, low costs, accessibility and environmentally friendly nature make this technique quite attractive, as it provides a procedure that is simple, robust, and readily reproducible in both clinical laboratory and household settings.

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