Simultaneous quantification of Cd, Cu, Pb and Zn in Thai fermented food by DPASV with a microwave digestion

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Abstract: A differential pulse anodic stripping voltammetry (DPASV) has been developed for the determination of Cd, Pb, Cu and Zn in the local traditional fermented foods of northeastern Thailand. An optimized reagent mixture for microwave digestion composed of 6 mL of HNO_3, 2 mL of H_2O_2 and 2 mL of HF used in microwave digestion. Detection limits of 1.0, 2.0, 0.5 and 0.5 µg/L for Cd, Cu, Pb and Zn, respectively, were obtained. Percentage recoveries obtained of each metal to the sample solution were in the range of 80.6–112.5. It was found that the content of Cd, Cu, Pb and Zn extracted from the samples were in the range < 1.0, 10.36-12.45, 18.50-20.75 and 309.65-335.80 µg/L, respectively. The method was successfully applied to determine Cd, Pb, Cu and Zn in Thai traditional fermented food. The results were in agreement with those of graphite furnace atomic absorption spectrometry (GFAAS).

Keywords: Fermented food analysis, microwave assisted digestion, trace metal, DPASV

Introduction

Fermentation is one of the oldest and most economical methods of producing and preserving food (Chavan *et al*., 1989; Billings, 1998). In addition, fermentation provides a natural way to reduce the volume of the material to be transported, to destroy undesirable components, to enhance the nutritive value and appearance of the food, to reduce the energy required for cooking and to make a safer product (Simango, 1997).

Heavy metals are natural components of the Earth's crust. They cannot be degraded or destroyed. To a small extent they enter our bodies via food, drinking water and air. As trace elements, some heavy metals (e.g. copper, zinc) are essential to maintain the metabolism of the human body. However, at higher concentrations they can be toxic. Heavy metal poisoning could result, for instance, from drinking-water contamination (e.g. lead pipes, Cd from glass-ceramic ware), high ambient air concentrations near emission sources, or intake via the food chain. Heavy metals are dangerous because they tend to bioaccumulate. Compounds accumulate in living things any time they are taken up and stored faster than they are broken down (metabolized) or excreted. Still, food poisoning from heavy metals is very rare and in most cases only occurs after environmental pollution (Woodbury, 1993; Oghenekaro *et al*., 2008). Toxic and persistent substances in the environment continuously increase owing to anthropic activities. In particular, the rapid diffusion of heavy metals as environmental contaminants has necessitated their determination at trace and ultra-trace levels. In fact, such elements tend to concentrate in all matrices involved in foods and food chains because of their irreversible deleterious effects on human (Merian, 1991; Munõz-Olivas *et al*., 2001). Therefore, great attention must be addressed to everything directly concerning the human healthy, especially diet and nutrition. As well-known, trace elements play a very important role in human nutrition. Their quantities in the human body vary so much that in many instances they are essential to life, while in others they are toxic even at very low concentrations (He *et al*., 2008).

The concern with possible heavy metal contamination of food has created a need for analytical methodology to detect these metals in trace amounts. Several techniques, e.g. atomic absorption spectrometry (AAS) (Durali *et al*., 2007),

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electrothermal atomic absorption spectrometry (ETAAS) (Falomir *et al.*, 1999; Alexiu *et al.*, 2003), inductively coupled plasma mass spectrometry (ICP-MS) (Hennebruder *et al.*, 2004) and inductively coupled plasma atomic emission spectrometry (ICP-AES) (Işıl, 2008), etc., have been proposed for the determination of trace element in different samples because of their sensitivity, high precision and accuracy, and also wide availability of instruments. AAS is a well-developed and relatively inexpensive technique. However, it is a single-element" analytical technique, since a specific light source is required for detection of each element. ICP-MS is a powerful technique for the "simultaneous multi-element" analysis. It used plasma as the excitation source and is capable of measuring low levels (μg/L) of refractory metals; however it is expensive, requires significant investment and functioning cost.

DPASV method is a sensitive electroanalytical technique for simultaneous determination of cadmium and lead at trace levels (Cao *et al.*, 2008). Metal ions were electrochemically deposited on a working electrode, e.g., a hanging mercury drop electrode (HMDE), Static Drop Mercury Electrode (SDME), a mercury film electrode (Scott *et al.*, 1994; Sherigara *et al.*, 2007), or a more environment friendly bismuth film electrode (Cao *et al.*, 2008). This technique consists of three steps. First, metal ions are deposited onto an electrode which is held at a suitable potential. The solution is stirred during this step to maximize the amount of metal deposited. Second, stirring is stopped so that the solution will become quiet. Third, the metal deposits are stripped from the electrode by scanning the potential. The observed current during the stripping step can be related to the amount of the metal in the solution. The deposited metal was occurred at a specific potential for each metal. However, this method can detect free metal ions only and is suffered from interferences such as surface active organic substances (Jakmunee *et al.*, 2005). Some modified electrodes have been developed for improving sensitivity and selectivity in the analysis of complicated samples (Hwang *et al.*, 2008). An electrolyte medium such as acetate buffer (Jakmunee *et al.*, 2005; Samo *et al.*, 2005; Hwang *et al.*, 2008) was widely utilized in DPASV determination of trace metal. This work, the acetate buffer (pH 4.6) has been used as an electrolyte in the DPASV analysis. Cu(II), Pb(II), Cd(II) and Zn(II) are frequently present in the chemical compounds employed during the agricultural practice before the food. For this reason, it seemed attractive to consider the setup of a sensitive analytical procedure for simultaneously determining these metals at trace and ultratrace level in meals

by differential pulse anodic stripping voltammetry (DPASV). Such a technique may be certainly a good alternative to spectroscopy, since it allows to carry out a multicomponent determination and it does not need too expensive equipment.

The goal of this work developed microwave assisted digestion method for simultaneous quantification of cadmium, copper, lead and zinc in Thai traditional fermented products. Analytical solutions were studied by a differential pulse anodic stripping voltammetry (DPASV). The results of the proposed method were compared with those obtained by Graphite Furnace Atomic Absorption Spectrometry (GFAAS).

Materials and Methods

Instrumentation

A voltammetric analyzer (VA 797, Metrohm, Switzerland) including a voltammetric cell with a HMDE as a working electrode (WE), a platinum rod electrode as an auxiliary electrode (AE), and a Ag/AgCl electrode (3M KCl) as a reference electrode (RE), was employed for differential pulse anodic stripping voltammetric analysis. The voltammetric analyzer was controlled by a personal computer, using a VA Computrace version 2.797.00X0, SR1 software (8.797.8013, 797 VA Computrace, Metrohm). The AAS method was used as a reference. The atomic absorption determination of trace concentrations of cadmium, lead and copper and zinc was made with GFAAS *(Spectra AA 880Z*; *Varian*, Darmstadt, Germany). The microwave digestion was carried out with a CEM Model MARS 5 (CEM Corporation, USA).

Reagents

All chemicals used were of analytical reagent grade. Deionized water (obtained from a system of RiOsTM Type I Simplicity 185 (Millipore Waters, USA) was used throughout the experiment. Stock standard solution (1000 ppm) of lead (II), cadmium (II), copper (II) and zinc (II) was purchased from Merck, Germany. The working standard solutions were prepared daily by diluting the stock standard solution of each metal with 0.025 M acetate buffer (pH 4.6). An oxygen free nitrogen (OFN) gas (99.9995%, TIG, Thailand) was used for purging the solution to remove dissolved oxygen.

Voltammetric analysis procedure

An aliquot of 10 mL acetate buffer (pH 4.6) was placed in a voltammetric cell and the solution was purged with pure Nitrogen for 300 second. Then, a

fixed potential of -1.15V was applied to the WE for a period of 90 s, while the solution was stirred at 2000 rpm (deposition step). After that the stirring was stopped and equilibrate for 5 s, followed by anodically scanning of the potential from −1.15 to 0.15 V, employing a square wave waveform with amplitude of 50 mV, step potential of 30 mV, and frequency of 50 Hz (stripping step). A voltammogram was recorded with a personal computer. Peak potential and peak current corresponding to each metal was calculated from the voltammogram. Amounts of the metal from sample were reported as μg/L. The metal contents were quantified by the standard addition method with four additions for each metal. The values obtained for the curve slopes were used to calculate the metal quantities in the sample solution and in the blank.

Microwave digestion procedures

Microwave PTFE vessels were cleaned with 65 % (v/v) HNO_3 (10 mL) and then rinsed with deionized water before next run. Samples were accurately weighed (0.100 g) and then directly transferred to the microwave vessels. The ratio of HNO_3, HF and H_2O_2 content was used as the following procedures in Table 1. The samples were digested in two steps: Firstly, the temperature was ramped to 110°C (maximum pressure 300 psi) within 20 min with the application of 600 W powers, followed by a hold time of 5 min at 110°C and an initial cooling in the microwave oven for 5 min. The vessels were then removed from the oven and cooled in a freezer -20°C for an hour, after which they were vented and opened. The second, the temperature was ramped to 200°C (maximum pressure 350 psi) within 10 min with the application of 1200W power, followed by a hold time of 10 min at 200°C. They were again cooled in the microwave oven for 5 min and in a freezer at -20°C for 1 h, after which they were vented and opened. A blank solution was prepare by digesting 2 mL of 18.2 MΩ cm deionized water and reagent using the same digestion procedure. The digested samples and reagent blank were diluted to 25 mL of 0.025 M acetate buffer (pH 4.6). All digestions were prepared in triplicate.

Table 1. Reagents and volumes used in microwave-assisted digestion procedures

	69% HNO_3 (mL)	30% H_2O_2 (mL)	48% HF(mL)
Procedure1	6.0	0.0	4.0
Procedure2	6.0	1.0	3.0
Procedure3*	6.0	2.0	2.0
Procedure4	6.0	3.0	1.0
Procedure5	6.0	4.0	0.0

* The optimized method.

Results and Discussion

Voltammetric analysis

DPASV method is a sensitive electro analytical technique for the determination of trace amounts of metals in solution. The technique consists of three steps. First, metal ions are deposited onto an electrode which is held at a suitable potential. The solution is stirred during this step to maximize the amount of metal deposited. Second, stirring is stopped so that the solution will become quiet. Third, the metal deposits are stripped from the electrode by scanning the potential. The observed current during the stripping step can be related to the amount of the metal in the solution. Conditions for deposition and stripping steps of ASV were investigated. A square wave waveform was employed in the stripping step, as it provided fast scanning and good sensitivity for the reversible redox reaction. A square wave waveform with amplitude of 50 mV, step potential of 30 mV, and frequency of 50 Hz was used. A solution of acetate buffer (pH 4.6) was used as an electrolyte. This electrolyte is also suitable for the voltammetric determination of Cd, Pb, Cu and Zn by DPASV (Sherigara *et al.*, 2007). Standard solutions of Cd, Pb, Cu and Zn were added to the electrolyte solution and voltammetric measurement was carried out. Effect of deposition potential was investigated in the range of -1.15 to 0.15 V. It was found that the more negative potential used the higher sensitivity was obtained. Deposition potential of -1.15V was chosen because too negative potential may lead to deposition of some interferences or evolving of hydrogen gas at the WE in the high acidic medium. Deposition time was studied for the determination of 50 μg/L of each metal. A plot of peak current versus deposition time is depicted in Figure 1.
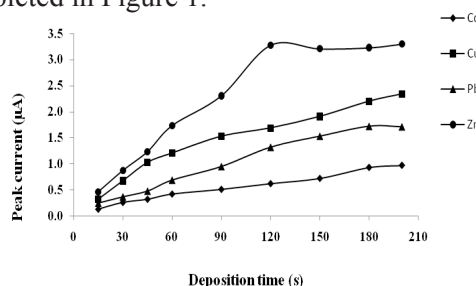

Figure 1. Effect of deposition time on peak current. Condition: deposition potential -1.15 V, stirring rate 2000 rpm, scan rate 0.01V s-1, potential scan range -1.15 to 0.15 V

It was found that peak currents of all the metals are linearly proportional to deposition time up to 200 s. At too long deposition time, the deposited metals may saturate at the HMDE so no further increase in peak current was observed. In this work, 90 s was selected as the preconcentration time. A solution of 0.025M acetate buffer providing pH 4.6 was used as

a supporting electrolyte. There are several reasons for this selection. Zinc and lead determinations are sensitive to the pH value. While Zn determination is not possible in strong acidic solutions, weak acidic media is not relevant for Pb determination due to its hydrolysis. Copper determination by DPASV with amalgamation also requires carefully selected electrolyte as the method uses chloride ions as complex forming agent (Nedeltcheva *et al.*, 2005).

Analytical characteristics and method validations

Under the optimized condition: acetate buffer (pH 4.6) as an electrolyte solution, deposition potential of -1.15 V, deposition time of 90 s, square wave waveform with amplitude of 50 mV, step potential of 10 mV, and frequency of 50 Hz, voltammograms were obtained as shown in Figure 2. The linear range of the stripping voltammetry for Pb(II), Cd(II) , Cu(II) and Zn(II) at concentration in the range 0-50 µg/L is demonstrated as the voltammograms in Figure 3. Limit of detection (LOD) and limit of quantification (LOQ) were calculated from three times and ten times standard deviation of blank/slope of the calibration graph (G.D. 2004), respectively.

Table 2. Linear equations, regression coefficients, LOD, LOQ, precision and recovery range of the studied metal

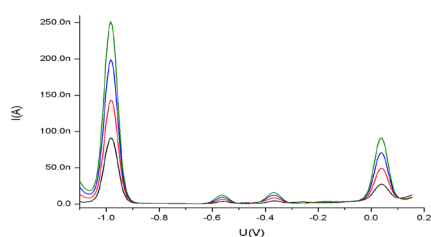| Metal	Linear equation	R^2	LOD (µg/L)	LOQ (µg/L)	Precision, RSD (%)		Recovery range (%)
					Intra-day (n =10) Peak current	Inter-day (n = 4x3) Peak current	
Cd	Y = 0.0203x + 0.0035	0.9967	1.00	10.0	3.5	4.6	80.6
Cu	Y = 0.074x + 0.0691	0.9971	2.00	5.0	2.6	2.5	94.7
Pb	Y = 0.0167x + 0.0006	0.9907	0.5	10.0	3.8	2.5	83.7
Zn	Y = 0.0337x + 0.0015	0.9994	0.5	5.0	1.8	3.5	112.5

Figure 2. Voltammograms of zinc, cadmium, lead and copper at the concentrations in the range 0 – 50 µg/L. Conditions: acetate buffer (pH 4.6), deposition potential of -1.15 V, deposition time 90 s, stirring rate 2000 rpm, scan rate 0.01Vs-1, potential scan range -1.15 to 0.15 V

The linearity of response, limit of detection (LOD), limit of quantitation (LOQ) and precisions (i.e. intra-day and inter-day reproducibility) were summarized in Table 2. The method shows good linearity in the range 0–50 µg/L with regression coefficient (R^2) higher than 0.99. LODs ranged from 2.0 to 10.0 µg/L and LOQs ranged from 5.0 to 10.0 µg/L were obtained. The reproducibility of the proposed method was studied by repeated determination of the metal mixtures 10 times in a day (intra-day precision), whereas inter-day precision was tested by triplicate quantifications for 4 days. The results show good precisions (Table 2) with relative standard deviation (RSD) of peak current less than 4.0 % (Intra-day precision) and lower than 5.0 % (inter-day precision). The results indicate that the proposed method has good precision for both qualitative and quantitative studies.

Recoveries obtained from spiking of the metal standard solutions (each concentration of 50 µg/L) into the individual fermented samples. The percentage recoveries of metal ions in all studied samples were in the range 80.6 – 112.5 as summarized in Table 2. The recoveries showed dependence on the origin of the sample and the sample matrix.

Analysis of samples

The described procedure was applied to analyze four fermented samples, numbered 1, 2, 3 and 4. Standard addition method was employed for determination in order to account for the effect of sample matrix. Each sample digest was split into two parts and one of them passed microwave digestion. Then the metal contents were determined by DPASV for both extract subsets. The GFAAS method was used as a reference. The results from standard addition method are given in Table 3. It was found that the content of Cd, Cu, Pb and Zn present in the samples were < 1.0 , 10.36 - 12.45, 18.50 - 20.75 and 309.65 - 335.80 µg/L, respectively. There are in good agreement for Zn, Cd, Cu and Pb content those obtained from DPASV (with microwave digestion) and GFAAS analysis. It is important to emphasize that the presence of organic matters in solutions does not interfere the voltammetric determination of Zn, Cd, Cu and Pb. However, the DPASV determination of metal does not provide reproducible results, even when the digestion has been subjected to without microwave digestion. Organic matters and chloride ions strongly deteriorate the anodic metal peak generated by DPASV (Jana *et al.*, 2008).

In contrast, the results obtained by DPASV with microwave digestion are well correlated with the GFAAS results. The results obtained by using calibration graph method were correlated well with those from the standard addition method. The results from standard addition method are summarized in Table 3. The developed method was convenient to be used and could be applied as an alternative method to the standard method for testing the releasing of heavy metal from fermented products.

Conclusion

A differential pulse anodic stripping voltammetric method was proposed for the determination of Cd,

Table 3. Comparative results from the analysis of heavy metals in fermented sample (in μg/kg dry sample)

| Analyte | fermented sample | DPASV | | GFAAS |
		Without microwave digestion *n*=3(*P* = 95%)	With microwave digestion *n*=3 (*P* = 95%)	*n*=1
Zn	1	317.50±0.5	309.56 ±0.1	306.40
	2	320.47±0.2	312.20±0.1	314.50
	3	330.40±0.7	335.80±0.02	335.65
	4	328.90±0.3	317.55±0.05	318.85
Cd	1	N.D.	N.D.	N.D.
	2	N.D.	N.D.	N.D.
	3	N.D.	N.D.	N.D.
	4	N.D.	N.D.	N.D.
Pb	1	31.50±0.7	23.42±0.1	21.50
	2	28.50±0.3	20.75±0.03	18.80
	3	27.60±0.1	18.50±0.1	20.65
	4	29.80±0.4	20.74±0.03	20.82
Cu	1	17.25±0.2	11.50±0.02	9.21
	2	21.60±0.1	10.36±0.1	12.85
	3	31.65±0.35	12.45±0.07	12.30
	4	15.50±0.2	12.05±0.1	10.97

N.D. = Not detectable, n is the number of determinations.
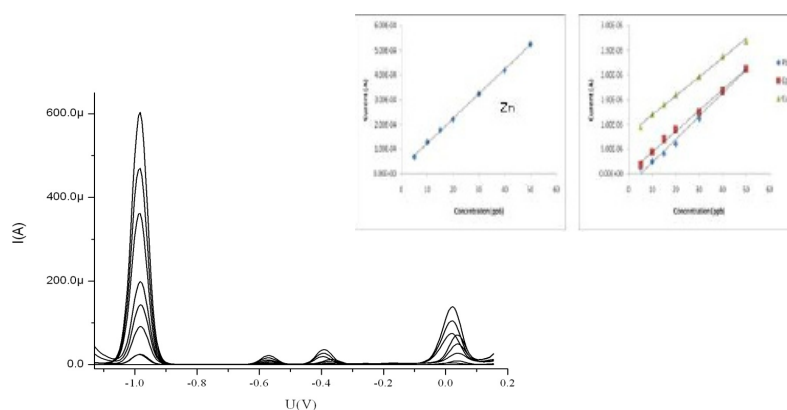1= fermented shrimp, 2= fermented fish (pla-jom), 3= fermented fish (pla-ra), 4= fermented crab

Figure 3. A series of voltammograms for increasing concentrations of Pb(II), Cd(II), Cu (II) and Zn(II) on an in situ plated .
From below: blank and successive additions of 5 μg/L of Pb(II), Cd(II), Cu (II) and Zn(II). Conditions as in Fig. 2

Cu Pb and Zn in the Thai traditional fermented products. A microwave digestion show to be the excellent sample preparation method for in the studied fermented products. The method is fast, sensitive and selective, and simultaneously determining for four metals analysis in the complicated samples. The proposed method opens to be as an alternative to conventional AAS method.

Acknowledgements

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