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Determination of Zinc, Cadmium, Lead and Copper in Kakade, Anise, Cumin, Caraway Black Pepper Extracts Using Differential Pulse Anodic Stripping Voltammetry with Hanging Mercury Drop Electrode

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Abstract: An extraction and sensitive differential pulse anodic stripping voltammetric (DPASV) method at a hanging mercury drop electrode (HMDE) is described for the determination of Zn, Cu, Pb (μg g^{-1}) and Cd (ng g^{-1}) metal ions in water and 0.1 M HCl extracts for kakade, anise, cumin, caraway and black pepper. The procedure in water extract is based on the determination of four trace metal ions in presence of protein and carbohydrate at natural pH's. The extracted species in water extract acts as an electrolyte. The influence of extraction times on the content of proteins, carbohydrates and trace metal ions as well as pH values is demonstrated. DPASV could be able to determine Zn, Cd, Pb and Cu for kakade or Zn and Pb for anise in water extract in presence of 42 or 37 % protein in dissolved organic matter (DOM). For cumin, caraway and black pepper, DPASV method could not be able to determine four metal ions in water extract in presence of 74-43 % protein in DOM. Two wet digestion procedures (HNO_3/H_2O_2 and HCl/H_2O_2) are applied to determine four metal ions in cumin, caraway and black pepper. Wet digestion process with HNO_3/H_2O_2 efficiently destroys dissolved organic matter, thus enabling voltammetric measurements in presence of <19 % protein. The proposed methods are shown to be applicable for Zn, Cd, Pb and Cu speciation before and after entering stomach

Key words: Herbs, Spices, Determination of Metal Ions, DPASV, AAS, Natural Organic Ligands

INTRODUCTION

Herb seeds have gained an important role in agronomy production and pharmacy because of their increased needs as a raw material for medicinal compounds, flavorings beverage and food additives. Previous information on chemical composition of organic ligands in herb extracts showed that soluble protein and carbohydrate (oil) represent the major (minor) constituents in aqueous extracts due to high (low) solubility in cold water and/or may partially be decomposed with mineral acids [1, 2]. With growing interest in this field by professional and general public alike, it was important to analyze metal ions that accompany on the aqueous herbs extract. Few methods have been reported to determine total concentration of metals in water extract viz; spectrophotmetric [3, 4], atomic absorption spectroscopy (AAS) [5], neutron activation analysis (NAA) and photon activation analysis (PAA) [6]. The use of electrochemical techniques is restricted to few papers, despite the high sensitivity of these methods and their ability for determining a number of metal ions. Inam *et al.* [7] determined Cd, Pb and Se in Medicago sativa after digestion with $HNO_3/HClO_4$ acids by DPASV. Jeong and Kim [8] determined germanium(IV) in hermit leaf by adsorption stripping voltammetry (ASV) using catechol reagent. Recent study suggested that cumin biomass had ability to bind with Zn and Cu metal ions in suspension system by active sites (COO^-) on cumin protein surface as a monodentate binding [9]. DPASV is a highly sensitive, precise, selective and cost-effective electroanlytical technique for determining metal ions. Most workers have preferred HMDE. It is more easily reducible than other electrodes. It is applied to distinguish whether the metal ions to be measured in the electroactive or bound to inert complex forms in real samples [10]. No direct method is applied to determine metal ions owing to interferences with organic component.

In the present study, DPASV at a HMDE is employed for the determination of Zn, Cd, Pb and Cu in five herbs after their extraction with water and 0.1 M HCl extracts. Effect of extraction times and pH values on the natural contents of protein, carbohydrate and metal ions is investigated. Two wet digestion are applied. DPASV results of Zn, Cd, Pb and Cu in aqueous extract are compared with those obtained by DPASV in 0.1 M HCl extract, digested solution and AAS in aqueous extract.

MATERIALS AND METHODS

Apparatus and Chemicals: An EG&G PAR Model 264A Polarographic Analyzer was used in combination with a 303 static mercury drop electrode stand equipped with A PM 8271 XYT recorder. A three-electrode combination was used, consisting of a HMDE, a saturated Ag/AgCl reference electrode and a platinum

wire auxiliary electrode. A Buck Scientific Model 210 VGB Atomic Absorption Spectroscopy (AAS), A Jasco Spectrophotometer Model V-530, CHN Elemental Analyzer Model Leco CHN 600 and an Orion pH electrode equipped with combined electrode were used. Suprapur grade HCl and HNO_3 acids from Merck and H_2O_2 (w/v 30%) from analytical reagent grade were used. Stock standard solutions were prepared by dilution of Ventron AAS standard (1000 mg L^{-1}) or Baker dilutes AAS solution standards. Water purified in Barnstead (Nano pure II water purification system) was used. All glassware and Teflon cells were cleaned by soaking in 1:1 HCl, in 1:1 HNO_3 and in ultrapure water for 3 days in each case.

Samples and Sample Preparation: Kakade, anise, cumin, caraway, and black pepper herbs were purchased from local market at Sohag city (Upper Egypt) then ground using a food blender with titanium blades. Place 2.5 g dry-weight sample in 500 mL flask containing 100 mL ultraupre water or 100 mL 0.1 HCl and shake the suspension at zero, 5, 10, 15, 20 and 30 min to prepare aqueous or 0.1 M HCl extracts. Centrifuge the suspension to separate the solid phase. Whereas the digested solution was prepared by placing 50 mL aqueous extract (30 min time) in 100 mL flask containing 10 mL ultrapur HNO_3 or HCl acid and 7 mL of H_2O_2. Evaporate until the appearance of a solid residue. This procedure is repeated twice for each sample. Fifty mL ultrapure water is added to dissolve the residue. Transferred water and 0.1 M HCl extracts or digested solution to 100 mL flasks and brought to volume.

Detection of CC, DOM, Protein and Carbohydrate at 30 Min. Extractable Time: To illustrate the extent of natural protein and carbohydrate in water extracts (at 30 min) on the determination of four metal ions, the complexation capacities (CC) were detected using voltammetric titration. Fig. 1 was given for Cu metal ion in five aqueous extracts, as an example. The other voltammograms for Zn, Pb and Cd metal ions are nearly similar in principle. The titration was performed by placing 10 mL extract in Teflon cup under the experimental votlammetric condition. The end point for the complexation titration was calculated by extrapolating the linear branch of the free metal ions and the linear branch of the bound metal concentrations. The point of the intersection (end point) of two linear branches was determined as the value of complexation capacity [11]. Contents of protein and carbohydrate in real aqueous extract or digested solution at 30 min were measured spectropotomtrically using folin phenol and anthrone reagents at $_{max}$ 490 and 750 nm respectively. Dissolved organic matter (DOM) in aqueous extract was measured with loss ignition [1].

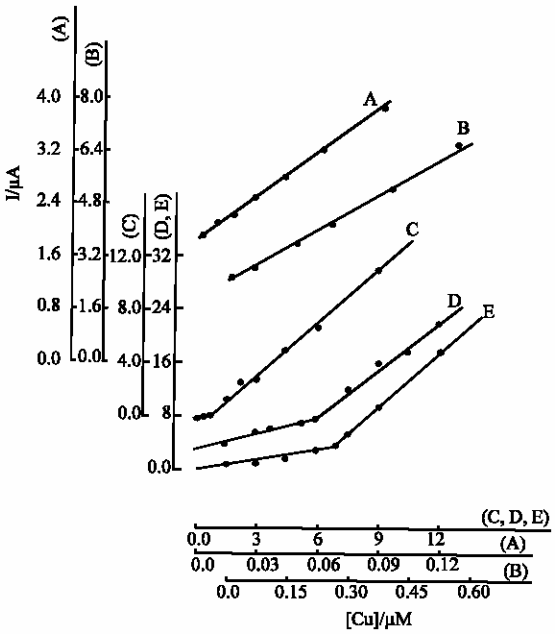

Fig. 1: Example of a Cu Titration in Aqueous Extracts for Kakade (A), Anise (B), Cumin (c), Caraway (D) and Black Pepper (E) at natural pH and 30 min Extraction Time

DPASV Measurement: Ten mL aqueous, 10 mL 0.1 M HCl or 1.0 mL digestion solutions, were pipette into the cell, adjust pH to 2, and purged with nitrogen for 4 min. The deposition potential of -1.2 V and the deposition time of 2 min were applied to a fresh mercury drop. After 15 s (equilibrium period), the voltammogram was recorded by applying a positive-going differential pulse scan (with 5 mV/s scan rate, 50 mV amplitude, fast stirring speed and drop size medium) to +0.05 V. Concentration of four metal ions was achieved by means of three standard additions [10].

AAS Measurements: Zn, Pb, Cu and Cd metal ions in herbs were measured in aqueous and 0.1 M HCl extracts at all six extractable time while digested solution at 30 min. Cd was measured in pyrolytically coated graphite tube ashing temperature of 900°C and an atomization temperature of 1350°C. Zn, Pb and Cu were measured with an air/acetylene flame. In this study, AAS result in aqueous extract was selected as a standard method to compare the results that obtained with DPASV in aqueous, 0.1 M HCl and digested solutions. Because, under each extraction period used, organic components or complexes in real extracts are destroyed and the element concentration found are nearly constant.

Blanks and Detection Limits: The blank of the procedure is evaluated from five repetitive measurements of metal ions in ultapure water or 0.1 M HCl solution.

Table 1: Chemical Characteristics of Five Herbs and Spices in Aqueous Extracts (30 min, n= 3)

	pH	DOM content (mg L^{-1})	Protein content (mg L^{-1})	Protein in DOM (%)	Crab content (mg L^{-1})	Crab in DOM (%)
Kakade	2.88 ----	38.3 ± 0.1	14.2 ± 0.15	37	24.8 ± 0.53	65
Anise	5.09 ± 0.04	50.7 ± 0.7	21.2 ± 0.92	42	2.5 ± 0.50	49
Cumin	5.80 ± 0.12	29.9 ± 0.5	22.1 ±1.00	74	7.20 ± 0.40	24
Caraway	6.30 ± 0.07	32.9 ± 0.8	23.9 ±0.23	73	6.00 ± 0.26	18
B. pepper	6.81 ± 0.09	32.0 ± 0.5	13.9 ±0.49	43	13.6 ± 0.53	42.5

B= black; Carb = carbohydrate; DOM = dissolved organic matter; n represent the number of measurements

Table 2: dpasv and aas Concentrations of Zn, Pb, Cu and Cd Metal Ions in Aqueous (a) and 0.1 M HCl (b) Extracts at Six Period Times for Five Herbs. Concentration of Zn, Pb and Cu = µg g^{-1} While Cd = ng g^{-1}

Time/ min	Zn a ass	Zn a dpasv	Zn b dpasv	Pb a aas	Pb a dpasv	Pb b dpasv	Cu a aas	Cu a dpasv	Cu b dpasv	Cd a aas	Cd a dpasv	Cd b dpasv	Zn a aas	Pb a aas	Pb b dpasv	Cu a aas	Cu b dpasv	Cd a aas
Kakade													**Cumin**					
0.0	3.79	3.80	3.82	2.99	2.95	2.96	1.64	1.62	1.65	3.04	2.98	3.10	1.36	0.72	0.025	0.92	0.57	nil
5.0	3.50	3.54	3.55	2.82	2.79	2.80	1.56	1.53	1.49	2.86	2.91	2.89	1.19	0.59	0.028	0.86	0.49	nil
10	3.41	3.39	3.42	2.56	2.52	2.55	1.45	1.47	1.48	2.48	2.43	2.49	1.07	0.53	0.271	0.76	0.48	nil
15	2.95	2.91	2.94	2.41	2.40	2.39	1.41	1.39	1.42	2.09	2.12	2.07	1.04	0.52	0.301	0.74	0.47	nil
20	2.59	2.56	2.57	2.29	2.25	2.31	1.38	1.36	1.39	2.02	2.01	1.97	0.86	0.47	0.307	0.65	0.46	nil
30	2.39	2.35	2.38	2.21	2.19	2.20	1.29	1.25	1.28	1.91	1.81	1.85	0.78	0.33	0.309	0.60	0.45	nil
x	3.10	53.092	3.11	2.547	2.517	2.535	1.455	1.437	1.452	2.40	2.377	2.395	1.05	0.527	0.207	0.755	0.487	nil
sd (±)	0.551	0.576	0.574	0.306	0.301	0.295	0.127	0.131	0.123	0.47	0.484	0.516	0.212	0.129	0.140	0.121	0.043	---
Anise													**Caraway**					
0.0	1.72	1.64	1.69	2.08	2.01	2.05	1.66	1.58	1.62	1.25	1.24	1.28	1.13	1.01	0.054	0.230	0.137	1.90
5.0	1.63	1.57	1.62	2.03	1.99	2.01	1.45	1.42	1.41	1.25	1.20	1.21	1.09	1.00	0.085	0.210	0.137	1.87
10	1.59	1.51	1.55	1.99	1.92	1.94	1.43	1.40	1.40	1.19	1.20	1.21	1.05	0.88	0.246	0.195	0.135	0.62
15	1.55	1.46	1.51	1.95	1.84	1.87	1.31	1.34	1.35	1.15	1.19	1.17	1.04	0.61	0.280	0.174	0.130	0.60
20	1.49	1.44	1.43	1.76	1.69	1.72	1.24	1.31	1.32	1.10	1.02	1.08	1.02	0.54	0.301	0.168	0.110	0.37
30	1.46	1.43	1.42	1.63	1.58	1.60	1.27	1.21	1.23	1.05	1.02	1.03	1.01	0.41	0.312	0.165	0.101	0.22
x	1.573	1.508	1.537	1.907	1.838	1.865	1.393	1.377	1.388	1.165	1.145	1.163	1.057	0.742	0.213	0.190	0.125	0.364
sd (±)	0.095	0.083	0.106	0.174	0.172	0.174	0.156	0.124	0.131	0.081	0.098	0.092	0.045	0.255	0.114	0.026	0.015	0.202
													Black pepper					
0.0													1.49	2.65	0.27	3.90	0.037	2.77
5.0													1.24	1.58	0.37	3.15	0.038	2.21
10													1.03	1.49	0.45	2.98	0.180	1.68
15													1.00	1.22	0.49	2.91	0.235	1.11
20													1.02	1.09	0.53	2.80	0.239	1.09
30													0 .96	1.04	0.53	2.71	0.340	0.92
x													1.123	1.512	0.440	3.075	0.178	1.63
sd (±)													0.205	0.598	0.102	0.431	0.120	0.735

x = mean value; sd = standard deviation

Table 3: Investigation of the Occurrence of Systematic Errors by ANOV Tested Procedures (a in Aqueous and b in 0.1 M HCl Extracts) Using aas and dpasv Techniques, $F_{1,4,\ 95\%} = 7.71$

Herb	Zn a(ass-dpasv)	Zn b(aas-dpasv)	Pb a(aas-dpasv)	Pb b(aas-dpasv)	Cu a(aas-dpasv)	Cu b(aas-dpasv)	Cd a(aas-dpasv)	Cd b(aas-dpasv)
				A- Regression parameters				
Intercept (a ± ts)								
Kakade	0.15±0.166	0.12±0.12	0.01±0.13	0.09±0.15	0.05±0.32	0.09±0.54	0.07±0.40	0.24±0.07
Anise	0.17±0.405	0.20±0.29	0.02±0.36	0.03±0.32	0.33±0.52	0.28±0.52	0.13±0.85	0.11±0.55
Slope (b ± ts)								
Kakade	1.04± 0.05	1.04±0.04	0.98± 0.05	0.96±0.06	1.03± 0.22	0.93±0.37	1.02±0.17	1.09±0.03
Anise	0.85±0.25	1.107±0.19	0.98±0.19	0.99±0.17	0.75±0.37	0.80±0.37	1.09±0.72	1.09±0.47
Correlation coefficient (r)								
Kakade	0.9993	0.9997	0.9993	0.9991	0.9882	0.9610	0.9932	0.9998
Anise	0.9774	0.9927	0.9905	0.9925	0.9432	0.9488	0.9034	0.9543
				B- Linearity test				
Variance ratio (F)								
Kakade	3016	5859	2746	2176	166.0	48.4	292.7	10615
Anise	85.63	271.5	207.3	263.5	32.25	36.12	17.76	40.77

The blank levels with their standard deviations (sd) were 0.76±0.11, 0.23±0.06, 0.21±0.04 and 0.018±0.02 or 1.05±0.14, 0.42±0.14, 0.79±0.043 and 0.021±0.025 μg L^{-1} in ultrapure water or 0.1 M HCl extract for Zn, Pb, Cu and Cd, respectively. The detection limits (3 sd of the blanks) for Zn, Cd, Pb and Cu were 0.33, 0.06, 0.18 μg L^{-1} in ultrapure water and 0.12 or 0.42, 0.075, 0.42 and 0.129 μg L^{-1} 0.1 M HCl extract.

RESULTS AND DISCUSSION

Chemical Characteristics of Herbs: Table 1 shows that the protein percent in DOM for cumin, caraway and black pepper aqueous extracts was higher than in anise and kakade whereas carbohydrate was higher for anise and kakade than in cumin, caraway and black pepper. These discrepancies might be due to the variations of organic species in each extract [12]. Previously, influence of saccharose content on standard solution of 100 μg L^{-1} Zn, 100 μg L^{-1} Cd and 50 μg L^{-1} Pb ions with DPASV at a HMDE was investigated [13] and they found that stripping peaks decreased with 50, 50 and 35 %, respectively. They also measured Zn, Cd and Pb concentrations successfully in untreated refined beet sugar samples by DPASV. Since, no results have been published, present results are compared with those results to asses the adsorption of carbohydrate onto mercury electrode. Clearly, aqueous extracts contain low content of carbohydrates than such previous results [13], indicating that carbohydrate has no influence on HMDE.

To elucidate the role of dissolved protein on dissolved metal ions in herb aqueous (real) extracts at 30 min extractable time, complxation capacity (CC) is examined. Results reveal that Zn-CC, Pb-CC, Cu-CC and Cd-CC for kakade and anise and Pb-CC and Cd-CC for cumin, caraway and black pepper extracts were practically equals zero, indicating no interaction can occur. While Zn-CC or Cu-CC was 2.0±0.45, 4.7±0.46 and 0.8±0.2 or 3.9±0.6, 0.07±0.03 and 10±0.26 μ mol for cumin, caraway and black pepper, respectively, indicating there is a strong interaction (Fig. 1). Moreover, results show that Cu-CC is higher than Zn-CC. Analysis of seeds, also, reveals that kakade, anise, cumin, caraway and black pepper contain 30.0±0.43.0, 41±0.43, 48.8±0.53, 28.3±1.15 and 44.3±0.47 % carbon, 22.1±1.4, 4.0±0.45, 2.2±0.03, 1.24±0.18 and 1.8±0.11 % nitrogen, respectively. Sulfur element (4.6±0.15 %) only found in black pepper. Accordingly, high values of Cu-CC and Zn-CC in cumin, caraway and black pepper may possibly due to high content of dissolved protein (organic ligand) in aqueous extracts which posses a large number of complexing groups such as NH_2 and COO^- groups. These groups have high ability to form complexes with Cu and Zn metal ions [11]. This lead to serious influenced on mercury electrode. To complete quantification, amounts of major cations were measured. It was found that Mn, Fe, Ca, Mg, K and Na were 4.0, 3.4, 92.9, 2.99, 111 and 62 mg L^{-1} for kakade, 0.22, 0.28, 48.2, 17.3, 131 and 8.2 mg L^{-1} for anise; 0.14, 0.1, 40.6, 13.1, 92 and 16.2 mg L^{-1} for cumin, 0.08, 0.1, 48.3, 22.3, 181 and 11.3 mg L^{-1} for caraway and 0.04, 0.03, 7.4, 8.02, 127 and 2.7 mg L^{-1} for black pepper, respectively. In this study, real organic ligands and major cations were considered as interfering ions during the DPASV of Zn, Cd, Pb and Cu measurements.

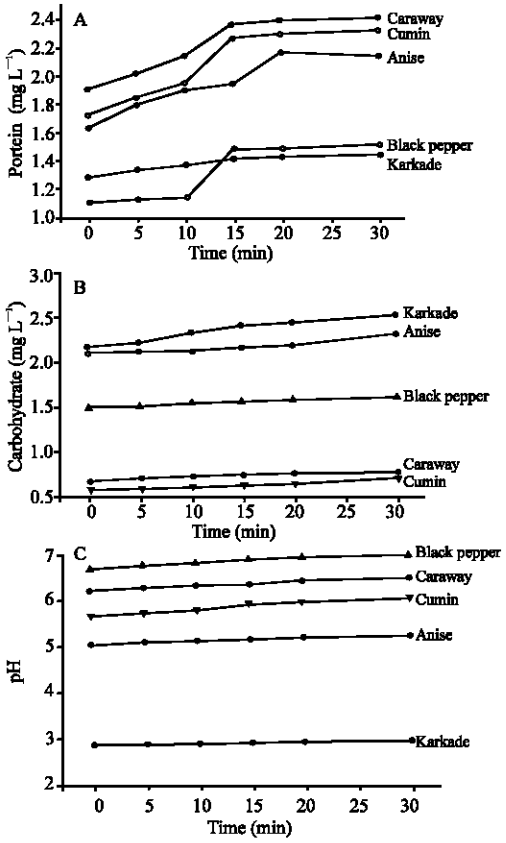

Fig. 2: Dependence of Protein (A), Carbohydrate (B), pH Value (C) on Extending Extraction Times for Cumin, Caraway, Black Pepper, Anise and Kakade Aqueous Extracts

Effect of Extraction Time on the Protein Content and Carbohydrates and pH in Aqueous Extracts: Fig. 2A shows that protein content in cumin, caraway, black pepper and anise aqueous extracts increases gradually with increasing extraction periods up to 15.0 min. At still increase extraction times up to 30 min, the proteins content remained nearly constant. Figure 2b shows carbohydrate content increases slightly with increasing extraction times up to 30 min. Finally, pH values increases slightly with increasing time of extraction for cumin, caraway and black pepper aqueous extracts. For kakade, increase in the extraction times does not influenced on the pH value.

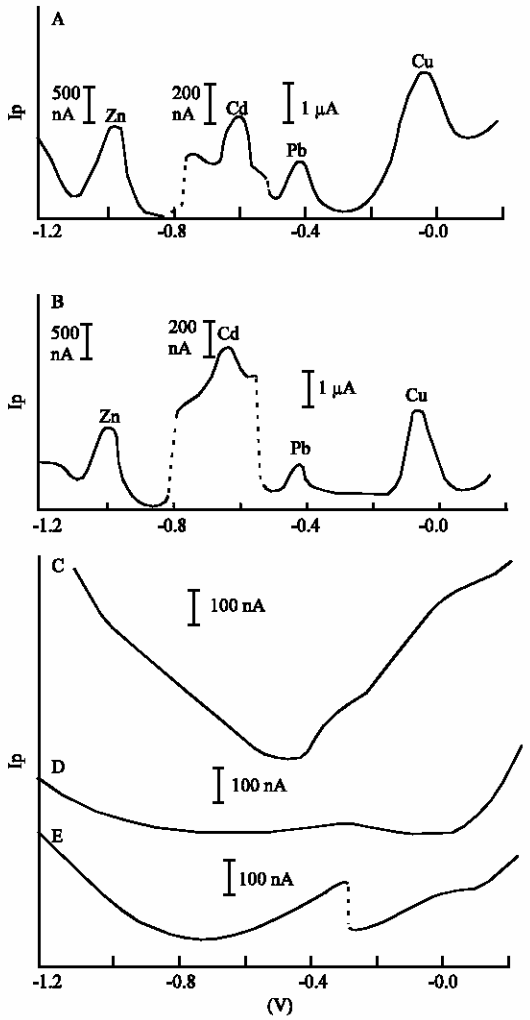

Fig. 3: Stripping Voltammograms of Zn, Cd, Pb and Cu in Presence of 14.2, 21.2, 22.1, 23.9 and 13.9 mg L^{1} Proteins and 24.8, 22.5, 7.2, 6.0 and 13.6 mg L^{1} Carbohydrates in Aqueous Extracts for Kakade (A), Anise (B), Cumin (C), Caraway (D) and Black Pepper (E)

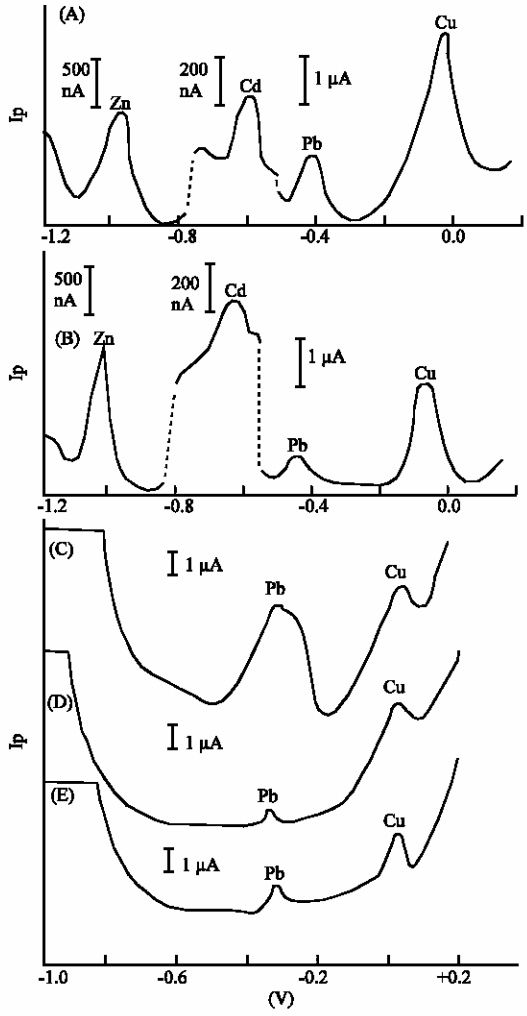

Fig. 4: Stripping Voltammograms of Zn, Cd, Pb and Cu in 0.1 M HCl Extracts for Kakade (A), Anise (Curve B), Cumin (Curve C), Caraway (Curve D) and Black Pepper (Curve E)

Detection of Metal Ions in Natural Extract by DPASV: Generally, results in Table 2 show that the concentration of Zn, Pb, Cu and Cd by DPASV and AAS in five aqueous extracts decreases slightly with increasing extraction times up to 30 min extractable time as a result of increasing content of protein in the five aqueous extracts. Similar behavior is also observed in 0.1 M HCl extract except Pb in cumin, caraway and black pepper as concentration of it increases with increasing time of extraction. This difference in behaviour may possibly is attributed to nature of interaction between heavy metals and natural organic ligands (e.g. protein) in real samples. The Irving-Williams series illustrated the order of stability constants for metal complexes in the following: Cu(II)>Zn(II)>Cd(II)>Pb(II) [1].

Table 2 reveals that there is an agreement between Zn, Pb, Cu and Cd concentration with DPASV in aqueous or 0.1 M HCl extract and with AAS. The relative errors were ranges 0.21-1.18 or 0.42– 0.96 % for kakade and 1.5-3.61 or 0.17-2.23 % for anise. Low relative errors may possibly attributed to low protein content in extracts. Comparing Fig. 3 and 4 show that stripping peaks of Zn, Cd, Pb and Cu in kakade and anise aqueous (curves A, B) and 0.1 M HCl (curves A, B) extracts are nearly identical with high resolution, suggesting that DPASV at HMDE may permit a separation of metal ion peaks in aqueous extract, so that an initial and problematic, samples treatment and addition of a supporting electrolyte were may be unnecessary. Hence, Zn, Pb, Cu and Cd concentration with DPASV in aqueous extracts for kakade and anise can be successfully detected.

Table 4: Results of Zn, Pb, Cd and Cu with AAS in Aqueous Extracts and DPASV in Five Aqueous Extracts Before and After Wet Digestion, at 30 min Extractable Time

M aas (aq) $\mu g\ g^{-1}$	dpasv (HNO_3/H_2O_2) $\mu g\ g^{-1}$	RE(%)	dpasv (HCl/H_2O_2) $\mu g\ g^{-1}$	RE(%)	M aas (aq) $\mu g\ g^{-1}$	dpasv (HNO_3/H_2O_2) $\mu g\ g^{-1}$	RE(%)	dpasv (HCl/H_2O_2) $\mu g\ g^{-1}$	RE(%)
Cumin									
Zn 0.78±0.01	0.793±0.02	1.67	0.805±0.07	3.20	Cu 0.60±0.03	5.930±0.01	1.17	0.51±0.04	15.0
Pb 0.33±0.01	0.351±0.05	6.36	0.140±0.02	57.6	Cd* nil	nil	----	nil	---
Caraway									
Zn 1.01±0.01	0.995±0.08	1.48	1.223±0.03	22.1	Cu 0.165±0.02	0.167±0.01	1.21	0.237±0.11	43.6
Pb 0.41±0.04	0.392±0.05	4.39	0.441±0.06	7.56	Cd* 0.220±0.01	0.228±0.06	3.64	0.210±0.06	4.54
Black pepper									
Zn 0.96±0.12	1.010±0.23	5.21	0.643±0.04	33.0	Cu 2.71±0.790	2.640±0.19	2.58	2.578±0.08	4.80
Pb 1.04±0.120	0.992±0.09	4.61	1.087±0.132	4.42	Cd* 0.92±0.05	0.890±0.02	6.52	0.055±0.09	14.7

* = concentration of cadmium = ng g^{-1}; RE = relative errors

Table 5: Distribution of Four Metal Concentrations in Five Herbs and Spices Extracts at 30 min (n=3)

	Kakade					Anise					Cumin			Caraway			Black pepper		
	(a)	(b)	(c)	(d)	(e)	(a)	(b)	(c)	(d)	(e)	(a)	(d)	(e)	(a)	(d)	(e)	(a)	(d)	(e)
Zn ($\mu g\ g^{-1}$)																			
(x)	2.39	2.35	0.04	2.38	0.01	1.46	1.43	0.03	1.42	0.04	0.78	--	--	1.01	--	--	0.96	--	--
(%)		98.3	1.70	99.6	0.40		97.9	2.10	97.3	2.70									
Cd ($ng\ g^{-1}$)																			
(x)	1.91	1.81	0.10	1.85	0.06	1.05	1.02	0.03	1.03	0.02	--	--	--	0.22	--	--	0.92	--	--
(%)		94.8	5.20	96.9	3.10		97.1	2.90	98.1	1.90									
Pb ($\mu g\ g^{-1}$)																			
(x)	2.21	2.19	0.02	2.20	0.01	1.63	1.58	0.05	1.60	0.03	0.33	0.309	0.021	0.41	0.312	0.098	1.04	0.53	0.51
(%)		99.1	0.90	99.54	0.45	96.9	3.10	98.2	1.80		93.6	6.40		76.1	23.9		51	49	
Cu ($\mu g\ g^{-1}$)																			
(x)	1.29	1.25	0.04	1.28	0.01	1.27	1.21	0.06	1.23	0.04	0.60	0.45	0.15	0.165	0.101	0.064	2.71	0.34	2.37
(%)		96.9	3.10	99.2	0.80		95.3	4.70	96.9	3.10		75.0	25.0		61.2	38.8		12.55	87.45

(a)- aas results; (b)- dpasv results in aqueous extract; (c)- bound metals in aqueous extract; (d)- dpasv results in 0.1 M HCl extract; (e)- bound metals in 0.1 M HCl extract; x = mean value; n = number of measurements

Table 2 shows that there is no response of Zn, Pb, Cu and Cd with DPASV aqueous extracts. Also Zn or or Cd has no response with DPASV in 0.1 M HCl extract with AAS was higher than in 0.1 M HCl with DPASV for cumin, caraway and black pepper with relative errors ranges 60, 70 and 71% for Pb and 35, 69 and 94 % for Cu in cumin, caraway and black pepper, respectively, indicating no agreement between the results. For Zn or Cd, measurements can be occurred only with AAS in aqueous extract for cumin, caraway and black pepper or caraway and black pepper. Finally, measurements of Cd by AAS in cumin aqueous extract is often lacking due to the low concentration, indicating that the detection limits with DPASV was high for useful comparison to be made. On the other hand, Fig. 3 and 4(C, D, E) show the masking effect of protein on peaks current of Zn, Pb, Cu and Cd in aqueous and Zn and Cd in 0.1 M HCl extracts for cumin, caraway and black pepper. Moreover, stripping peaks for Pb and Cu in 0.1 HCl were still poorly resolved, resulting high content of dissolved protein in aqueous and HCl extracts which forms metal-protein complexes. Hence, the DPASV measurements of Zn, Pb and Cu in aqueous and 0.1 M HCl extracts were rendered the metal oxidation irreversibility by creating a barrier to ion diffusion/or retard chemical steps prior to electron transfer [14], i.e. metal ions could not approach the electrode. On the other hand, high concentration of four metal ions with AAS in aqueous extracts may possibly attributed to high energy applied which can overcome the interaction of analyte with the matrix [15]. Thus, DPASV did not permit a successful determination of Zn, Pb, Cu and Cd metal ions in aqueous and 0.1 M HCl extracts for cumin, caraway and black pepper.

Comparison between DPASV and AAS Measurements: In order to validate the best procedure for the determination of Zn, Cd, Pb and Cu in kakade and anise in aqueous or 0.1 M HCl extracts, linear regression analysis was applied to the data represented in Table 2, which it takes into account the systematic errors associated with variables x and y, according to the model:

$$y = a + bx$$

Where y is the results produced by DPASV in aqueous and 0.1 M HCL extracts and x is the results obtained with AAS standard method. More detailed on this procedure was described elsewhere [16]. The results were listed in Table 3. Clearly, F values measurement of Zn, Pb, Cu and Cd, for kakade, Zn and Pb for anise in either aqueous or 0.1 M HCl and Cd for anise in 0.1

M HCl extracts were found to be higher than critical value ($F_{(1,4;95\%)}$=7.71) with high correlation coefficients, thus confirming the linearity of the regression model for the concentration range tested, i.e. there are agreement between DPASV measurements of Zn, Pb, Cu and Cd in kakade and Zn and Pb in anise aqueous and 0.1 M HCl extracts. Table 3, also, shows that F value measurement of Cu metal ion in aqueous and 0.1 M HCl and Cd in aqueous extracts for anise is slightly higher than critical values with rather slightly poor r (0.9034) indicating, the linearity is not confirmed.

Table 3 shows that DPASV measurements of Zn, Cd, Pb and Cu in kakade aqueous and 0.1 M HCl extracts, Zn and Pb in anise aqueous and 0.1 M HCl as well as Cd in 0.1 M HCl anise aqueous extracts do not differ significantly from the expected theoretical values of 1.0 (slope) and 0.0 (intercept), i.e. no systematic errors is found. This indicates that DPASV could be used accurately to determine Zn, Pb, Cu and Cd for kakade, Zn and Pb or Cd in aqueous and 0.1 M HCl or 0.1 M HCl extracts for anise. Whereas, DPASV measurement of Cd in anise aqueous extract, did not differ significantly from 1.0 (slope) but differ significantly from 0.0 (intercept =0.13) with poor r (0.9034); systematic error. This means, concentration independent of the concentration level of Cd occurs. This is probably due to sample contamination during sample extraction with water extract occurs. Finally, DPASV of Cu in anise aqueous and 0.1 M HCl extracts was affected by systematic errors as both the slopes and intercept differ slightly from their theoretical values, therefore, hindering the accurate determination. Above finding reveals unreliable DPASV measurements of Cu in anise aqueous and 0.1 M HCl due to high contents of proteins and/or volatile oil that dissolved in the extract [12].

Determination of Metals Ions in Digested Extracts: Table 4 gives the concentrations of Zn, Pb, Cu and Cd with DPASV in digested solution containing 18, 19 and 14 % protein and 10, 11 and 4 % carbohydrate in DOM for cumin, caraway and black pepper, respectively and AAS in aqueous extract. It is clear that concentration of Zn, Pb, Cu and Cd in caraway and black pepper and Zn, Pb and Cu in cumin extracts with HNO_3/H_2O_2 is better than HCl/H_2O_2 suggesting that former wet digestion is reliable. Relative errors between DPASV and AAS with HNO_3/H_2O_2 were found to be lower than HCl/H_2O, suggesting there is a satisfactory agreement between concentration metal ions. High concentration of metal ions in digested extract with DPASV is attributed to high ability of HNO_3/H_2O_2 mixture to destroy most of metal-protein compounds and/or organic ligands in cumin, caraway and black pepper extracts, thus complete resolution of stripping peaks can occurs. Again, Cd metal ions in cumin can not be detected in cumin digested extract due to low concentration of metal in the solution (Table 4).

Extent of Zn, Cd, Pb and Cu Metal Ions in Aqueous and 0.1 M HCl Extract: Elucidation of the speciation of Zn, Cd, Pb and Cu forms is considered as one of the significant task in drinks or food research. Because, the extent of Zn. Pb Cu and Cd metal ions in five aqueous extracts was affected by the respective chemical fractions or speciation of metal ions. So, DPASV technique was used to determine free ions and any reducible complexes of four metal ions in five aqueous and 0.1 M HCl extracts, respectively. AAS response should yield the total metal concentrations. Such differences in the concentrations between aqueous or 0.1 M HCl extracts with DPASV and AAS allows estimating the bound metals before and after the extracts entering the stomach. The results are listed in Table 5.

Table 5 shows that the abundant Zn, Cd, Pb and Cu in kakade and anise aqueous and 0.1 M HCl extracts are mostly in free fractions (>94.8%) resulting low content of protein in DOM (<42.0%) for kakade and anise and/or high acidity particularly for kakade (pH 2.88) extract. This observation reveals the following: (i) dissolved protein or metal organic complexes in aqueous extract is decomposed or dissociated at low pH, i.e. complete protonation of the active sites and release the metal freely, particularly, in kakade aqueous extract, (ii) 0.1 M HCl medium has a slightly significant influence on decomposition of protein in kakade and anise extracts. Table 5 also shows that the abundant Zn and Cd in 0.1 M HCl extract in cumin, caraway and black pepper are in bound fractions (100%). For Pb and Cu, there are a large discrepancies between free and bound fractions in cumin, caraway and black pepper aqueous and 0.1M HCl extracts resulting different chemical composition in extracts. In this case, 0.1 M HCl medium has a significant influence on the type of fractions of metal ions in cumin, caraway and black pepper extracts.

CONCLUSION

DPASV has advantage of allowing the determination of Zn, Pb, Cu and Cd for kakade and Zn and Pb for anise in aqueous extracts in absence of an electrolyte at natural pH. In 0.1 M HCl extract, DPASV of Zn, Pb, Cu and Cd for kakade and Zn, Pb and Cd for anise can also measured successfully. No DPASV responses are obtained of Zn, Pb, Cu and Cd in aqueous and Zn, Cd in 0.1 M HCl extracts for cumin, caraway and black pepper. Satisfactory results obtained of Zn, Cd, Pb and Cu in kakade and Zn and Pb in anise aqueous extracts with DPASV at HMDE could be an indication of the possible applicability to other herbs in water extracts under the following condition: (i) percentage of protein and carbohydrate in aqueous extract should be <42% and <65% in DOM, respectively, (ii) herbs seeds should not contains high amounts of volatile fatty acids (such as oleic, inoleic and plamatic acids). Most

fractions of four metal ions in kakade and anise water extracts are presented in the free species whereas, the dominant species in cumin, caraway and black pepper present are existed in bound fractions. 0.1 HCl extract (medium in stomach) has a slightly significant (no significant) influence on water extracts for cumin, caraway and black pepper (kakade and anise) after entering stomach.

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