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Validation and quantitative analysis of cadmium, chromium, copper, nickel, and lead in snake fruit by Inductively Coupled Plasma-Atomic Emission Spectroscopy

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ABSTRACT

Snake fruit or known as *Salak* is one of the favourite fruits and is consumed not only in Indonesia but also in the worldwide. The fruit may be contaminated with heavy toxic metals such as cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), and lead (Pb). The purpose of this study was to validate inductively coupled plasma-atomic emission spectroscopy (ICP-AES) for determination of Cd, Cr, Cu, Ni, and Pb in snake fruit. ICP-AES was validated by determining several validation parameters which included linearity and range, limit of detection and limit of quantification, precision, and accuracy. The validated method was further used for assay of these metals in snake fruit. The results showed that ICP-AES was linear over the concentration ranges of 0.025–1.0 µg/mL for Cd, Cr, and Ni and 0.050–1.0 µg/mL for Cu and Pb, respectively. The values of coefficient of determination (r^2) obtained for these regressions were higher than 0.99. The values of limit of detection (LoD) of Cd, Cr, Cu, Ni, and Pb found were at 0.0010 µg/mL, 0.0024 µg/mL, 0.0047 µg/mL, 0.0037 µg/mL, and 0.0091 µg/mL respectively, while limits of quantitation were of 0.0030 µg/mL (Cd), 0.0073 µg/mL (Cr), 0.0143 µg/mL (Cu), 0.0113 µg/mL (Ni), and 0.0274 µg/mL (Pb), respectively. The validated method was accurate and precise enough for determination of these heavy metals in snake fruit samples as indicated by acceptable values of recovery and low relative standard deviation (RSD) values. The developed method has been successfully used for determination of Cd, Cr, Cu, Ni, and Pb levels commercially available in the markets and farms.

INTRODUCTION

Fruits are parts of human diet sources. Snake fruit (*Salacca zalacca*), also locally in Indonesia known as "salak", is one of the favourite fruits for Indonesian people. "Pondoh" cultivar which originally grown in Yogyakarta province is the most popular snake fruit cultivar due to its high aroma intensity and sweetness (Supriyadi *et al*., 2002). Snake fruit contains various nutritional compounds such as fibers, proteins, fats, and carbohydrates and possesses high level of antioxidant (Goristein *et al*., 2009). Snake fruit also positively affected plasma lipid levels in cholesterol fed rats (Leontowicz *et al*., 2007).

Heavy toxic metals can be accumulated in fruits with various concentrations depending on the harvesting sites of fruits (Wagner, 1993). The accumulation of the heavy metals can decline the physical health and mental cognitive of the individual (Sandeep *et al*., 2012). Cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), and lead (Pb) are heavy metals which are harmful for human health. Cd has toxic effects on many organs and tissues, especially on kidneys, bones, and lungs (ATSDR, 2012a). Cr may cause bad effects on gastrointestinal tract, such as abdominal pain, vomiting, peptic ulcer, hemorrhage and necrosis, and bloody diarrhea (ATSDR, 2012b). Accidental ingestion of large doses of Cu causes gastrointestinal bleeding, haematuria, and acute renal failure amongst other symptoms. The lower doses of Cu have similar effects, which caused headache, nausea, vomiting, and

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diarrhoea (Agarwal *et al*., 1993). Ni may cause gastrointestinal and cardiovascular disorder, liver damage, and carcinogenic effect (ATSDR, 2005). Pb nephrotoxicity is characterized by proximal tubular nephropathy, glomerular sclerosis and interstitial fibrosis (Goyer, 1989; Loghman-Adham, 1997).

Inductively coupled plasma-atomic emission spectroscopy (ICP-AES) is a method of choice for multi-elements analysis, especially for analysis of metal in trace levels. This method has some advantages over the other techniques of spectrophotometric methods, atomic absorption spectrometry, and atomic fluorescence spectrometry, namely high sensitivity (very low limit of detection), simple instrumentation, rich spectrum (more choices of spectral lines), and can analyze multiple elements at one time (Velez, 2009). The presences of Cd, Cr, Cu, Ni, and Pb in various sample such as in soil, sediment, and geological materials (Moor *et al*., 2001) and methanolic leaf extract (Pednekar and Raman, 2013) have been reported. However, using literature review, there was no available report related to the determination of Cd, Cr, Cu, Ni, and Pb in snake fruit. Therefore, in this study, we developed and validated fast and reliable analytical technique of Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES) for quantitative analysis of Cd, Cr, Cu, Ni, and Pb in snake fruit.

MATERIALS AND METHODS

Materials

Snake fruits were collected from markets and farms in Yogyakarta, Indonesia. Cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), and lead (Pb) standard solution (1000 mg/L) was purchased from Merck (Darmstadt, Germany) in form of $Cd(NO_3)_2$, $Cr(NO_3)_3$, $Cu(NO_3)_2$, $Ni(NO_3)_2$, $Pb(NO_3)_2$. Nitric acid 65% and perchloric acid 70–72% were also purchased from Merck (Darmstadt, Germany). All reagents and solvents used were pro analysis (p.a) grade. Destilled and deionized water were used as solvent. Inductively Coupled Plasma-Atomic Emission Spectrometer ICPE-9820® (Shimadzu Corp., Japan) was used for measuring the analytical response.

Digestion procedure

One kilogram of fresh snake fruit was peeled and cut into small pieces. This sample was then subjected to digestion process. Digestion procedue was carried out according to Eka *et al*. (2012) with slight modification. A-5 g of snake fruit sample was accurately weighed into 125 mL Erlenmeyer flask and added with 10 mL nitric acid-perchloric acid mixture in a volume ratio of 1:1. The mixture was subsequently heated at temperature of 110–120ºC until the solution was clear. The sample solution was then cooled, filtered with filter paper, and diluted to 25 mL in volumetric flask with distilled water.

Determination of Cd, Cr, Cu, Ni, and Pb using ICP-AES

ICP-AES instrument ICPE-9820® was operated under the following conditions: radio frequency power was adjusted at 1.2 kW, plasma gas flow at 10 L/min, auxiliary gas flow at 0.6 L/min, and carrier gas flow was set at 0.7 L/min. Approximately of 10.0 mL sample solution was introduced into sample container and analyzed at wavelength of 226.502 nm (Cd), 205.552 nm (Cr), 324.754 nm (Cu), 231.604 nm (Ni), and 220.353 nm (Pb).

Method validation

Analytical method validation of ICP-AES for analysis of trace metals was assessed by determining several analytical parameters according to International Conference on Harmonization (ICH, 2005).

RESULTS AND DISCUSSION

Validation of ICP-AES

ICP-AES is method of choice for analysis of heavy metals in food and pharmaceutical products because of its low detection limits and its high degree of selectivity (Gaur *et al*., 2011). Before being used for quantitative analysis of heavy metals (Cd, Cr, Cu, Ni, and Pb) in snake fruit, ICP-AES was validated by determining some analytical parameters, namely linearity and range, sensitivity which is expressed by limit of detection (LoD) and limit of quantitation (LoQ), precision, and accuracy. The linearity of analytical response was assessed by plotting the intensity values (y-axis) of diluted series of Cd, Cr, Cu, Ni, and Pb standard solution versus its final concentration (x-axis). The dynamic concentration ranges used were 0.025–1.000 µg/mL for Cd, Cr, and Ni and 0.050–1.000 µg/mL for Cu and Pb. The linear relationship was established for all five regression equations with acceptable coefficient of determination (r^2) values (Table 1). The analytical response was linear over certain concentration ranges, if the r^2 value obtained is higher than 0.995 (Eurachem, 1998). Figure 1 revealed the example of releationship between concentration (x-axis) and the corresponding intensity values (y-axis) of Cd and Cr. In addition, the percentage of y-intercept was low which indicated that the linear regression was free from systematic error.

Table 1: Linear regression data of cadmium, chromium, copper, nickel, and lead calibration curves.

Parameters	Cd	Cr	Cu	Ni	Pb
Linear range	0.025–1.000 µg/mL	0.025–1.000 µg/mL	0.050–1.000 µg/mL	0.025–1.000 µg/mL	0.050–1.000 µg/mL
R^2	0.9997	0.9986	0.9996	0.9963	0.9979
Slope	9677.8126	1717.9694	23798.1373	3070.7428	540.0743
Intercept	58.3341	20.5148	344.3711	13.8504	8.7384
% Intercept	1.6218%	3.1627%	3.3750%	1.2186%	3.7587%

The analytical sensitivity of ICP-AES was evaluated by determining the values of limit of detection (LoD) and limit of quantitation (LoQ). The values of LoD and LoQ were calculated as 3.3 SD/b and 10 SD/b respectively, where SD is the standard deviation of analytical responses and b is the slope of calibration curve. When the responses of the sample blanks

were still high and had good precision, the sample blanks need to be diluted until its reached the lowest concentration of analyte that still showed analytical response. The values of LoD found were of 0.0010 µg/mL (Cd), 0.0024 µg/mL (Cr), 0.0047 µg/mL (Cu), 0.0037 µg/mL (Ni), and 0.0091 µg/mL (Pb). Meanwhile, the LoQ values found were of 0.0030 µg/mL, 0.0073 µg/mL, 0.0143 µg/mL, 0.0113 µg/mL, and 0.0274 µg/mL for Cd, Cr, Cu, Ni, and Pb, respectively. Based on LoD values, ICP-AES was sensitive enough for analysis of these heavy metals because LoD values were lower than maximum values of heavy metals allowed to be present in fruit products, i.e. 0.5 µg/g (Roba *et al.*, 2016).

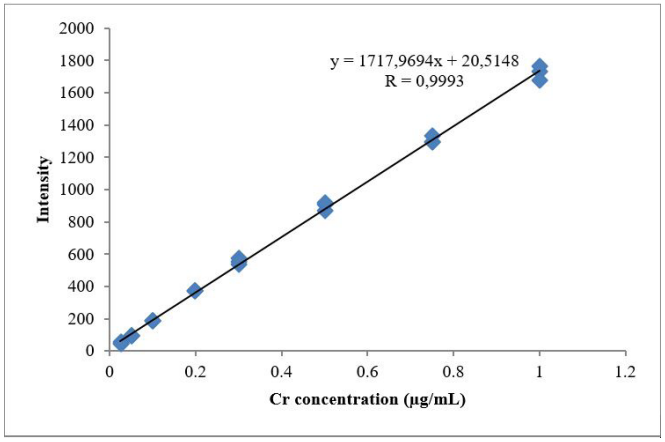

Fig. 1: The relationship between concentration (x-axis) and the corresponding intensity values (y-axis) of chromium (top) and cadmium (down).

Precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions (ICH, 2005). Precision is typically evaluated by measuring the values of relative standard deviation (RSD) of a set of data. The assessments were done by determining RSD under the conditions of repeatability and intermediate precision (different day of measurement). Repeatability was evaluated by measuring 10 blank sample solutions spiked with the standard solutions of Cd, Cr, Cu, Ni, and Pb, each at concentration of 0.2 µg/mL, under similar conditions (day, analyst, instrument, sample). The RSD values obtained for repeatability tests were 3.10% for Cd, 3.88% for Cr, 4.36% for Cu, 1.96% for Ni, and 4.56% for Pb. Furthermore, the same blank samples were measured again in 2 other different days to determine the intermediate precision. The RSD values obtained during the intermediate precision were 3.96% (Cd), 6.87% (Cr), 4.63% (Cu), 3.56% (Ni), and 5.77% (Pb), as shown in Table 2. According to Horwitz, the maximum RSD values acceptable for the analyte level of 1 µg/mL is 16% (Gonzales and Herrador, 2007). AOAC Peer Verified Methods set the maximum acceptable RSD value at 11% for the same analyte level. Therefore, it can be stated that the ICP-AES method showed good precision based on RSD values obtained.

Table 2: The relative standard deviation (RSD) values for precision studies of ICP-AES during determination of cadmium, chromium, copper, nickel, and lead in snake fruit.

Cadmium			
Day	**Calculated analyte conc. (µg/mL)**	**RSD values (%)**	
		Repeatability	**Intermediate precision**
1	0.2484 ± 0.0077	3.10	
3	0.2622 ± 0.0078	2.97	3.92
8	0.2479 ± 0.0070	2.84	
Chromium			
Day	**Calculated analyte conc. (µg/mL)**	**RSD values (%)**	
		Repeatability	**Intermediate precision**
1	0.1866 ± 0.0072	3.88	
3	0.2060 ± 0.0080	3.88	6.87
8	0.2146 ± 0.0071	3.32	
Copper			
Day	**Calculated analyte conc. (µg/mL)**	**RSD values (%)**	
		Repeatability	**Intermediate precision**
1	0.4761 ± 0.0208	4.36	
3	0.4820 ± 0.0212	4.40	4.63
8	0.4583 ± 0.0178	3.89	
Nickel			
Day	**Calculated analyte conc. (µg/mL)**	**RSD values (%)**	
		Repeatability	**Intermediate precision**
1	0.1979 ± 0.0039	1.96	
3	0.2074 ± 0.0041	1.96	3.56
8	0.2125 ± 0.0043	2.03	
Lead (Pb)			
Day	**Calculated analyte conc. (µg/mL)**	**RSD values (%)**	
		Repeatability	**Intermediate precision**
1	0.2509 ± 0.0114	4.56	
3	0.2667 ± 0.0142	5.34	5.77
8	0.2700 ± 0.0132	4.88	

Table 3: The recovery values for accuracy studies of ICP-AES during determination of cadmium, chromium, copper, nickel, and lead in snake fruit.

Cadmium (Cd)			
Actual analyte conc. (µg/mL)	**Calculated analyte conc. (µg/mL)**	**Recovery percentage (%)**	**RSD (%)**
0.1	0.0941 ± 0.0085	94.08 ± 8.49	9.03
0.2	0.1946 ± 0.0042	97.30 ± 2.12	2.18
0.3	0.2883 ± 0.0091	96.08 ± 3.02	3.14
Chromium (Cr)			
Actual analyte conc. (µg/mL)	**Calculated analyte conc. (µg/mL)**	**Recovery percentage (%)**	**RSD (%)**
0.1	0.0851 ± 0.0045	85.13 ± 4.53	5.32
0.2	0.1876 ± 0.0036	93.80 ± 1.82	1.94
0.3	0.2899 ± 0.0104	96.62 ± 3.58	3.58
Cuprum (Cu)			
Actual analyte conc. (µg/mL)	**Calculated analyte conc. (µg/mL)**	**Recovery percentage (%)**	**RSD (%)**
0.1	0.0990 ± 0.0071	99.02 ± 7.11	7.18
0.2	0.2086 ± 0.0097	104.28 ± 4.84	4.64
0.3	0.3107 ± 0.0097	103.57 ± 3.23	3.12
Nickel (Ni)			
Actual analyte conc. (µg/mL)	**Calculated analyte conc. (µg/mL)**	**Recovery percentage (%)**	**RSD (%)**
0.1	0.0922 ± 0.0030	92.19 ± 3.00	3.26
0.2	0.1789 ± 0.0015	89.43 ± 0.73	0.82
0.3	0.2762 ± 0.0054	92.08 ± 1.80	1.95
Timbal			
Actual analyte conc. (µg/mL)	**Calculated analyte conc. (µg/mL)**	**Recovery percentage (%)**	**RSD (%)**
0.1	0.0996 ± 0.0057	99.63 ± 5.69	5.71
0.2	0.1988 ± 0.0040	99.43 ± 2.00	2.01
0.3	0.3021 ± 0.0059	100.71 ± 1.96	1.95

Accuracy of the developed method was assessed using standard addition method and is expressed as recovery. Accuracy can determine the lack of analyte levels due to the losses or contamination during sample preparation, and matrix interferences during the measurement step (Ertas and Tezel, 2004). The recovery determination was carried out by spiking technique. Known concentration of standard solutions (Cd, Cr, Cu, Ni, and Pb) were added to snake fruit, and the resulting spiked samples were measured, calculated, and compared to the known value of standard solutions added. As suggested by ICH (2005), the analytical steps were performed in three different levels of analyte concentration, with three replicates for each level of concentration. The recovery values for accuracy were shown in Table 3. According previous published study (Huber, 1998), the acceptable recovery percentage range is 80–110% for the analyte level of 1 µg/mL. Therefore, the developed method was accurate for quantitation of Cd, Cr, Cu, Ni, and Pb in snake fruit.

Determination of Cd, Cr, Cu, Ni, and Pb in snake fruit

The levels of Cd, Cr, Cu, Ni, and Pb in some marketed snake fruit samples were quantified using the developed method. The levels of Cd, Cr, Cu, Ni, and Pb in snake fruit were shown in Table 4.The levels of Cd, Cr, Cu, Ni, and Pb from six marketed snake fruit samples were found in the range of 0.2449–0.2962 mg/kg, 0.0658–0.1230 mg/kg, 0.4063–1.3982 mg/kg, 0.1157–0.1624 mg/kg, and 0.4097–0.5970 mg/kg, respectively.

According to Indonesian National Standard (2009), the maximum levels of Cd and Pb permissible in fruit were 0.2 mg/kg and 0.5 mg/kg, respectively. National Standard of the People's Republic of China (2012) set the maximum levels of Cr and Ni in foods were 0.5 mg/kg and 1.0 mg/kg, respectively. Meanwhile, maximum level of Cu in fruit was 5 mg/kg according to Romanian Ministry of Agriculture Food and Forestry Order (Roba *et al.*, 2016). The results showed that the levels of Cr, Cu, and Ni in commercially snake fruit samples were acceptable. Meanwhile, the levels of Cd in those snake fruit samples were higher than the level permitted by Indonesian National Standard. One of the snake fruit sample also contained unacceptable Pb level at 0.5970 mg/kg.

Table 4: The levels of Cadmium, chromium, copper, nickel, and lead content in marketed snake fruits.

Sample	Cd (mg/kg)	Cr (mg/kg)	Cu (mg/kg)	Ni (mg/kg)	Pb (mg/kg)
Salak 1	0.2449 ± 0.0147	0.0681 ± 0.0033	0.4063 ± 0.0554	0.1242 ± 0.0055	0.4097 ± 0.0131
Salak 2	0.2765 ± 0.0054	0.0814 ± 0.0063	0.6697 ± 0.0320	0.1453 ± 0.0069	0.4258 ± 0.0214
Salak 3	0.2560 ± 0.0126	0.0658 ± 0.0050	1.3982 ± 0.0464	0.1157 ± 0.0121	0.4308 ± 0.0298
Salak 4	0.2930 ± 0.0127	0.0879 ± 0.0088	0.9620 ± 0.1447	0.1467 ± 0.0178	0.4542 ± 0.0340
Salak 5	0.2812 ± 0.0027	0.0960 ± 0.0045	0.7583 ± 0.0374	0.1456 ± 0.0070	0.4327 ± 0.0692
Salak 6	0.2962 ± 0.0137	0.1230 ± 0.0137	0.7447 ± 0.0622	0.1624 ± 0.0060	0.5970 ± 0.0093

CONCLUSION

Analytical method development of Cd, Cr, Cu, Ni, and Pb in snake fruit using ICP-AES has been developed. Evaluation of analytical method parameters including linearity, sensitivity, precision, and accuracy showed acceptable results. Furthermore, the developed method can be successfully used for determination of Cd, Cr, Cu, Ni, and Pb in snake fruits available in markets and farms. The levels of Cr, Cu, and Ni reported were lower than the maximum levels permitted. In

the other hand, the levels of Cd in all those samples and Pb in one of the sample were unacceptable.

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