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Determination of major-to-trace elements in hot chilli and tomato varieties economically grown in the Northeast of Thailand by ICP-OES following microwave assisted digestion

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Abstract

Multi-element species in hot chilli and tomato fruits at a red-ripe stage were determined using microwave assisted digestion (MAD) followed by inductively coupled plasma-optical emission spectrometry (ICP-OES). The ground sample (0.5 g) was digested with 5 mL of concentrate nitric acid by MAD programmed at 900 W for 35 min, and diluted to 25-mL with 10%(v/v) HCl prior to measurement by the validated method under the optimum conditions of ICP-OES. In this study, major elements (Ca, K, Mg, Na), minor elements (Fe, Zn, Mn, Cu, Si) and trace elements (Cr, Mo, Al, Ni, Pb, Ba) were comparatively classified among each group between hot chilli and tomato samples. It was found that the amounts of Ca (177.2-395.3 mg/kg) were predominant among the major ones for both kinds of the fruit samples. While those of Fe (20.7-43.3 mg/kg) and Zn (11.6-46.9 mg/kg) was comparable among the minor ones. For trace elements, Cr was also predominant for both fruit samples, while those of Mo and Al were considerably distributed among their varieties. From these minerals patterns, some of them available in these economic plants would be a useful database for daily mark of their consumption.

Introduction

There are micronutrients and minerals that concern us because of foods and food products consumption and/or occupational or residential exposure (Nnorom *et al.*, 2007; Demirbas, 2010; Bakircioglu *et al.*, 2011). Some minerals include alkali and alkaline earth, and heavy metal elements i.e. Sb, As, Cd, Cr, Co, Cu, Fe, Pb, Mn, Hg, Ni or Zn. Small amounts of the elements are common in our environment and diet and are actually necessary for good health, but large amounts of any of them may cause acute or chronic toxicity (Hamurcu *et al*., 2010; Ismail *et al*., 2011). The toxicity of some heavy metals can reduce mental and central nervous function, and damage to blood composition, lungs, kidneys, liver, and other vital organs (Reykdal *et al*., 2011).

Almost alkali and alkaline earth elements, which are the major ones such as Ca, Mg, Na, K, are useful for physiological functions of plants and animals. However, among those minerals, trace metals are still interesting ones that widely distributed in the environment, originating either from soil materials or from discharge of heavy metals onto land as a result of human activities. Consequently, people and livestock are being exposed to heavy metals via contamination of drinking water and consumption of foods grown in the contaminated soils or irrigated with toxic metals-contaminated water (Al-Lahham *et al.*, 2007; Lawal and Audu, 2011). Understanding how trace metals are taken up by plants and subsequently transformed in plant tissue is, therefore, essential for estimating the risks posed to human and wildlife populations (Mihali *et al.*, 2012). However, the residual contents of trace metals and its inorganic salts in such common fruits like hot chilli and tomato varieties, which are daily consumed, have not been reported. Thus, simple analytical procedures for sample preparation and measurement of minerals species are needed for routine analysis with high sensitivity, selectivity, accuracy and precision including short analysis time (Bakkali *et al*., 2009; Castro *et al*., 2009).

Several methods for the determination of trace metals have been commonly conducted by flame atomic absorption spectrometry (FAAS) (Bakkali *et al*., 2009; Lawal and Audu, 2011; Hina *et al*., 2011), graphite furnace atomic absorption spectrometry (GFAAS) (Bakkali *et al*., 2009; Ekinci and Koklu, 2000), inductively coupled plasma-atomic/optical emission spectroscopy (ICP-AES/OES) (Mikuła and Puzio, 2007; Fallah *et al.*, 2011; Jaric *et al*., 2011) or inductively coupled plasma-mass spectrometry

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(ICP-MS) (Llorent-Martinez *et al.*, 2011). Those are available for the determination of trace metals with sufficient sensitivity for most of applications. In recent years, each of them is still being used because it combines fast analysis time, relative simplicity, cheaper cost, low sample volume requirements and good analytical performance. All of these features have been responsible for its broad utilization in the determination of trace elements in different samples (Hamilton *et al*., 2008; Tokalioglu, 2012).

Some of them are briefly reviewed as following. The heavy metal contents including of Pb, Cd, Cu, Zn, Cr, Ni, Co, and Fe in herbal drugs were determined (Hina *et al*., 2011). The samples were digested with HNO_3 and 70% $HClO_4$. The heavy metals (ug g^{-1}) in herbal drugs were found in the range of 3.26-30.46 for Pb, 1.60-4.91 for Cd, 0.65-120.21 for Cu, 83.74-433 for Zn, 1.61-186.76 for Cr, 0.48-76.97 for Ni, 5.54-77.97 for Co and 65.68-1652.89 for Fe. The heavy metals (Cd, Cu, Mn, Cr and Pb) in tomato, pepper and onion were determined (Bakkali *et al.*, 2009). The sample digestion was carried out by microwave oven using 6 mL of conc. HNO_3 and 2 mL H_2O_2. The limit of detection was ranged from 0.05-2.20 ug kg^{-1} and limit of quantitation was found to be 0.15-7.37 µg kg^{-1}. The method recovery of the vegetable samples was 96-105%. The heavy metals (Co, Cu, Zn, Cr, Ni and Pb) in spinach, okra, tomato and onion samples were also determined (Lawal and Audu, 2011). Digestion of the samples by Kjaedahl method with H_2SO_4, H_2ClO_4 and HNO_3 in the ratio of 1:4:40 and left stand overnight. Thereafter, heated at 70°C for 40 min and then increased heating to 120°C. The contents of Co, Cu, Zn, Cr, Ni and Pb were found to be 1.14, 7.50, 18.89, 0.85, 2.20 and 1.60 mg kg^{-1}, respectively. Na, K, Ca and Mg in milk products were determined (Noel *et al*., 2008). The sample preparation was carried out using dry ashing or wet digestion with HNO_3. The repeatability expressed as relative standard deviation were 1.7-6.4%, 1.8-10.0%, 0.9-5.5% and 1.1-4.7% for Na, K, Ca, and Mg, respectively. The reproducibility in terms of relative standard deviations was 3.7-9.2%, 2.7-8.9%, 2.1-7.9% and 1.3-7.4% for Na, K, Ca, and Mg, respectively.

As mentioned above, many analytical methods for trace element determination in the sample materials require the decomposition of the sample (Santos *et al.*, 2010; Cindric *et al*., 2011). Hence, the sample separation is of great importance for obtaining desirable results for the analytes. The wet digestion and dry ashing procedures are quite slow (Masson *et al.*, 2006). Also these procedures are difficult to follow consistently. Microwave assisted digestion (MAD) is a rapid and efficient method for sample decomposition prior to the determination of trace metals (Mingorance, 2002; Qing-hua *et al*., 2012). In recent years, the microwave-assisted leaching technique has become a simple, rapid and powerful sample preparation method for extraction of heavy metals from environmental samples such as soils and sediments. This technique needs low consumption of reagent and time. On the other hand, digestion in sealed containers also reduces the risk of external contamination. These are all the advantages of microwave induced sample preparation method over the conventional hot-plate digestion method. The microwave oven heats the contents to a high temperature very rapidly and the closed vessel helps in preventing losses due to volatilization of elements.

The aim of this study was to investigate some minerals contents usually found in fruits and vegetables such as hot chilli and tomato samples, which are economically important plants grown in the Northeast of Thailand. The sample was digested with concentrate nitric acid using MAD prior to multi-elements determination by ICP-OES. By comparison, the multi-elements classified as major, minor and trace elements were confined according to their amounts found as residue and/or background contamination in the plant samples analyzed.

Materials and Methods

Chemicals

All chemicals used were of analytical grade. Stock standard solutions of each element were prepared by dissolving its salt in 10%(v/v) HCl including zinc nitrate hexahydrate (QREC, New Zealand), lead sulfate, manganese chloride (Ajax Chemicals, Australia), chromium chloride hexahydrate, aluminum chloride hexahydrate (Fluka, Italy), sodium chloride (BDH laboratory supplies, England), copper sulfate, potassium chloride and iron sulfate (Carlo Erba, Italy). The standard solutions of Mg, Ca, Ni, Si, Mo and Ba (Carlo Erba, Italy) were prepared from their commercial stocks (1000 mgL^{-1}). Hydrochloric acid and nitric acid (Lab Scan Asia, Thailand) were also used. Aqueous solutions were prepared with de-ionized water (Milli Q Millipore 18.2 $M\Omega cm^{-1}$ resistivity) by Simplicity water purification system, Simplicity 185, Millipore Corporation (USA).

Instruments

An inductively coupled plasma-optical emission spectrometry (Optima 2100 DV ICP-OES, Perkin Elmer Instruments, USA) equipped with WinLab 32, CCD detector and Echelle optical system was used.

Table 1. ICP-OES operational conditions

Parameter	Condition
RF power (W)	1300
Plasma gas flow rate (L/min)	15.0
Auxiliary gas flow rate (L/min)	0.20
Nebulizer gas flow rate (L/min)	0.80
Sample flow rate (L/min)	1.50
Element	Wavelength (nm)
Mo	202.031
Zn	206.604
Pb	220.353
Ni	231.604
Ba	233.270
Fe	238.204
Mn	257.610
Si	261.611
Cr	267.716
Mg	285.213
Ca	317.933
Cu	327.393
Al	396.153
Na	589.952
K	766.490

Some descriptions of the operational conditions and analytical lines used are summarized in Table 1. Microwave assisted digestion (Anton Paar, Multiwave 3000, Austria) equipped with a rotor holding for 8 PTFE cuvettes was used for sample preparation.

Sample materials

Hot chilli and tomato samples were collected randomly from natural cultivars which had been planted in horticulture fields without any supplement of chemical fertilizers at Department of Plant Science and Agricultural Resources, Khon Kaen University, Thailand. Six varieties of hot chilli commonly consumed, the so-called local Thai names; Jindanil 80, Numkaew Thong 80, Super Hot, Yodson Khem 80, Yodson Korat and Huay Sithon, are belong to the same species of *Capsicum annuum* L. Seven varieties of tomato at red-ripe stage (consisting of three kinds of the plant breeding code as PD09, PS07, GD, and four local Thai names as Cherry Kham Kaen, Puang Thong, Morrakot Daeng and Manee Siam) were obtained from pedigree selection of Lycopersicon esculentum Mill.). These plants were grown during September 2011 - February, 2012. The plant samples were dried in an oven at 60°C for 48 h and ground by a kitchen grinder (Philips, Indonesia) to pass a 35-mesh sieve. The ground samples were stored in a plastic bag in desiccator before use. Moisture content of the sample was determined by a drought oven set at 105°C until a constant weight was obtained. Dry matter of the samples was calculated as dry weight from the moisture content. The results were reported based on dry weight (DW) basis.

Sample preparation

The ground fruit sample (0.5 g) was accurately weighed into a high pressure PTFE vessel. Five-mL of concentrate nitric acid was added. The vessel was closed and placed inside the microwave oven unit. It was then heated following a one-stage digestion programmed at 900 W for 35 min. The acid digested solution was cooled and diluted to 25 mL final volume in volumetric flask with 10% (v/v) HCl.

Results and Discussion

Under the ICP-OES operating conditions for multi-elements measurement with their analytical lines used, differences in the suitable calibration ranges of these elements were established with $r^2 > 0.999$. The limit of detection (LOD) and the limit of quantification (LOQ) for fifteen metals were determined by analyzing five portions of standard solutions simultaneously following the general procedure. The LOD values in μgL^{-1} defined as 3σ where σ is the standard deviation were listed in Table 2. The LOQ values (10σ) were also determined to confine that trace amount of some elements could be certainly detectable, although some of other elements had been tried to be analyzed as well.

Major elements

For the six varieties of hot chilli samples, the results of major elements, i.e. Ca, K, Mg and Na are shown in Table 3. The average concentrations ($mgkg^{-1}$) among these varieties were 342.6, 175.6, 179.9 and 57.20 for Ca, K, Mg, and Na, respectively. It was found that Ca content is the highest, while those of K and Mg are comparable and well distributed among these varieties (Figure 1), except K and Mg which peak in Namkeawtong 80, and Huay sithon and Jindanil 80, respectively.

For the major elements that found in seven varieties of tomato samples, the content of Ca is also the highest (Table 4) and is comparable with that of hot chilli, while those of K, Mg and Na show the same pattern. These major elements are distributed well in these varieties of tomato samples (Figure 2), except in the Morrakotdaeng variety which contains quite low contents of the elements.

Minor elements

In six varieties of hot chilli samples, the average contents of Fe, Zn, Mn, Cu and Si are 26.42, 14.44, 7.99, 6.72 and 2.63 mg/kg, respectively (Table 5 and Figure 3). Also, the distribution of these elements among the chilli varieties is not much different. The minor elements of seven varieties of tomato samples are found in the same pattern (Table 6 and Figure 4), except their distribution of Zn. However, the lowest content of Fe is found in the Golden Princess variety.

Table 2. LOD, LOQ and calibration data of the elements determined by ICP-OES

Element	LOD (µgL^{-1})	LOQ (µgL^{-1})	Calibration range	r^2
Na	1.91	6.35	0.5-8 mgL^{-1}	0.9990
Mg	0.75	2.52	1-5 mgL^{-1}	0.9997
Ca	0.71	2.36	1-5 mgL^{-1}	0.9996
K	0.89	2.95	1-5 mgL^{-1}	0.9999
Al	0.66	2.18	1-20 µgL^{-1}	0.9993
Cr	0.33	1.09	1-20 µgL^{-1}	0.9996
Pb	0.08	0.25	1-20 µgL^{-1}	0.9998
Ni	0.03	0.11	1-20 µgL^{-1}	0.9997
Cu	0.07	0.24	0.05-0.25 mgL^{-1}	0.9993
Si	0.02	0.07	0.05-0.25 mgL^{-1}	0.9996
Mn	0.07	0.23	0.1-0.5 mgL^{-1}	0.9993
Mo	0.04	0.12	1-20 µgL^{-1}	0.9997
Fe	0.16	0.52	0.2-1.0 mgL^{-1}	0.9997
Zn	0.15	0.51	0.2-1.0 mgL^{-1}	0.9999
Ba	0.10	0.33	1-20 µgL^{-1}	0.9998

Table 3. The major elements contents found in six varieties of hot chilli samples

Sample	Concentration (mg/kg), mean ± SD; n = 5			
	Ca	K	Mg	Na
Huay sithon	272.0 ± 3.4	187.9 ± 2.1	265.1 ± 3.7	70.22 ± 0.75
Yodsonkorat	332.9 ± 2.4	152.6 ± 2.7	140.5 ± 2.0	17.40 ± 1.71
Superhot	386.2 ± 2.3	154.5 ± 2.7	128.5 ± 2.4	69.17 ± 2.1
Yodsonkhem	319.6 ± 3.5	145.0 ± 1.7	113.7 ± 1.5	64.10 ± 1.7
Jindanil 80	371.3 ± 3.1	145.8 ± 2.6	287.6 ± 2.9	68.51 ± 1.0
Num keawtong 80	373.3 ± 3.0	267.5 ± 1.3	143.8 ± 1.4	53.80 ± 1.3
Range	114.2	122.5	173.9	52.8
Average, n = 6	342.6	175.6	179.9	57.2

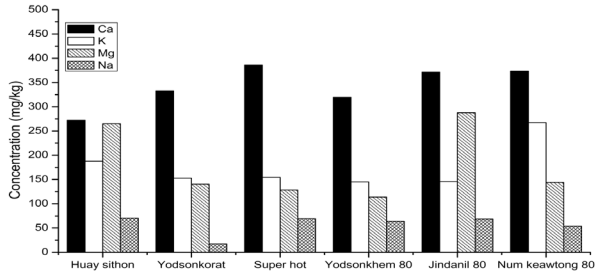

Figure 1. Illustrating graph showing bar plot of the major elements contents in six varieties of chilli samples

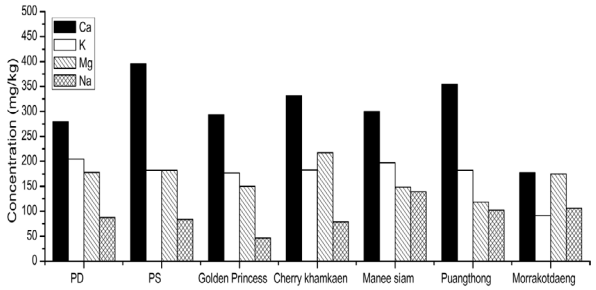

Figure 2. Illustrating graph showing bar plot of the major elements contents in seven varieties of tomato samples

Trace elements

In this study, trace element is defined at µgkg^{-1} level. It was found that the average contents of Cr, Mo, Al, Ni, Pb and Ba found in six varieties of chilli pepper samples were 763.8, 310.7, 354.8, 140.7, 155.7 and 80.36 µgkg^{-1}, respectively (Table 7 and Figure 5), indicating that the contents of Cr, Mo and Al are rather high and comparable among the chilli varieties. However, the Al content peaks in the varieties of Super hot and Numkeawtong 80, while other trace elements are equally distributed among the chilli varieties. For tomato samples, the average values of the trace elements are lower than those found in hot chilli, and the contents of Cr, Mo and Al are not laid in the same direction as shown in Table 8

Table 4. The major elements contents found in seven varieties of tomato samples

Sample	Concentration (mg/kg), mean ± SD; n = 5			
	Ca	K	Mg	Na
PD	280.0 ± 2.3	204.9 ± 2.4	178.0 ± 3.2	87.79 ± 1.6
PS	395.3 ± 2.4	182.2 ± 1.5	182.1 ± 1.9	83.64 ± 2.4
Golden Princess	293.6 ± 3.6	177.0 ± 2.3	150.0 ± 2.7	46.18 ± 0.31
Cherry khamkaen	331.8 ± 3.4	182.9 ± 1.7	217.3 ± 2.6	78.60 ± 1.7
Manee Siam	300.0 ± 3.2	197.2 ± 2.3	148.6 ± 1.8	139.1 ± 1.9
Puangthong	354.3 ± 3.0	182.1 ± 2.6	118.4 ± 1.3	101.9 ± 2.4
Morrakotdaeng	177.2 ± 1.5	91.0 ± 1.3	174.9 ± 2.4	106.2 ± 1.2
Range	218.1	113.9	98.9	92.9
Average, n = 7	304.6	173.9	167.0	91.92

Table 5. The minor elements contents found in six varieties of hot chilli samples

Sample	Concentration (mg/kg), mean ± SD; n = 5				
	Fe	Zn	Mn	Cu	Si
Huay sithon	28.94 ± 0.46	13.66 ± 0.41	8.620 ± 0.046	6.612 ± 0.33	2.595 ± 0.042
Yodsonkorat	20.69 ± 0.27	18.17 ± 0.61	8.057 ± 0.081	10.82 ± 0.13	2.663 ± 0.026
Superhot	27.74 ± 0.97	15.66 ± 0.53	6.670 ± 0.069	5.172 ± 0.13	2.657 ± 0.021
Yodsonkhem	22.80 ± 0.24	11.56 ± 0.27	7.927 ± 0.130	5.205 ± 0.038	2.586 ± 0.03
Jindanil 80	23.93 ± 0.76	13.79 ± 0.22	8.128 ± 0.039	4.814 ± 0.034	2.621 ± 0.041
Num keawtong 80	34.41 ± 0.84	13.79 ± 0.34	8.563 ± 0.032	7.710 ± 0.31	2.658 ± 0.005
Range	13.72	6.61	1.95	6.01	0.08
Average, n = 6	26.42	14.44	7.99	6.72	2.63

Table 6. The minor elements contents found in seven varieties of tomato samples

Sample	Concentration (mg/kg), mean ± SD; n = 5				
	Fe	Zn	Mn	Cu	Si
PD	42.71 ± 3.6	15.71 ± 1.1	8.901 ± 0.11	2.925 ± 0.044	2.649 ± 0.03
PS	43.28 ± 0.88	27.35 ± 0.73	11.74 ± 0.21	3.770 ± 0.038	2.837 ± 0.017
Golden Princess	22.23 ± 0.85	18.25 ± 0.93	7.681 ± 0.14	2.993 ± 0.041	2.609 ± 0.16
Cherry khamkaen	31.25 ± 1.4	18.89 ± 0.97	8.733 ± 0.14	3.358 ± 0.068	2.700 ± 0.052
Manee Siam	28.63 ± 1.3	46.88 ± 1.6	18.45 ± 0.55	8.680 ± 0.11	3.080 ± 0.094
Puangthong	23.56 ± 0.96	27.15 ± 0.49	13.49 ± 0.82	5.939 ± 0.068	2.818 ± 0.079
Morrakotdaeng	30.65 ± 0.72	36.03 ± 0.5	18.99 ± 0.42	6.200 ± 0.084	2.931 ± 0.086
Range	21.05	31.17	11.31	5.755	0.431
Average, n = 7	31.76	27.18	12.57	4.838	2.803

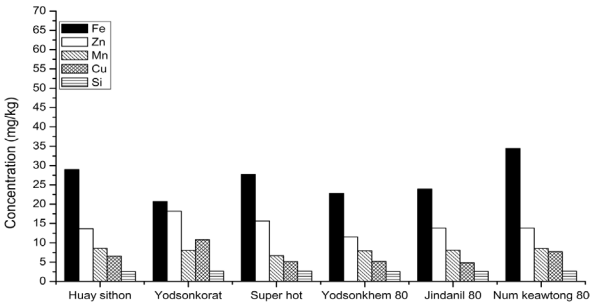

Figure 3. Illustrating graph showing bar plot of the minor elements contents in six varieties of hot chilli samples

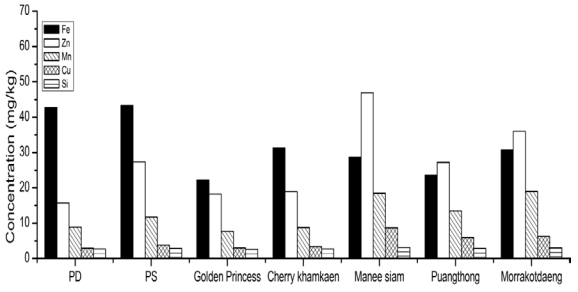

Figure 4. Illustrating graph showing bar plot of the minor elements contents in seven varieties of tomato samples

Table 7. The trace elements contents found in six varieties of hot chilli samples

Sample	Concentration (µg/kg), mean ± SD; $n = 5$					
	Cr	Mo	Al	Ni	Pb	Ba
Huay sithon	722.3 ± 0.013	401.7 ± 0.017	196.6 ± 0.047	164.9 ± 0.04	121.3 ± 0.021	82.78 ± 0.015
Yodsonkorat	757.1 ± 0.032	374.4 ± 0.016	138.2 ± 0.039	174.0 ± 0.009	165.1 ± 0.012	67.18 ± 0.01
Super hot	734.3 ± 0.018	284.5 ± 0.013	525.8 ± 0.154	162.5 ± 0.005	173.1 ± 0.02	65.79 ± 0.011
Yodsonkhem 80	786.0 ± 0.010	457.0 ± 0.007	255.2 ± 0.075	176.9 ± 0.009	160.5 ± 0.028	81.55 ± 0.008
Jindanil 80	735.0 ± 0.025	346.4 ± 0.02	332.1 ± 0.076	165.6 ± 0.015	137.8 ± 0.012	94.13 ± 0.004
Num keawtong 80	848.1 ± 0.009	388.9 ± 0.02	681. 0±0.03	213.9 ± 0.004	176.3 ± 0.013	90.74 ± 0.011
Range	125.8	172.5	542.8	51.4	55.0	26.95
Average, $n = 6$	763.8	310.7	354.8	140.7	155.7	80.36

Table 8. The trace elements contents found in seven tomato samples

Sample	Concentration (µg/kg), mean ± SD; $n = 5$					
	Cr	Mo	Al	Ni	Pb	Ba
PD	347.0 ± 0.022	277.7 ± 0.027	544.1 ± 0.112	140.4 ± 0.051	194.0 ± 0.042	63.23 ± 0.068
PS	634.2 ± 0.032	366.3 ± 0.012	73.61 ± 0.013	146.3 ± 0.024	88.71 ± 0.042	92.05 ± 0.005
Golden Princess	547.5 ± 0.049	290.4 ± 0.036	152.3 ± 0.017	112.9 ± 0.027	110.3 ± 0.007	61.52 ± 0.01
Cherry khamkaen	619.8 ± 0.044	192.6 ± 0.015	675.4 ± 0.04	107.6 ± 0.014	192.2 ± 0.038	90.42 ± 0.003
Manee siam	545.2 ± 0.035	121.2 ± 0.008	64.88 ± 0.015	91.22 ± 0.015	166.2 ± 0.017	84.70 ± 0.011
Puangthong	551.1 ± 0.038	52.85 ± 0.009	87.05 ± 0.017	85.23 ± 0.006	260.5 ± 0.079	82.70 ± 0.011
Morrakotdaeng	597.9 ± 0.076	95.89 ± 0.014	58.70 ± 0.008	354.7 ± 0.034	197.9 ± 0.019	89.11 ± 0.013
Range	287.2	313.5	616.7	269.5	171.8	30.53
Average, $n = 7$	549.0	199.6	236.6	148.3	172.8	80.53

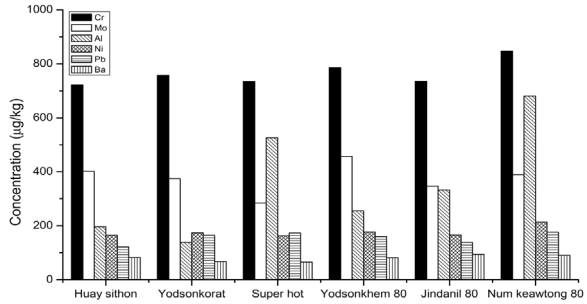

Figure 5. Illustrating graph showing bar plot of the trace elements contents in six varieties of hot chilli samples

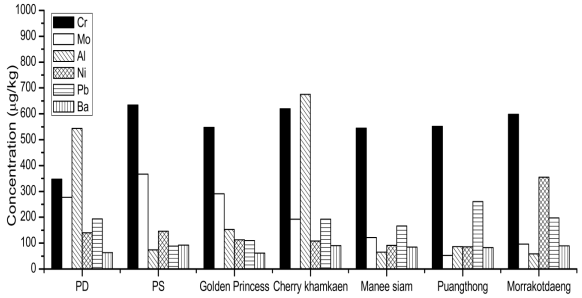

Figure 6. Illustrating graph showing bar plot of the trace elements contents in seven varieties of tomatoes

and Figure 6. The content of Al peaks in the PD and Cherry khamkaen varieties, while those of other trace elements are not much different among the tomato varieties.

Conclusion

The microwave digestion method with 0.5 g sample and 5 mL conc. HNO_3 gave faster, more safety and accurate results in association with simultaneous multi-element measurement by ICP-OES. The analytical method was successfully applied to determine multi-elements in these economic plants, both hot chilli and tomato varieties generally grown in Thailand. Since the measurement of intakes of various elements by man is of importance in nutrition, the results obtained can, therefore, be useful information with respect to their minerals consumption, because both kinds of the spicy chilli and sour or sweet tomato are very popular food ingredients in Asian foods and food products, especially in the mixture of the papaya salad with sticky rice available throughout Thailand.

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