RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2014.40.053

ScienceAsia 40 (2014): **53–59**

Determination of arsenic in chilli and tomato grown in North East Thailand

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Received 4 Apr 2013
Accepted 14 Dec 2013

ABSTRACT: Heavy metals in edible fruits constitute a health hazard. We present an analytical procedure for determining inorganic arsenic (As) species in hot chilli pepper and tomato fruits at the red-ripe stage using microwave assisted digestion (MAD) followed by flow injection-hydride generation atomic absorption spectrometry (FI-HGAAS). The optimum conditions for the acid digestion method and arsenic hydride (AsH_3) determination were studied in detail. The plant sample (0.5 g) was digested with 5 ml of concentrated nitric acid by MAD programmed at 900 W for 35 min. Arsenite, As(III), in the acid digests could only be analysed by FI-HGAAS using 1% (v/v) HCl as a carrier solution and 0.5% (w/v) $NaBH_4$ in 0.04% (w/v) NaOH as a reducing agent, while total As content was determined after pre-reduction of arsenate, As(V), to As(III) with 2% (w/v) thiourea prior to measurement. The concentration of As(V) was then calculated as the difference between total As and As(III). Detection limits for As(III) and As(V) were 0.004 and 0.006 µg/l, respectively. The relative standard deviation of the data was less than 5% ($n = 10$). The recovery of the samples spiked with 10 µg/l As was 98–103%. The proposed method was applied to determine traces of As in six varieties of hot chilli pepper (0.55–0.88 µg/g As(III) and 0.22–0.93 µg/g As(V)) and seven varieties of tomato (0.36–0.64 µg/g As(III) and 0.22–0.60 µg/g As(V)) samples.

KEYWORDS: speciation analysis, hydride generation, microwave digestion, atomic absorption spectrometry

INTRODUCTION

Heavy metals concern us because of occupational or residential exposure. Small amounts of the elements are common in our environment and diet and are necessary for good health, but large amounts of any of them may cause acute or chronic toxicity. The toxicity of some heavy metals can reduce mental and central nervous function, and deteriorate blood components, lungs, kidneys, liver, and other vital organs. Long-term exposure may result in slowly progressing physical, muscular, and neurological degenerative deficits [1,2].

Among toxic metals, arsenic (As) is still an interesting one as it is widely distributed in the environment, originating either from As in the soil parent material or from discharge of As onto land as a result of human activities. Consequently, people and livestock are being exposed to As via contamination of drinking water and consumption of food grown in the As-contaminated soil or irrigated with As-contaminated water. Understanding how As is taken up by plants and subsequently transformed in plant tissue is, therefore, essential for estimating the risks posed to human and wildlife populations [3]. In aerobic soils, arsenate, As(V), is the most thermodynamically stable and hence dominant species. The uptake of As(V) by plants has been studied extensively. The transformation of As(V) in the plant tissue and its controlling factors and the subsequent translocation of As species from roots to shoots are, however, not well understood. In terrestrial plants, both organic and inorganic As species have been found with the inorganic species of arsenate, As(V) and arsenite, As(III), being the most dominant [4–8]. Biosorption of As(III) and As(V) from aqueous solution by various sources of algae, fungus, or lichen biomass has been reported in terms of equilibrium and kinetics studies [9–12]. This transformation of the inorganic arsenic species can be implemented into the plant tissue. Generally, only small levels of organic As species have been detected in plant tissue; however, it is unclear whether these species are a product of transformation in plants or whether they are simply taken up from the soil [3,8]. The residual contents of As and its inorganic speciation in common edible fruits like hot chilli pepper and

tomato varieties have not been reported. This novel finding was achieved by an optimization study on the sample treatment using microwave assisted digestion (MAD) prior to measurement by flow injection-hydride generation atomic absorption spectrometry (FI-HGAAS). Thus simple analytical procedures for sample preparation and measurement of inorganic arsenic species are needed for routine analysis with high sensitivity, selectivity, accuracy and precision including short analysis time.

This study aimed to investigate optimum conditions for the determination of As(III) and As(V) using FI-HGAAS and suitable procedure for sample preparation using MAD method to determine inorganic As species in hot chilli pepper and tomato samples [13, 14].

MATERIALS AND METHODS

Chemicals

All reagents and solutions were prepared from analytical reagent grade of chemicals using de-ionized water (Milli Q Millipore 18.2 M cm^{-1} resistivity) by water purification system (Simplicity 185, Millipore Corporation, USA). The atomic absorption spectroscopy standard solution (1000 mg/l) of As(III) was obtained from Spectrosol (England). The stock standard solution of As(V) was prepared by dissolving $AsHNa_2O_4 \cdot 7\,H_2O$ from Sigma-Aldrich (USA) in 2% (v/v) HNO_3. Sodium tetrahydroborate from Ajax Fine Chem (Australia) dissolved in sodium hydroxide from Carlo Erba (Italy) was employed. Hydrochloric acid solution was prepared from 37% (w/v) HCl (Lab Scan Asia, Thailand). Nitric acid (Lab Scan Asia, Thailand), thiourea (BDH laboratory supplies, UK), potassium iodide (Carlo Erba, Italy), potassium bromine (Asia Pacific Specialty Chemicals Ltd., Australia), ascorbic acid (Carlo Erba, Italy) were also used.

Instruments

Measurement of As was performed using an atomic absorption spectrometer of the Perkin Elmer Instruments AAnalyst 100 equipped with a flow injection analysis system (FIAS-100, Perkin Elmer Instruments, USA), used for continuous flow-hydride generation. The FIAS-100 consists of a peristaltic pump, five-port valve with 500 µl sample loop and a regulated gas control. Argon gas was used as carrier gas for the transposition of arsine (AsH_3) from the gas-liquid separator to the electrically heated quartz tube (900 °C). For AAS using As electrodeless discharge lamp (EDL, 380 mA), arsenic absorption line was detected at 193.7 nm with 0.7 nm slit width. Microwave assisted digestion (Anton Parr 3000, Austria) with 8 units of a high pressure PTFE vessel was used.

Plant 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 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 in a kitchen grinder (Philips, Indonesia) to pass a 35-mesh sieve. The ground samples were stored in plastic bags and stored in desiccator [15, 16]. Moisture content of the samples 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 are reported based on dry weight basis [17].

Microwave assisted digestion

The ground plant sample (0.5 g) was accurately weighed into a high pressure PTFE vessel and 5 ml of concentrated HNO_3 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-digests solution was cooled and diluted to 25 ml final volume in a volumetric flask with 10% (v/v) HCl. The method recovery was done by spiking 10 µg/l each of As(III) and As(V) into the ground sample before digestion by MAD.

Analytical methods for As(III), As(V), and total As

Standard solutions (10 µg/l) of As(III) and As(V) were prepared by a step dilution with 10% (v/v) HCl from its stock solution (1 g/l). The working solution for both As species was prepared daily. The calibration curve (1.0, 2.0, 3.0, 4.0, and 5.0 µg/l) for As(III) determination was established, using the standard solution prepared in 10% (v/v) HCl, by plotting the absorbance obtained versus their concentration. Also for As(V), the standard curve was done in the same

manner using the working solution of As(V) including 1 ml of 2% (w/v) thiourea as a pre-reduction agent. In addition, total inorganic As standard solution was prepared by a mixture of each appropriate contents of both As(III) and As(V) standard solution including 2% (w/v) thiourea.

Optimization conditions for As determination by FI-HGAAS

An HCl solution used as a carrier solution was prepared by an appropriate dilution of concentrated HCl with de-ionized water. The carrier solution was investigated by varying the concentration of HCl in the range 0.5–9% (v/v). A $NaBH_4$ solution was prepared fresh daily by dissolving an accurate amount of $NaBH_4$ in dilute NaOH solution and used as a reducing agent. The reducing agent was a major factor for the arsine formation which was studied by varying the concentration of 0.1–1% (w/v) of $NaBH_4$ dissolved in the base solution. In addition, the concentration of NaOH for stabilizing the reducing agent was also optimized between 0.01 and 0.09% (w/v).

Effect of pre-reduction agents on As(V) and total As determination

Some selected pre-reducing agents including potassium iodide (KI), potassium bromine (KBr), thiourea, ascorbic acid solutions in the concentration range 1.0–6% (w/v) were prepared by dissolving a required amount of KI, KBr, thiourea, and ascorbic acid in de-ionized water. For mixture of the pre-reducing agents, KI plus ascorbic acid and KI plus thiourea were prepared by dissolving KI with 0.3% (v/v) ascorbic acid and 2% (v/v) thiourea, respectively. For the determination of As(V), aliquots (2 ml) of the acid digested solution was pipetted into a 10 ml volumetric flask. Then 1 ml of 2% (w/v) thiourea solution was added and diluted with 10% (v/v) HCl to the final volume. This solution was left to stand for 15 min prior to analysis. The concentration of total inorganic As, As(III) plus As(V), was obtained, if the As(III) originally presented in the solution. Thus the As(V) content in the sample can be calculated by the difference between the original content of As(III) and sum of total inorganic As one. It is noted that in case of the acid digestion of the plant sample, organoarsenic species were negligible.

RESULTS AND DISCUSSION

Optimization conditions for FI-HGAAS

Firstly, experiments were carried out to optimize the hydride generation (HG) conditions for the speciation analysis, since the performance of FI-HGAAS system will give rising in ultra-trace level with high sensitivity and selectivity. Such parameters for the HG conditions were optimized including various concentrations of HCl as carrier solution and $NaBH_4$ in NaOH as reducing agent for both As(III) and As(V). Working conditions of As determination by FI-HGAAS are summarized in Table 1. Pre-reduction of As(V) to As(III) was necessary for the determination of As(V) and total inorganic arsenic.

Table 1 Instrumental parameters and working conditions of As determination by FI-HGAAS.

Parameter	Condition
Measurement mode	Absorbance
Slit width	0.70 nm
Wavelength	193.7 nm
Quartz tube temperature	900 °C
Lamp current	380 mA
Energy	48
HCl concentration	1% (v/v)
$NaBH_4$ concentration	0.5% (w/v) in 0.04% (w/v) NaOH
Purging gas	70 ml/min argon
Sample loop	500 µl

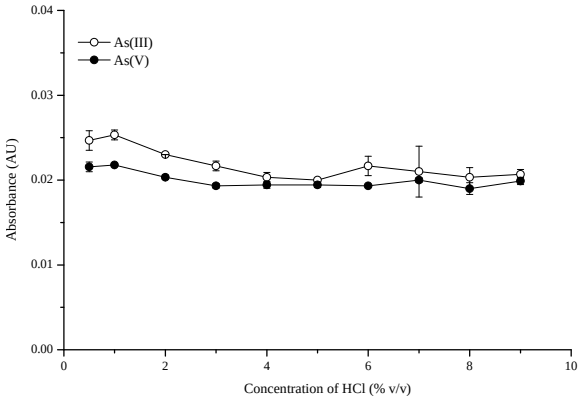

Fig. 1 Effect of HCl concentration on the absorbance of As(III) and As(V) in the presence of 0.5% (w/v) $NaBH_4$ in 0.04% (w/v) NaOH.

Effect of HCl concentration as carrier solution

The influence of HCl concentration in carrier solution was investigated in the range 0.5–9% (v/v) (Fig. 1). It was found that varying concentration of HCl did not produce much difference on absorbance. Lower concentration of HCl between 0.5 and 1% (v/v) gave considerably higher absorbance, while higher concentrations of the acid did not improve the analytical signal and it would cause severe effervescence and splashing of solution droplets on the gas-liquid separator inner walls to fast reaction. Then about 1% (v/v) HCl solution was chosen as the acid medium.

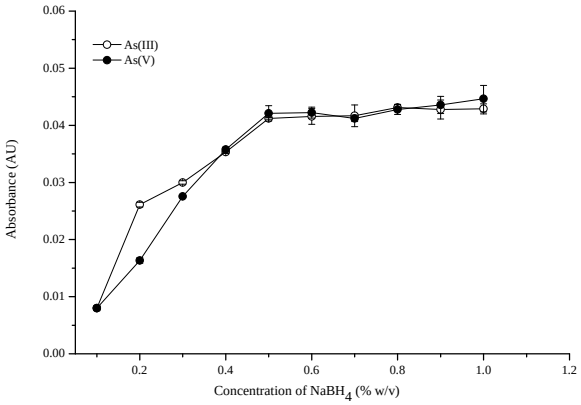

Fig. 2 Effect of $NaBH_4$ concentration on the absorbance of As(III) and As(V) in the presence of 0.04% (w/v) NaOH and 0.1% (w/v) HCl as carrier solution.

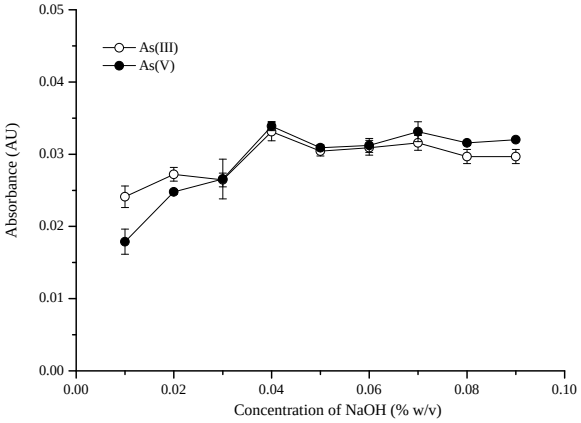

Fig. 3 Effect of NaOH concentration on the absorbance of As(III) and As(V) in the presence of 0.5% (w/v) $NaBH_4$ and 0.1% (w/v) HCl as carrier solution.

Effect of $NaBH_4$ concentration as reducing agent

The concentration of $NaBH_4$ is an important parameter for arsine generation because it is formed in the present of hydrogen generated by $NaBH_4$ in an acidic medium. It was found that the absorbance for As determination increased linearly when increasing the $NaBH_4$ concentrations from 0.1–0.5% (w/v), after that it tended to be constant (Fig. 2). Thus 0.5% (w/v) $NaBH_4$ was preferably used for further experiment.

Effect of NaOH concentration as auxiliary stabilizing the reducing agent

To stabilize the $NaBH_4$ solution, the concentration of NaOH was also investigated in the range 0.01–0.09% (w/v). From Fig. 3, the absorbance increased slightly when increasing of NaOH concentration between 0.01 and 0.04% (w/v), beyond that it had no change. Hence, 0.04% (w/v) NaOH was also important one as an auxiliary reagent for the reducing system.

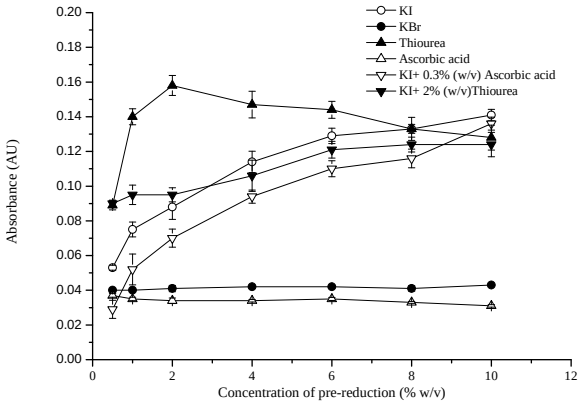

Fig. 4 Effects of pre-reducing agents on the absorbance of As(III) in the presence of 0.5% (w/v) $NaBH_4$ in 0.04% (w/v) NaOH as reducing agent and 0.1% (v/v) HCl as carrier solution.

Effect of pre-reduction agent on As(V) to be As(III)

Various types of pre-reducing agents had been used to reduce As(V) to be As(III) including KI[18], KBr, thiourea[18], ascorbic acid, KI plus ascorbic acid[19,20], and KI plus thiourea[21] solutions. The concentration of the pre-reducing agent was investigated in the range 0.5–10% (w/v) (Fig. 4). It was found that 2% (w/v) thiourea gave the highest absorbance among others in this experiment.

The optimized conditions for FI-HGAAS were therefore obtained as follows: 1% (v/v) HCl as a carrier solution, and 0.5% (w/v) $NaBH_4$ in 0.04% (w/v) NaOH as reducing agent. Pre-reduction of As(V) prior to the determination of As(III) was demonstrated by using 2% (w/v) thiourea. Under this optimum condition of the instrument, limit of detection (LOD) and limit of quantification (LOQ) were calculated by showing the probability density function for normally distributed measurements of the blank, at the LOD defined as 3 standard deviations of the blank, and at the LOQ defined as 10 standard deviations of the blank. LOD and LOQ of FI-HGAAS for As detection were found to be 0.006 µg/l and 0.022 µg/l, respectively.

Sample preparation by MAD

The sample preparation for chilli and tomato samples by using microwave assisted digestion was carried out. The optimum conditions for MAD were also investigated in detail in order to reduce the plant

Table 2 Effect of the sample amounts on the recovery of spiked 10 µg/l As(III) in chilli pepper and tomato samples.

Amount of sample (g)*	Chilli pepper	Tomato
0.25	$86 \pm 4\%$	$116 \pm 5\%$
0.50	$99 \pm 4\%$	$101 \pm 4\%$
0.75	$97 \pm 5\%$	$90 \pm 6\%$
1.00	$94 \pm 3\%$	$90 \pm 5\%$

* with 5 ml concentrated HNO_3 and at 900 W for 35 min.

Table 3 Effect of microwave powers on the recovery of spiked 10 µg/l As(III) in chilli pepper and tomato samples.

Microwave power (W)	Chilli pepper*	Tomato*
700	$111 \pm 3\%$	$95 \pm 2\%$
800	$119 \pm 5\%$	$87 \pm 3\%$
900	$98 \pm 2\%$	$100 \pm 1\%$
1000	$93 \pm 4\%$	$87 \pm 5\%$

* 0.5 g sample in 5 ml concentrated HNO_3 and at 700–1000 W for 35 min.

matrices which might affect the digestion efficiency by varying sample amount, microwave power and digestion time by a simple optimization method comparing its response in terms of recovery (%) of the As content in selected real sample. The obtained results are shown in Tables 2–4. It was found that using 900 W microwave current for 35 min digestion time can be used for both tomato and chilli samples with 0.5 g sample weight and 5 ml concentrated HNO_3.

Analytical performance

The calibration curve and its regression equation obtained for both As(III) and As(V) standard solutions are shown in Table 5. The calibration graphs are linear up to a concentration of 10 µg/l for As(III) and As(V), but their linear analytical range for sample determination is selected between 1.0 µg/l and 5.0 µg/l. The detection limits based on $3\sigma/m$, where σ is the standard deviation of 10 measurements of a blank and m the slope of the calibration graphs, were 0.004 µg/l for As(III) and 0.006 µg/l for As(V).

Table 4 Effect of digestion times on the recovery of spiked 10 µg/l As(III) in chilli pepper and tomato samples.

Digestion time (min)	Chilli pepper*	Tomato*
25	$69 \pm 5\%$	$103 \pm 2\%$
30	$73 \pm 6\%$	$107 \pm 2\%$
35	$98 \pm 2\%$	$116 \pm 5\%$
40	$89 \pm 5\%$	$84 \pm 5\%$

* 0.5 g sample in 5 ml concentrated HNO_3 and at 900 W.

Table 5 Analytical characteristics for both As(III) and As(V) determination under the optimized conditions of MAD and FI-HGAAS methods.

Analytical characteristic	As(III)	As(V)
Calibration equation	$y = 0.0099\,x + 0.00007$	$y = 0.0153\,x + 0.0069$
Correlation coefficient (r^2)	0.9995	0.9988
Linear analytical range (µg/l)	1.0–5.0	1.0–5.0
RSD (%, $n = 10$)	4.99	5.32
Detection limit (µg/l)	0.004	0.006

Table 6 Recovery of spiked 10 µg/l of As(III), As(V) and total As in three chilli varieties.

Sample	As(III)	As(V)	Total As
Yodson Khem	$98 \pm 2\%$	$121 \pm 3\%$	$98 \pm 4\%$
Jindanil 80	$98 \pm 5\%$	$91 \pm 5\%$	$107 \pm 2\%$
Super Hot	$93 \pm 5\%$	$105 \pm 4\%$	$84 \pm 4\%$

Recovery study

The recovery was studied by spiking each of 10 µg/l standard solution of As(III), As(V) and total As into the ground chilli and tomato samples. Under the optimized conditions for MAD, the recovery methods of As(III) found in both plant samples were obtained (Tables 2–4) as mentioned above. Emphasizing on some varieties of the hot chilli, the recoveries (%) of As(III) for three kinds of the samples used were found in the range 93–98%. Those of As(V) were ranged 91–122% and total As were also investigated and found to be 84–107% as summarized in Table 6. In this case, the method recovery study of which an ultra-trace amount of each of standard solution of As(III) and As(V) was used to spike into the ground sample mixture. The variable ranges of the obtained results were still found due to difference in these sample matrices, although an organoarsenic species present in the plant samples was negligible.

Real sample analysis

The proposed method was applied for the trace determination of inorganic As species in hot chilli and tomato samples under the optimum conditions for MAD and FI-HGAAS. The contents of both As(III) and As(V) including total As in six varieties of hot chilli and seven varieties of tomato are summarized in Table 7 and Table 8, respectively. The contents of As(III), As(V) and total arsenic in chilli samples were in the range 0.547–0.878 µg/g, 0.219–0.930 µg/g and 0.766–1.58 µg/g, respectively. And those of As(III), As(V) and total arsenic in tomato samples were in the range 0.358–0.637 µg/g, 0.218–0.596 µg/g and 0.583–

Table 7 The contents of inorganic As species (μg/g) in some chilli varieties (mean ± SD; $n = 5$).

Sample	As(III)	As(V)	Total As
Jindanil 80	0.79 ± 0.08	0.38 ± 0.20	1.17 ± 0.25
Super Hot	0.55 ± 0.06	0.22 ± 0.06	0.77 ± 0.09
Yodson Khem 80	0.65 ± 0.17	0.30 ± 0.18	0.96 ± 0.29
Numkaew Thong 80	0.88 ± 0.17	0.25 ± 0.11	1.12 ± 0.17
Yodson Korat	0.76 ± 0.09	0.63 ± 0.17	1.39 ± 0.20
Huay Sithon	0.65 ± 0.07	0.93 ± 0.34	1.58 ± 0.32

Table 8 The contents of inorganic As species (μg/g) in some tomato varieties (mean ± SD; $n = 5$).

Sample	As(III)	As(V)	Total As
PD 09	0.36 ± 0.06	0.23 ± 0.17	0.59 ± 0.12
PS 07	0.55 ± 0.14	0.22 ± 0.10	0.76 ± 0.20
GD	0.64 ± 0.18	0.60 ± 0.27	1.23 ± 0.45
Cherry Kham Kaen	0.41 ± 0.11	0.32 ± 0.10	0.73 ± 0.07
Puang Thong	0.41 ± 0.20	0.34 ± 0.19	0.75 ± 0.05
Morrakot Daeng	0.36 ± 0.16	0.23 ± 0.16	0.58 ± 0.15

1.233 μg/g, respectively.

In conclusion, highly sensitive and simple method for the determination of inorganic As species by FI-HGAAS was obtained. The method exhibited very low detection limit of 0.006 ng/l and limit of quantitation of 0.022 ng/l. The calibration curve of As was linear in the range 1.0–5.0 μg/l with an $r^2 > 0.999$. This method was then applied for the determination of As(III), As(V) and total arsenic in some hot chilli and tomato samples after an optimized sample preparation MAD. The MAD method with small amount of 0.5 g sample and 5 ml concentrated HNO_3 gave faster, more safe, and accurate results. The proposed analytical method was successfully applied to determine As in these economic plant samples.

Acknowledgements: This study was supported by the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission, through the Food and Functional Food Research Cluster of Khon Kaen University. The Centre of Excellence for Innovation in Chemistry (PERCH-CIC), Commission on Higher Education, Ministry of Education is also gratefully acknowledged.

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