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Identification of kinetin and kinetin riboside in coconut (*Cocos nucifera* L.) water using a combined approach of liquid chromatography–tandem mass spectrometry, high performance liquid chromatography and capillary electrophoresis

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Abstract

Kinetin (free base and riboside), which was assumed by many scientists to be a synthetic cytokinin plant growth hormone, has been detected for the first time in the endosperm liquid of fresh young coconut fruits ("coconut water"). To facilitate the study, we developed a sensitive liquid chromatography–tandem mass spectrometry (LC–MS/MS) method for the identification and quantification of kinetin and kinetin riboside in purified coconut water extract sample. Following a solid-phase extraction of cytokinins in coconut water using C_{18} columns, the samples were further purified by Oasis MCX columns and analyzed by LC–MS/MS for kinetin and kinetin riboside. Detection by mass spectrometry was carried out using selected reaction monitoring (SRM) mode, by identifying the putative kinetin and kinetin riboside based on their characteristic fragments. Based on a signal-to-noise ratio of 3, the limits of detection in SRM mode were 0.02 μM and 0.005 μM for kinetin and kinetin riboside, respectively. Furthermore, optimal conditions for a baseline chromatographic separation of 18 cytokinin standards by high performance liquid chromatography (HPLC) were developed. The HPLC method had been employed for the confirmation and further fractionation of kinetin in coconut water extracts. The confirmation and fractionation of kinetin riboside was carried out using a further modified HPLC program due to the presence of other interfering material(s) in the sample matrix. Finally, fractions of putative kinetin and kinetin riboside collected from HPLC eluate of coconut water sample were further authenticated by independent capillary zone electrophoresis (CZE) experiment.
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Keywords: Kinetin; Kinetin riboside; Liquid chromatography; Tandem mass spectrometry; Selected reaction monitoring; Capillary electrophoresis; Coconut water

1. Introduction

Kinetin (N^6-furfuryladenine) was first isolated and identified in 1955 [1,2] from DNA as an artifactual rearrangement product of the autoclaving process. Since then this compound has been widely used as a synthetic cytokinin in various aspects of plant research, including applications in biotechnology and cell biology [3–5]. Most importantly, kinetin also exerts anti-ageing effects in plants [6–8], as well as in human skin cells and fruitflies [9–11]. Although the biological significance of endogenous kinetin and the molecular mechanisms of its action are not completely understood at present, several lines of evidence indicate that kinetin may act directly as an antioxidant or indirectly as a regulator of antioxidants in vitro and in vivo, with potential beneficial uses in agriculture and human healthcare [12–14]. For example, kinetin has been shown to (i) have a direct effect on superoxide dismutase activity in plants [15]; (ii) prevent oxidation of unsaturated acids in plant membranes [16]; (iii) have effective free radical-scavenging activity in vitro and antithrombotic activity in vivo [17,18]; (iv) pro-

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tect DNA against oxidative damage [19] and (v) rescue human mRNA splicing defect [20]. Previously, sources of kinetin in biological samples were from DNA preparations [21,22], human urine [23], highly processed coconut kernel tissues [21], and roots of casuarina and palmarosa with symbiotic associations [24,25].

The extensive use of coconut water (coconut liquid endosperm) as a growth-promoting component in tissue culture goes back more than half a century, when Van Overbeek et al. first introduced coconut water as a new component of nutrient medium for callus cultures in 1941 [26]. Coconut water, which contains many uncharacterized biochemicals that can act as growth factors individually or synergistically [27], is commonly used at 2–30% (v/v) for plant tissue culture [27–30]. A number of attempts to identify the cytokinins in coconut water have been reported [31–35], but there is no known report for the natural occurrence of kinetin or its riboside in coconut water. Recently, we reported on the identification and quantification of two isoprenoid cytokinins (*trans*-zeatin *O*-glucoside and dihydrozeatin *O*-glucoside) and one aromatic cytokinin (*ortho*-topolin) in young coconut water using a combination of capillary electrophoresis (CE), LC and LC–MS [36,37]. Our previous studies add to the growing list of cytokinins present in coconut water which now includes zeatin, zeatin riboside, *trans*-zeatin *O*-glucoside, dihydrozeatin *O*-glucoside and *ortho*-topolin. Unlike the earlier study [21], where highly processed coconut kernel tissues were used for experiment, the use of endosperm liquid of fresh young coconut fruits ("coconut water") is a good way to ensure that there is no artifact generated during the entire extraction and purification process of the putative kinetin and its related compounds from the coconut water.

In addition to the previously described CE method, the most common techniques utilized in cytokinin analyses are gas and liquid chromatography combined with mass spectrometry (GC–MS, LC–MS) [38–41] as well as enzyme-linked immunosorbent assay or radioimmunoassays (ELISA, RIA) [42,43] of LC fractionated samples. However, even when a suitable chromatographic separation of cytokinin standards is available, the chromatograms of naturally-occurring cytokinin sample always contain a large number of peaks, of which the identity cannot be properly established. In contrast to the common techniques, tandem mass spectrometry is an attractive technique, allowing for the identification of cytokinins in natural products without special chromatographic method development.

In this paper, we describe a sensitive and selective LC–MS/MS method for the qualitative identification and quantification of kinetin and kinetin riboside in coconut water. Based on the LC–MS/MS results, we detected the presence of kinetin free base and kinetin riboside in the endosperm liquid of young fresh coconut fruits. Further, high performance liquid chromatography (HPLC) methods have been developed for the confirmation and further fractionation of kinetin and kinetin riboside in the coconut water sample. The identity of kinetin and kinetin riboside in coconut water is further orthogonally confirmed by capillary zone electrophoresis (CZE) which operates on different separation mechanism from that of HPLC.

2. Experimental

2.1. Chemicals and materials

The standards: kinetin (K), kinetin riboside (KR), *trans*-zeatin (Z), *trans*-zeatin *O*-glucoside (ZOG), *trans*-zeatin 9-glucoside (Z9G), *trans*-zeatin 7-glucoside (Z7G), *cis*-zeatin (cZ), dihydrozeatin *O*-glucoside (DZOG), isopentenyladenine (iP), *ortho*-topolin (oT) and *ortho*-topolin riboside (oTR) were obtained from OlChemIm Ltd., Czech Republic; *trans*-zeatin riboside (ZR), *cis*-zeatin riboside (cZR), *trans*-zeatin *O*-glucoside riboside (ZROG), isopentenyladenine riboside (iPR), benzylaminopurine (BA), *meta*-topolin (mT) and *meta*-topolin riboside (mTR) were gifts from Prof. Stuart Letham at the Australian National University (Table 1). Methanol (HPLC grade) was from J.T. Baker (Phillipsburg, NJ, USA); triethylamine (HPLC grade) was from BDH (England, UK). All other chemicals were of analytical reagent grade. Acetic acid and formic acid were purchased from Fisher Scientific; ammonia solution (28%) was purchased from APS Finechem (NSW, Australia). Ammonium phosphate, monobasic, ammonium phosphate, dibasic and phosphoric acid were purchased from Sigma (St. Louis, MO, USA). Water purified with a Milli-Q system (Waters, Milford, MA, USA) was used throughout the study. The pH of the buffer solutions was monitored using a pH meter (CORNING 440, Corning Glass Works; NY, USA).

2.2. Coconut water sample pretreatment and the isolation of cytokinins

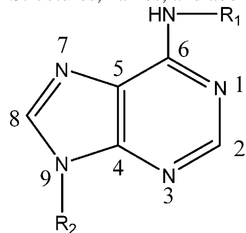
Coconut water was obtained from fresh coconut fruits (Emanate Agricultural Industries Ltd., Selangor, Malaysia) purchased from a supermarket. The procedure for the extraction and purification of putative cytokinins from coconut water using C_{18} and mixed mode SPE columns (Oasis MCX, Waters, Milford, MA, USA, 3 mL) [44] for further analyses, was adapted from the method described by Ge et al. [36].

A blank experiment was carried out to ensure that kinetin and kinetin riboside did not originate from the multi-steps sample pretreatment and purification procedure. Therefore same volume of acidified Milli-Q water (pH adjusted to 3 by acetic acid) was subjected to the same sample pretreatment and purification procedure [36], and subsequently analyzed using LC–MS/MS experiment (see Section 2.4).

2.3. Recovery study of the multi-steps SPE procedure

The extraction recoveries of kinetin and kinetin riboside using C_{18} and mixed mode SPE columns were also investigated. Unlabeled kinetin and kinetin riboside standards (6 nmol each) were added to 600 mL coconut water, and extracted according to the same sample preparation procedure [36]. The analysis of kinetin and kinetin riboside in the spiked extracted sample was then processed using LC–MS/MS measurement.

Table 1
Structures, names, and abbreviations of cytokinins used to develop HPLC process



R_1	R_2	R_3	Compound	Abbreviation
	H	H	*trans*-Zeatin	Z
	R	H	*trans*-Zeatin Riboside	ZR
	H	G	*trans*-Zeatin *O*-glucoside	ZOG
	G	H	*trans*-Zeatin 9-glucoside	Z9G
	–	H	*trans*-Zeatin 7-glucoside[a]	Z7G
	R	G	*trans*-Zeatin *O*-glucoside Riboside	ZROG
	H	H	*cis*-Zeatin	cZ
	R	H	*cis*-Zeatin Riboside	cZR
	H	G	Dihydrozeatin *O*-glucoside	DZOG
	H	–	Isopentenyladenine	iP
	R	–	Isopentenyladenine Riboside	iPR
	H	–	Benzylaminopurine	BA
	H	–	*meta*-Topolin	mT
	R	–	*meta*-Topolin Riboside	mTR
	H	–	*ortho*-Topolin	oT
	R	–	*ortho*-Topolin Riboside	oTR
	H	–	Kinetin	K
	R		Kinetin Riboside	KR

H: hydrogen; R: β-D-ribofuranosyl; G: β-D-glucopyranosyl.
[a] In Z7G, β-D-glucopyranosyl group is substituted at N-7.

2.4. LC–MS/MS conditions

The LC–MS/MS system consisted of an Agilent 1100 series liquid chromatograph coupled to LC/MSD Trap XCT (Agilent Technologies, Palo Alto, CA, USA) equipped with an electrospray ionization interface. ESI-MS/MS analysis was performed with positive mode with the scan range of m/z 100–400. The maximum accumulation time for the ion trap was set at 200 ms and the target count was set at 10,000. In preliminary experiments, the mass spectrometer was tuned for kinetin and kinetin

riboside standards (10 μM) individually by direct flow injection. The source parameters (including nebulizing gas pressure, the drying gas temperature, flow rate and capillary voltage) were optimized to obtain more abundant protonated molecular ions for both compounds and kept constant through out the whole this study. The nebulizing gas (nitrogen) pressure, the drying gas (nitrogen) temperature, flow rate and capillary voltage were set to 40.0 psi, 350 °C, 8.0 L min^{-1}, and 4.5 kV, respectively. The selected reaction monitoring (SRM) mode was performed by monitoring the transition between the precursor ion (protonated molecular ion) and most abundant product ion (the collision gas was helium).

Samples were dissolved in mobile phase (water–methanol–formic acid (20:80:0.1, v/v/v), filtered through a 0.45 μm Whatman glass microfiber filter and injected into a Zorbax Narrow-Bore column (2.1 mm × 30 mm, Agilent Technologies, CA, USA) with a injection volume of 2 μL. The column thermostat was set at 25 °C. The isocratic mobile phase used after degassing was consisted of water–methanol–formic acid (20:80:0.1, v/v/v) with total running time of 12 min and a flow rate of 0.25 mL min^{-1} throughout the whole separation. Prior to each analysis, the column was washed with the solvent (methanol–water–formic acid, 95:5:0.1, v/v/v). The data were processed by the accompanying system software (LC/MSD Chemstation).

2.5. HPLC instrumentation and procedure

The further analysis of the kinetin and kinetin riboside content was performed using a HPLC system (2695 Separations Module, Waters; Milford, MA, USA) linked simultaneously to a photodiode array detector (PDA 2996 detector, Waters; Milford, MA, USA). Data were processed by the accompanying system software (Millennium™ software, Data Handling System for Windows, version 4.0).

Samples were dissolved in mobile phase (initial conditions), filtered through a 0.45 μm Whatman glass microfiber filter and injected into a C_{18} column (Platinum 100 Å 5 μm, 250 mm length, 4.6 mm diameter, Alltech, Deerfield, IL). Compared with the dimension of column (30 mm length, 2.1 mm diameter) used in LC–MS/MS, a relatively large dimensional column was used in HPLC experiment for the following two reasons: (i) to achieve high separation resolution among a large number of peaks in the real sample; (ii) to achieve high throughput of the fractionation. The column thermostat was set at 25 °C. Solvent (A) consisted of 40 mM acetic acid, pH adjusted to the range of 3.78–3.80 with triethylamine [45]. Solvent (B) was methanol. Injection volume was 10 μL for all the sample analyses and 60 μL for fraction collections. The flow-rate was 1 mL min^{-1} throughout the whole separation. Prior to each analysis, the column was washed with mobile phase (methanol–acetic acid buffer, 95:5, v/v) for 5 min. The absorbance of the column effluent was monitored at 269 nm with the programmable PDA detector.

The putative kinetin and kinetin riboside fractions corresponding to single peaks, previously characterized by HPLC were collected manually based on the retention time. The fractions from 20 separate runs were pooled together and dried under vacuum at room temperature. Finally, the dried putative kinetin and kinetin riboside fractions were re-dissolved in 0.1 mL of CE running buffer individually for CZE analysis.

2.6. CE Instrumentation and procedure

The analyses of kinetin and kinetin riboside were also carried out using a capillary electrophoresis system (P/ACE™ MDQ; Beckman Coulter Inc., CA, USA) equipped with a PDA detector. Instrumentation, system control and data analyses were carried out using a PC with the accompanying software (P/ACE™ System MDQ Software, Version 2.3). The electrophoretic separations were carried out in an uncoated fused-silica capillary (76 μm i.d., 363 μm o.d., Polymicro Technologies Inc.; Phoenix, Arizona, USA) using 100 mM ammonium phosphate buffer (pH 2.5) solution. The wavelength for detection was set at 269 nm. The capillary had an effective length of 30 cm (total length: 40 cm) and was operating at an applied voltage of 15 kV. Sample introduction was accomplished by vacuum injection for 5 s under a pressure of 0.5 psi. All samples were filtered through a 0.45 μm Whatman glass microfiber filter before injecting into the CE instrument. At the beginning of each working day, the capillary was rinsed sequentially with phosphoric acid (0.1 M), water and the separation buffer solution. Each rinsing step was about 5 min, and between each sample injection, the above rinsing procedure was adopted except the time taken for each rinsing step was 3 min. These standard conditions were used for all experiments.

3. Results and discussion

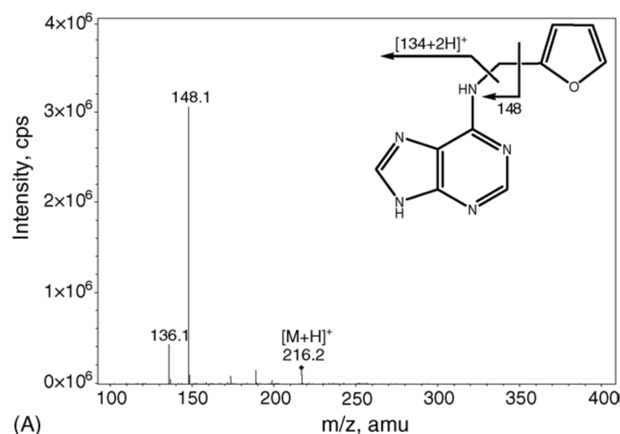
3.1. Identification of kinetin and kinetin riboside in coconut water using LC–MS/MS

3.1.1. LC–MS/MS optimization

The SRM transitions and optimized MS conditions (see Section 2.4 for details) were determined through direct flow injection of kinetin and kinetin riboside standards. Based on MS/MS spectra of kinetin and kinetin riboside (Fig. 1), the mass spectrometer was operated in SRM mode by monitoring the following precursor/product ion pairs: 216 → 148 and 348 → 216 for kinetin and kinetin riboside, respectively. The chromatographic conditions were optimized for analysis speed and sensitivity, with special emphasis on the good separation resolution of kinetin and kinetin riboside.

3.1.2. Method validation

The reproducibility of the retention time of the kinetin and kinetin riboside under optimum LC–MS/MS conditions was investigated by doing repeated injections ($n = 6$) of a mixture of the standards at a concentration of 5 μM. The R.S.D.s for K and KR were 0.12% and 0.18%, respectively (Table 2). The good reproducibility in retention time indicated that this method is accurate, robust and would probably be reliable for screening kinetin and kinetin riboside in purified plant samples. Based on a signal-to-noise ratio of 3, the limits of detection in SRM mode were 0.02 μM and 0.005 μM for kinetin and



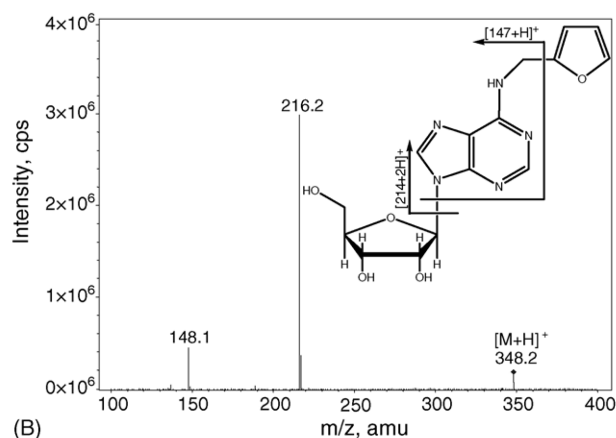


Fig. 1. The positive product ion spectra of (A) kinetin and (B) kinetin riboside, each with the protonated molecule ($[M+H]^+$) as a precursor ion. (♦) indicates the precursor ion. The chemical structures of the compounds are displayed and their cleavage sites are indicated with arrows pointing to the product ions and their respective *m/z*.

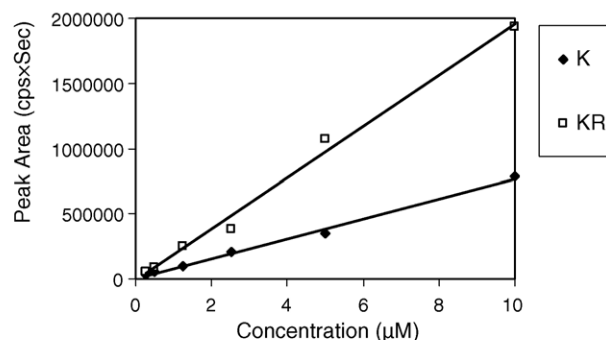


Fig. 2. Standard calibration curves of peak areas against the concentrations for kinetin and kinetin riboside using LC–MS/MS analysis.

kinetin riboside, respectively. Good linearity of the assay was found over the investigated calibration range of 0.25–10 µM, based on 6-level calibration curves (Fig. 2). Three independent injections were carried out for every calibration point. Typically the coefficients of correlation (r^2) of this method were above 0.993. The quantitative data obtained from the analysis of kinetin and kinetin riboside by LC–MS/MS were summarized in Table 2.

3.1.3. Analyses of cytokinins in coconut water

Under optimum LC–MS/MS conditions, the presence of kinetin and kinetin riboside in the purified coconut water extract was successfully screened based on ion transition used for the SRM detection as well as their retention times. Representative SRM chromatograms of kinetin and kinetin riboside obtained from standards and the coconut water extract sample were shown in Fig. 3. The selectivity for the analysis was shown by symmetrical resolution of the peaks with no significant chromatographic interference from plant matrix. No interfering matrix signals were observed for both SRM transitions. Kinetin as well as kinetin riboside, which was previously assumed to be a synthetic cytokinin plant growth hormone was found in young coconut water based on their characteristic fragments (Fig. 3(A2) and (B2)). On the basis of calibration curves, the presence of kinetin and kinetin riboside in the coconut water was quantified. The estimated concentration of kinetin and kinetin riboside in the coconut water were 2.3×10^{-4} µM and 1.8×10^{-4} µM disregarding the loss during the purification steps. The recovery yield of kinetin and kinetin riboside was about 68% and 62%, respectively, which is considered reasonably high for a multi-steps purification procedure. Based on the recovery, the estimated original concentrations of kinetin and kinetin riboside in coconut water were 3.4×10^{-4} µM and 2.9×10^{-4} µM, respectively. The concentrations of kinetin and kinetin riboside in coconut water are below the detection limits of LC–MS/MS, while the use of SPE as a powerful preconcentration and purification method definitely overcame this limitation.

No peaks were observed in the SRM chromatograms of blank acidified water extract, when monitored for kinetin and kinetin riboside (data not shown). This implied that kinetin and kinetin riboside were absent from the purification columns and filter paper used in the multi-steps sample pretreatment procedure.

Table 2
Response characteristics of kinetin and kinetin riboside using LC–MS/MS

Compounds	Retention time (min)		Equation of calibration curve[a]	R^2	Linear range (µM)	Detection limit (µM)[b]
	Mean	R.S.D. (%)				
K	3.9	0.12	$y = 75998x + 8257$	0.995	0.25–10	0.02
KR	8.8	0.18	$y = 196871x - 8987$	0.993	0.25–10	0.005

[a] In the calibration equations, x represents concentration of the analyte (µM) and y represents the peak area (cps × s).
[b] The limits of detection were estimated based on the signal/noise = 3.

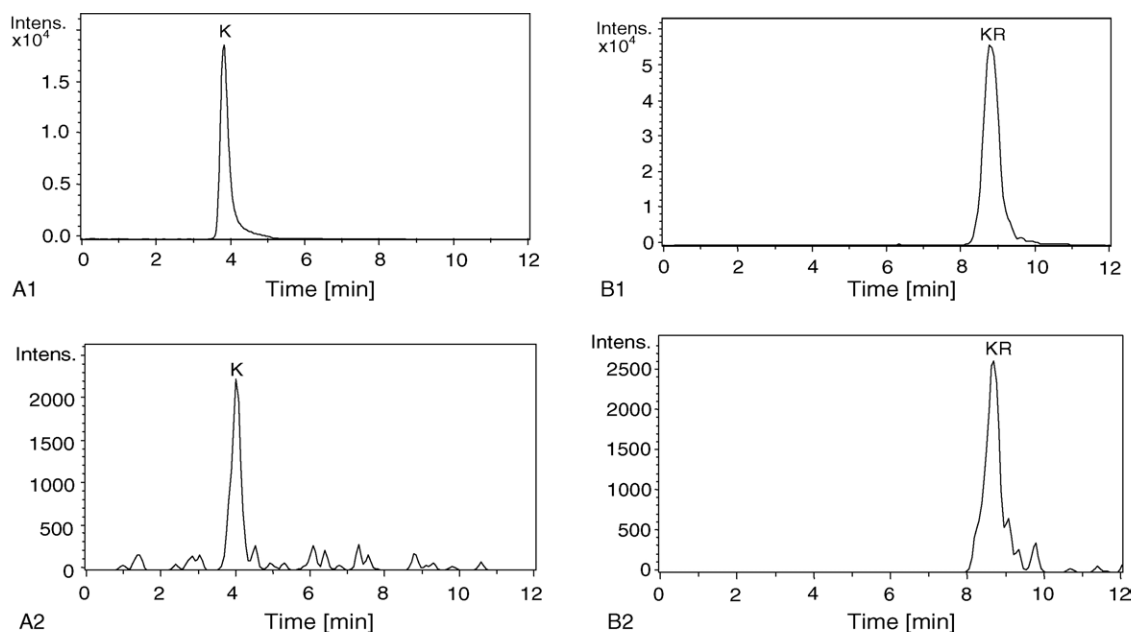


Fig. 3. Representative SRM chromatograms of (A1) kinetin standard; (B1) kinetin riboside standard; (A2) putative kinetin in coconut water extract sample; and (B2) putative kinetin riboside in coconut water extract sample determined by LC–MS/MS method. All other LC parameters and MS conditions were as described in Section 2.4.

3.2. Separation and fractionation of kinetin and kinetin riboside using HPLC

3.2.1. Separation of cytokinin standards

The baseline separation of cytokinin standards (K, KR, Z, ZR, ZOG, Z9G, Z7G, ZROG, cZ, cZR, DZOG, iP, iPR, BA, oT, mT, oTR and mTR), containing 5 μM of each compound was achieved by multiple gradient elution approach with a flow-rate of 1 mL min^{-1}. The utilized mobile phase elution gradient is shown in Table 3. All changes in the composition of the mobile phase were linear. This program has the ability to separate almost all the analytes (Fig. 4(A)), except the overlapping of the Z and DZOG peaks. This fact has little impact on our result quality, since both Z and DZOG would not be further analyzed. However, this situation should be taken in the account when these two compounds will be analysed by our method. Despite the many efforts taken to shorten the time taken for HPLC separation, the total run time typically exceeds 120 min due to the need for column washing (methanol–acetic acid buffer (95:5, v/v) for 5 min) and column equilibration (15 min). Since purification is still the most time consuming part of the entire analysis, chromatographic run duration is not crucial.

Table 3
Chromatographic gradient conditions for the analysis of cytokinins

Time (min)	Methanol (%)	40 mM acetic acid buffer (%)
0	10.0	90.0
35	20.0	80.0
45	20.0	80.0
65	25.0	75.0
70	25.0	75.0
100	34.0	66.0

3.2.2. Analysis of putative kinetin in coconut water

In a first attempt to analysis kinetin and kinetin riboside in coconut water extracts, the program (Table 3) allowed a baseline separation for cytokinins was used to screen for the putative kinetin and kinetin riboside in the coconut water. Fig. 4(B) shows the chromatogram of coconut water extracts purified using C_{18} and mixed mode SPE columns. Qualitative analysis of kinetin was successfully performed by spiking the sample with the appropriate standard to observe the growth of the peak (Fig. 4(C)), and by comparing the retention time with the standard (Fig. 4(A) and (B)).

Unfortunately, the analysis of kinetin riboside could not be accomplished by this HPLC program due to the presence of endogenous interferences in the matrix. In addition, a large unknown peak was observed in the coconut water sample at the retention time of kinetin riboside on the chromatogram, interfering with the further fractionation of kinetin riboside.

3.2.3. Analyses of putative kinetin riboside in coconut water

To overcome the problem of kinetin riboside analysis described above, the main changes of the HPLC program were performed based on the separation kinetin riboside with the large unknown peak. Under the new chromatographic conditions (Table 4), the large unknown peak appears in an area where it does not interfere with the resolution of kinetin riboside peak on the chromatogram (Fig. 5(B)). Qualitative analysis of kinetin riboside was successfully performed by spiking the sample with appropriate standard to observe the growth of the

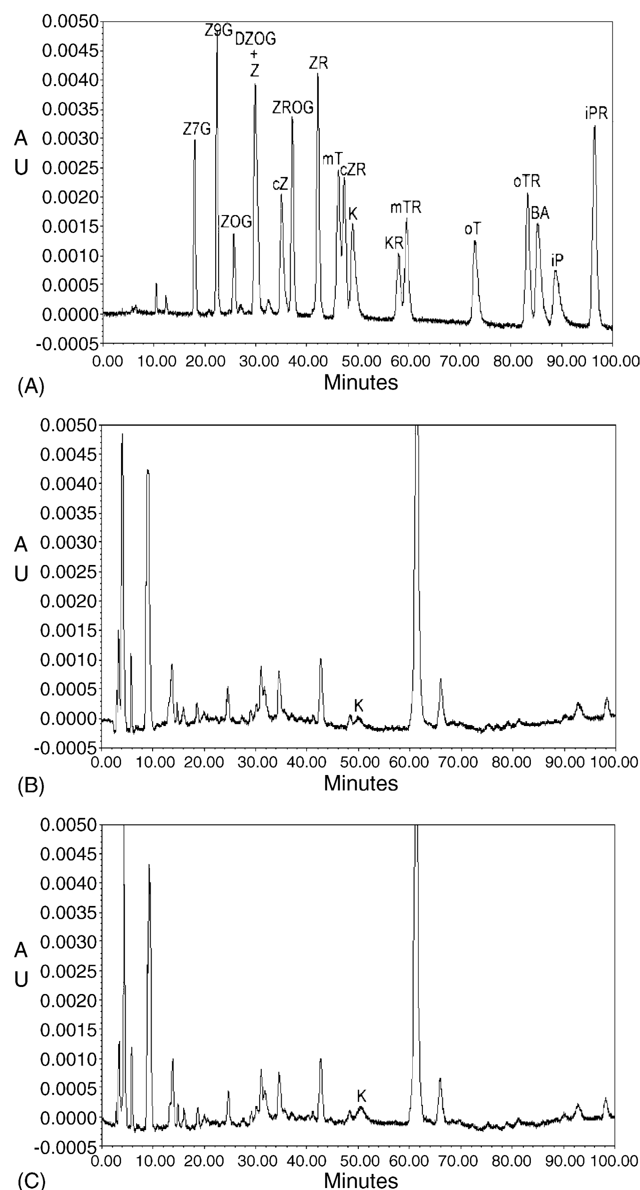


Fig. 4. HPLC chromatograms of (A) cytokinin standards mixture; (B) coconut water extracts; and (C) spiked coconut water extracts by kinetin. Chromatography was performed as described in Table 3.

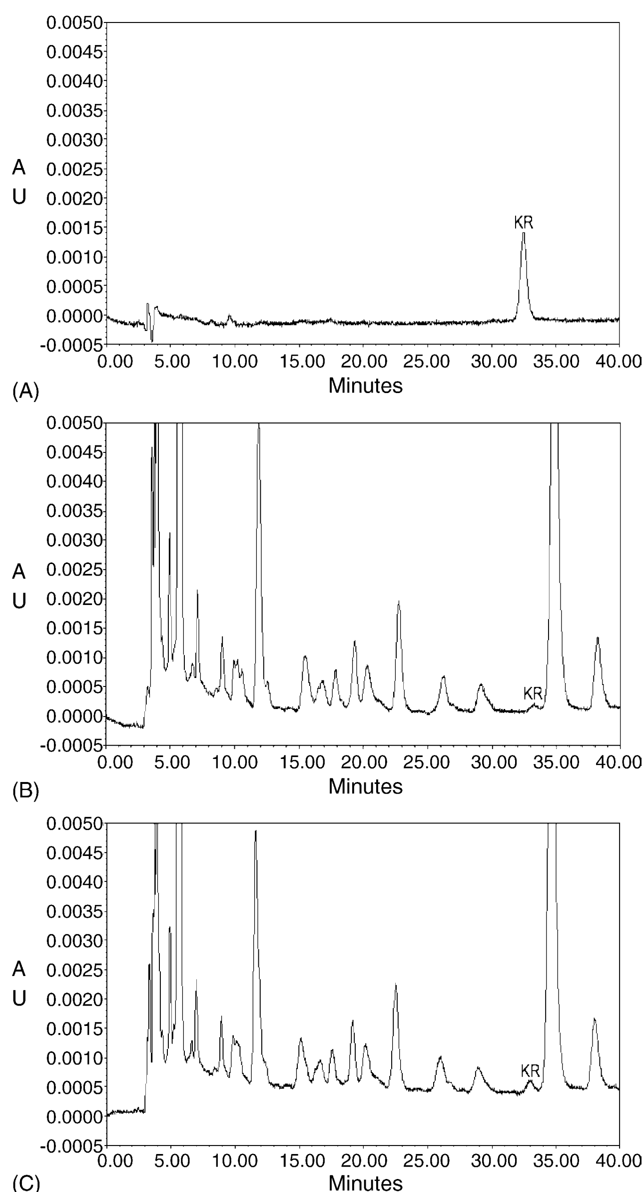


Fig. 5. HPLC chromatograms of (A) kinetin riboside standard; (B) coconut water extracts; and (C) spiked coconut water extracts by kinetin riboside. Chromatography was performed as described in Table 4.

peak (Fig. 5(C)), and by comparing the retention time with the standard (Fig. 5(A) and (B)).

3.3. *Confirmation of kinetin and kinetin riboside identity using CZE*

CE, which offers orthogonal complementary analyses with different separation mechanisms from chromatography, is a useful tool for the analysis of cytokinins [46–48]. Baseline separation of kinetin and kinetin riboside by CZE was accomplished with an uncoated fused-silica capillary of 30 cm effective length and multifunctional electrophoretic media of simple composition, i.e., only 100 mM ammonium phosphate buffer at pH 2.5, in less than 5 min (Fig. 6(A)).

Chromatographic fractions containing either putative kinetin or kinetin riboside were successfully confirmed using CZE by their migration times (Fig. 6(B) and (C)) as well as by using standard addition method. The CZE results also indicated the kinetin and kinetin riboside fractions were well separated and clean enough for further confirmation of identities.

Table 4
Chromatographic gradient conditions for the analysis of kinetin riboside

Time (min)	Methanol (%)	40 mM acetic acid buffer (%)
0	20.0	80.0
10	20.0	80.0
40	30.0	70.0

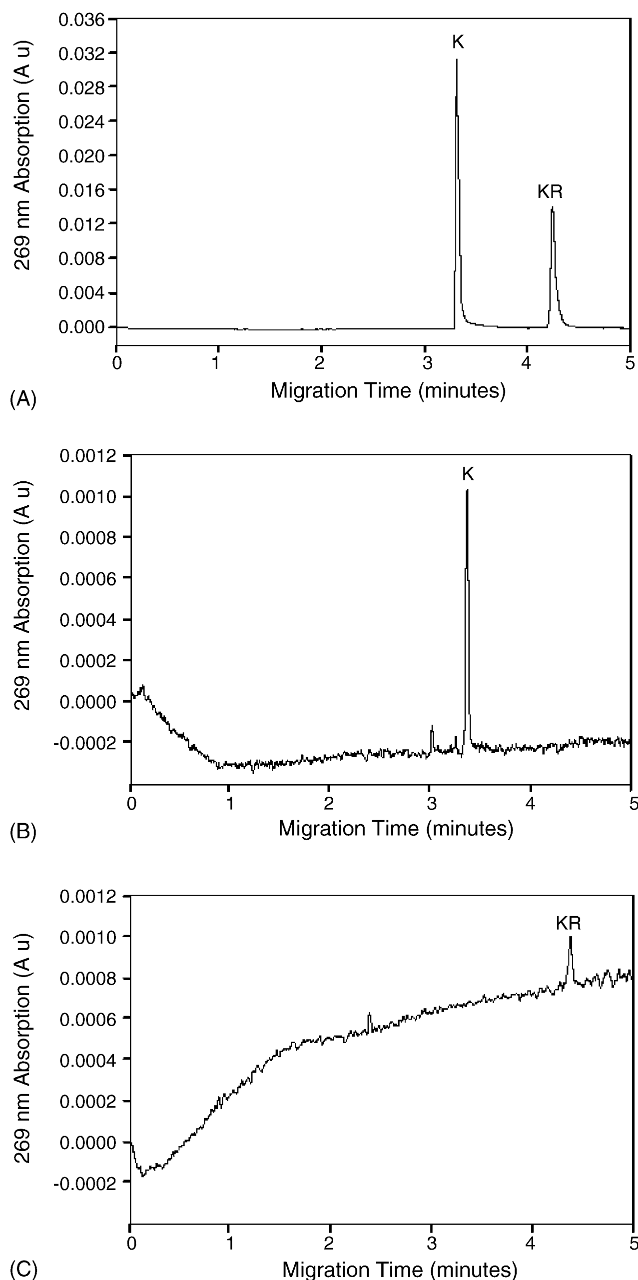


Fig. 6. Electropherograms of (A) kinetin and kinetin riboside standards; (B) putative kinetin chromatographic fraction; and (C) putative kinetin riboside chromatographic fraction. Note: running buffer solution contained 100 mM phosphate ammonium salts (pH 2.5). Other experimental conditions were as described in Section 2.6.

4. Conclusions

We have detected and quantified both the free base and riboside of kinetin for the first time in the endosperm liquid of fresh young coconut fruits. Unlike the previous study [21] where kinetin was detected in the highly processed coconut kernel tissues, it is interesting to note that kinetin riboside is also present naturally in the young coconut water. This study adds two more cytokinins to the growing list of endogenous cytokinins present in coconut water. The presence of kinetin and kinetin riboside (in addition to the isoprenoid and aromatic cytokinins [32–37]) in coconut water is of significant biological importance as it may in part provide an adequate biochemical explanation to the effectiveness of coconut water as a growth supplement in regulating growth and development in plant tissue culture. Since kinetin has been reported to have therapeutic medicinal and anti-aging effects [17–20], and kinetin free base and kinetin riboside are now confirmed to be present in young coconut water, the benefits of drinking coconut water as a healthy beverage should be further explored. Based on the findings in this paper, further investigation concerning the inherent relationship of kinetin and kinetin riboside present in coconut kernel and coconut water as well as the stability of these two compounds will be carried out in the near future. This will provide insights about the possible biosynthesis pathway and metabolism of these cytokinins.

Meanwhile, the present work shows a validated, highly sensitive, and selective LC–MS/MS method for the determination of kinetin and kinetin riboside in coconut water. Based on SRM transitions, the optimized LC–MS/MS method could be adapted for identification and quantification of kinetin and kinetin riboside in coconut water extract sample. The estimated original concentrations of kinetin and kinetin riboside in coconut water were 3.4×10^{-4} μM and 2.9×10^{-4} μM, respectively. Moreover, the presence of kinetin and kinetin riboside in coconut water were further confirmed by HPLC. The identities of the kinetin and kinetin riboside detected in coconut water were further authenticated by orthogonal complementary CZE experiments.

Combination of LC–MS/MS, HPLC and CE has been successfully applied for the identification of kinetin and kinetin riboside in the coconut water. CE is a very powerful analytical tool, which can be used as a confirmatory analysis for LC, due to the orthogonal separation mechanisms. The following advantages, such as shorter analysis time and lower consumption of chemicals, make CE more attractive as a tool for the analysis of cytokinins. For future work, it will be interesting to couple the CE approach to MS for the direct analyses of cytokinins in biological samples.

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