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Characterization of Antioxidants and Change of Antioxidant Levels during Storage of *Manilkara zapota* L.

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Antioxidants found in fruits and vegetables play an important role via their protective effects against the onset of aging-related chronic diseases. Our previous research has indicated that unripe ciku fruits (*Manilkara zapota* L.) are an excellent source of antioxidants, with over 3000 mg of L-ascorbic acid equivalent antioxidant capacity (AEAC) per 100 g of fresh sample. In this study, 24 antioxidants in an extract of ciku king were characterized through a free radical spiking test. Their chemical structures were proposed using high-performance liquid chromatography−mass spectrometry (HPLC-MS) and tandem MS (HPLC/MS^n). The antioxidant capacity of ciku king fruits was mainly attributed to polyphenolics with basic blocks of gallocatechin or catechin or both. The changes of total antioxidant capacity (TAC) and total phenolics content (TPC) of ciku king fruits with storage time were also investigated. It was found that the TAC and TPC decreased significantly as the fruits gradually changed from the unripe to the overripe stage. The best time for one to consume ciku king fruits at a flavorful stage with high amounts of antioxidants with AEAC values ranging from 600 to 1200 mg per 100 g fresh sample is suggested. The change of the content of major antioxidant peaks was also consistent with changes of antioxidant levels during storage.

KEYWORDS: Ciku king fruit; HPLC/MS; antioxidants, storage

INTRODUCTION

The consumption of fruits and vegetables is an essential part of a balanced diet. Fruits and vegetables are excellent sources of dietary fiber, vitamins, and minerals and are usually low in calories and fat and contain no cholesterol, making them healthy additions to diets.

Having an abundance of fruits and vegetables in the daily diet can help to reduce one's risk of developing some cancers, coronary diseases, inflammation, arthritis, immune system decline, brain dysfunction, and cataracts (*1*−*7*). These protective effects are considered, in large part, to be related to the presence of various phytonutrients, especially antioxidants that such foods contain. Antioxidants inhibit or delay the oxidation of vital biomolecules in a chain reaction triggered by free radicals in vivo, which is important in the prevention and progression of many chronic diseases (*8*−*13*).

The ciku fruit (*Manilkara zapota* L.), also known as the sapodilla, is a small evergreen tree native to Mexico and tropical America and now is well spread throughout the tropics. It ripens within 9 days at ambient temperature and spoils within 13 days after harvest (*14*). In Singapore, fruits are normally eaten fresh, but the pulp has been reported to be incorporated into sherbets, milkshakes, and ice cream. Unripe ciku are found to possess an extremely high antioxidant capacity that is not attributed to L-ascorbic acid, a constituent that is partly responsible for the antioxidant capacity of many fruits (*15*).

In a study by Ma and co-workers, 4-*O*-galloylchlorogenate and 4-*O*-galloylchlorogenic acid were reported to be found in ciku extracts (*17*). The same author and co-workers also reported that ciku possesses a high antioxidant capacity using the 1,1′-diphenyl-2-picryl hydrazyl scavenging assay and showed cytotoxicity in the HCT-116 and SW-480 human colon cell lines. The compound 5-caffeoyl-quinic acid was also detected in the peel and pulp of ripe ciku fruits (*18*). In previous studies, a high amount of tannins, which may be responsible for the high antioxidant capacity, was found in the unripe fruits of ciku. The tannin content was reported to decrease with natural ripening (*14*, *19*).

Ciku king is a ciku cultivar that is popular in the Singapore market, probably due to its bigger size, better texture, and taste. In this study, it was found that the unripe fruits of ciku king possess an extremely high antioxidant capacity. At present, detailed information on the composition of antioxidant compounds in ciku fruit is sparse. The contribution of L-ascorbic acid to the total antioxidant capacity (TAC) in ciku fruit is very low (<0.1%) (*15*), which suggested that most of the antioxidant activity of ciku fruit could be due to polyphenolic compounds. Therefore, one objective of this study is to investigate the antioxidant profile of ciku fruit. In addition, the amount of antioxidants that would be ingested from the consumption of the fruit is still unclear. In this study, an attempt was undertaken

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to estimate the best time to consume ciku king fruit with reasonable sensory attributes and still be able to benefit from the high antioxidant capacity of the fruit.

MATERIALS AND METHODS

Chemicals and Materials. 2,2′-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), l-ascorbic acid, (−)-epicatechin, (−)-gallocatechin, (−)-epigallocatechin gallate, and potassium persulfate were purchased from Sigma (St. Louis, MO); (+)-catechin hydrate and chlorogenic acid were purchased from Aldrich Chem. Co. (Madison, WI); gallic acid was purchased from Acros Organics (New Jersey); Folin−Ciocalteu reagent was purchased from Merck (Darmstadt, Germany); anhydrous sodium carbonate was purchased from J. T.Baker (New Jersey). Mature, green, wholesome, and unripened ciku king fruits, of roughly equal size that were directly imported from Vietnam, were purchased from a local supermarket. Fruits were then stored in an incubator with its temperature set at 25 °C.

Sample Preparation. Preliminary high-performance liquid chromatography−mass spectrometry (HPLC-MS) analysis indicated that tannins were the major antioxidants found in ciku king fruit. The fruit was peeled, and pulp was extracted with different solvents (w/v, 1:20) at different temperatures and extraction times. Optimized extraction conditions used for the extraction of antioxidants from the pulp of ciku king fruit, which were similar to those used in the study of antioxidants in star fruit, were 50% aqueous acetone for 45 min at 90 °C (*20*). After 45 min, the extracts were rapidly cooled to room temperature using an ice bath, after which the extracts were centrifuged at 14000*g* in an Eppendorf 5804R centrifuge, and the supernatant was used without further treatment for the determination of TAC and total phenolic content (TPC). A portion of the supernatant was also dried by rotary evaporation (Buchi Rotavapor R-200, Switzerland) at 35 °C, redissolved in 20% aqueous methanol, and centrifuged again. The antioxidant capacity of the supernatant was measured. Less than 5% of TAC was lost after evaporation and reconstitution. The solution obtained was kept in the refrigerator at −18 °C for HPLC-MS and HPLC-MS/MS assays.

TAC Assay. The free radical scavenging activity of ciku king was determined using the Ultraspec 3000 UV/visible spectrophotometer (Pharmacia Biotech Ltd., Cambridge, United Kingdom) according to the method reported previously (*15*). $ABTS^{\bullet+}$ were generated using 7.4 mM ABTS and 2.45 mM potassium persulfate. The reaction mixture, which served as a stock solution, was kept at room temperature overnight for complete reaction. The stock was diluted accordingly to obtain an absorbance value of about 2.00 at 730 nm [$\epsilon = 1.5 \times 10^4$ mol^{-1} L cm^{-1}, (*21*)] with HCl solution at pH 4.5 before use. The pH of the diluted solution remains at 4.5. An aliquot (20 μL) of the extract, with appropriate dilution, if necessary, was added to 3 mL of $ABTS^{\bullet+}$ solution. The change in absorbance at 730 nm was measured at 30 min. The TAC of the extract was expressed in terms of mg of L-ascorbic acid equivalents (AEAC) per 100 g fresh weight.

TPC Assay. The TPC of the extracts was determined using the popular Folin−Ciocalteau reagent (*21*). Forty microliters of the extract was mixed with 1.8 mL of Folin−Ciocalteu reagent (previously diluted 10-fold with distilled water) and kept at room temperature (about 28 °C) for 5 min. After 5 min, 1.2 mL of sodium bicarbonate (7.5%) was added to the mixture and mixed well. After 60 min, the absorbance of the reaction mixture was measured at 765 nm. Forty microliters of distilled water was used as a blank following the same procedure as above. TPCs of the ciku fruit extract were obtained by comparing its increase in absorbance at 765 nm with that of gallic acid via a calibration curve. The total phenolics present in the extract was expressed as mg gallic acid equivalents per 100 g fresh weight.

HPLC and Tandem Mass Spectrometry. A Finnigan/MAT LCQ ion trap mass spectrometer (San Jose, CA) equipped with TSP 4000 HPLC system, which included UV6000LP PDA detector, P4000 quaternary pump, and AS3000 autosampler, was used. The instruments were set to measure the following events: (i) UV chromatogram at 280 nm, (ii) UV spectra of individual peaks, and (iii) total ion chromatograms (TICs). Chromatographic separations were done on a 250 mm × 4.6 mm i.d. Shim-Pack VP-ODS column (Shimadzu, Kyoto, Japan) with a 10 mm × 4.6 mm i.d. GVP-ODS guard column under the following elution conditions: flow rate, 600 μL/min; room temperature, 27 °C; solvent A, 0.1% formic acid in water; solvent B, methanol, starting from 20 to 50% B in 20 min, from 50 to 90% B in 25 min, and from 90 to 20% B in 5 min for washing and reconditioning of the column. The heated capillary and spray voltage were maintained at 250 °C and 4.5 kV, respectively. Nitrogen was operated at 80 psi for the sheath gas flow rate and 20 psi for the auxiliary gas flow rate. The full scan mass spectra from *m*/*z* 50−2000 were acquired both in positive and in negative ion mode with a scan speed of 1 s per scan. Tandem mass spectrometry was performed using helium as the collision gas, operated at 0.8 mTorr.

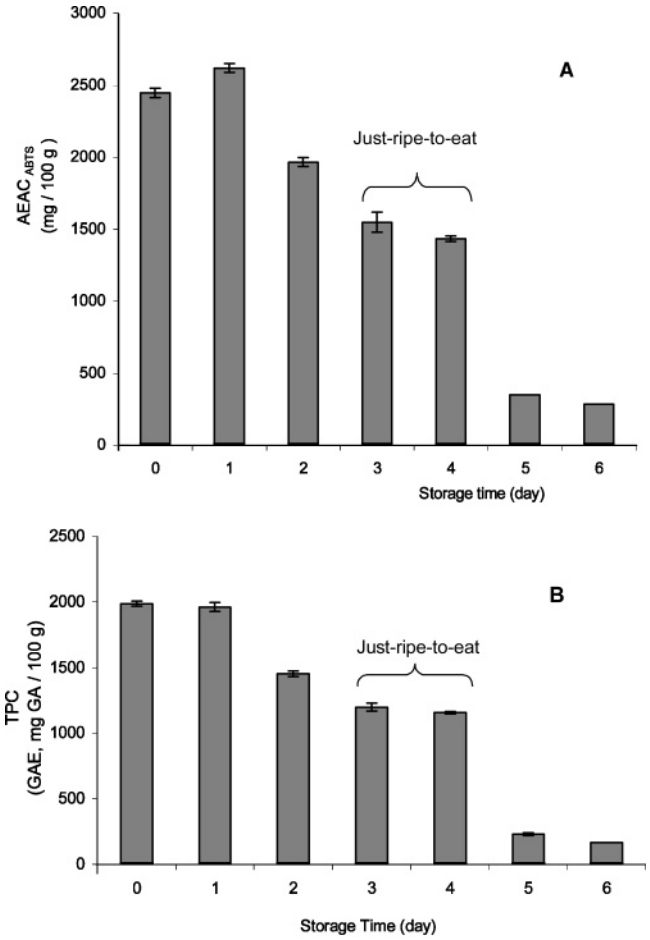

Figure 1. Variation of TAC (**A**) and TPC (**B**) of ciku king fruits with storage time (mean ± SD, $n \geq 3$).

One milliliter of extract and 1 mL of undiluted $ABTS^{\bullet+}$ stock solution were mixed to react for 1 h (*15*) and then passed through a 0.45 μm filter and injected for HPLC assay. A blank of extract with water was used as the control. For characterized antioxidant peaks, the collision energy was set from 50 to 80% to obtain fragment ions from their corresponding parent ions.

RESULTS AND DISCUSSION

Changes of TAC and TPC during Storage. The TAC of various batches of ciku king fruits was found to follow a general trend where it decreased with storage time and progression of natural ripening (**Figure 1A**). The variation in the TAC of fruits for different batches could be due to natural variation, especially when cultivation conditions and stages at harvest are hard to ascertain, as the fruits were randomly obtained from a supermarket. Hence, it is not unusual for consumers to encounter fruits of a large variation of antioxidant capacity although the fruits are of the same size and similar appearance. A trend

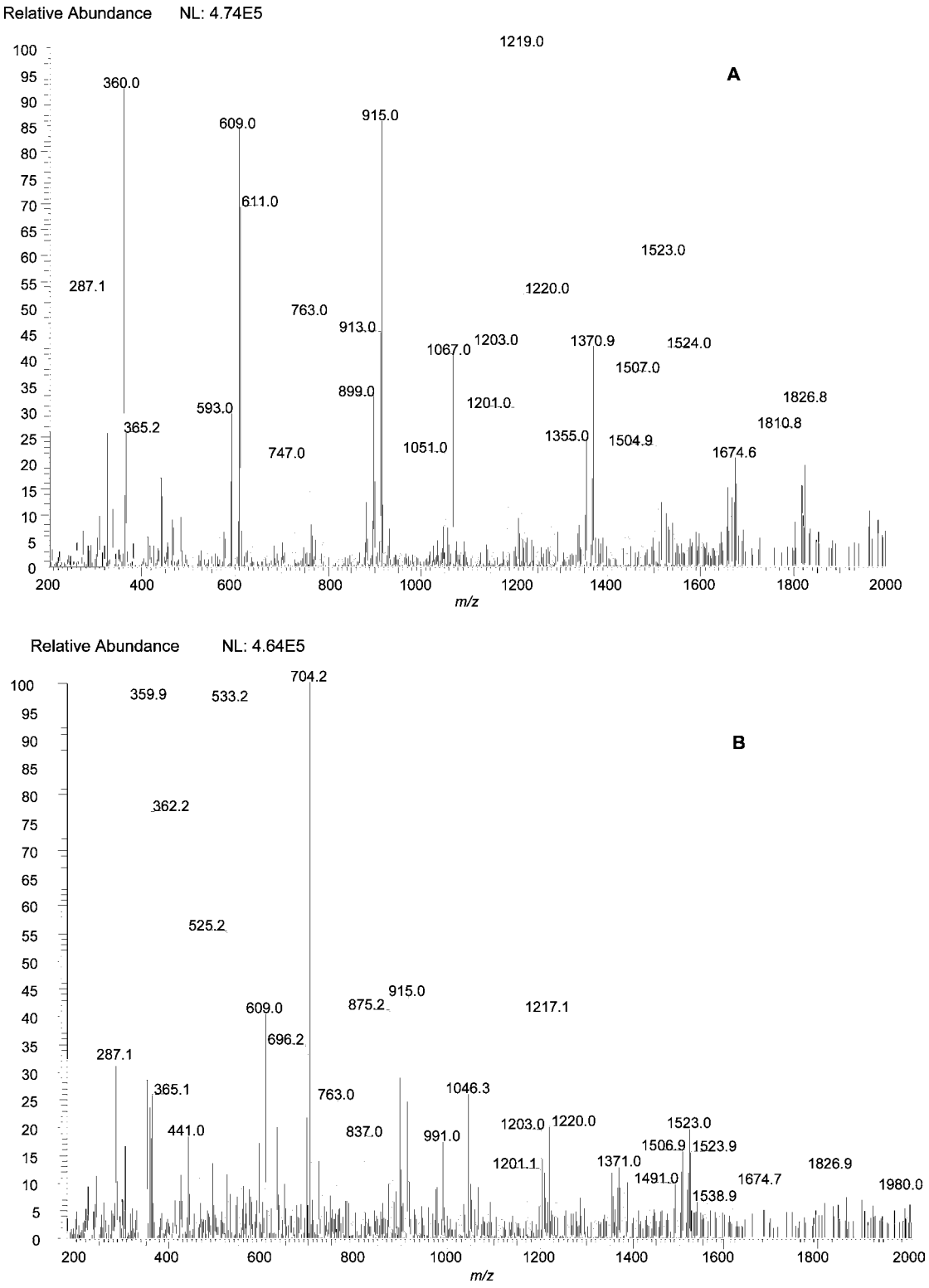

Figure 2. Averaged ESI-MS profiles of antioxidant elution period (6–19 min). (**A**) Sample with water; (**B**) sample with $ABTS^{\bullet+}$.

similar to that of variation of TAC was also observed for the TPC of ciku king fruits with storage time (**Figure 1B**). This trend is also similar to the variation of tannin content in ciku fruits during ripening, which had been reported by Mohamed et al. (*14*) and de Brito and Narain (*19*) before.

It was also observed that within 1−2 days after the fruits started to ripe, a sudden fall in TAC and TPC occurred. **Figure 1A**,**B** shows that this happens between day 4 and day 5. During days 0−2, the mature ciku king fruits have a greenish-brown appearance and are firm to the touch, but fruits at this stage are unsuitable for consumption as they are extremely astringent. The astringency of unripe fruits is probably due to the high tannin and polyphenolic compounds that it contains (*16*). Although unripe fruits possess high antioxidant capacities, they are not edible as they are sappy due to an abundant latex content and the flesh of the fruits is also tough. When the fruit start to ripen, as observed at around days 3−4 in this study, the texture of the ciku king fruit becomes softer due to the breakdown of pectin (*14*). During ripening, the color of the flesh also changes from light yellow in mature, green fruits to an orange shade at

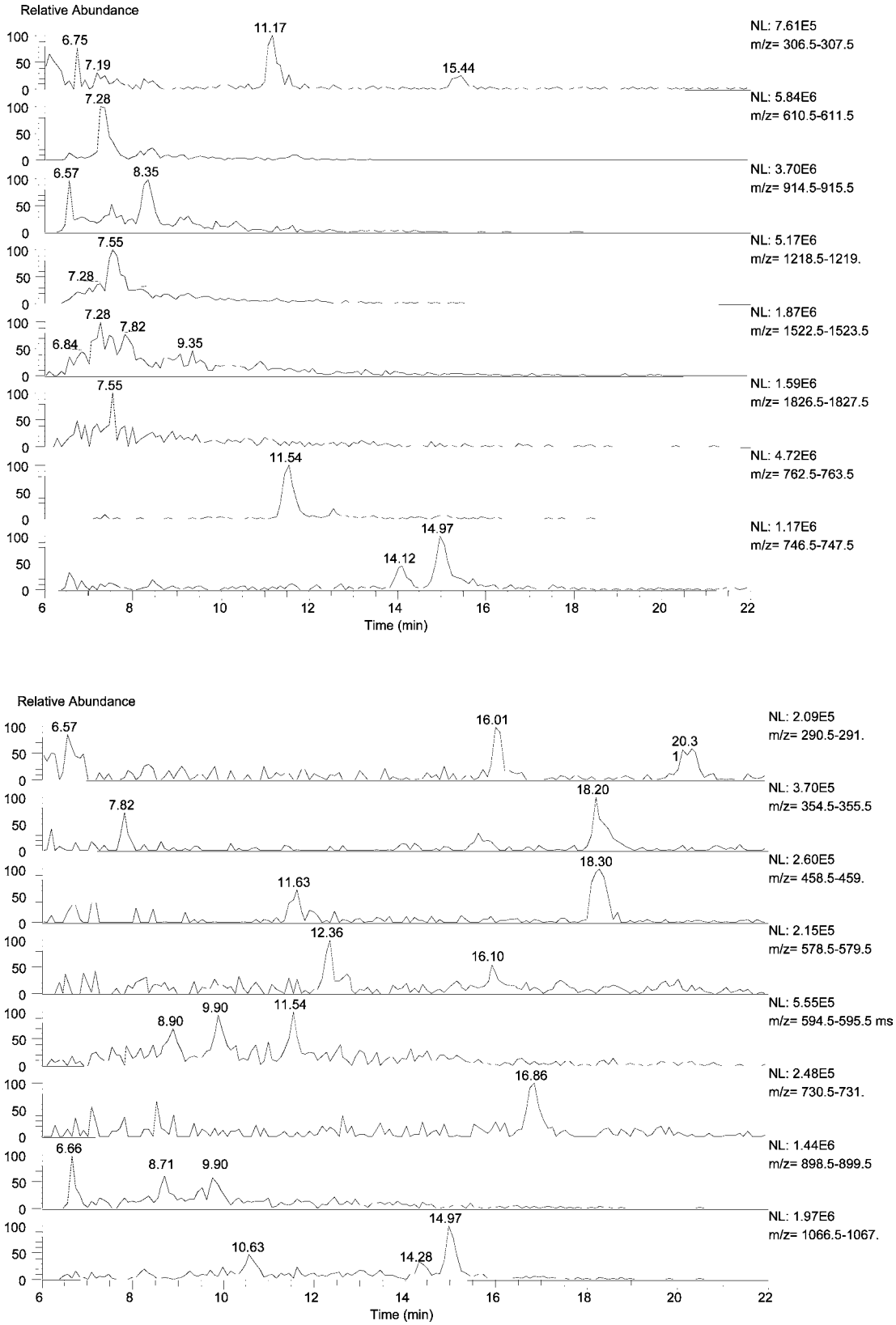

Figure 3. Extracted chromatograms of antioxidant ion peaks in ciku king extract.

the just-right-to-eat stage. Overripe fruits are mushy in texture, and the color of the flesh turns brown. Ciku king fruits are best eaten while the flesh is still firm and sweet to taste. Hence, leaving the fruits to ripen is often necessary so that the amount of reducing sugars increases, thereby increasing the sweetness while allowing the astringency due to tannins and acidity caused by organic acids to decrease with time (*19*).

An excellent correlation between TAC and TPC was obtained ($R^2 = 0.9784$). This excellent correlation indicated that phenolic compounds were likely to be the major antioxidants in the extract of ciku king fruits.

From the results obtained in this study, it was found that the best time for one to consume this batch of ciku king fruits at a flavorful stage with a high amount of antioxidants and total

phenolics would be approximately the third or fourth day during storage at room temperature, i.e., the just-right-to-eat stage. During this time, ciku king fruits have an extremely high antioxidant capacity ranging from 600 to 1200 mg L-ascorbic acid equivalents per 100 g flesh, which is comparable to that of blueberry, a fruit known for its very high antioxidant capacity, and here whose AEAC value was found to be around 800 mg per 100 g fresh sample. Overripe ciku king fruits only had antioxidant capacity levels with an AEAC value of around 200 mg L-ascorbic acid equivalents per 100 g flesh, which is around only 20% AEAC of fruit at the just-ripe stage. However, this time, where the fruits are at their most flavorful and nutritious stage may not be exactly the same for all ciku king fruits because of the difference of cold storage time, harvest time, etc. For example, it was found that the best time for one batch of fruits was found to be two or three days of storage while three or four days of storage was the best for the other four batches.

Identification of Antioxidants in Ciku King Using HPLC/MS^n. Probably because of low molar extinction coefficients of antioxidants in ciku king fruit, previously developed methods (*20*, *23*) failed to identify antioxidant peaks by spiking with free radicals using HPLC-diode array detection under the conditions specified. A recently developed approach was applied for characterization and identification of antioxidants in the extract of ciku king fruit (*24*). Briefly, free radical active compounds could be monitored by observing the difference of peak intensity of individual ions between reactants and reaction products using a mass spectrometric detector. A mass detector combined with a HPLC separation system will not only serve as an ideal tool to monitor free radical active components but also provide their possible chemical structures in a biological sample (*24*).

As can be seen from **Figure 2A**,**B**, the averaged mass spectrometric profile of the reaction solution was significantly different from that of the extract. By comparing the mass spectrometric profile of the extract to that of the reaction solution, a number of ion peaks were identified as antioxidant-related peaks as intensities of these peaks were significantly reduced. Those major antioxidant-related ion peaks were found to be eluted with retention times from around 6 to 19 min except that a very small amount of (−)-epicatechin was eluted around 20.3 min. **Figure 3** presents the extracted ion chromatograms for these possible antioxidant ions. The extracted peaks of each antioxidant-related ion from both the extract and the reaction solution of the extract with free radical were further compared to characterize the antioxidant peaks. It was found that not all of the extracted peaks of ions at selected *m*/*z* values were reduced after reacting with free radical. Some peaks, although they had the same *m*/*z* values as some antioxidant peaks, were not considered as antioxidants. They might be other compounds or different isomers of some antioxidants with the same *m*/*z* signals. Only those peaks showing lowered intensities after mixing with $ABTS^{\bullet+}$ as compared to the control are considered as antioxidants. **Table 1** summarizes the retention times of antioxidants, *m*/*z* of their parent ions, and their corresponding fragment ions from collision-induced dissociation (CID).

Initial TICs of ciku king extract, which were obtained by HPLC-ESI-MS at both positive and negative modes, indicated that **C1** has a molecular mass of 610 [306 × 2 − (2 − 1) × 2] Da. As shown in **Table 1** and **Figure 4A**, the parent ion $[M + H]^+$ at *m*/*z* 611 gave main daughter ion peaks at *m*/*z* 443, 425, and 307. Further MS^3 results showed that daughter ions from ions at *m*/*z* 307 gave the same fragmentation pattern as gallocatechin, which indicated that **C1** was a dimer of gallocatechin. **Figure 5** gives the proposed fragmentation mechanism for gallocatechin dimers, a similar fragmentation pattern to proanthocyanidins and catechins (*20*, *25*). Briefly, main fragment ions at *m*/*z* 443 $[M + H - 168]^+$ might arise from retro-Diels−Alder fission (RDA-F) of the heterocyclic rings and an ion at *m*/*z* 425 possibly from RDA-F of the heterocyclic rings and loss of water. As mass spectrometry cannot distinguish between stereoisomers, for the convenience of explanation, all ions that gave similar CID spectra to (+)-gallocatechin will be addressed as gallocatechins. Thus, compound **C1** was assigned as a gallocatechin dimer. Similarly, **C2** had a molecular of 1218 [306 × 4 − (4 − 1) × 2] Da. **Figure 4C** and **Table 1** show the fragment ions of its parent ion at *m*/*z* 1219. Its main fragment ions at *m*/*z* 611 $[M + H - 608]^+$ and 915 $[M + H - 304]^+$ might arise from interflavanic bond cleavage, 1051 $[M + H - 168]^+$ might arise from RDA-F of the heterocyclic rings, 443 and 747 most likely might arise from interflavanic bond cleavage and followed by RDA-F of the heterocyclic rings, and 609 $[M + H - 610]^+$ might arise from interflavanic bond cleavage following the quinone−methide mechanism. Therefore, compound **C2** was assigned to be a tetramer of gallocatechin. Compounds **C3** and **C4** have a molecular mass of 914 [306 × 3 − (3 − 1) × 2] Da. **Figure 4B** and **Table 1** show the fragment ions of its parent ion at *m*/*z* 915, similar fragmentation patterns to those of compounds **C1** and **C2**. Therefore, compounds **C3** and **C4** were assigned to be two trimers of gallocatechin. Compound **C5** and a few of its isomers with a molecular mass

Table 1. Positive and Negative Ions and Corresponding CID Ions of Some Antioxidants

R_t (min)	ESI-MS *m*/*z* mode	ESI-MS *m*/*z* $[M \pm H]^{+/-}$	major CID ion peaks of $[M \pm H]^{+/-}$
7.22 (**C1**)	+	611	443, 425, 317, 307, 299
	−	609	
7.46 (**C2**)	+	1219	611, 443, 915, 1051, 747, 745, 609
	−	1217	
6.57 (**C3**), 8.33 (**C4**)	+	915	611, 443, 747
	−	913	
7.28 (**C5**)	+	1523	609, 611, 913, 915, 1217, 1219, 1353, 1355
	−	1521	
7.55 (**C6**)	+	1827	913, 915, 1219, 1523, 1521, 1657, 1659, 611
	−	1825	
6.66 (**C7**), 8.71 (**C8**), 9.90 (**C9**)	+	899	595, 593, 731, 611, 443, 427, 425
	−	897	
11.59 (**C10**)	+	763	425, 443, 595, 459, 317, 299
	−	761	
14.20 (**C11**)	+	747	595, 427, 409, 579, 459, 301, 247
	−	745	
15.08 (**C12**)	+	747	443, 425, 579, 299, 287, 317
	−	745	
10.63 (**C13**), 14.28 (**C14**), 14.97 (**C15**)	+	1067	763, 609, 899, 609, 443, 459, 425
	−	1065	
16.80 (**C16**)	+	731	409, 579, 427, 443
	−	729	
18.35 (**C17**)	+	459	289, 151, 307, 271
	−	457	169, 331, 305, 287, 269
16.17 (**C18**), 20.33 (**C19**)	+	291	139, 165, 123, 151, 273
	−	289	
11.17 (**C20**), 15.42 (**C21**)	+	307	139, 169, 181, 289, 151
	−	305	
12.34 (**C22**), 16.10 (**C23**)	+	579	427, 409, 453
	−	577	
18.20 (**C24**)	+	355	163
	−	353	191, 179, 135

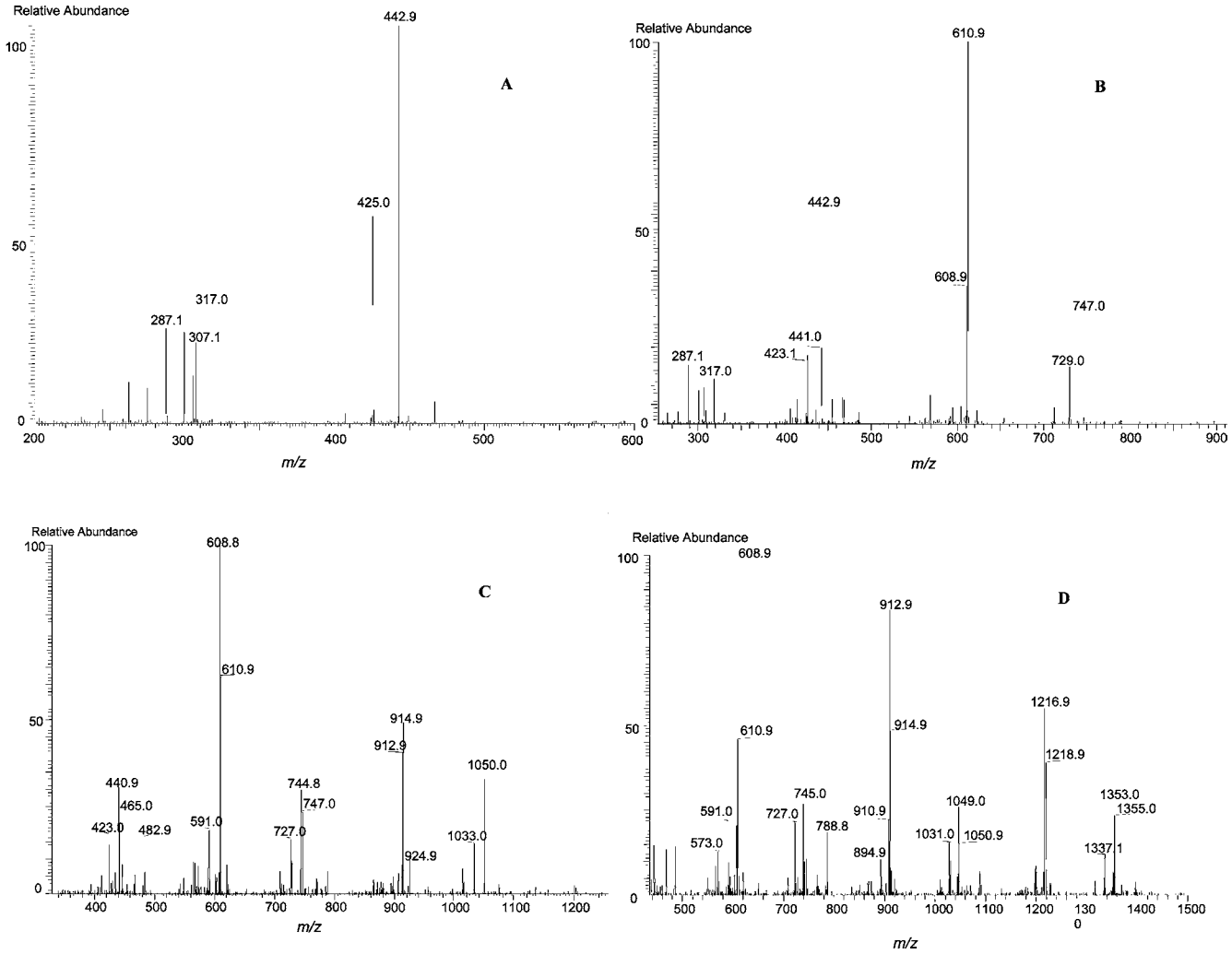

Figure 4. ESI-MS/MS mass spectra of gallocatechin dimers through pentamers at positive mode. (**A**) The CID spectrum corresponds to the positive parent ion(s) at *m*/*z* 611 (MS/MS scan). (**B**) The CID spectrum corresponds to the positive parent ion(s) at *m*/*z* 915 (MS/MS scan). (**C**) The CID spectrum corresponds to the positive parent ion(s) at *m*/*z* 1219 (MS/MS scan). (**D**) The CID spectrum corresponds to the positive parent ion(s) at *m*/*z* 1523 (MS/MS scan). Collision energy, 70%.

of 1522 [306 × 5 − (5 − 1) × 2] Da might exist in the extract of ciku king fruit although no significant extracted ion peaks were observed. **Figure 4D** and **Table 1** show the fragment ions of its parent ion at *m*/*z* 1523, which indicated that compound **C5** and a few of its isomers were pentamers of gallocatechin. Similarly, compound **C6** had a molecular mass of 1826 [306 × 6 − (6 − 1) × 2] Da and was assigned to a hexamer of gallocatechin.

As shown in **Table 1**, compounds **C7**, **C8**, and **C9** had the same molecular mass of 898 [306 × 2 + 290 − (3 − 1) × 2] Da, and their similar CID spectra from parent ions at *m*/*z* 899 included *m*/*z* 611 $[M + H - 288]^+$, 595 $[M + H - 304]^+$, 593 $[M + H - 306]^+$, 731 $[M + H - 168]^+$, 443 $[M + H - 288 - 168]^+$, and 427 $[M + H - 304 - 168]^+$. Their fragmentation patterns were similar to those of gallocatechin ytrimers. Therefore, compounds **C7**, **C8**, and **C9** were assigned to be three trimers constituted of two gallocatechins and one catechin. Compound **C10** had a molecular mass of 762 [306 + 458 − 2] Da. Its CID spectra from parent ions at *m*/*z* 763 were shown in **Table 1**. The main fragment ions were *m*/*z* 443 $[M + H - 168 - 152]^+$, possibly from RDA-F of the heterocyclic rings and loss of gallyol part; *m*/*z* 425 $[M + H - 168 - 152 - 18]^+$, possibly from RDA-F of the heterocyclic rings and losses of gallyol part and water; and *m*/*z* 459 $[M + H - 304]^+$, possibly from interflavanic bond cleavage. Therefore, compound **C10** was assigned to be a dimer constituted of one gallocatechins and one gallocatechin gallate. Compound **C11** had a molecular mass of 746 Da. Its main fragment ions at *m*/*z* 595 $[M + H - 152]^+$ might arise from RDA-F of the heterocyclic ring of catechin moiety, *m*/*z* 459 $[M + H - 288]^+$ might arise from interflavanic bond cleavage, *m*/*z* 579 $[M + H - 168]^+$ might possibly arise from RDA-F of the heterocyclic ring of gallocatechin gallate moiety or from RDA-F of the heterocyclic ring of catechin moiety and loss of water, and *m*/*z* 427 might arise from RDA-F of the heterocyclic ring of catechin moiety from ions at *m*/*z* 579. Therefore, compound **C11** was assigned to be a dimer constituted of one catechin and one gallocatechin gallate. Compound **C12** also had a molecular mass of 746 Da. Its main fragment ions at *m*/*z* 443 $[M + H - 304]^+$ might arise from interflavanic bond cleavage, *m*/*z* 579 $[M + H - 168]^+$ might possibly arise from RDA-F of the heterocyclic ring of the gallocatechin moiety, and *m*/*z* 425 might possibly arise from RDA-F of the heterocyclic ring of the gallocatechin moiety plus a loss of H_2O. Therefore, compound **C12** was tentatively assigned to be a dimer constituted of one gallocatechin and one catechin gallate. Compounds **C13**, **C14**, and **C15** had molecular masses of 1066 [306 × 2 + 458 − (3 − 1) × 2] Da. Their main fragment ions at *m*/*z* 763 $[M + H - 304]^+$ might arise

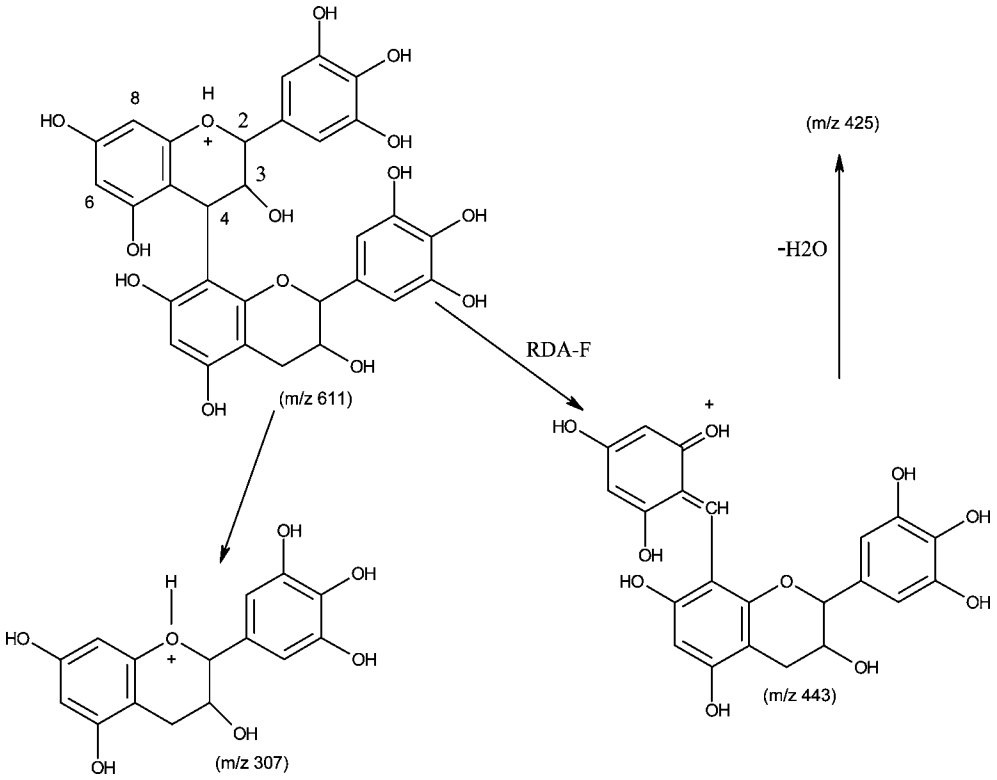

Figure 5. Proposed fragmentation patterns of the gallocatechin dimer.

from interflavanic bond cleavage, *m*/*z* 899 $[M + H - 168]^+$ might possibly arise from RDA-F of the heterocyclic rings, and *m*/*z* 609 $[M + H - 306 - 152]^+$ might arise from interflavanic bond cleavage following the quinone−methide mechanism. Therefore, compounds **C13**, **C14**, and **C15** were assigned to be three trimers constituted of two gallocatechins and one gallocatechin gallate. Compound **C16** had a molecular mass of 730 [290 + 442 − × 2] Da. Its main fragment ion at *m*/*z* 579 $[M + H - 152]^+$ might arise from loss of gallyol moiety or RDA-F of the heterocyclic ring of catechin moiety, *m*/*z* 427 $[M + H - 152 - 152]^+$ might possibly arise from RDA-F of the heterocyclic ring of the catechin gallate moiety, *m*/*z* 409 might possibly arise from RDA-F of the heterocyclic ring of the gallocatechin moiety plus a loss of H_2O. Therefore, compound **C16** was tentatively assigned to be a dimer constituted of one catechin and one catechin gallate.

Compound **C17** had a molecular mass of 458 Da. Its main fragment ions at *m*/*z* 289 $[M + H - 152 - 18]^+$ might arise from losses of a gallyol moiety and water, and *m*/*z* 307 $[M + H - 152]^+$ might arise from losses of a gallyol moiety, etc. It was assigned to be (−)-epigallocatechin gallate by further spiking and comparing its CID spectra with an authentic standard. Compounds **C18** and **C19** were assigned to be (+)-catechin and (−)-epicatechin, respectively, by spiking and comparing its CID spectra with standards. Compounds **C20** and **C21** have a molecular mass of 306 Da. By spiking and comparing their CID spectra with authentic standards, compounds **C20** and **C21** were assigned to be (−)-gallocatechin and (−)-epigallocatechin, respectively. Compounds **C22** and **C23** had molecular masses of 578 Da. Their main fragment ions at *m*/*z* 427 $[M + H - 152]^+$ might arise from RDA-F of the heterocyclic rings, and *m*/*z* 409 $[M + H - 170]^+$ might arise from RDA-F of the heterocyclic rings and a loss of water. Their fragmentation patterns indicated that they were two catechin dimers. Compound **C24** had a molecular mass of 354 Da. Its parent ion at *m*/*z* 353 gave fragment ions at *m*/*z* 191 $[\text{quinic acid} - H]^-$ and 179 $[\text{caffeic acid} - H]^-$, which were generated by cleavage of the ester bond and consistent with the structures of caffeoylquinic acid derivatives. Compound **C24** was assigned to be chlorogenic acid (5-*O*-caffeoyl quinic acid) by spiking and comparing its CID spectra with an authentic standard.

Other than the above compounds, which had at least one distinct ion peak, the intensities of several other ion peaks, e.g., ions at *m*/*z* 609, 913, and 593, were also significantly reduced after reacting with $ABTS^{\bullet+}$; however, no obvious corresponding ion peaks were observed in ion-extracted chromatograms. They might not come from a compound itself but from fragment ions of other larger molecular ions such as ions at *m*/*z* 915, 1067, 1219, etc. Similarly, no obvious peaks were observed for antioxidant ions at *m*/*z* 1051, 1203, 1355, 1371, 1507, 1675, and 1811, which showed quite relatively high peak intensities in **Figure 2**. These ions might arise from fragmentation of other larger molecular ions. The relatively high intensities of these ions might also be an overall reflection of the sum of a number of isomers of each ion. Their corresponding CID spectra from parent ions at *m*/*z* $[M + H]^+$ indicated that they were proanthocyanidins with basic units of gallocatechin or catechin or both. In addition, although three antioxidant ions at *m*/*z* 595 with low peak intensities and retention times of 8.90, 9.90, and 11.54 were observed, they were tentatively considered as fragment ions of other main antioxidant ions at *m*/*z* 899, 899, and 763, respectively. They might also arise from dimers constituted of one catechin and one gallocatechin but need to be confirmed under improved chromatographic separation such as using normal phase HPLC.

Except for those compounds **C17**−**C21** and **C24**, which have been confirmed with authentic standards, the chemical structures and stereochemistries of other compounds still need to be elucidated by isolation of individual compounds for the NMR assay. The peak intensities of major antioxidants were also found to decrease with storage time (data no shown). This was

consistent with the above results, which indicated that both antioxidant capacity and TPC decreased with storage time.

Here, we did not observe 4-*O*-galloylchlorogenate and 4-*O*-galloylchlorogenic acids in ciku king fruit, which are identified in ciku fruit by Ma and co-workers (*17*). This might be due to different fruit varieties used as ciku king fruit was investigated for this study.

It has been claimed that a high consumption of ciku fruit might be effective in the prevention of cardiovascular disease. This might be partly attributed to the high antioxidant capacity and high polyphenol content of ciku king fruit. However, as there is no direct evidence currently, further studies need to be carried out to verify the healthy effects imparted by the consumption of ciku fruit at the just-ripe-to-eat stage. In addition, quantification of those individual compounds is also important for the initial understanding of the possible dietary intake of these compounds. Furthermore, to understand the stereochemistry of most antioxidants in this study, further separation and isolation of those compounds by large-scale column separation or preparative HPLC needs to be carried out as soon as possible.

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