

# Ảnh hưởng của giai đoạn chín và điều kiện bảo quản đến tính chất lý hóa của trái giác (*Cayratia trifolia*) từ các vùng địa lý khác nhau ở Việt Nam

## TÓM TẮT

Nghiên cứu này nhằm khảo sát ảnh hưởng của vị trí lấy mẫu, giai đoạn chín và điều kiện bảo quản đến tính chất hóa lý, bao gồm hàm lượng polyphenol tổng số trong trái giác (*Cayratia trifolia*) (CT) được thu thập từ 09 tỉnh Đồng bằng sông Cửu Long tại Việt Nam như Kiên Giang, An Giang, Đồng Tháp, Long An, Bạc Liêu, Cà Mau, Sóc Trăng, Trà Vinh và Bến Tre. Thêm vào đó, kỹ thuật sắc ký lỏng hiệu năng cao (HPLC) được sử dụng để định lượng hàm lượng quercetin và resveratrol trong mẫu CT. Kết quả cho thấy trái CT có hàm lượng polyphenol cao từ 12,14 đến 19,51 mg GAE/g, cao nhất ở mẫu thu thập tại Đồng Tháp (DT1) (19,51 mg GAE/g). Trong giai đoạn chín từ lúc chưa chín đến chín hoàn toàn, hàm lượng polyphenol tổng số của mẫu CT thu ở AG3, TV4, DT1, DT2, CM1 và CM2 tăng lên trong khi hàm lượng này lại giảm ở các mẫu thu thập tại KG1, DT3, DT4, CM3 và CM4. Sự khác biệt này có thể là do sự thay đổi trong quá trình tích lũy hợp chất phenolic ở quá trình chín của trái, vị trí địa lý và điều kiện trồng trọt. Bên cạnh đó, trái CT đông lạnh có hàm lượng polyphenol tổng số không thay đổi đáng kể (18,62 mg GAE/g) trong thời gian bảo quản 5 ngày, trong khi giá trị này tăng lên 19,05 mg GAE/g sau 7 ngày bảo quản. Kết quả phân tích HPLC cũng cho thấy hàm lượng quercetin và resveratrol trong mẫu DT lần lượt là  $0,14 \pm 0,012$  mg và  $1,46 \pm 0,035$  mg/g trọng lượng trái tươi.

**Từ khóa:** *Cayratia trifolia*, polyphenol, quercetin, resveratrol

# Effect of maturation stages and storage conditions on physicochemical properties of three-leave cayratia (*Cayratia trifolia*) berries from different geographical regions in Vietnam

## ABSTRACT

This study aims at investigating the influence of sampling locations, maturation stages, and storage conditions on total polyphenol content in threeleaf cayratia (*Cayratia trifolia*) (CT) berries collected from 09 provinces of the Mekong Delta River in Vietnam, including Kien Giang, An Giang, Dong Thap, Long An, Bac Lieu, Ca Mau, Soc Trang, Tra Vinh, and Ben Tre. In addition, High Performance Liquid Chromatography technique (HPLC) was used to quantify quercetin and resveratrol contents in CT samples. The results showed that CT berries had a high polyphenol content from 12.14 to 19.51 mg GAE/g, being highest in samples collected in Dong Thap (DT1) (19.51 mg GAE/g). During the ripening stages from unripened to fully ripened, the total phenolic content of CT samples from AG3, TV4, DT1, DT2, CM1, and CM2 increased whereas it decreased in those collected in KG1, DT3, DT4, CM3, and CM4. This variation could be due to changes in phenolic compound accumulation during fruit maturation, geographical location, and growing conditions. Besides, total polyphenol content in frozen CT berries frozen showed no significant change (18.62 mg GAE/g) during 5 days of storage, while increased to 19.05 mg GAE/g after 7 days. The results on HPLC further showed that the amount of quercetin and resveratrol in DT samples were  $0.14 \pm 0.012$  and  $1.46 \pm 0.035$  mg/g fresh weight.

**Keywords:** *Cayratia trifolia*, polyphenol, quercetin, resveratrol

## 1. INTRODUCTION

*Cayratia trifolia* (CT), a tropical plant belonging to the Vitaceae family, is mainly found in India, Asia, and Australia. It is a perennial climber, trifoliated leaves with 2-3 cm long petioles and ovate to oblong-ovate leaflets, and small white and brown-greenish flowers<sup>1</sup>. Berries are fleshy, juicy, dark purple, nearly spherical, and about 1-2 cm in diameter<sup>1,2</sup>. *Cayratia trifolia*, commonly known as Giac in Vietnam, is widely distributed in the Mekong Delta provinces<sup>2</sup>. The whole plant contains tannins, alkaloids, flavonoids, polyphenols, steroids, and yellow waxy oil<sup>1-3</sup>. The extract of CT possesses various pharmacological benefits such as antioxidant, antimalarial, gastric anti-ulcer, antiviral, antibacterial, antiprotozoal, anti-inflammatory, anticancer, and diuretic activities<sup>1-8</sup>.

Three-leaf cayratia berries are rich in polyphenol compounds, exhibiting health and anti-aging potential due to their antioxidant capacity<sup>2,9</sup>. However, a large degree of variability in polyphenol content may exist concerning variety, geographic location, environmental conditions, ripening status, and storage condition<sup>10</sup>. The differences in bioactive compound contents during berry maturation are well documented<sup>11-13</sup>. For instance, the total

phenolic content (TPC) of plums decreased during development, while anthocyanin content increased in plums as a function of maturity stages<sup>11</sup>. Similarly, the highest TPC of kiwis is identified at the immature stage<sup>13</sup> and young kiwi stage<sup>14</sup>. Also, the effects of maturation on the amounts of bioactive compounds in grapes<sup>15</sup>, strawberries<sup>16</sup>, redcurrants<sup>17</sup>, and raspberries<sup>18-19</sup> have been demonstrated.

Additionally, the storage conditions are the other factors that influence the polyphenol content of perishable berries<sup>20-22</sup>. Therefore, this study aims to investigate the effect of sampling regions, ripening stages, and storage conditions on the polyphenol contents of *Cayratia trifolia* in Vietnam. The quercetin and resveratrol contents were quantified in appropriate CT sample.

## 2. CONTENTS

### 2.1. Materials and Methods

#### 2.1.1. Materials

Samples: *Cayratia trifolia* berries samples were collected in 9 provinces of the Mekong Delta, Vietnam (Table 1). The provincial names were recorded as they existed at the time of sampling, as they have changed since July 1<sup>st</sup> 2025.

Chemicals: Sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) was purchased from Xilong Scientific (Guangdong, China). Acid gallic was purchased from HiMedia (Maharashtra, India). Folin-Ciocalteu and methanol were purchased from Merck (Darmstadt, Germany).

### 2.1.2. Effects of the collected locations

A total of 36 dark purple and soft pulpy CT berry samples were collected in 9 provinces (namely 4 districts for each province) of the Mekong Delta River in Vietnam (Table 1). These provinces were divided into the marsh ecosystem (An Giang, Dong Thap, and Long An) and the mangrove ecosystem (Ca Mau, Bac Lieu, Soc Trang, Tra Vinh, Ben Tre, Kien Giang).

**Table 1.** *Cayratia trifolia* sample locations

Provinces	Locality	Sample names
Kien Giang 9.8250° N, 105.1259° E	Giong Rieng	KG1
	Ha Tien	KG2
	Kien Luong	KG3
	Hon Dat	KG4
An Giang 10.5216° N, 105.1259° E	Cho Moi	AG1
	Tri Ton	AG2
	Tinh Bien	AG3
	Phu Tan	AG4
Dong Thap 10.4938° N, 105.6882° E	Chau Thanh	DT1
	Lai Vung	DT2
	Cao Lanh	DT3
	Dong Thap Muoi	DT4
Long An 10.6851° N, 106.2051° E	Can Duoc	LA1
	Ben Luc	LA2
	Chau Thanh	LA3
	Thanh Hoa	LA4
Bac Lieu 9.2899° N, 105.5005° E	Hoa Binh	BL1
	Hong Van	BL2
	Gia Rai	BL3
	Phuoc Long	BL4
Ca Mau 9.1527° N, 105.1961° E	Cai Nuoc	CM1
	Nam Can	CM2
	Tran Van Thoi	CM3
	U Minh	CM4
Soc Trang 9.6025° N, 105.9739° E	Ke Sach	ST1
	My Xuyen	ST2
	My Tu	ST3
	Nga Nam	ST4
Tra Vinh 9.7627° N, 106.4406° E	Cau Ngang	TV1
	Chau Thanh	TV2
	Duyen Hai	TV3
	Tieu Can	TV4
Ben Tre	Ba Tri	BT1

Provinces	Locality	Sample names
10.1082° N, 106.4406° E	Mo Cay Bac	BT2
	Thanh Phu	BT3
	Ben Tre	BT4

### 2.1.3. Effects of the ripening stage

CT berries with a high TPC based on results from section 2.1.2 were selected for further investigation of the effect of berries maturation on TPC. Three stages of ripeness were selected, namely unripe (M1, green and hard pulpy), barely ripe (M2, red-purple and slightly soft pulpy), and fully-ripe (M3, dark purple and soft pulpy) (Figure 1A).

### 2.1.4. Effects of the storage conditions

The sample was obtained from the 2.2.2 experiment results regarding TPC. The experiment was performed at three different temperatures including room temperature (28–33°C), cooling temperature (4°C), and freezing temperature (−0.5°C). TPC of the CT berry samples were measured at 3, 5, and 7 days of duration storage.

### 2.1.5. Analysis of physicochemical properties

The pH value was measured using a pH meter (Hanna, USA) and the total soluble solid content was recorded using a manual refractometer (ATAGO, 0–33 °Brix, France).

TPC was determined following the Folin–Ciocalteu colorimetric method<sup>23</sup>. In detail, the CT berries were washed thrice under tap water and finally rinsed with distilled water. After draining for 30 mins, 10 g samples were completely crushed and allocated 25 µL into a test tube, and 975 µL methanol was added to the sample. Subsequently, 200 µL of Folin–Ciocalteu reagent 10% and 2500 µL  $\text{Na}_2\text{CO}_3$  (20% w/v) were added to the mixture. Then, the mixture was homogenized with a vortex for 15 seconds and incubated at room temperature for 45 min in the dark. The absorbance of the sample was measured at 765 nm using a UV-Vis spectrophotometer (ACCURIS SmartReader; Edison, NJ, USA). Gallic acid was used to build a standard curve using a linear regression equation with the relationship between gallic acid concentrations (20, 40, 60, 80, 100, 120 µg/mL) expressed as the X axis and the magnitude of the absorbance results of the reaction of gallic acid with Folin–Ciocalteu reagent stated as the Y axis. TPC estimation was expressed as milligram gallic acid equivalents per gram of sample fresh weight (mg GAE/g). Additionally, experimental samples were chosen

for the quantification of quercetin and resveratrol using high performance liquid chromatography (HPLC). The samples were extracted with a mixture of methanol/water/acetonitrile (30:35:35). Methanol (100%) have been used as mobile phase solution for separation and quantification of the target components. The flow rate was at 0.5 mL/min, the processed temperature in the column was kept stable at 40°C, and the injection volume was 10 µL. UFLC HPLC system (Shimadzu, Kyoto, Japan) was used. Reverse-phase HPLC process were performed using a Shimadzu C18 column (250 x 4.6 mm).

### 2.1.7. Data analysis

All the experiments were conducted in triplicate. Data were expressed as means  $\pm$  standard deviation (SD). SPSS 20.0 software was used for data analysis including one-way analysis of variance (ANOVA) test and Fisher LSD analysis for comparison of means.

## 2.2. Results and Discussion

### 2.2.1. *Physicochemical* properties of CT berries collected in 09 different provinces of the Mekong Delta in Vietnam

The TPC of CT berries varies between 12.14 and 19.51 mg GAE/g while the pH values ranges from 2.94 to 3.64, and the total soluble solids fall within 7.0 to 9.0 °Brix (Table 2). These data indicate the deviation in the flavor of CT berries.

CT berries collected in the different ecological regions in Vietnam exhibit significant differences in TPC ( $p \leq 0.05$ ). The high TPC was identified in CT berries collected in Dong Thap (19.23-19.51 mg GAE/g), followed by those from Ca Mau (17.75-18.13 mg GAE/g), Tra Vinh (16.07-17.52 mg GAE/g), Soc Trang (17.01-17.48 mg GAE/g), Kien Giang (14.59-17.34 mg GAE/g), Long An (14.89-16.45 mg GAE/g), An Giang (12.14-16.57 mg GAE/g), Ben Tre (13.51-16.26 mg GAE/g), and Bac Lieu (13.13-13.80 mg GAE/g). Noblely, DT1 had the highest TPC of 19.51 mg GAE/g, and the lowest TPC of 12.14 mg GAE/g was found in AG2, significantly different from other samples at the 95% confidence level. All CT samples in this study had higher TPC than those extracted by methanol ( $4.6 \pm 0.3$  mg GAE/g sample) and water ( $2.9 \pm 0.1$  mgGAE/g) in Malaysia<sup>9</sup>.

**Table 2.** pH, Brix, and TPC of CT berries collected in 9 provinces of the Mekong Delta River

Province	Sample	pH	°Brix	TPC (mgGAE/g)
Kien Giang	KG1	3.07	7.0	17.34 <sup>fg</sup>
	KG2	3.05	7.5	14.59 <sup>pq</sup>
	KG3	3.45	8.0	15.63 <sup>m</sup>
	KG4	2.98	7.0	16.29 <sup>jk</sup>
An Giang	AG1	3.64	8.0	15.01 <sup>n</sup>
	AG2	2.94	7.0	12.14 <sup>u</sup>
	AG3	3.59	8.5	16.57 <sup>i</sup>
	AG4	3.20	8.0	14.72 <sup>op</sup>
Dong Thap	DT1	3.35	7.0	19.51 <sup>a</sup>
	DT2	3.46	7.5	17.79 <sup>d</sup>
	DT3	3.39	9.0	18.05 <sup>c</sup>
	DT4	3.30	8.0	19.23 <sup>b</sup>
Long An	LA1	3.45	8.0	14.89 <sup>no</sup>
	LA2	3.30	7.0	16.45 <sup>ij</sup>
	LA3	3.48	8.0	15.67 <sup>m</sup>
	LA4	3.38	7.0	16.06 <sup>l</sup>
Bac Lieu	BL1	3.79	8.5	13.80 <sup>r</sup>
	BL2	3.81	10.0	13.13 <sup>t</sup>
	BL3	3.86	9.0	14.53 <sup>q</sup>
	BL4	3.65	9.0	13.62 <sup>s</sup>
Ca Mau	CM1	3.09	6.0	17.99 <sup>c</sup>
	CM2	3.74	7.0	18.13 <sup>c</sup>
	CM3	3.15	8.0	18.03 <sup>c</sup>
	CM4	3.59	7.0	17.75 <sup>d</sup>
Soc Trang	ST1	3.50	8.0	17.05 <sup>h</sup>
	ST2	3.37	6.0	17.48 <sup>ef</sup>

Province	Sample	pH	°Brix	TPC (mgGAE/g)
Tra Vinh	ST3	3.31	7.0	17.24 <sup>g</sup>
	ST4	3.60	7.0	17.01 <sup>h</sup>
	TV1	3.44	9.0	16.28 <sup>ik</sup>
	TV2	3.37	9.0	16.35 <sup>ik</sup>
Ben Tre	TV3	3.85	8.0	16.07 <sup>l</sup>
	TV4	3.88	9.0	17.52 <sup>e</sup>
	BT1	3.68	6.0	13.51 <sup>s</sup>
	BT2	3.29	7.0	16.26 <sup>k</sup>
	BT3	3.72	7.0	16.39 <sup>ik</sup>
	BT4	3.26	6.0	14.47 <sup>q</sup>

The average values in a group with the same letter were not significantly different at the 95% confidence level. KG, AG, DT, LA, BL, CM, ST, TV, and BT are the abbreviations of provinces where the samples were collected; 1-4: number of collected samples of each province

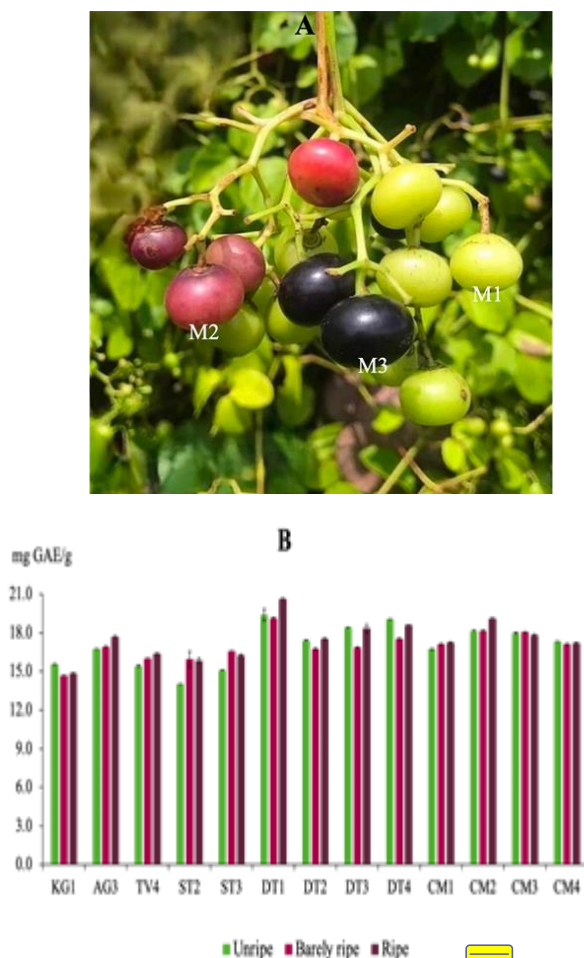
Likewise, the TPC of CT berries was higher than that of other tropical berries extracted by H<sub>2</sub>O, such as pineapple (34.7 mgGAE/100g), banana pisang mas (27 mgGAE/100g), guava (153 mgGAE/100g)<sup>24</sup>. Compared to grape species (another genus of the Vitaceae family), CT berries had higher TPC than both white and red varieties<sup>25</sup>. However, it was lower than that of species of Myrtaceae (49.21 mg GAE/g)<sup>26</sup>, *Myrciaria cauliflora* (31,6 mg GAE/g) and *Eugenia aggregate* (25,3mg GAE/g)<sup>27</sup>. Thirteen CT berries with a high TPC were selected for further study on the changes in polyphenol content during fruit maturation in the next experiment.

#### 2.2.2. TPC of CT berries during maturation

CT berries collected in 13 regions showed significant differences in TPC ( $p < 0.05$ ) as affected by stages of ripeness (Table 3). Most fully ripened CT berries acquired the highest TPC, followed by the unripened and barely ripened samples (Figure 1). This trend is partly in accordance with our previous report on the sampling region in Can Tho (Vietnam), where the fully ripened samples were the mutual best option in terms of TPC<sup>28</sup>.

Interestingly, TPC gradually increased from unripened to fully ripened berries in AG3, TV4, DT1, DT2, CM1, and CM2. By contrast, this value reduced from unripened to fully ripened in samples collected in KG1, DT3, DT4, CM3, and CM4. This difference might be due to the variation of each compound and its concentration (acid gallic, anthocyanin, quercetin, ellagic acid, etc.) in the total polyphenolic content. According to Guofang, the gallic acid content of all rabbiteye blueberry cultivars increased at the beginning of berry formation and decreased at the berry ripening stage<sup>29</sup>. Meanwhile, the ferulic acid contents of Powderblue and Gardenblue cultivars gradually increased during ripening, and the ellagic acid content of Powderblue blueberries increased at the ripening stage but decreased at this stage for Baldwin blueberries<sup>29</sup>. Tahir Mahmood (2017) documented an increasing trend in TPC (201– 2287 mg/100 g GAE) of different species (*Morus alba*, *M. nigra*, *M. macroura*, and *M. laevigata*) of mulberry as the maturity of berries progressed<sup>30</sup>. In this study, the TPC was highest in ripe DT1 and lowest in unripe ST2 samples, at 20.66 mg GAE/g and 14.02 mg GAE/g, respectively.





**Figure 1.** The effect of the ripeness stage on polyphenol content of CT berries. A: The picture of color changes in CT berries during the ripening stage: full-green (M1), purple (M2), and dark purple (M3).

**Table 3.** pH, Brix, and TPC of threeleaf cayratia berries collected at 3-different ripening stages

Ripening stage	Sample	pH	°Brix	TPC (mgGAE/g)
Unripe (Green berries)	KG1-M1	2.78	3.0	15.57 <sup>s</sup>
	AG3-M1	2.78	3.0	16.75 <sup>op</sup>
	TV4- M1	2.73	3.0	15.44 <sup>s</sup>
	ST2- M1	2.92	3.0	14.02 <sup>v</sup>
	ST3- M1	2.86	4.0	15.11 <sup>t</sup>
	DT1- M1	2.82	3.0	19.41 <sup>b</sup>
	DT2- M1	2.80	3.5	17.42 <sup>kl</sup>
	DT3- M1	2.79	3.0	18.43 <sup>de</sup>
	DT4- M1	2.77	3.5	19.06 <sup>c</sup>
	CM1- M1	2.81	3.0	16.74 <sup>op</sup>
	CM2- M1	2.76	4.0	18.18 <sup>fg</sup>
	CM3- M1	2.83	4.0	18.00 <sup>gh</sup>

B: the variation in the total polyphenol content of *Cayratia trifolia* berries at three different ripeness stages. KG, AG, TV, ST, DT, and CM are the abbreviations of provinces where the samples were collected; 1-4: number of collected samples of each province.

The fluctuation in TPC in this study might depend greatly on the complex alteration of polyphenol composition in different genotypes of the same *Cayratia trifolia* L. variety and the development of individual phenolic compounds, requiring further adequate investigation as maturity progresses. In the food production industry, the ripening stage of berries greatly affects the flavor, color, and nutritional content of the product due to the presence of mineral components and biological compounds<sup>31</sup>. CT wine and cider are two fermented products from full-ripened CT berries developed by our research<sup>32,33</sup>. To further diversify CT-based products and improve their quality and yield, a comprehensive assessment of berry quality and origin is essential. Moreover, because CT is a wild species widely distributed across the Mekong Delta region of Vietnam, the centralized transportation and storage of raw materials for fruit processing, underutilization of this resource represents a significant limitation. The fully ripened CT berry (DT1-M3) was chosen for the storage stability experiment.

	CM4- M1	2.80	3.5	17.32 <sup>lm</sup>
Barely ripe	KG1- M2	2.78	4.0	14.69 <sup>u</sup>
(Purple berries)	AG3- M2	2.70	4.5	16.93 <sup>no</sup>
	TV4- M2	2.74	4.0	16.00 <sup>r</sup>
	ST2- M2	2.88	3.5	15.95 <sup>r</sup>
	ST3- M2	2.87	4.0	16.61 <sup>p</sup>
	DT1- M2	2.78	3.5	19.16 <sup>c</sup>
	DT2- M2	2.79	4.5	16.77 <sup>op</sup>
	DT3- M2	2.88	4.0	16.88 <sup>o</sup>
	DT4- M2	2.87	4.0	17.55 <sup>jk</sup>
	CM1- M2	2.97	4.0	17.14 <sup>mn</sup>
	CM2- M2	2.98	3.0	18.15 <sup>fg</sup>
	CM3- M2	2.93	3.0	18.06 <sup>g</sup>
	CM4- M2	2.96	3.5	17.13 <sup>mn</sup>
Ripe	KG1- M3	2.95	5.0	14.85 <sup>u</sup>
(Dark purple berries)	AG3- M3	3.39	7.0	17.72 <sup>ij</sup>
	TV4- M3	3.04	5.0	16.38 <sup>q</sup>
	ST2- M3	3.04	5.0	15.84 <sup>r</sup>
	ST3- M3	3.12	6.0	16.27 <sup>q</sup>
	DT1- M3	3.29	9.0	20.66 <sup>a</sup>
	DT2- M3	3.45	7.5	17.54 <sup>jk</sup>
	DT3- M3	3.42	7.0	18.35 <sup>ef</sup>
	DT4- M3	3.54	9.0	18.61 <sup>d</sup>
	CM1- M3	3.22	7.0	17.25 <sup>lm</sup>
	CM2- M3	3.62	7.0	19.13 <sup>c</sup>
	CM3- M3	3.36	9.0	17.83 <sup>hi</sup>
	CM4- M3	3.48	8.0	17.15 <sup>lm</sup>

The average values in a group with the same letter were not significantly different at the 95% confidence level. KG, AG, DT, LA, BL, CM, ST, TV, and BT are the abbreviations of provinces where the samples were collected; 1-4: number of collected samples of each province; M1: unripened green berry; M2: barely ripened purple berry; M3: full-ripened dark purple berry.

### 2.2.3. Effects of the temperature and storage duration on TPC of CT berries

The influence of storage temperature (cooling, freezing, and room temperature) and storage time (3, 5, and 7 days) on TPC of CT berries are shown in Table 4. Room temperature (28-33°C) caused adverse effects in polyphenol retention in CT berries, representing the lowest amount of

TPC. At this temperature, the CT berries decayed during 7-day storage since high temperature might promote microbial spoilage, respiration level, and oxidative and nonoxidative reactions<sup>22</sup>.

**Table 4.** The effects of temperature and duration storage on TPC of *Cayratia trifolia* berries



Temperature (°C) - days	Samples	TPC (mgGAE/g)
33°C - 3	DT1-RT3	18.34 <sup>c</sup>
33°C - 5	DT1-RT5	18.42 <sup>c</sup>
33°C - 7	DT1-RT7	-
4°C - 3	DT1-CT3	16.49 <sup>e</sup>
4°C - 5	DT1-CT5	16.18 <sup>d</sup>
4°C - 7	DT1-CT7	16.16 <sup>d</sup>
-5°C - 3	DT1-FT3	18.66 <sup>b</sup>
-5°C - 5	DT1-FT5	18.62 <sup>b</sup>
-5°C - 7	DT1-FT7	19.05 <sup>a</sup>

Prolong storage duration seemed to degrade polyphenols in samples stored at tropical temperatures while unexpectedly advanced such content in those kept at freezing condition. The total phenolic compound content in frozen samples obtained the highest value of 19.05 mg GAE/g in 7-day storage. In our previous study, CT berries stored at the frozen temperature represented maximal TPC<sup>28</sup>. The best storage temperature to preserve polyphenols in berry was freezing to -20 °C, as evidenced by many authors<sup>22,25</sup>. Freezing temperatures crystallize water, reducing water activity, biochemical changes, and microbial growth<sup>34</sup>.

#### 2.2.4. Quantification of resveratrol and quercetin in the berries

Concentration of resveratrol and quercetin was quantified by using RP-HPLC method, at wavelength of 306 nm and 368 nm, respectively. Resveratrol in CT samples appears in a sharp peak, with retention time at around 5.4-5.6 minutes. Quercetin peaks in the CT sample had the retention time at 5.5-5.8 minutes. The average concentrations of resveratrol in the samples was 1.46 mg/g wet weight while lesser amount counted for quercetin by 0.14 mg/g. A paired sample test showed that the amount of resveratrol was  $1.32 \pm 0.046$  mg higher than that of quercetin, with significant difference ( $p < 0.001$ ).

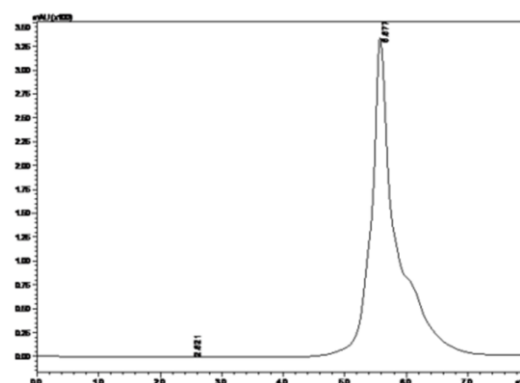


Figure 2. Chromatogram of resveratrol in CT berries

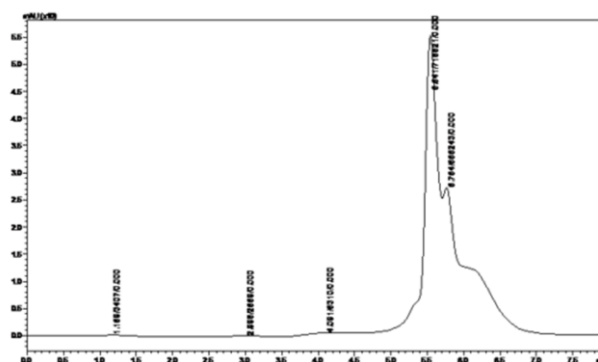


Figure 3. Chromatogram of quercetin in CT berries



**Table 5.** Concentrations of resveratrol and quercetin in CT berries

Compounds	Retention time (minutes)	Concentration (mg/g wet weight)
Resveratrol	5.4-5.6	1.46 ± 0.35***
Quercetin	5.5-5.8	0.14 ± 0.11

The result values are expressed as means ± SD, n = 3. The stars (\*\*\*) indicates a significant difference at  $p < 0.001$ .

Resveratrol and its glucosides was found in the CT roots culture<sup>35</sup>. Additionally, resveratrol and quercetin are rich stilbenes and flavonols found the fruit berries<sup>36</sup>. Quercetin and other flavonoid components have been extensively found in different types of fruit berries and plant species around the globe<sup>37-38</sup>.

### 3. CONCLUSIONS

Wild three-leaf cayratia in Vietnam can be classified into berries with high polyphenol content from 12.14 to 19.51 mg GAE/g. The growing regions, ripening stages, and storage conditions affected the level of bioactive compounds. Dong Thap and Ca Mau provinces highly nourished three-leaf cayratia compared to other sampling locations. The berries exhibited the optimal content of phenolic compounds at the fully ripened stage. The frozen storage favorably accumulated the highest polyphenols in 7 days. The results of this study provided essential information for determining the location, harvesting time and storage conditions for processing products from the three-leaf cayratia with high biological activity due to high polyphenol content. Additionally, the higher content of resveratrol (1.46 mg/g wet weight) than that of quercetin (0.14 mg/g wet weight) has been found in the CT berries. Further researchs on other polyphenol targets in the CT products are important to confirm its valuable source for food and health.

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