

COMPARISON OF BIOACTIVE COMPOUNDS AND ANTIOXIDANT ACTIVITY DURING DEVELOPMENT AND STORAGE OF *DAU HA CHAU* (*BACCAUREA RAMIFLORA* LOUR.) FRUIT OF VIETNAM

Nguyen Hong Xuan^{1✉}, Nguyen Cong Ha²

¹Faculty of Biological, Chemical and Food Technology, Department of Food Technology, Can Tho University of Technology
256 Nguyen Van Cu Street, Ninh Kieu District, Can Tho City, 90000, Vietnam

²Department of Food Technology, College of Agriculture, Can Tho University
Campus 2, 3/2 Street, Ninh Kieu District, Can Tho City, 90000, Vietnam

ABSTRACT

Background. *Dau Ha Chau* is a seasonal fruit which has originated from selecting and propagating the local varieties of *Baccaurea ramiflora* Lour. in Vietnam. However, the time to harvest, the quality of the fresh fruit, and the ability to store it have not been evaluated sufficiently. The aim of this study was to assess the changes in bioactive compounds and antioxidant activity during fruit development and storage stages to evaluate the quality of fruit after harvest and under storage conditions in order to stabilize the fruit sources for the processing of *dau Ha Chau* products during the off-season.

Materials and methods. Three development stages of *dau Ha Chau* (100, 115, and 130 days after fruit set) and four storage conditions (at 30, 4 and –20°C (whole fruit) and –20°C (peeled fruit)) were analyzed, as well as ascorbic, gallic acid (HPLC method), total phenolics (Folin-Ciocalteu), flavonoids content (AlCl₃ complexation), and antioxidant activity (DPPH method).

Results. Over three stages, the external color of fresh *dau Ha Chau* peel altered from light yellowish green (day 100) to pale yellow (day 130). The bioactive compounds and antioxidant activity (EC₅₀) showed a concentration decline, with figures on day 130 reaching 826.26, 78.17 µg/mL, 157.89 mgGAE/L, 76.69 mgQE/L, and 614.36 mg/mL (EC₅₀), respectively. While fresh *dau Ha Chau*, after being picked on day 130, retained their quality to 20 days at 30°C, with 407.66, 78.12 µg/mL, 117.07 mgGAE/mL, 55.29 mgQE/mL, and 1122.62 mg/mL (EC₅₀), respectively, on day 20, those parameters at 4°C were kept to 30 days. The frozen storage at –20°C showed a slight decline in fruit quality after 6 months, but whole fruit preserved the quality better than peeled fruit.

Conclusion. The results of this study are the first report on bioactive compounds and antioxidant activity of *dau Ha Chau* fruit during preharvest and postharvest stages and fruit storage capacity. This will be a source material based on which further assessments of fruit nutrition and health benefits for development in research, fresh fruit consumption, or the food-drink processing industry can be done.

Keywords: Antioxidant activity, *Baccaurea ramiflora* Lour., bioactive compounds, *dau Ha Chau*, storage

INTRODUCTION

Dau Ha Chau – DHC (*Baccaurea ramiflora* Lour. – *B. Ramiflora*) is one of the delicious, seasonal speciality

fruits that has been recognized as a trademark by the Intellectual Property Office of Viet Nam in 2006.

✉nhxuan@ctu.edu.vn

DHC trees were selected and propagated from the local varieties of *B. Ramiflora* in Phong Dien district, Can Tho city, Vietnam. The color of DHC peels changed from green (early fruiting stage) to milky white (or pale yellow) (ripening stage) after about 130 days. Although DHC fruit is mainly eaten raw, information on the quality attributes of fresh fruit has not been evaluated clearly during storage after being harvested.

The quality of fruit varies at different development stages and with some horticultural techniques (Moneruzzaman et al., 2013). Color values are experienced as L^* , Hue angle, and Chroma by conversion from a^* and b^* (McLellan et al., 1995). The total dissolved solids (TSS) are quite important for fruit taste development (Villanueva et al., 2004). The nutritional value of ripe *B. ramiflora* fruits was due to the high value of vitamin C (a soluble antioxidant), water, carbohydrates, fiber, magnesium, potassium, phosphorus, iron, and other elements that could be essential for human health (Sundriyal and Sundriyal, 2004). In addition, *B. ramiflora* juice also contains high levels of phenolic and flavonoid compounds that have potential natural antioxidant activity (Uddin et al., 2018). With this polyphenolic characteristic, flavonoids have been of interest to researchers because of their strong antioxidant properties that allow the prevention of cancer-inducing free radical damage to the body (Zitka et al., 2011).

For seasonal fruits, storage is necessary to provide information about the shelf life of fruit after harvest and ensure the fruit materials for the off-season processing product. The freezing process has been considered to be one of the best methods to preserve the quality characteristics of food by slowing spoilage and preventing microbial growth (Erickson and Hung, 2012). Furthermore, it better preserves the flavor, color, and nutritional values of the food than canning or drying processes (Sun, 2005).

The aim of this study was to evaluate the effects of three development stages and four storage conditions of DHC fruits on bioactive compounds and antioxidant activity in order to select the right time to harvest and assess the ability to store fresh DHC for research and processing of DHC products during the off-season.

MATERIALS AND METHODS

Raw material and preparation of fruit samples

Bunches of fresh DHC fruit were harvested three times at three development stages (100, 115, and 130 days after fruits set) from the local garden in Phong Dien District, Can Tho City. These bunches were packed in plastic bags covered with some DHC leaves inside and transported quickly to the laboratory of Can Tho University, Vietnam. After being rinsed in tap water to eliminate foreign matter and dust, the individual fruits in each stage were chosen with uniform size (visual observation), pH and TSS (instrumental measurements), and color (determination of peel color) for analysis of the bioactive compounds and antioxidant activity in the juice.

Postharvest storage

Fruit samples at the maturity stage (130 days after the fruits set) were divided into four groups. Group I (GI) was stored in ambient laboratory conditions ($30 \pm 2^\circ\text{C}$ as control) away from sunlight. In Groups II (GII) and III (GIII), each pouch for storage weighing 200 ± 5 g was stored at $4 \pm 2^\circ\text{C}$ and $-20 \pm 2^\circ\text{C}$. The fruits in Group IV (GIV) had their peels removed using the stainless steel knife. The pulp weighed between 200 ± 5 g and was placed in each pouch for freezing at $-20 \pm 2^\circ\text{C}$. Pouches were polyethylene zipper bags and the air was pressed out before sealing and freezing. The fruits were stored for various lengths of time at these temperatures. Thawing was carried out by submerging fruit pouches in flowing tap water until the fruit separated. After removing the peels, the pulps were wrapped in a cotton cloth and hand-squeezed to separate the juice for bioactive compounds and antioxidant activity evaluation.

Total soluble solids (TSS)

TSS was measured in triplicate using the ATAGO Hand-held Refractometer (ATAGO CO., LTD, Japan). The readings were taken at room temperature ($28\text{--}30^\circ\text{C}$). Data were expressed as equivalent °Brix.

Peel color

The colors of the peels were measured by using visible spectrophotometer sph870 (Germany) to record L^* , a^* , and b^* values parameters. The L^* value indicates

the lightness/darkness. The a^* is a color scale ranging from red to green value and b^* is a color scale ranging from yellow to blue. The color intensity (Chroma – C value) and visual color appearance (hue angle – H°) (McLellan et al., 1995) were calculated by equations $C = \sqrt{a^{*2} + b^{*2}}$ and $H^\circ = \arctan\left(\frac{b^*}{a^*}\right)$; where 0° – 360° was red-purple; 90° was yellow; 180° was green, and 270° was blue.

HPLC analysis of gallic acid and ascorbic acid

High Performance Liquid Chromatography (HPLC) analysis was carried out by (Arceusz and Wesolowski, 2013) with modifications to determine the content of gallic and ascorbic acid on a Shimadzu HPLC system equipped with a UV-VIS detector (SPD-20A), autosampler, and Ascentis® C18 column (25 cm × 4.6 cm, 5 μ m). The column temperature was maintained at 40°C and the flow rate at 1.0 mL/ min. Separations were performed using isocratic elution with methanol (solvent A) and trifluoroacetic acid (0,05%) (solvent B). The mobile phase ratios were fixed at 70A:30B (volume ratio) in 5 minutes for the determination of gallic acid and 50A:50B (volume ratio) in 10 minutes for the determination of ascorbic acid. The UV detection wavelength of ascorbic acid and gallic acid were 254 nm and 280 nm, respectively.

Total phenolics content

The total polyphenols content (TPC) was determined by the Folin-Ciocalteu method described by (Singleton et al., 1999) with slight modifications using gallic acid (GAE) as a standard. The absorbance was set at 765 nm using UV-1800 Spectrophotometer (Shimadzu, Japan). Different concentrations from the standard stock solution of gallic acid (1 mg/mL) in the range of 1, 2, 4, 6, 8 and 10 $\mu\text{g/mL}$ were prepared. The total polyphenols content in the juice was expressed as gallic acid equivalent (mgGAE/mL).

Total flavonoids content

The total flavonoids content (TFC) was determined by the AlCl_3 complexation method described by Bag et al. (2015) with slight modifications using quercetin (QE) as a standard. The absorbance was set at 510 nm using a UV-1800 Spectrophotometer (Shimadzu, Japan). Different concentrations from the standard

stock solution of quercetin (1 mg/mL) in the range of 10, 20, 40, 60, 80 and 100 $\mu\text{g/mL}$ were prepared. The total flavonoids content in the juice was expressed as quercetin equivalent (mgQE/mL).

Antioxidant activity

1,1-Diphenyl-2-picrylhydrazyl (DPPH) Assay: the DPPH assay was determined using the method of Sharma and Bhat (Sharma and Bhat, 2009) with suitable modifications using Trolox as a standard. A 40 μL DPPH (1000 $\mu\text{g/mL}$) reagent solution was added to 960 μL of the extract. A blank control was 960 μL of methanol and 40 μL DPPH solution. After leaving it in the dark for 30 minutes, the mixture was spectrophotometrically measured at 517 nm by UV-VIS Thermo Scientific (Miltiskan GO/Cat No: 51119200, Finland). DPPH scavenging activity was calculated as in the equation: $\%AA = [(A_o - A_s)/A_o] \times 100\%$, where A_s is the absorbance of the sample, A_o is the absorbance of the blank sample and $\%AA$ is the percentage of inhibition of DPPH. The EC_{50} (minimum concentration required for the antioxidant to reduce the initial concentration of the DPPH by 50%) was the term used to express the antioxidant activity. The higher the EC_{50} value was, the lower the antioxidant activity of the juice got.

Statistical analysis

All methods of analysis in this study were done via the Minitab 16 software (Minitab Inc. USA). The assay was repeated three times for each extract concentration. One-way analysis of variance and Tukey tests were used to determine significant group differences. Results were expressed as mean \pm standard deviation and considered to be statistically significant if $p < 0.05$.

RESULTS AND DISCUSSION

Effect of fruit development stages on pH, total soluble solids (TSS), peel color, bioactive compounds, and antioxidant activity of DHC

Changes in pH and TSS. According to the three harvesting stages of DHC (Table 1), pH and TSS increased from 2.83 to 3.1 and 9.67 to 17.50°Brix, respectively. During the advancement of fruit in maturity, while organic acids declined due to consumption in the process of respiration and the acidity of the fruit, the soluble

Table 1. Changes in parameter values of DHC at three development stages

Parameters	Day 100	Day 115	Day 130
pH	2.83 ±0.06 ^b	2.93 ±0.06 ^{ab}	3.10 ±0.10 ^a
Total soluble solids (TSS) (°Brix)	9.67 ±0.58 ^c	15.17 ±0.76 ^b	17.50 ±0.50 ^a
<i>Hunter parameter</i>			
<i>L</i> *	80.48 ±1.08 ^c	91.56 ±1.54 ^b	95.17 ±0.59 ^a
<i>a</i> *	-31.84 ±1.25 ^c	-10.81 ±0.46 ^b	0.75 ±0.09 ^a
<i>b</i> *	19.74 ±0.73 ^c	28.23 ±0.58 ^a	22.03 ±1.24 ^b
Chroma value, C	37.47 ±1.20 ^a	30.24 ±0.56 ^b	22.04 ±1.23 ^c
Hue angle, H°	148.19 ±1.26 ^a	110.95 ±0.92 ^b	88.04 ±0.28 ^c
Peel color	Light yellowish green	Light yellow green	Pale yellow
<i>Bioactive compounds</i>			
Ascorbic acid, µg/mL	1329.33 ±100.24 ^a	1031.63 ±49.11 ^b	826.26 ±44.27 ^c
Gallic acid, µg/mL	124.68 ±5.40 ^a	95.40 ±14.70 ^b	78.17 ±11.79 ^b
Total phenolics content, mgGAE/L	284.12 ±14.60 ^a	174.13 ±9.23 ^b	157.89 ±6.54 ^c
Total flavonoids content, mgQE/L	123.93 ±11.03 ^a	128.42 ±13.09 ^a	76.69 ±4.97 ^b
<i>Antioxidant activity</i>			
EC ₅₀ , mg/mL	337.23 ±29.401 ^b	298.32 ±23.70 ^b	614.36 ±19.81 ^a

Different letters (a, b, c) in the same row indicate significant differences between development stages.

sugars rose, leading to an increase in pH, but became relatively stable at the ripening stage (Akhtar and Rab, 2015). This was shown in the data that there was no statistical difference in pH between day 115 and day 130. In the studies of different fruit types, an improvement in TSS during the ripening period was reported in cherimoya fruits because of loss in fruit firmness (Tandon and Kalra, 1986) or in strawberry fruits due to the conversion of starch into sugars (Martínez et al., 2004).

Changes in peel color. The average duration from early fruiting to the mature fruiting stage was 130 days with the color change to pale yellow by visual observation. While the lightness value (*L**) gradually increased between 80.48 and 95.17 and the red-green value (*a**) rose rapidly from -31.84 to 0.75, the yellow-blue value (*b**) climbed in the period from day 100 to 115 and then decreased appreciably to day 130

(Table 1). This led to the fruit's color getting brighter, the fruit's green color fading, and the yellow color getting lighter. In addition, the analysis of Hue angle (H°) and Chroma value (C) has been effective in assessing colors accurately (Kelly and Judd, 1976; McLellan et al., 1995). The results showed that the Chroma and Hue angle values of the fruit were significantly affected by the levels of fruit ripeness (Table 1 and Fig. 1). As DHC fruit ripened, both C and H° values declined substantially. This demonstrated that the peel color of fresh DHC altered from light yellowish green (Fig. 1A) to pale yellow (Fig. 1C). This could be explained by the reduction of green compounds (chlorophyll) because of the activity of enzymes such as chlorophyllase, chlorophyll oxidase, and peroxidase. The study of Tapre and Jain (2012) on the ripening process of banana also showed that the riper the fruit was, the lighter the green color and the higher the amount of carotenoids became.

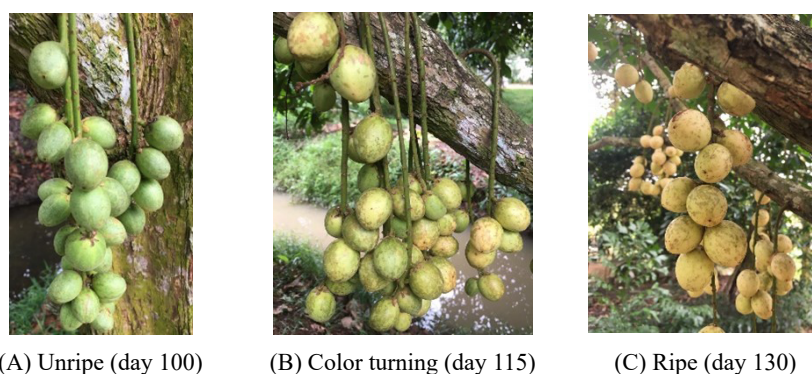


Fig. 1. Changes in the color of DHC fruits during harvesting stages

Changes in ascorbic acid and gallic acid. The results of both ascorbic acid and gallic acid values in Table 1 fell significantly from the first (1329.33 and 124.68 $\mu\text{g}/\text{mL}$) to the last levels with 37.84% and 37.3%, respectively. During the levels of fruit ripeness, ascorbic acid or vitamin C contents could vary according to different fruits. While the contents of ascorbic acid in ripe DHC decreased in a manner similar to that in apple and mangoes, an opposite picture could be seen in the cases of watermelon and papayas. (Muhammad et al., 2014).

Changes in TPC, TFC and antioxidant activity (EC_{50}). In Table 1, the great influence of the levels of DHC ripeness on TPC and TFC contents was presented. Both of them declined significantly by about 44.43% (TPC) and 38.12% (TFC) from unripe to ripe stage. The differences in the content of phenolic compounds (phenolic acids and flavonoids) could be mainly due to differences in various fruit species, growing conditions or fruit growth stages (Dixon and Paiva, 1995). Because of the downward trend of antioxidative compounds on development stages of DHC, the antioxidant activity in the fruit reduced sharply, corresponding to the two-fold increase in EC_{50} compared to unripened fruit. The accumulation of various phenolic compounds was related to the physiological state of the fruit as a result of an equilibrium between biosynthesis and metabolism, including anabolism and catabolism. The most important control mechanisms in phenolic metabolism include the number of enzymes, the differential distribution of enzymes, and the availability of

precursors and integration mediators in different parts of the growth stage (Harborne, 2013).

Effect of storage conditions on bioactive compounds and antioxidant activity of DHC

Over the survey period, DHC fruit stored at 30°C (GI) showed signs of damage with the appearance of a range of symptoms outside of the pericarp, such as dark brown spots and black lesions. Regarding natural dehydration, pericarp also gradually became soft, wilted, and hardened. In the 20 days' period, all the fruits stored were damaged because of stem rot and black or green-blue mould. While GII at 4°C experienced a longer storage time than GI at 30°C for about 10 days, the fruit were also damaged and no longer used until 30 days' storage. Only the fruit preserved in groups III and IV were relatively stable during 180 days of storage.

Evolution of ascorbic acid. As can be seen from Table 2, the content reached the highest level in the first storage time (789.43 $\mu\text{g}/\text{mL}$), after which it fell to the lowest point at 407.66 $\mu\text{g}/\text{mL}$ for 20 days at 30°C. Although the fruit was damaged slower at 4°C than 30°C, GII at 4°C witnessed a sharp drop, with figures decreasing by 45% at 30 days of storage. Despite being frozen at -20°C, the ascorbic acid content in GIII and GIV fell significantly by 27.76 and 30.23% over 6 months. There were no statistically significant differences between GIII and GIV in ascorbic acid losses. Sahari et al. (2004) reported that the major losses of ascorbic acid in Iranian strawberries occurred over

Table 2. Variation of ascorbic acid ($\mu\text{g/mL}$) in DHC in different storage conditions

Storage times	Storage temperatures			
	30 \pm 2°C (GI)	4 \pm 2°C (GII)	-20 \pm 2°C (fruit) (GIII)	-20 \pm 2°C (pulp) (GIV)
Day 0	789.43 \pm 18.77 ^a	789.43 \pm 18.77 ^a	789.43 \pm 18.77 ^a	789.43 \pm 18.77 ^a
Day 5	600.93 \pm 3.12 ^{bB}	726.59 \pm 12.80 ^{bA}	732.31 \pm 10.25 ^{bA}	722.26 \pm 37.63 ^{abA}
Day 10	475.32 \pm 12.76 ^{cC}	652.65 \pm 14.54 ^{bB}	731.86 \pm 4.03 ^{bA}	728.60 \pm 26.55 ^{abcA}
Day 15	430.03 \pm 6.61 ^{dC}	606.66 \pm 6.03 ^{bB}	730.23 \pm 14.29 ^{bA}	730.57 \pm 36.72 ^{abcA}
Day 20	407.66 \pm 23.22 ^{dC}	586.69 \pm 11.94 ^{bB}	725.14 \pm 23.10 ^{bA}	719.65 \pm 7.23 ^{abA}
Day 25		491.60 \pm 7.01 ^{cB}	718.65 \pm 6.28 ^{ba}	710.52 \pm 24.40 ^{bcA}
Day 30		434.33 \pm 16.54 ^{dB}	715.55 \pm 1.397 ^{bcA}	689.77 \pm 11.66 ^{bcA}
Day 60			692.29 \pm 4.182 ^{bcA}	662.05 \pm 3.35 ^{bcA}
Day 90			670.73 \pm 15.85 ^{cA}	662.05 \pm 12.31 ^{cA}
Day 135			618.85 \pm 27.25 ^{dA}	584.82 \pm 28.21 ^{dA}
Day 180			570.31 \pm 22.33 ^{cA}	550.75 \pm 20.58 ^{dA}

Different uppercase letters (a, b, c) in the same column indicate significant differences between storage temperatures. Different lowercase letters (A, B, C, D) in the same row indicate significant differences between storage times. (GI) whole fruits were stored in ambient laboratory conditions at 30 \pm 2°C, (GII) whole fruits were in a pouch and stored at 4 \pm 2°C, (GIII) whole fruits were in a pouch and stored at -20 \pm 2°C, (GIV) peeled fruits was in a pouch and stored at -20 \pm 2°C.

the first 15 days of three months of storage and the percentages were 64.5, 10.7, and 8.9% at -12, -18, and -24°C, respectively. The oxidation of ascorbic acid caused ascorbic acid loss during storage into dehydroascorbic acid and 2,3-diketogulonic. This was due to the presence of not only oxygen, but also exposure to light, heat peroxides, and enzymes such as ascorbate oxidase and peroxidase (Guadagni and Kelly, 1958).

Evolution of gallic acid. In Table 3, depending on the storage condition, the gallic acid content in GIII and GIV fluctuated slightly with no statistical significances for the first 3 months of storage. During the next 3 months of frozen storage, this value declined slightly by 14.93% and 19.87%, respectively. Unlike ascorbic acid degradation at 30 and 4°C, the gallic acid reached the highest point of 81.91 $\mu\text{g/mL}$ on the 5th day at 30°C and 82.10 $\mu\text{g/mL}$ on the 10th day at 4°C. This can be explained by the fact that the enzyme was released from the cellular wall to alter gallic acid derivatives of the fruits during storage. For example, hydrolysable

tannins (polyphenolic plant) and gallic acid esters were hydrolysed into gallic acid and glucose by tannase catalyzes (Lekha and Lonsane, 1997). However, the amount of gallic acid dropped considerably when the storage time until damage appeared as dark brown or black marks on the skin of fruit was prolonged.

Evolution of TPC. As presented in Fig. 2A, storage time and temperature significantly affected total phenolic content in DHC at four conditions. The level of phenolic compounds reduced by 25.85% and 29.20% after 20 days at 30°C in GI and 30 days at 4°C in GII, respectively. Although frozen storage during 180 days at -20°C slightly reduced TPC content in GIII (6.42%) and GIV (11.26%), there were no significant differences. The percentage loss of phenolic compounds during the frozen storage depended on the enzyme of polyphenol oxidase (PPO) released by the broken cells, and the presence of molecular oxygen oxidized the polyphenolics to quinones (Türkben et al., 2010). The decrease of TPC at 30°C and 4°C may be due

Table 3. Variation of gallic acid ($\mu\text{g/mL}$) in DHC at different storage conditions

Storage times	Storage temperatures			
	30 \pm 2°C (GI)	4 \pm 2°C (GII)	-20 \pm 2°C (fruit) (GIII)	-20 \pm 2°C (pulp) (GIV)
Day 0	77.69 \pm 2.32 ^b	77.69 \pm 2.32 ^b	77.69 \pm 2.32 ^a	77.69 \pm 2.32 ^a
Day 5	81.91 \pm 1.21 ^{aA}	79.96 \pm 0.08 ^{abAB}	75.51 \pm 0.79 ^{abC}	76.18 \pm 2.80 ^{abC}
Day 10	78.51 \pm 0.90 ^{abAB}	82.10 \pm 1.94 ^{aA}	76.21 \pm 1.70 ^{ab}	75.74 \pm 1.13 ^{abC}
Day 15	79.12 \pm 1.01 ^{abA}	80.06 \pm 0.56 ^{abA}	76.13 \pm 0.62 ^{ab}	75.97 \pm 1.00 ^{abC}
Day 20	78.12 \pm 1.05 ^{baB}	79.79 \pm 0.45 ^{abA}	76.42 \pm 1.18 ^{abC}	75.35 \pm 0.73 ^{abC}
Day 25		79.41 \pm 0.88 ^{abA}	75.31 \pm 0.50 ^{ab}	75.18 \pm 1.45 ^{bcB}
Day 30		79.33 \pm 0.75 ^{abA}	75.64 \pm 1.50 ^{ab}	73.58 \pm 1.05 ^{bcB}
Day 60			75.47 \pm 0.58 ^{aA}	72.01 \pm 0.60 ^{bcB}
Day 90			75.54 \pm 1.51 ^{aA}	67.68 \pm 3.50 ^{cB}
Day 135			70.15 \pm 2.23 ^{ba}	66.18 \pm 1.68 ^{da}
Day 180			66.09 \pm 2.28 ^{ba}	62.25 \pm 2.94 ^{da}

Different uppercase letters (a, b, c) in the same column indicate significant differences between storage temperatures. Different lowercase letters (A, B, C, D) in the same row indicate significant differences between storage times. (GI) whole fruits were stored in ambient laboratory conditions at 30 \pm 2°C, (GII) whole fruits were in a pouch and stored at 4 \pm 2°C, (GIII) whole fruits were in a pouch and stored at -20 \pm 2°C, (GIV) peeled fruits were in a pouch and stored at -20 \pm 2°C

to increased oxidation of the bioactive components (Moldovan et al., 2016).

Evolution of TFC. According to Fig. 2B, although the contents of flavonoids remained slightly stable at freezing temperature from the beginning of storage to 180 days in GII and 90 days in GIV, they decreased significantly to 21.40 mgQE/L after 20 days of storage time at 30°C and 21.34 mgQE/L after 30 days of storage time at 4°C. According to Irina and Mohamed (2012), the degradation of flavonoids was affected by not only temperature, pH, and structure of biologically active compounds but also the presence or absence of oxygen.

Evolution of antioxidant activity (EC₅₀). According to Fig. 2C, the effects of storage condition on antioxidant properties in DHC were shown to result in a clear fall of antioxidant activity or a climb of EC₅₀ values. The highest EC₅₀ at 1122.62 mg/mL at 30°C at 20 days of storage was synonymous with the lowest antioxidant activity of DHC. After 30 days, at 4°C, the

antioxidant activity of GII reduced and was equivalent to the EC₅₀ value which increased by 1.82 times at 1118.72 mg/mL. Freezing GIII and GIV at -20°C did not show differences in this value during 180 days (GIII) and 135 days (GIV). Therefore, stable antioxidant activity in DHC during storage could be due to freezing conditions.

The above-mentioned results showed that although secondary metabolism in DHC remained active in tissues under room and cooling conditions, there was a stable trend in freezing conditions. However, due to very limited storage time in 6 months and chemical analysis selection of bioactive substances, information about changes in the nutritional content of fruit was not observed thoroughly with long-term frozen storage. According to Kramer (1979), wherever the temperature is low, there will be certain enzymatic and non-enzymatic changes at slower rates, thus the storage life of frozen foods is limited. The ascorbic acid, gallic acid, TPC, TFC changes could affect the bioavailability of these compounds and antioxidant activity of DHC.

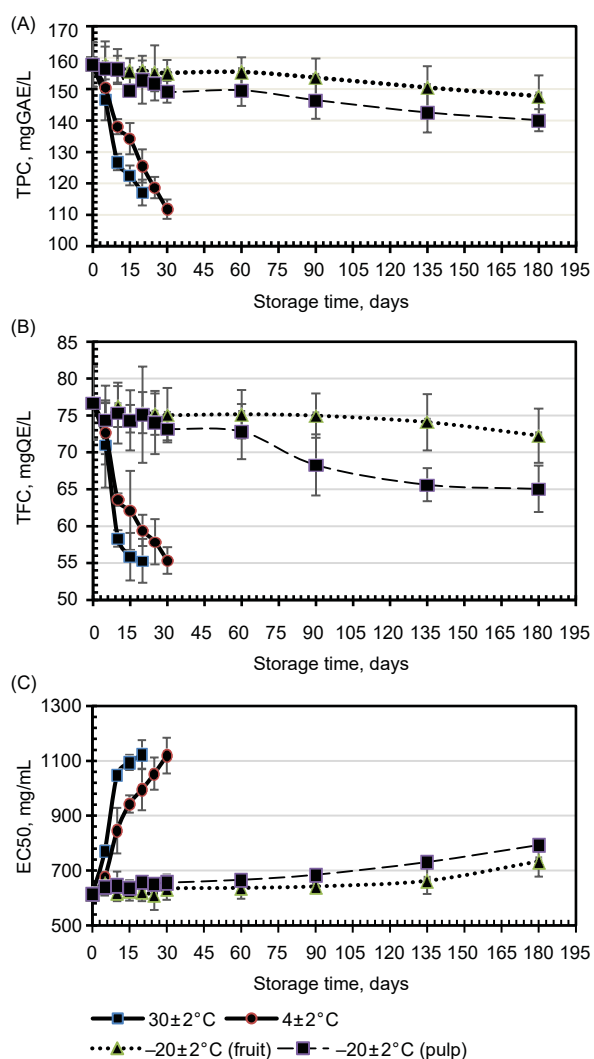


Fig. 2. Evolution of total phenolics content (TPC) (A), total flavonoids content (TFC) (B), antioxidant activity (EC₅₀) (C) in DHC under storage conditions with the error bars (mean ± standard deviation). (30 ± 2°C) whole fruits were stored in ambient laboratory conditions (GI), (4 ± 2°C) whole fruits were in pouch and stored at 4 ± 2°C (GII), (-20 ± 2°C (fruit)) whole fruits were in pouch and stored at -20 ± 2°C (GIII), (-20 ± 2°C (pulp)) peeled fruits were in pouch and stored at -20 ± 2°C (GIV)

CONCLUSIONS

In conclusion, ripe DHC has a pale yellow color and a perfect combination of sweet and sour. Fresh fruits could serve as potential natural sources of ascorbic

acid, gallic acid, total phenolics content, total flavonoids content, and antioxidant activity that could be good for human health-promoting compounds. Moreover, this is a seasonal fruit, so storing DHC is necessary to provide information about the shelf life of the fruit after harvest or compare the quality of fresh and frozen fruit to choose the right type of fruit storage conditions to provide fruit materials for the processing of DHC products.

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