STUDY ON CHANGES IN CHEMICAL COMPOSITIONS AND BIOACTIVE COMPOUNDS IN POUTERIA CAMPECHIANA FRUIT DURING STORAGE

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Abstract. Pouteria campechiana, a fruit cultivated in many provinces of the Mekong Delta, is typically eaten fresh and has many benefits to human health. Therefore, quality control during storage of this fruit is very important for fresh consumption and food processing. This study was conducted to investigate the effects of postharvest maturity on the quality of the fruit stored at room temperature, mainly on its chemical composition and bioactive compounds content. The results showed that storage time greatly affected the amounts of carotenoids, tannin, phenolic and flavonoids: the highest content of carotenoids, polyphenols, flavonoids were obtained at day 10th of storage while tannin content gradually declined. The greatest antioxidant activity of fruit extracts was also obtained after 10 days storage. It was recommended that the highest nutritional value of Pouteria campechiana can be achieved from 8 to 10 days after harvest.

Keywords: ABTS, DPPH, carotenoids, flavonoids, polyphenols, tannin.

Classification numbers: 1.2.1, 1.3.4, 1.4.4.

1. INTRODUCTION

Pouteria campechiana is a fruit grown in Peru, Ecuador, Chile and Mexico, which is an important of the Spaniards diet [1-4]. In Viet Nam, harvesting season is from July to November [5]. The flesh is orange-yellow, specially aroma and natural sweetness. Pouteria campechiana fruit is usually eaten fresh, frozen powder, ice cream, candy and Jam products [4, 6]. The composition of Pouteria campechiana fruit consists of peel (7-17 wt.%), pulp (64-82 wt.%), membranes (2-3 wt.%), Seeds (8-15 wt.%) [4]. The pulps contain many nutritional ingredients as antioxidant especially, which is necessary for the body's activities. Therefore Pouteria
campechiana fruit can increase erythropoiesis in the blood, stimulating the activity of the nervous system, anti-depression, reducing cholesterol and triglycerides in the blood, preventing cardiovascular diseases and obesity, limiting myocardial infarction, increasing the immune systems and energy efficiency [5-9]. Currently, scientists have paid to create natural products from bioactive compounds to apply in medicine and agriculture by extraction and concentration or semi-synthetic methods. In the modern life, human food requirements are higher than before. Consuming food is not enough, but also good healthy and more beautifully. Recently, scientists are studying foods that contain antioxidants, a good healthy compound. They can prevent cardiovascular diseases and anti-aging, significant effects on beauty people. It is also a plant - great source - contains antioxidants [10]. For a long time, plants have become a food source, a main source of medicinal materials in the folk and have helped people to choose good plants that have contained both high nutritive and preventing diseases. Many studies have shown that plants contain antioxidants such as phenolics, flavonoids, carotenoids, anthocyanins, tannins, vitamins, quinines, coumarins, lignans, lignin [11-13]. Phenolics were high bioactive compound because they have delayed lipids oxidant, reducing risks of cancer and cardiovascular diseases, improving qualities life and nutritive value of food [14-17]. These compounds were attracted more and more because they are potential useful for human [18]. The first compounds are polyphenol and flavonoid that were demonstrated to scavenge free radical, prevent and treat many diseases such as inflammation, allergy and bacteria [19]. Results were shown that natural antioxidant contents of fruit and its by-products could prevent cancer, cardiovascular, declining nervous and soon aging - the main reason is stress oxidant (it is not balance free radical and antioxidants activities in the body). Therefore, plants will be potential sources that may contain bioactive compounds and applied in the life. In Viet Nam, there are many kinds of plants which are raw materials to produce medicines, functional foods including Pouteria campechiana fruit – rich sources. Using Pouteria campechiana fruit to produce food products will be advantaged in Viet Nam. Study on the chemical composition of Pouteria campechiana is necessary and suitable for tendency of social development nowadays. The present objective is to create Pouteria campechiana fruit with high qualities and safety for human requirements by maintaining its qualities after harvesting that is the first interest in manufacturers and consumers. Besides, Pouteria campechiana fruit is harvested on time for suitable processing.

2. MATERIALS AND METHODS

2.1. Materials

Pouteria campechiana fruits were collected directly in the morning (from 7-9 am) from My Khanh, Phong Dien District, Can Tho City. The fruits were harvested at 120-125 days after fructification. Fruit weight ranged from 200-250 g each (harvest 20 fruits/tree). After harvest, Pouteria campechiana fruits were wrapped in sponge paper and preserved in carton box at room temperature for further study. All chemicals and reagents were of analytical grade and purchased from commercial sources. Total phenolic content (TPC), total flavonoid content (TFC), carotenoid, tannin, DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS (2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) were monitored and analyzed each two days during storing time.

2.2. Preparation of fruit extract
**Study on changes in chemical compositions and bioactive compounds in Pouteria Campechiana**

*Pouteria campechiana* fruit after stored at suitable time was washed, split, removed seeds and put into the scrub equipment to get paste *Pouteria campechiana*. Proceeding with extraction by the following extraction method: the sample was soaked in ethanol 40 % (v/v), ratio of paste *Pouteria campechiana* to ethanol of 1:20 (g/ml), extraction time of 60 minutes and temperature of 45 °C. The filtrate (crude extract) was diluted in ethanol at an appropriate ratio for further analysis.

### 2.3. Determination of bioactive compounds content in fruit extract

**Determination of total phenolic content:** TPC of *Pouteria campechiana* fruit extracts were identified by Folin-Ciocalteu assay using garlic acid as external standard [20]. Typically, 20 µl of fruit extract was mixed with 1.5 ml of 10 % Folinfer Ciocalteu reagent (FCR) and incubated for 5 min. Then 2 ml of 20 % (w/v) sodium carbonate solution was added to the solution and was incubated in the dark at 25 °C for 2 h. After incubation, UV–visible spectrophotometer at 760 nm was used to measure the absorbance. TPC of the samples was expressed as milligrams gallic acid equivalents (GAE) per gram of dry matter (mg GAЕ/g).

**Determination of total flavonoid content:** The amount of TFC was determined by colorimetric method as described by Ozsoy [21]. Fruit extracts (2 ml) were diluted with 5 ml of distilled water and mixed with 5 wt.% NaNO₃ solution. The solution was incubated for 6 min. Then 150 µl of 10 % AlCl₃ was added and incubated for 5 min. Finally, 1 ml of 1 M NaOH was added and the mixture was mixed thoroughly. The absorbance of the mixture was measured immediately at 510 nm. The results were expressed as milligrams of quercetin equivalents (QE) per g of dry matter sample (mg QE/g). All the determinations were performed in triplicates.

**Determination of carotenoid content** (AOAC Official Method 941.15): Determine A of solution as soon as possible with spectrophotometer at 436 nm. Calibrate these instruments first with solution of high purity carotenoid as shown by characteristic absorption curve. Prepare calibration chart and convert A of solution to be determined to carotenoid concentration from chart.

**Determination of tannin content:** Tannin content was determined by Folin-Denis method [22]. Each crude extract (0.5 ml) and distilled water (0.5 ml) were mixed in a test tube. The sample was then treated with 0.5 ml of freshly prepared Folin-Denis reagent followed by 20 % sodium carbonate (2 ml). The mixture was well-shaken and heated in boiling water-bath for 1 minute then cooled to room temperature. Absorbance of the colored complex was measured at 700 nm. Tannin concentration was quantified based on calibration curve of tannic acid in ethanol. The tannin content was expressed as milligrams of tannic acid equivalents (TAE) per gram dry weight (DW).

### 2.4. Antioxidant activity

DPPH radicals scavenging of *Pouteria campechiana* extracts were estimated according to Anshu [23]. This assay measures by spectrophotometer the ability of antioxidants to reduce DPPH. 200 µl of each extract with 2.5 ml of DPPH and 2 ml of methanol solvent was added to the test tube. The mixture was incubated for 1.5 h. DPPH and methanol was used as the control. The incubated samples were determined by their absorbance at 517 nm by UV- Vis spectroscopy.

The ABTS assay was carried out according to the method of Nikolaos [24]. An ABTS stock solution was diluted with methanol for working solution until the absorbance
reached 0.70 ± 0.02 at 734 nm. 20 μl sample was added to 200 μl ABTS working solution, incubated at 25 °C in darkness for 4 min. All measurements were carried out at least three times and absorbance was recorded at 734 nm. The activity was expressed as mmol of Trolox/g fruit extract.

2.5. Statistical analysis

All the assays were conducted at least triplicate and the results were expressed as mean ± SD. Experimental data were analyzed using Portable Stat graphics Centurion software (Version 15.2.11.0). Analysis of variance (ANOVA) with LSD test was used to determine the significant differences (P < 0.05) between means.

3. RESULTS AND DISCUSSION

3.1. Variation of total polyphenol content (TPC) and flavonoid (TFC)

Polyphenols are antioxidant groups that are able to prevent chain reactions by directly reacting to create more stable free radical or combine with metal ions to become a transitional complex that catalyzes for new free radical process [25]. In medicine field, polyphenols are one of the natural compounds with many effects such as antioxidant, anti-inflammatory, antibacterial, anti-allergy, anti-aging as well as its role in reducing the risk of chronic disease such as cancer, cardiovascular and neurodegenerative. The role of these compounds is to change intestine bacteria to beneficial one [14-15]. Total polyphenol content is an important indicator to evaluate the oxidase resistance of a raw material.

TPC of Pouteria campechiana fruit during storage (2-12 days) was presented in Figure 1A. It was observed that TPC of Pouteria campechiana fruit decreased during storing time. TPC decreased from 7.05 to 6.83 mg GAE/g after 2-6 days preserving. TPC kept decrease to 6.16 mg GAE/g at day 10th and then declined significantly to 5.88 (mg GAE/g db) at day 12th (P < 0.05).

Flavonoids are group of plant metabolites to provide health benefits through cell signaling pathways and antioxidant effects. Flavonoids are antioxidant compounds belonging to polyphenol groups with strong antioxidant activity due to the presence of aromatic hydroxyl groups [26]. TFC of Pouteria campechiana fruit during storage (2-12 days) was shown in Figure 1B. It can be seen that TFC contents was also decreased form 7.15-7.01 mg QE/g db after 6 days.
preserving. TFC continued to decrease to $6.32 \pm 0.169$ mg QE/g db at day 10th and decline to $6.03 \pm 0.100$ mg QE/g db at day 12th. It can be stated that when *Pouteria campechiana* fruit changes from yellow green to yellow during storing, its TPC and TFC tend to decrease and the difference is statistically significant at the 95% level. The results of this study are consistent to those of papaya, camellia and guava fruits during ripening [27-28] and peaches when refrigerated [29].

### 3.2. Variation of total carotenoid and tannin content

In addition to polyphenols and flavonoids, carotenoids are also effective antioxidant compounds, which are important in maintaining healthy health and preventing human diseases such as cardiovascular, cancers as well as other chronic diseases [30-34]. It can be observed from Figure 2A that carotenoid content increased from 82.93 to 113.27 (mg/g) from 2 to 8th day storage and reached the peak at $124.27 \pm 3.05$ (mg/g) on day 10th. During ripening, the chlorophyll decreased whilst carotenoids gradually increased, leading to the change of colour of ripe fruit [35]. However, at day 12th, carotenoid content reduced to $119.28 \pm 3.05$ (mg/g). This can be explained due to the entering of light and oxygen to the cracked skin of fruit during ripening. In addition, at the same time, the presence of intrinsic lipoxydase enzyme would also lead to the decrease of carotenoid content [35]. This result is similar to the increase in carotenoid content of some tomato varieties in the study [36-37] and ripening of tomatoes over time of storage [38].

Tannins are natural and common polyphenol compounds of plants. Fruit was change from green to ripe, polyphenol oxidase enzyme catalyzed the oxidation reaction of the polyphenol compound to quinone which combined with amino acids to produce color compounds [39]. In contradiction to carotenoid, tannin content tent to decrease with storage time from day 4th-8th (295.27 to 211.16 mg TAE/g db), and started to decrease rapidly at day 10th ($66.87 \pm 2.83$ mg TAE/g db) (Figure 2B).

![Figure 2](image)

*Figure 2. Effect of storing time on carotenoids (A) and tannin (B) contents of Pouteria campechiana fruit.*

Thus, it can be observed that tannin contents of *Pouteria campechiana* fruit decreased gradually when fruits color changed from green to yellow. The results of this study was also in agreement with those of Sancho [28] and Nguyen Van Khoa [40] on papaya and cashew nuts ripening, respectively. This result suggested that selecting soft yellow ripe *Pouteria campechiana* fruit (usually after 8-10 days storing) as raw material for food processing would provide better nutrition values.
3.3 Antioxidant activity

To evaluate antioxidant activity of *Pouteria campechiana* fruit during preserving, radical scavenging activities and reducing power of fruit extracts were analyzed. These methods are simple, quickly, stable and can be used for the screening effect of antioxidants [41]. Experimental results in Figure 3 showed that inhibition percent of DPPH and ABTS of *Pouteria campechiana* fruit during preserving gradually increased from 66.81 to 68.67 and from 67.58 to 69.63, respectively after 6 days of storing. Highest inhibition percent of DPPH and ABTS were obtained at day 10th (71.17 and 71.11). However, after 12 days, the inhibition percent of DPPH and ABTS suddenly reduced to 70.72 and 70.85. The results of this study indicated that 8-10 days is the best storing period for *Pouteria campechiana* fruits.

![Graphs showing DPPH and ABTS inhibition](image)

Figure 3. Change of DPPH (A) and ABTS (B) *Pouteria campechiana* fruit during preserving

4. CONCLUSIONS

Study on the transformation of bioactive compounds (polyphenols, flavonoids, carotenoids, tannin) and antioxidant activity (DPPH, ABTS) of *Pouteria campechiana* fruit at different stage of maturity was evaluated. The results from this study indicated that highest quality of *Pouteria campechiana* fruit was obtained at 8 to 10 days storage after harvesting.

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