

Validated UV Spectrophotometric Method for Quantitative Analysis of Carotenoid Content and Antioxidant Activities of Pluk Mai Lie Papaya Fruits

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Abstract

The ultraviolet (UV) spectrophotometric method for analysis of total carotenoid content in the flesh of Pluk Mai Lie papaya ripe fruits collected from the cultivated field (FP) and the local market (MP) was developed. Papaya flesh from each sample was also determined for total phenolic contents and free radical scavenging activities using Folin-Ciocalteu and DPPH scavenging assay, respectively. The validated UV spectrophotometric method was simple, accurate and precise. Total carotenoid contents in the flesh of papaya fruits FP and MP were 7.57 ± 0.53 and 5.39 ± 0.20 mg in 100 g fresh weight, respectively. The aqueous extract from FP possessed higher antioxidant capacity than that of MP (9.58 ± 0.05 and 7.28 ± 0.16 mg vitamin C equivalent (VCE) per 100 g ripe fresh pulp). Total phenolic contents of extracts from FP and MP were 59.44 ± 1.99 and 44.33 ± 3.16 mg gallic acid equivalent (GAE) in 100 g fresh weight, respectively. Pluk Mai Lie papaya sample collected from the cultivated field exhibited higher carotenoid content with better antioxidant properties including free radical scavenging effects and total phenolic contents than sample from the local market. The results suggested that Pluk Mai Lie, the new cultivated papaya cultivar in Thailand contained high chemical and biological qualities that could be used as raw material for medicinal and pharmaceutical purposes in the future. The validated UV spectrophotometric method can be applied for further quantitative analysis of carotenoid content.

Keyword: *Carica papaya*, Carotenoids, Phenolic content, DPPH scavenging activity, Pluk Mai Lie

INTRODUCTION

Papaya (*Carica papaya L.*) is a plant in Caricaceae family. It is a tree that can reach up to 9 metres height tall with the hollow trunk and the latex. Papaya has alternate, palmate-composite leaves with the petiole up to 0.5 metres long. The flowers emerge from the leaf axils and have 5 white, fleshy, waxy lobes¹. Papaya is a sexually variable plant that can be divided as dioecious (male and female flowers on separate plants), monoecious (male and female flowers on the same plant) or hermaphroditic (having bisexual or “perfect” flowers)². The fruits of this plant are berries that can reach 70 cm

long with a tasty flesh and black seeds surrounded by gelatinous layer inside. The ripe fruits are soft and the color of the flesh changes from light green to yellow, orange or red². Papaya is a native plant in South America such as Brazil, Colombia, Bolivia, Mexico and Argentina³. In traditional medicine, several parts of papaya including the roots are used as diuretic and to treat gonorrhoea, the leaves are used to treat dermatophytosis and insect bites. The fruits are tonic, digestant and laxative while the latex has been used to treat dermatophytosis and as digestant and emmenagogue⁴. Various phytochemicals and nutrients were reported from papaya including carotenoids^{5,6} such as

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β -carotene, lycopene and β -cryptoxanthin, sugars such as glucose, fructose and sucrose⁷, polyphenols and flavonoids such as caffeic acid, *p*-coumaric acid, chlorogenic acid, kaempferol and quercetin⁸ and enzyme papain⁹. Papaya is cultivated for its fruits, which are popularly consumed by the people of the tropical area as fresh fruits and as the ingredients in jellies, preserves, or cooked in various recipes. The juice can be made as a beverage while young leaves, shoots and raw fruits can be cooked as vegetables¹⁰. Papain, the proteolytic enzyme in papaya has been used as meat-tenderizers, chewing gums, dentifrices, shampoos, and cosmetic preparations⁹. Moreover, papaya fruits were reported to promoted immuno-stimulating and anti-oxidant effects^{11,12}. Papaya is one of the important economic plants in Thailand that promote high agricultural products for domestic usages and import purposes. Fresh and dried products from the fruits of papaya are highly required. Even though there are some reports about nutritional and chemical contents in papaya fruits cultivated in oversea countries^{13,14}, there is still lack of the information about the carotenoid

contents in the fruits of papaya varieties cultivated in Thailand. Carotenoids are hydrophobic terpenoidal compounds which promoted the protective effects against some types of cancer¹⁵, aging-related molecular degeneration¹⁶ and heart disease¹⁷, while some clinical studies revealed that phenolic compounds also play important roles in prevention of chronic diseases related to oxidative stress such as cancer, diabetes and cardiovascular disease¹⁸.

Among various types of papayas, Holland or Pluk Mai Lie (Figure 1) is a new papaya cultivar that has been bred from Red Maradol papaya cultivar from Mexico, which promotes the firm flesh, preferable flavor and odor¹⁹. However, there is no data regarding antioxidant activity and active chemical constituent contents, particularly carotenoids and phenolics contents of Pluk Mai Lie papaya fruits. Therefore, this study was setup in order to quantitative analysis of the total carotenoid and total phenolic contents using spectrophotometric methods and free radical scavenging activity of Pluk Mai Lie papaya ripe fruits collected from different locations were investigated.

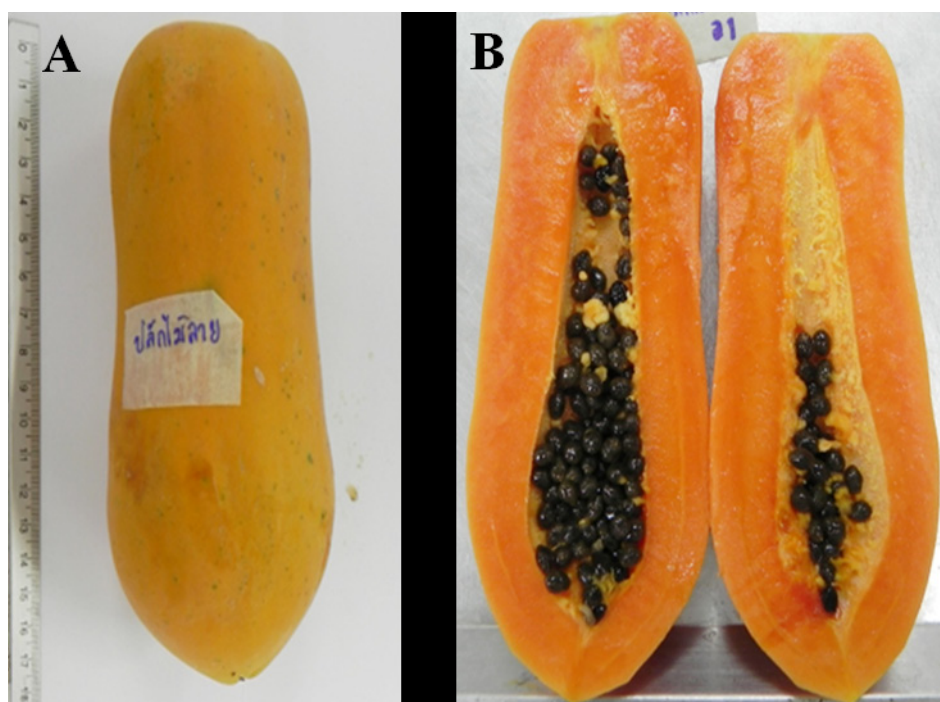


Figure 1. Physical characteristics of Pluk Mai Lie papaya fruit; A = whole fruit, B = longitudinal section

MATERIALS AND METHODS

Preparation of papaya fruit samples

The ripe fruits of Pluk Mai Lie papaya were collected from Kampangsan district, Nakhon Pathom province, while other Pluk Mai Lie papaya fruit samples were purchased from local markets in Bangkok in June 2011, around 3–4 kg of Pluk Mai Lie fresh fruits were used. The fruits were cleaned, peeled and each ripe fruits were longitudinally cut into two parts. first part was used for the extraction of carotenoid, and the second part for analysis of phenolic contents and antioxidant activities

Extraction of carotenoid in papaya fruit flesh

The ripe fruit flesh from each papaya was finely ground in dried ice. A single composite of each sample was prepared by combining together about 10 g of single grounded sample, and then the sample was re-homogenized to obtain a single uniform composite of each sample. Carotenoid was extracted following the method of Serino *et al*²⁰. One gram of each sample (n = 3) was extracted by agitation (vortex, at maximum speed) and centrifugation (10,000 rpm, 4°C, Kubotamodel 6930, Japan) in the following order: addition of 1 ml of saturated aqueous sodium chloride (NaCl) solution and 0.5 ml of *n*-hexane (Hex), agitation for 5 min, and centrifugation for 5 min; addition of 2 ml of dichloromethane (DCM), agitation for 5 min, and centrifugation for 5 min; addition of 10 ml of Ethyl Acetate (EA), agitation for 5 min, and centrifugation for 10 min. An aliquot of the organic fraction (upper phase) was collected. Five milliliters of the organic phase was transferred to 10-ml volumetric flask and diluted with the mixture of EA:DCM:Hex (80:16:4, v/v/v).

Preparation of standard β -carotene solution

To prepare a primary solution of β -carotene (PS, 100 μ g/ml), the standard β -carotene (Sigma, St. Louis, MO, USA)

was dissolved in *n*-hexane. The PS concentration of β -carotene was precisely determined by spectrophotometry at 450 nm (Shimadzu UV-1800 ultraviolet spectrophotometer, Kyoto, Japan), using the specific absorption coefficients of 2,592. The secondary solution (SS, 16.77 μ g/ml) was obtained by diluting PS with the mixture of EA:DCM:Hex (80:16:4, v/v/v).

Method validation for quantitative analysis of β -carotene content

Method validation was evaluated in term of linearity, accuracy and precision. Linearity of the method was performed in a range of 0.84-5.03 μ g/ml of β -carotene. Linear regression and correlation coefficient (r^2) were calculated using Microsoft Excel[®] version 2010. Accuracy was performed by standard addition. Three different volumes (1, 1.5 and 2 ml) of PS were added to the sample extract and absorption at 454 nm of the spiked and unspiked samples was measured (n = 3). Percent recovery (%R) was calculated from (amount found/amount added) x 100. Precision was determined by repetitive measurement of absorption of standard β -carotene solution at 1.67 μ g/ml (n = 6) on the same day for intra-day precision and on three different days for inter-day precision. The percent relative standard deviation (%RSD) was calculated.

Analysis of total carotenoid content

The single uniform composite of each sample was extracted (n = 3) as described earlier. The absorption of each extract was performed at 454 nm in duplicate. Total carotenoid content was calculated base on the calibration curve and expressed as mg β -carotene equivalent (BE) in 100 g fresh fruit (mg% BE).

Preparation of papaya fruit flesh for determination of total phenolic content and free radical scavenging activity

The second portion of ripe flesh fruit from each Pluk Mai Lie papaya collected from different locations was homogenized using a mechanical blender. A single com-

posite of each sample was prepared by combining about 10 g of each single homogenized sample to obtain a single uniform composite sample. Then one gram of the fruit flesh from each sample ($n = 3$) was mixed with 10 ml distilled water and centrifuged at 5,000 rpm for 10 min. The supernatant was collected and filtered through a 0.45 μm filter. The filtrate was diluted with distilled water (1/2-fold), yielded the aqueous extract (AQ)

Determination of antioxidant activities of papaya fruit flesh by DPPH scavenging assay

Free-radical-scavenging effects of AQ from each Pluk Mai Lie papaya collected from different locations as well as standard ascorbic acid were carried out using a modified method of Sithisarn *et al.*²¹. Two milliliters of the extract or ascorbic acid (concentrations of 4, 5, 6, 7 $\mu\text{g}/\text{ml}$ in methanol) were added to 2 ml of DPPH in methanol solution (152 mM). After incubation at room temperature for 30 min, the absorbance of each solution was determined at 517 nm. A control consisted of 2 ml of distilled water and 2 mL of 152 mM DPPH solution. The percentage of scavenging activity (%SA) was calculated as $\%SA = (C-X) 100/C$, where C was absorbance of control and X was absorbance of extract. In order to express antioxidant activities of extract in the general term, antioxidant capacity as mg vitamin C equivalent (VCE) in 100 g of fresh fruit flesh was introduced²². A standard curve of ascorbic acid was obtained from % SA (X) plotted against various vitamin C concentrations (Y).

Determination of total phenolic contents of papaya fruit flesh

Using the method applied by Naithani *et al.*²³, 2 ml of AQ was transferred and reacted with 2 ml of 0.2 N Folin-Ciocalteu reagent in 10-ml volumetric flask. After 5 min., 3.2 ml of 7.5% of sodium carbonate was added and mixed, and the content of the flask was adjusted to volume with distilled water. Solution was heated in a $40 \pm 5^\circ\text{C}$ water bath for 30 min. The blue

color was developed and absorbance was measured at 760 nm. The standard curve was prepared using 0, 100, 200, 300, 400 and 500 μg of gallic acid stock solution (200 $\mu\text{g}/\text{ml}$) in 10-ml volumetric flask. Each sample was prepared in duplicate. Total phenolic content were expressed as mg gallic acid equivalent (GAE) in 100 g fresh fruit flesh (mg% GAE).

Statistical analysis

All data are reported as means \pm standard deviation of triplicates. Least significant difference was used to compare means ($p < 0.05$). All analyses were performed using SPSS for Windows, version 16.0 (SPSS Inc., USA).

RESULTS AND DISCUSSION

The absorption spectra of β -carotene and the extract of Pluk Mai Lie papaya were scanned from 300-600 nm, and a maximum absorption was obtained at 454 nm for β -carotene and the extract (Figure 2). Because of their chemical structure, carotenoids possess characteristic absorption spectra in the visible region, determined by the long conjugated double bond system. Lycopene, β -cryptoxanthin and β -carotene have been identified as the major carotenoids in papaya (Figure 3), which β -carotene was used as a marker in this study. Method validation data of the spectrophotometric method are shown in Table 1.

The method was found to be linear, accurate and precise for the analysis of the total carotenoid contents of papaya under the experimental conditions used.

Total carotenoid, total phenolic contents and free radical scavenging activities of the extracts from two Pluk Mai Lie papaya samples are shown in Table 2. The extracts from the fruits of Pluk Mai Lie collected from the cultivated field (FP) significantly contained higher amount of carotenoid and phenolic contents than extracts from the fruits of Pluk Mai Lai papaya purchased from local market (MP). The extracts from FP also significantly exhibited stronger DPPH scavenging activities. However, there could

be other biological and environmental factors that promote the effect to the analytical results such as age of the plant, age of the fruits, cultivating temperature, humidity and mineral

contents in soil. Therefore, further experiments with the control of these related factors and higher numbers of collected samples should be performed.

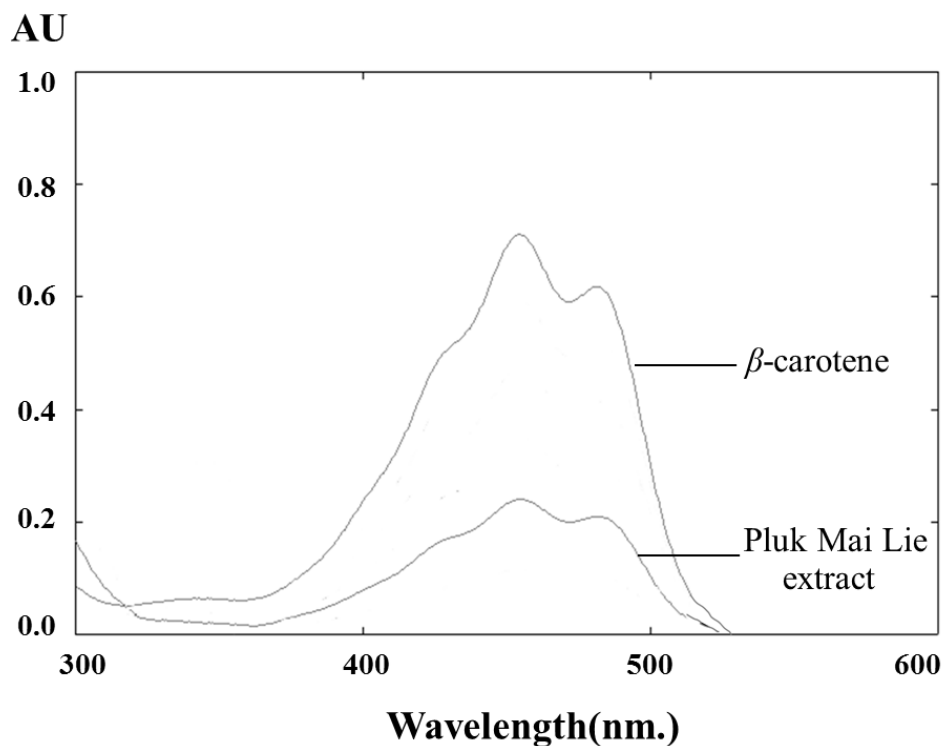


Figure 2. UV spectra of β -carotene standard solution (5.03 $\mu\text{g/ml}$) and the extract of Pluk Mai Lie papaya

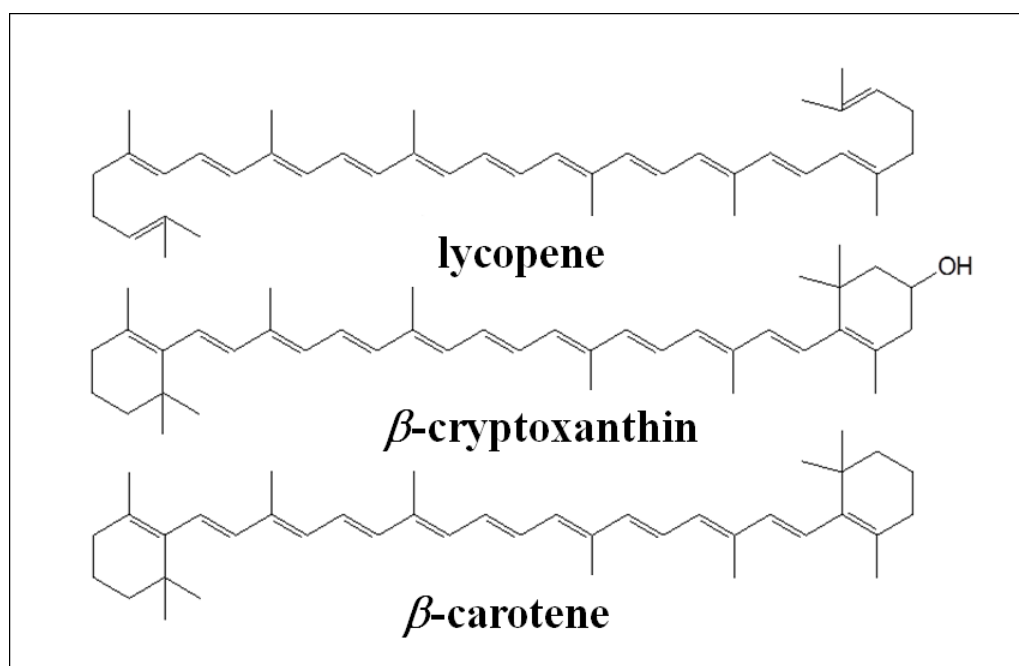


Figure 3. Structure of some carotenoids in papaya fruits

Table 1. Method validation data of the proposed spectrophotometric method

Validation parameter	Results
Linearity	$y = 0.2007x + 0.0091$ ($r^2=0.9998$)
Range	0.84-5.03 $\mu\text{g/ml}$
Accuracy (% R)	107.1-110.2
Intra-day precision (% RSD, n = 6)	0.28-0.54
Inter-day precision (%RSD, n = 3)	2.97

Table 2. Total carotenoid, total phenolic contents and free radical scavenging activities of the fruit extracts from Pluk Mai Lie papaya collected from different locations

Papaya sample	Total carotenoid content (mg BE)	Total phenolic content (mg GAE)	DPPH scavenging activity (mg VCE)
MP	$5.39 \pm 0.20\text{a}$	$44.33 \pm 3.16\text{a}$	$7.28 \pm 0.16\text{a}$
FP	$7.57 \pm 0.53\text{b}$	$59.44 \pm 1.99\text{b}$	$9.58 \pm 0.05\text{b}$

The data was expressed as the mean \pm SD of each result in 100 g fresh fruit flesh. Different letters in the same column indicated significant differences ($p < 0.05$)

CONCLUSION

Extracts from ripe fruit flesh of Pluk Mai Lie papaya, especially the fruits collected from the cultivated field significantly promoted stronger free radical scavenging activities with higher amount of total carotenoid and total phenolic contents than extracts from the flesh of Pluk Mai Lie papaya purchased from local market. The validated UV spectrophotometric method was validated, which could be used for quantitative analysis of carotenoid contents in papaya samples. This study is beneficial for further development of the high quality papaya fruit products and for standardization of papaya fruit extract which could be used in pharmaceutical and medicinal purposes in the future.

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