Quantitative Evaluation On The Anthocyanins And Vitamin C Content Of *Carissa carandas* Fruit At Various Stages Of Ages And Storage Time

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ABSTRACTS

Anthocyanins at five different stages of fruit development, *i.e* immature, mature, ripening, over ripening and storage ripening were quantified using high performance liquid chromatography (HPLC) at λ = 520nm and two dimensional chromatography (using BAW and 15% Acetic acid as a solvent system. Three biopigments were identified i.e. pelargonidin-3-O-glucoside, cyanidin-3-O-rhamnoside and cyanidin-3-O-glucoside. The highest percentages of pelargonidin 3-O-glucoside were detected at the ripening stage. The same method was also used to detect the phenolic substances i.e. aesculetin and chrysoeriol-7-O-glucoside.

The level of vitamin C in the five samples was measured using 2,6-dichloindophenol (DCPIP) method. The highest content of vitamin C was detected during the mature stage of its fruit ripening. The level decreased during the storage ripening as compared with the natural ripening. The content of sugar and citric acid were also quantified. The content of sugar and citric acid were the highest at immature stage.

INTRODUCTION

Carissa carandas is native to India and has been cultivated in a limited way in the tropical, subtropical and Mediterranean regions. It is well suited climatically to be cultivated in the Asian region. It produces abundant berries through out the year in the monsoon tropical climate especially in the northern part of Malaysia.

in the monsoon tropical climate especially in the northern part of Malaysia. *Carissa carandas* is a perennial plant and very easily maintained, hardy shrub. A one-year-old tree can produce abundant of berries. Pruning of the plant seemed to enhance the production of berries, and being a shrub harvest of the berries is not labour intensive.

The ripe berries are very rich in anthocyanidin. The uses of natural biopigments, such as anthocyanins in food product are excellent alternative to the synthetic colours. They are non toxic by nature and inherent value added advantageous. However, until now lack of research have been conducted on this plant. Ithe ultimate goal of this research is to determine the content of anthocyanidin, vitamin C, phenolic compounds, sugar and citric acid in five stages of fruit ripening of *Carissa carandas*. This is considered as the first step towards commercialization of its healthy fruit juice.

MATERIALS AND METHODS

Five different stages of fruit developments, *i.e* immature (2 weeks), mature (4 weeks), ripening (6 weeks), over ripening (8 weeks) and storage ripening (picked after 4 weeks development and were stored in the room temperature for 2 weeks for them to ripe) were collected from the same plant in Minden Height, Penang. The samples were weighted and the juices of the weighted samples were freshly extracted.

Sugar quantification

Each of the samples was weighted 10g. The 10g fruits were grinded dan 100 ml of distilled water were added. The extracts were then filtered. 50 ml of the extracts were filled into a biuret and they were titrated using 25 ml of benedict reagent until the benedict turned brown or yellow in colour.

Citric acid quantification

Each sample was weighted 40g and they were grinded in 100 ml of water and were filtered. 50ml extracts were transferred to the conical flask. A few drop of phenoltalin reagent were then added. The extracts were titrated using 0.1N sodium hydroxide solution until red to orange red colours were performed.

Quantification of anthocyanins and phenolic compounds

The quantification of anthocyanins and phenolic compounds were made using high performance liquid chromatography (HPLC) at λ = 520nm and λ = 350nm, respectively.

The determinations of types of anthocyanins were made using paper chromatographies based on the rf values of the aglycones and glycosides in various solvent systems. The identified compounds were then injected to the HPLC to detect their retention times in to different solvent systems. They are considered as the marker compounds.

Quantification of vitamin C

The content of vitamin C was determined by the 2,6-dichloindophenol titrimetic method (JAOAC, 1990). Ascobic acid reduces oxidation-reduction indicator dye, 2,6-dichloindophenol, to colourless solution. At end point, excess unreduced dye is rose pink in acid solution. Extraction and titration of vitamin C is performed in the presence of Metaphosphoric acid-acetic acid solution to maintain proper acidity for reaction and to avoid autooxidation of ascorbic acid at high pH.

RESULTS AND DISCUSSION

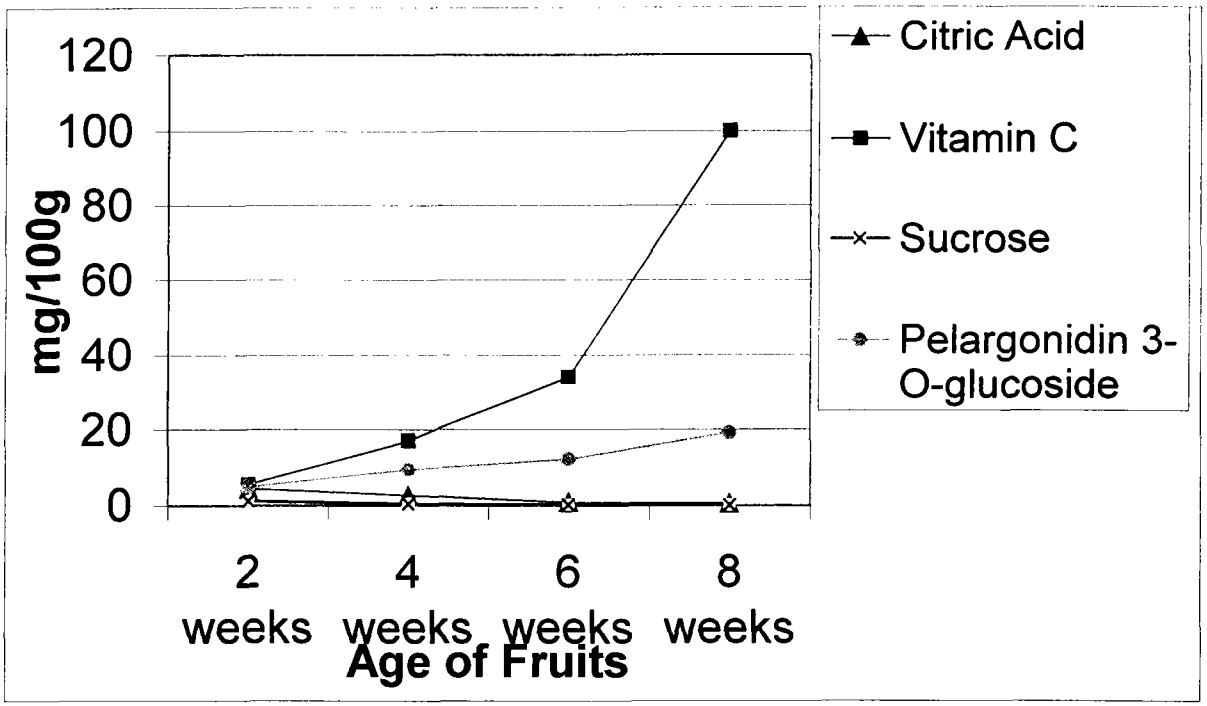


Figure 1: Correlation between the different stages of berries ripening and the production of citric acid, vitamin C, sucrose and pelargonidin 3-*O*-glucoside.

Table 1: The percentages of sugar (mg/100g) in 5 different stages of fruit ripening

Fruit stages	Percentages of sugar (mg/100g)
immature	1.25%
mature	0.53%
ripe	0.13%
Over ripe	0.23%
Storage ripe	0.16%

Table 2: The percentages of citric acids (mg/100g) in 5 different stages of fruit ripening

Fruit stages	Percentages of citric acid (mg/100g)
immature	4.50%
mature	2.71%
ripe	0.62%
Over ripe	0.77%
Storage ripe	2.08%

Table 3: Percentages of anthocyanins in 5 different stages of fruit ripening

Retention time	Anthocyanin type	Percentages of anthocyanins (%)				
		Immature	Mature	Ripe	Over ripe	Storage ripe
2.30	Cyanidin 3-O-rhamnoside	30.02	17.00	2,77	34.28	24.30
2.65	Cyanidins 3-O glucoside	45.35	42.15	13.14	46.95	45.52
3.50	Pelargonidins 3-O-glucoside	24.63	40.85	84.10	18.78	30.18

Table 4: Percentages of phenolic compounds in 5 different stages of fruit ripening

Retention time	Phenolic type	Percentages of phenolic compounds (%)				
		Immature	Mature	Ripe	Over ripe	Storage ripe
2.30	aesculetin	48.11	47.45	46.82	38.28	35.63
2.65	chrysoeriol-7-O-glucoside	46.16	48.02	39.71	56.00	61.93

Table 5: The percentages of vitamin C (mg/100g) in 5 different stages of fruit ripening

Fruit stages	Percentages of vitamin C (mg/100g)
immature	5.60%
mature	5.00%
ripe	34.70%
Over ripe	30.20%
Storage ripe	32.70%

The content of sugar was the highest at immature stage. This might be due to the mature stage of fruit do not need extra energy for the metabolism processes. So the extra sugars were stored in carbohydrate form. The sugar content is found higher in the over ripening fruit compared with the natural ripening and storage ripening fruits may be caused by the increment of respiration and transpiration during ripening that reduced the water content.

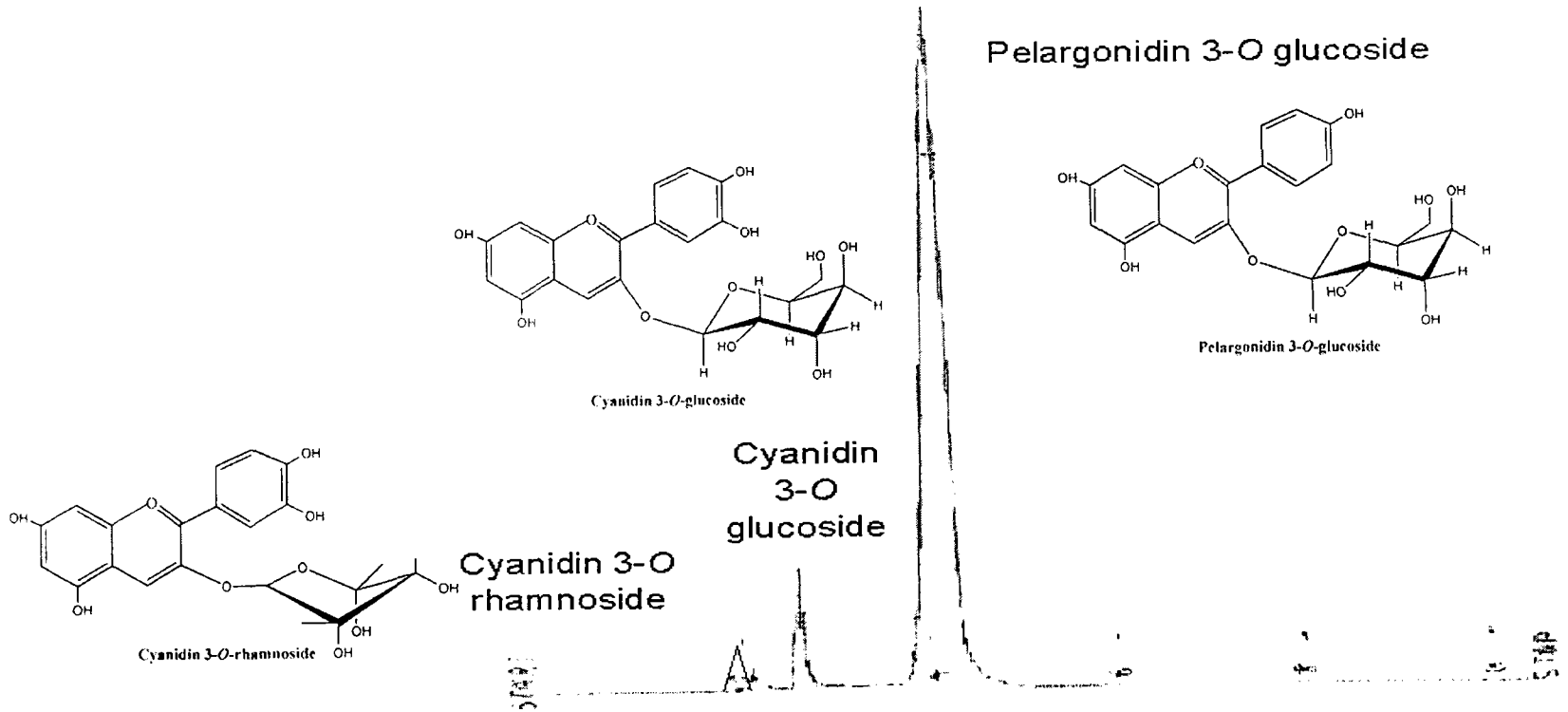
The highest percentage of citric acid was also observed in the immature fruits and the percentage reduced drastically during ripening. Citric acid is one of the component involved in the Krebs Cycle, hence the increment of this compound can be correlated with the metabolism process during ripening. The storage ripening fruit exhibited higher percentage of citric acid compared with the natural ripening fruit. This reduction of citric acid during natural ripening might be due to the exponential increment of climacteric respiration rate. The storage ripening fruit might not undergo the exponential increment of climacteric respiration rate.

Three biopigments were identified i.e. pelargonidin-3-O-glucoside, cyanidin-3-O-rhamnoside and cyanidin-3-O-glucoside. The highest percentages of pelargonidin 3-O-glucoside were detected at the ripening stage. The content of cyanidin-3-O-glucoside and cyanidin-3-O-rhamnoside were quite constant during the early stages of development but the percentages reduced in the over ripening fruit. In the storage fruit ripening, the pelargonidin 3-O-glucoside percentage was

found lower compared with the natural fruit ripening. This might be due to the gene that stimulate the synthesis of pelargonidin 3-O-glucoside was not expressed when the fruit was harvested from the tree.

Two major phenolic substances i.e. aesculetin and chrysoeriol-7-O-glucoside were detected. Aesculetin reduced during the fruit ripening, while chrysoeriol-7-O-glucoside increased. It is suggested that aesculetin is needed for ripening and chrysoeriol-7-O-glucoside is needed after ripening. The storage ripening fruit showed a lower percentage of aesculetin compared with the natural ripening fruit. There might be degradation of aesculetin in the storage condition. On the other hand, the storage ripening fruit has higher content of chrysoeriol-7-O-glucoside than the natural ripening. This fruit might be stressed during harvesting at the mature stage and induced the production of chrysoeriol-7-O-glucoside to overcome the stress.

The level of vitamin C in the five samples was measured using 2,6-dichloindophenol (DCPIP) method. The highest content of vitamin C was detected during the mature stage of its fruit ripening. The level decreased during the storage ripening as compared with the natural ripening. The reduction might be due to the degradation of vitamin C during ripening in the storage condition.

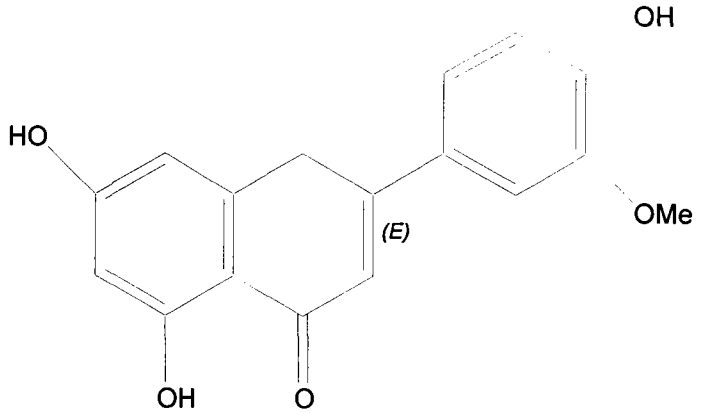


Cyanidin 3-*O*-glucoside

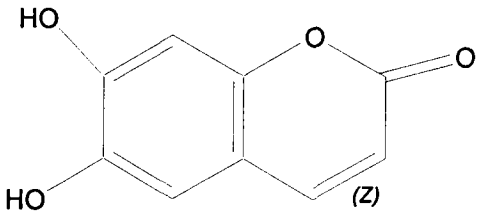
Pelargonidin 3-*O*-glucoside

Cyanidin 3-*O*-rhamnoside

Flavonoids in *Carissa carandas*



Flavone: Chryseriol



Hydrocoumarins: Aesculetin