

Metabolite correlation with antioxidant activity in different fruit maturation stages of *Physalis peruviana*

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Abstract. Mubarok S, Wicaksono FY, Budiarto R, Rahmat BPN, Khoerunnisa SA. 2021. Metabolite correlation with antioxidant activity in different fruit maturation stages of *Physalis peruviana*. *Biodiversitas* 22: 2743-2749. Fruit development influences the metabolites contents and then its biological activity, however, such report is still limited in goldenberry (*Physalis peruviana* L.). This work aimed to evaluate metabolite variability and its correlation to antioxidant activity in several stages of fruit maturation in goldenberry originating from Sumedang, Indonesia. The research was laid out in a completely randomized design with six different treatments in term of fruit maturation stages. Only fresh and pest-disease-free fruits collected from local farm were prepared for several metabolites assay, i.e pH, total soluble solid (TSS), lycopene, β -carotene, polyphenol, flavonoid and its antioxidant activity. The statistical analysis resulted in a significant improvement of all fruit metabolite variables in response to fruit maturation process, except polyphenol. Compared to the oldest fruit stage of S6 (orange berry covered by full wilt white calyx), the youngest fruit of S1 (green berry covered by fresh green calyx) increased the pH, TSS, lycopene, β -carotene and flavonoid by about 7%, 67.5%, eight-fold, nine-fold and two-fold, respectively. Pearson correlation analysis showed a significant and positive correlation between all metabolite variables (except polyphenol) to antioxidant activity of goldenberry fruit.

Keywords: β -carotene, flavonoid, goldenberry, lycopene, polyphenol, TSS

INTRODUCTION

Goldenberry (*Physalis peruviana* L.) is an exotic perennial tropical shrub native to South America, specifically Peruvian Andes as the center of origin (Legge 1974). Goldenberry has a wide growing location in numerous countries worldwide, leading to ecotypes phenomenon as indicated by various fruit size, color, taste, and plant size in each specific location (Puente et al. 2011). Goldenberry is classified into husk tomato as the most diverse and important genera in Solanaceae family (Sadiyah et al. 2021).

The rapid interest to grow goldenberry is associated with its low-calorie but high-nutritious content (Mayorga et al. 2001; Joshi and Joshi 2015; Yildiz et al. 2015). Goldenberry can be consumed in raw form of salad or served as processed products, such as candy, cake, jam, juice, raisin, pomace and other desserts (Ramadan and Moersel 2007; Ramadan and Moersel 2009; Sharoba and Ramadan 2011). Numerous pharmacological properties of goldenberry are analgesic, antispasmodic, antiseptic, antimicrobial and sedative (Januário et al. 2000; Puente et al. 2011). These pharmacological characters associated with metabolite content and composition.

In case of fruit, metabolites content could be influenced by its maturation stages. As the fruit getting mature, there are several alterations in terms of chemical, physical (e.g. color) and biological properties (Gil et al. 1995; Trincherro

et al. 1999; Ramadan and Morsel 2003; Serrano et al. 2005; Ersoy 2011). Fruit maturation process allowed the occurrence of metabolite degradation processes leading to the alteration of the nutritional value, thus the evaluation of metabolites in relation to fruit ripening stage becomes an interesting issue (Etzbach et al. 2018).

One of important pharmacological properties of natural products of fruit was antioxidant activity. Among 97 plants collected from Gunung Gede Pangrango national park in West Java, the highest antioxidant activity and the most potential cervix anticancer plants were goldenberry (Arbiastutie et al. 2017). Numerous studies on antioxidant activity and metabolite variation related to fruit ripening have been conducted in mangoes (Islam et al. 2013), God's crown (Soeksmanto et al. 2007), goldenberry (Bravo et al. 2015). Correlation analysis previously reported to display the relationship among morphological characters (Budiarto et al. 2021); among pigmentation content such as chlorophyll- α , chlorophyll- β , and carotene (Yora et al. 2018), and physical characters of goldenberry fruit (Yildiz et al. 2015), opening the possibility to use this approach to reveal the metabolites and antioxidant relationship. Because of the major influence of phenotype (genotype and growing location) on metabolite content (Khodadadi et al. 2015; Yusnawan 2016; Calvindi et al. 2020), further study specifically for goldenberry from Indonesia was needed. Therefore, this study aimed to evaluate metabolite variability and its correlation to antioxidant activity in

several stages of fruit maturation in goldenberry originating from Sumedang, Indonesia.

MATERIALS AND METHODS

Preparation of plant materials

Goldenberry fruits were harvested from Waida Farm Sumedang (-6.87359; 107.82360; 946 meters above sea levels), in the morning using hand picking technique. Only selected goldenberry fruits with fresh and pest-disease-free conditions used in present work. This work was arranged in a completely randomized design, with 6 different maturation stages in term of fruit maturation stages, i.e stage 1 (unripe green berry covered by green calyx), stage 2 (yellow berry covered by whitish-green calyx), stage 3 (yellow berry covered by greenish-yellow calyx), stage 4 (yellow berry covered by yellowish-white calyx), stage 5 (yellow berry covered by semi wilting yellowish-white calyx), stage 6 (yellow berry covered by wilting white calyx) (Figure 1). Each treatment consisted of four replications, so that there were 24 experimental units involved, with 20 goldenberry fruits for each experimental unit. Each sample was measured in triplicate

Analysis of pH and total soluble solids (TSS)

Goldenberry fruits with a fresh weight of around 5 g were ground to collect the fruit juice for pH analysis by using a pH meter. For TSS analysis, the 5 mL fruit juice was prepared in microtube. The microtube was then centrifugated at 1000 rpm. The 1000 μ L supernatant was then collected using a micropipette and transferred to refractometer.

Analysis of antioxidant activity (DPPH free radical scavenger)

The 20 mg of dried sample of goldenberry in a dark bottle was added with 2 mL methanol and then homogenized. The 0.6 mL supernatant was then transferred to a test tube. The 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) solution was prepared by combining 0.8 mg DPPH

with 20 mL methanol inside an Erlenmeyer flask covered by an aluminum foil to prevent oxidation. The 2.5 mL DPPH solution was added into the test tube containing the supernatant sample and incubated for 15 min prior to the measurement by using UV-Vis spectrophotometer.

Analysis of lycopene and β -carotene

Analysis of lycopene and β -carotene was followed previous study by Mubarok et al. (2015). 1 g fresh goldenberry fruit sample was ground prior to transfer to dark bottle for preventing any light penetration.—7 mL acetone-hexane- previously prepared from acetone and hexane with a ratio 4:6 - was transferred into dark bottle of sample and then homogenized. After 10 minutes, there were two separated layers formed, i.e supernatant and debris. The supernatant was transferred into a new bottle and then measured its absorbance by using UV-Vis spectrophotometer at various wavelengths; 663 nm (A663), 645 nm (A645), 505 nm (A505), and 453 nm (A453). The content of lycopene (CLYL) and β -carotene (CCAR) was then measured using the following formula:

$$\begin{aligned} \text{CLYC} &= -0.04584(\text{A663}) + 0.204(\text{A645}) + \\ &0.372(\text{A505}) - 0.0806(\text{A453}) \\ \text{CCAR} &= 0.216(\text{A663}) - 1.22(\text{A645}) - 0.304(\text{A505}) + \\ &0.452(\text{A453}) \end{aligned}$$

The results of lycopene and β -carotene content were then expressed in $\mu\text{g g}^{-1}$ of fresh weight (FW).

Analysis of polyphenol

0.3 grams of dried goldenberry sample in the microtube was added with 1 mL methanol and then centrifugated at 6000 rpm for 5 minutes. The 50 μ L of supernatant was transferred into a test tube and added with 2 mL of 7.5% Na_2CO_3 prior to incubation at 45°C for 15 minutes. The absorbance was measured using UV-Vis spectrophotometer at 765 nm. The result was plotted into a standard curve and then used for calculation of polyphenol content ($\mu\text{g g}^{-1}$ FW).



Figure 1. Various *Physalis peruviana* L. fruit maturation stages (from left to right), i.e. stage 1 (green berry covered by fresh green calyx), stage 2 (greenish-yellow berry covered by fresh pale green calyx), stage 3 (pale yellow berry covered by fresh greenish-yellow calyx), stage 4 (pale yellow berry covered by fresh pale yellow calyx), stage 5 (yellow berry covered by semi wilt pale yellow calyx), stage 6 (orange berry covered by full wilt white calyx), (unripe green berry covered by green calyx), stage 2 (greenish-yellow berry covered by whitish-green calyx), stage 3 (pale yellow berry covered by greenish-yellow calyx), stage 4 (pale yellow berry covered by yellowish-white calyx), stage 5 (yellow berry covered by semi wilting-yellowish white calyx), stage 6 (yellow berry covered by wilting white calyx)

Analysis of flavonoid

Analysis of flavonoid on dried goldenberry sample followed the AlCl_3 method. 0.5 grams of dried sample in the 25 mL volumetric flask was added with 10 mL ethanol and then homogenized by using a sonicator bath for 5 minutes. The 4 mL clear homogenized solution that had been separated from its sediment was placed in microtube prior to centrifuge at 6000 rpm for 5 minutes. Two mL supernatant was then put into a test tube and added with 2 mL of 2% AlCl_3 that dissolved in ethanol and then re-homogenized by using vortex. The sample was then incubated at 45°C for 30 minutes. The measurement of absorbance by using UV-Vis spectrophotometer was done at 415 nm. The result was plotted into a standard curve and used to calculate flavonoid content ($\mu\text{g g}^{-1}$ FW).

Analysis of data

Analysis of variance (ANOVA) and Pearson correlation analysis was performed by using Statistical Tool for Agricultural Research (STAR) version 2.0.1. For any significant differences between treatments, the Duncan Multiple Range Test (DMRT) was further evaluated at level of confident of 5%.

RESULTS AND DISCUSSION

Fruit juice pH and TSS

The result showed that goldenberry fruit juice pH level was significantly influenced by the fruit maturation stages (Figure 2). The pattern was the older the stages, the increase the pH level. The lowest pH was found in the stage of green berry covered by fresh green calyx (S1), whereas the highest result was measured in fruit from the stage of orange berry covered by full wilt white calyx. The increase of pH in S1 compared to S6 was caused by the loss of acidic compounds due to hydrolysis that continuously happened during the fruit ripening process. In addition, some fruit organic acid was apparently converted into monosaccharides such as glucose and fructose during the fruit ripening process (Mahmood et al. 2012). This finding was in agreement with previous studies in pomegranate (Zarei et al. 2011; Ben-Arie et al. 1984), tomato (Monerruzzaman et al. 2008), and goldenberry (Puente et al. 2011; Salazar et al. 2008).

Fruit maturity stages also affected fruit juice TSS level significantly. The TSS is a simple and feasible variable of refractometric index that showed the proportion of dissolved solids in a solution and expressed in % units. In terms of fruit juice, most of dissolved solid were sugars, including sucrose and hexose (Beckles 2012). The highest TSS was measured in S5 (yellow berry covered by semi wilt pale yellow calyx) that was no significant different than S6 (orange berry covered by full wilt white calyx). At the same time, the lowest result was observed in S1 (green berry covered by fresh green calyx) (Figure 3). The rate of TSS improvement in S6 was 67.5% compared to S1. The breaking down of starch and polysaccharide from the cell wall to be monosaccharide (glucose, fructose, sucrose) that happened during the ripening process was the main cause

of higher TSS content on ripened fruit (Crouch 2003; Balaguera-Lopez et al. 2015). In similar to our finding, previous study by Gutierrez et al. (2008) also showed the increasing pattern of TSS during goldenberry ripening.

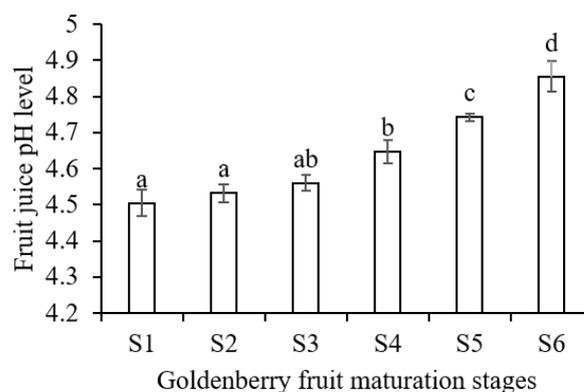


Figure 2. Effect of six different goldenberry maturation stages - S1 (green berry covered by fresh green calyx), S2 (greenish-yellow berry covered by fresh pale green calyx), S3 (pale yellow berry covered by fresh greenish-yellow calyx), S4 (pale yellow berry covered by fresh pale yellow calyx), S5 (yellow berry covered by semi wilt pale yellow calyx), S6 (orange berry covered by full wilt white calyx) – on fruit juice pH level. Note: the different alphabet above the rectangular bar is significantly different based on DMRT at α 5%; the error bar represents the standard deviation.

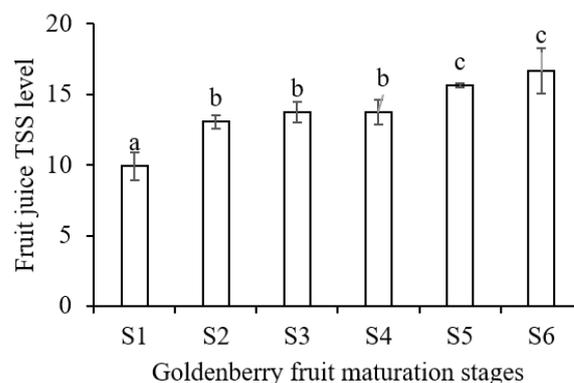


Figure 3. Effect of six different goldenberry maturation stages - S1 (green berry covered by fresh green calyx), S2 (greenish-yellow berry covered by fresh pale green calyx), S3 (pale yellow berry covered by fresh greenish-yellow calyx), S4 (pale yellow berry covered by fresh pale yellow calyx), S5 (yellow berry covered by semi wilt pale yellow calyx), S6 (orange berry covered by full wilt white calyx) – on fruit juice TSS ($^{\circ}\text{Brix}$). Note: the different alphabet above the rectangular bar is significantly different based on DMRT at α 5%; the error bar represents the standard deviation.

Lycopene and β -carotene

Both β -carotene and lycopene are carotenoids that have already well-known for their antioxidant properties (Başkan et al. 2013; Suwanaruang 2016; Burri 1997). The result from statistical analysis revealed that both lycopene and β -carotene content of goldenberry fruits continuously increased during the ripening process. The younger fruit, the lower content of lycopene (Figure 4a) and β -carotene (Figure 4b). The S6 fruit, as the oldest fruit stage, had the highest lycopene and β -carotene content for more than eight-fold and nine-fold greater than S1 fruit as the youngest one (Figure 4). Earlier works by Etzbach et al. 2018 and Hdider et al. (2013) showed similar results. The transformation of chlorophyll into chromoplast due to the deposition of lycopene and β -carotene caused the changes in fruit color from green into yellow-red (Mubarok et al. 2015). In addition to ripening stages (Ilahy et al. 2011), the

content of carotenoids was possibly determined by plant genetic factors (Ordonez-Santos and Ledezma-Realpe 2013; Davies 2000).

Polyphenol and flavonoid

This work revealed that polyphenol content of goldenberry was not significantly affected by the fruit maturation stages (Figure 5.A). The polyphenol in present work varied from 0.01356- 0.01361 $\mu\text{g g}^{-1}$ FW. A previous study reported that the polyphenol increased as the fruit getting mature (Butkhup and Samappito 2011), however numerous other studies reported the opposite result (Fawole and Opara 2013; Fredes et al. 2012). The polyphenol content in fruit was associated with the activity of polyphenol oxidase (PPO), where the increase of PPO caused the decline of polyphenol in the ripe fruit (Ortega-Garcia et al. 2008).

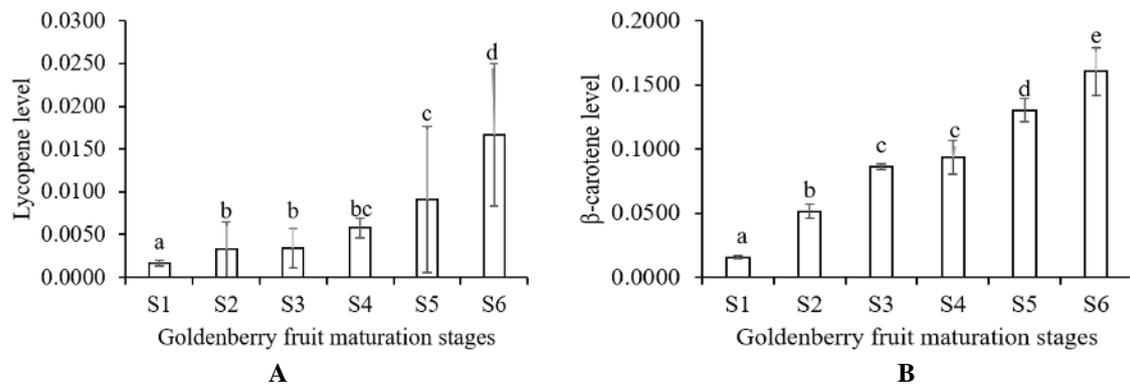


Figure 4. Effect of six different goldenberry maturation stages - S1 (green berry covered by fresh green calyx), S2 (greenish-yellow berry covered by fresh pale green calyx), S3 (pale yellow berry covered by fresh greenish-yellow calyx), S4 (pale yellow berry covered by fresh pale yellow calyx), S5 (yellow berry covered by semi wilt pale yellow calyx), S6 (orange berry covered by full wilt white calyx) – on (A) lycopene level ($\mu\text{g g}^{-1}$ FW) and (B) β -carotene level ($\mu\text{g g}^{-1}$ FW). Note: the different alphabet above the rectangular bar is significantly different based on DMRT at α 5%; the error bar represents the standard deviation.

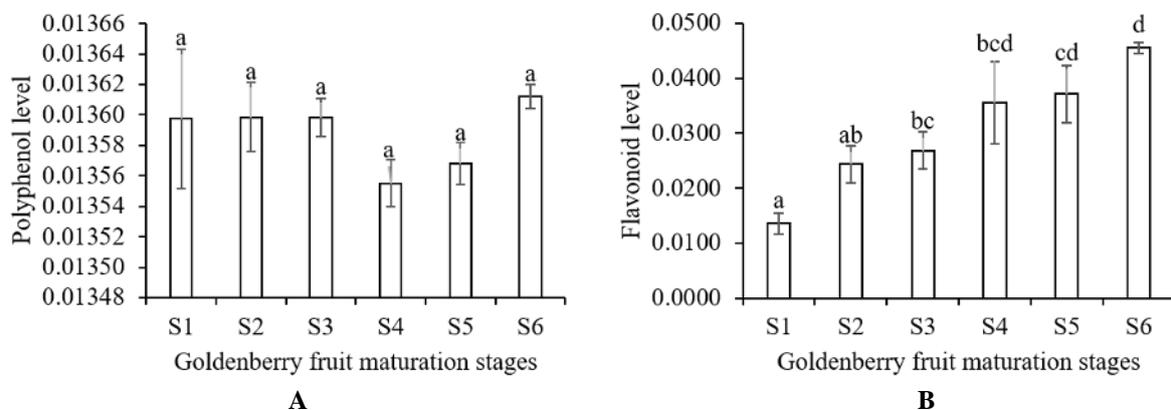


Figure 5. Effect of six different goldenberry maturation stages - S1 (green berry covered by fresh green calyx), S2 (greenish-yellow berry covered by fresh pale green calyx), S3 (pale yellow berry covered by fresh greenish-yellow calyx), S4 (pale yellow berry covered by fresh pale yellow calyx), S5 (yellow berry covered by semi wilt pale yellow calyx), S6 (orange berry covered by full wilt white calyx) – on (A) polyphenol level ($\mu\text{g g}^{-1}$ FW) and (B) flavonoid level ($\mu\text{g g}^{-1}$ FW). Note: the different alphabet above the rectangular bar is significantly different based on DMRT at α 5%; the error bar represents the standard deviation

Table 1. Pearson correlation coefficient among fruit juice pH, TSS, antioxidant activity, lycopene, β -carotene, polyphenol and flavonoid level in six different goldenberry fruit maturation stages

Variable	pH	TS	AT	LC	CR	PP
TS	0.8076*					
AT	0.8528*	0.8636*				
LC	0.7227*	0.6262*	0.6882*			
CR	0.9082*	0.866*	0.9709*	0.7188*		
PP	-0.0611	0.0551	0.0048	0.0614	-0.0507	
FL	0.8435*	0.8499*	0.8818*	0.6907*	0.8868*	-0.011

Note: TS: total soluble solids, AT: antioxidant activity, LC: lycopene, CR: β -carotene, PP: polyphenol, FL: flavonoid

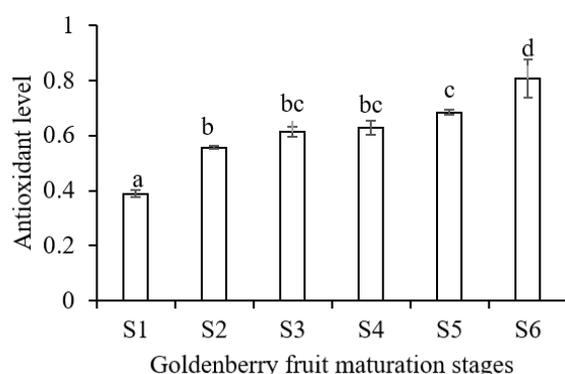


Figure 6. Effect of six different goldenberry maturation stages - S1 (green berry covered by fresh green calyx), S2 (greenish-yellow berry covered by fresh pale green calyx), S3 (pale yellow berry covered by fresh greenish-yellow calyx), S4 (pale yellow berry covered by fresh pale yellow calyx), S5 (yellow berry covered by semi wilt pale yellow calyx), S6 (orange berry covered by full wilt white calyx) – on antioxidant level (%). Note: the different alphabet above the rectangular bar is significantly different based on DMRT at α 5%; the error bar represents the standard deviation

In contrast to polyphenol, the flavonoid content was significantly affected by the fruit maturation stages. There was an improvement of flavonoid content as the goldenberry fruit getting mature. Green berry that is still covered by fresh green calyx had the lowest flavonoid content and it was increased up to more than two-fold when the berry becomes orange ones that covered by full wilt white calyx (Figure 5b). The result of present experiment was in agreement with earlier study by Bhandari and Lee (2016) on tomatoes. The increase of flavonoid concentration was associated with the increasing activity of chalcone synthase as the precursor of flavonoid biosynthesis (Lewinsohn et al. 1989). Aside maturation stage, the flavonoid content of fruit could also be affected by genotype and modification of the growing environment (Ridwan et al. 2021; Omidi et al. 2018; Golkar and Taghizadeh 2018; Pan and Guo 2016).

Correlation between antioxidant activity and metabolites content during fruit maturation

Present finding highlighted the significant difference in antioxidant activity among several fruit maturation stages (Figure 6). There was an increasing pattern of antioxidant activity as the fruit getting mature. The fruit in S1 stage (green berry covered by fresh green calyx) increased its antioxidant activity up to 107% when they reached S6 stage (orange berry covered by full wilt white calyx). This finding was similar to Yan et al. (2006) in guava and Chang et al. (2008) in goldenberry. The increase of antioxidants could be explained by the correlation analysis among phytochemicals observed. Pearson correlation analysis showed that all phytochemical variables observed, except polyphenol have a positive and significant antioxidant activity (Table 1). In agreement to present finding, previous studies have been reported the biological activity of lycopene, β -carotene and polyphenol as antioxidants (Calvindi et al. 2020; Hussain et al. 2019; Goodarzi et al. 2018; Sayahi and Shirali 2017; Setyawan and Darusman 2008; Burda and Oleszek 2001), however, the opposite result was not proved only in term of polyphenol. The correlation results in the present work enriched previous studies that reported a significant correlation among the physical characters of the golden straw (Yildiz et al. 2015).

In conclusion, this work revealed significant improvement of fruit juice pH, TSS, lycopene, β -carotene, and flavonoid content as the fruit gets mature. The fruit maturation stages showed no significant effect on polyphenol level. The antioxidant activity of goldenberry was positive and significantly correlated by all observed variables, except polyphenol content.

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