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Flavonols and antioxidant activity of *Physalis peruviana* L. fruit at two maturity stages

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ABSTRACT. Since the characteristics of the fresh fruit of cape gooseberry (*Physalis peruviana* L.) are little known, its valorization and use are impaired. The fruit's bioactive compounds at two stages of maturity, start and end of maturity, are evaluated, with differentiating colors between green-yellow and orange for two sizes of the fruit. The ratio between sugars and acids increased from the beginning to the end of maturity. Quercetin was not found in the samples. Nevertheless, rutin was predominant in small and large size mature sample, followed by greenish yellow (start of maturity) color of the small size fruit, with values ranging between 6.904 and 6.761 $\mu\text{g g}^{-1}$ and 5.891 to 4.465 $\mu\text{g g}^{-1}$, respectively. Myricetin rates ranged between 1.085 and 1.170 $\mu\text{g g}^{-1}$ and 1.110 to 1.309 $\mu\text{g g}^{-1}$ for greenish yellow and orange fruits, respectively. These results characterize the fruit of *Physalis peruviana* L. as a source of phenolic compounds in food. Antioxidant activity, influenced by the different stages of fruit ripening, is correlated to a higher content of the flavonols rutin and myricetin. Maturity degree and fruit size affect the fruit's chemical characteristics.

Keywords: phenolic compounds, cape gooseberry, Rutin, Myricetin.

Flavonóis e atividade antioxidante do fruto *Physalis peruviana* L. em dois estádios de maturação

RESUMO. As características do *Joá do capote* (*Physalis peruviana* L.) consumido na forma "in natura", são pouco conhecidas, o que dificulta sua valorização e aproveitamento. Dessa forma, este trabalho teve como objetivo avaliar os compostos bioativos do fruto em dois estádios de maturação, início e final do amadurecimento, com a coloração diferenciando-se entre verde-amarelo e laranja, para dois tamanhos de frutos. A relação entre os açúcares e os ácidos (ratio) teve incremento do início para o final da maturação. A quercetina não foi encontrada nas amostras analisadas. Entretanto, a rutina predominou na amostra madura nos tamanhos pequeno e grande, seguida da verde-amarela (início da maturação) no tamanho pequeno, com valores que variaram entre 6,904 - 6,761 $\mu\text{g g}^{-1}$ e 5,891 - 4,465 $\mu\text{g g}^{-1}$, respectivamente, enquanto que o teor de miricetina foi de 1,085 a 1,170 $\mu\text{g g}^{-1}$ e 1,110 a 1,309 $\mu\text{g g}^{-1}$ para o fruto laranja e verde-amarelo respectivamente, indicando que o *Physalis* também é fonte de compostos fenólicos na alimentação. A atividade antioxidante sofreu influência dos distintos estádios de amadurecimento do fruto e está correlacionado com o maior conteúdo dos flavonóis rutina e miricetina. Assim, concluiu-se que tanto o grau de maturação como o tamanho têm influência nas propriedades químicas do fruto.

Palavras-chave: compostos fenólicos, *Joá do capote*, Rutina, Miricetina.

Introduction

The genus *Physalis* L. of the Solanaceae family, has approximately 120 species distributed throughout the planet's temperate zones, from southern North America to South America, with its taxonomical diversity focus in Mexico, United States and Central America (NURIT SILVA; AGRA, 2005). *Physalis peruviana* L. is the species most commonly found in Brazil. The southern State of Rio Grande do Sul is the main producer of the fresh fruit (RUFATO et al., 2008). The species's chief characteristics is the fruit in the shape of a

small, round and exotic berry, with a pulp ranging from yellow to dark orange, similar to a tomato in form and structure. The fruit may contain between 150 and 300 seeds, with a diameter ranging between 12.5 and 25.0 mm and weighing from 4 to 9 g each. The seeds are partially or totally enclosed in a papery husk in the shape of a balloon, known as the calyx (AVILA et al., 2006; CCI, 2000; NURIT SILVA; AGRA, 2005).

The fruit consists of a berry with a fleshy pulp with numerous seeds enclosed by a calyx, commonly called a balloon.

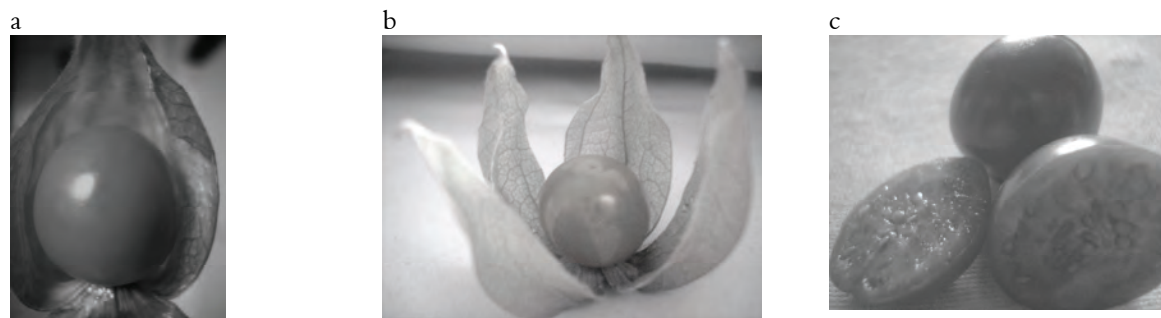


Figure 1. *Physalis peruviana* L. (a and b) fruit enclosed by the calyx; (c) seeds of the fruit.

Its chemical composition features low acidity and an important source of vitamins A and C with sugars ranging between 11 and 15 °Brix, according to maturity stage (AGUILAR et al., 2006; CARRASCO; ZELADA, 2008; LANCHERO et al., 2007).

Physalis peruviana is widely used in folk medicine for the treatment of malaria, asthma, hepatitis, dermatitis and rheumatism (FRANCO et al., 2007; TOMASSINI et al., 2000), coupled to other proven medicinal remedies as a diuretic and the reduction of bad cholesterol and glucose level (RUFATO et al., 2008).

Several chemical compounds may be found within the genus's diversity, such as simple flavonoids or glycosides (kaempferol, quercetin, rutin), linear-chain fatty acids (C6 to C4), ascorbic acid, carotenoids, alkaloids and terpenes (such as Withaesteroids) (ANGELO; JORGE, 2007; HUBER et al., 2007).

Flavonoids, hydroxybenzoic and hydroxycinnamic acids and stilbenes are phenolic compounds which have currently been highlighted owing to their beneficent biological effect to health and because of their anti-oxidant activities that combat free radicals (BARREIROS; DAVID, 2006; CHIRINOS et al., 2010; MONAGAS et al., 2005). Flavonoids are important polyphenols that occur naturally in food consumed daily and highly common worldwide. They are secondary metabolites of plants and may be divided into six main classes: flavons, flavanons, flavonils, isoflavones, antocianines and flavanols (MANACH et al., 2004; YAO, 2004). The first three types are commonly found in citric fruits (CALABRO et al., 2004; DI MAJO et al., 2005) and they differ from each other because of the hydroxyl (position 3) and carbonyl (position 4) groups on the C ring (Figure 2). As a rule, flavonols occur in food, such as O-glycosides, in which one or more hydroxyl flavonoid groups link with mono-, di- or tri-saccharides. Glucose is the most frequently sugar type, followed by galactose, rhamnose, xylose and arabinose (ROBARDS; ANTOLIVIC, 1997).

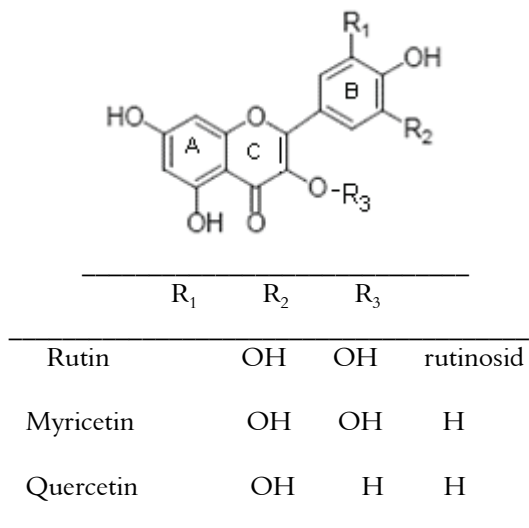


Figure 2. Basic structure of flavonols in fruits.

The term 'flavonoid' is also used to include a group of phenolic compounds in plants which contributes towards the fruits' sensorial quality, also comprising astringency and bitterness (VASCO et al., 2008; VENDRAMINI; TRUGO, 2004).

Brazil has a great variety of native and non-native fruits which require quantification and characterization of their potential sources of compounds, especially the bioactive ones. Current analysis studies the characteristics of small and big size *Physalis peruviana*, at the start and end of maturity so that the fruit's compound contents and characteristics may be defined.

Material and methods

Prime matter

Fruits of the species *Physalis peruviana* L. from the 2009/2010 harvest and produced in the region of Vacaria, Rio Grande do Sul State, Brazil, were provided. They were harvested in the first semester of 2010 and donated by the firm Italbraz® established in the mountain region of the State of Rio Grande Sul, Brazil. Samples were transported in

a refrigerator so that the fruit's characteristics could be maintained and then stored at $7.0 \pm 0.5^\circ\text{C}$ till analyses. Fruits were divided in bunches by their maturity stages: greenish-yellow (start of maturity) and orange (end of maturity) colored, and by size: small-sized, ranging between 16.17 and 16.89 mm, and big-sized, ranging between 20.42 and 22.55 mm diameter, or rather, four groups under analysis.

The fruit's physical and chemical characteristics

The samples' humidity rate was determined by placing the fruits in a buffer at $105 \pm 2^\circ\text{C}$ up to constant weight (AOAC, 2000).

Water activity rates were determined by a digital hygrometer *Aqualab* 3TE (Decagon Devices, USA) and pH was calculated in a disintegrated sample aliquot by a pH meter (ORION 710A). Titrated total acidity of samples was calculated by titration with a standardized solution of sodium hydroxide 0.1 N by an alcohol solution of phenolphthalein 1% as indicator. Results were given in percentages of citric acid (AOAC, 2000 total acidity - 920.92).

Ascorbic acid (Vitamin C) rate was determined by calculating the volume of oxy-reduction with titration of samples with a solution of 2,6-dichlorophenolindophenol sodium (DCFI) (AOAC, 2000 - 967.21).

Total soluble solids rates in samples was determined by refractometer (RL3 - Polskie Zaklady Optyczne S.A.), scales from 0 to 90°Brix ; results were given in $^\circ\text{Brix}$ (AOAC, 2000, soluble solids - 973.21).

Color rates of the fruit *Physalis* were measured by a colorimeter HunterLab MiniScan XE Plus (Hunter Associates Laboratory Inc., Reston, VA, USA), calibrated and equipped with Illuminant $D_{65}/10^\circ$, which established the chromatic space in rectangular coordinates (L^* , a^* , b^*). Data were used in triplicate for each coordinate.

Flavonols per high performance liquid chromatography

Rutin, myricetin and quercetin standards used in current analysis were acquired from Sigma Chemicals Co[®] (St. Louis, USA) and chromatographic degree methanol and analytic degree formic acid were acquired from Merck (Darmstadt, Germany). Milli-Q system from Millipore (Bedford, USA) were used to purify reagent water and $0.45\ \mu\text{m}$ filtering membranes from Millipore[®] were used for filtering samples and during the mobile phase.

Extract was prepared from 15 g of the fresh fruits of *Physalis* and 0.13% ascorbic acid (as anti-oxidant), ground in a mortar till total trituration. Further, 12 mL methanol were added to the sample until a volume of 25 mL was completed with Milli-Q

water. Extraction was undertaken for 20 min. in ultrasound and the extract filtered. Another filtration with $0.45\ \mu\text{m}$ Millipore was undertaken for the chromatographic analysis prior to injection. All determinations in samples were done in duplicate.

Chromatographic separation was done in an Agilent 1200 liquid chromatograph, controlled by software EZ Chrom Elite, with an automatic liquid sampler (ALS), diode array detector (DAD) and Quad pump. Column was Zorbax Eclipse XDB-C18 ($4.6 \times 150\ \text{mm}$, $5\ \mu\text{m}$) filled with $1.8\ \mu\text{m}$ stationary phase of double endcapped C18 (MALDANER; JARDIM, 2009).

Mobile phase of gradient elution consisted of solvent A - water/formic acid (99.55:0.45, v/v) and solvent B - methanol/formic acid (99.55:0.45, v/v). Gradient started in the proportion 80:20 (A:B), for 5 min., up to 58:42 after 7 min. and maintained up to 25 min., returning to 80:20 after 26 min. and maintained up to 36 min. for column reconditioning. Discharge was $1.0\ \text{mL min}^{-1}$ and the volume of injected sample was $20\ \mu\text{L}$. Phenolic compounds were quantified at 370 nm by external standardization. Identification of components was foregrounded on specters between 200 and 600 nm in DAD with regard to respective retention time of injected standards.

Determination of anti-oxidant activity

Reagents acetone, methyl alcohol and potassium persulfate, used in current analysis, were bought from Merck (Darmstadt, Germany) and DPPH (2,2-Diphenyl-1-picryl-hydrazyl), ABTS (2,2 azino-bis-3-ethylbenzthiazoline-6-sulfonic acid) and Trolox (6-Hidroxi-2,5,7,8-tetramethylchroman-2-carboxylic acid) from Sigma Chemicals Co.[®] (St. Louis, USA).

Fruits were then weighed and ground in a mortar till complete disintegration. Further, 40 mL of methanol 50% were added to the sample, followed by homogenization and rest during 60 min. at room temperature. The sample was then centrifuged during 15 min. and supernatant was transferred to a 100 mL balloon. Second extraction with 40 mL acetone 70% was repeated from the residue of the first extract, and then centrifuged for 15 min. Supernatant was transferred to a volumetric balloon with the first supernatant and the volume completed to 100 mL with distilled water.

Determination of anti-oxidant activity by DPPH method

Method followed Brand-Williams et al. (1995), modified by Miliauskas et al. (2004), by employing stable radical DPPH as standard. Reduction

occurred with anti-oxidants when there was a change from Violet to yellow, proportional to the concentration of the sample's reducing compound. Results were given in g fruit μM^{-1} DPPH.

Determination of anti-oxidant activity by the ABTS method

Anti-oxidant activity was analyzed by ABTS method according to methodology by Re et al. (1999), modified by Kuskoski et al. (2005). Results were given in μM trolox g^{-1} fruit.

Statistical analysis

Analyses were randomized and undertaken in duplicate, with the exception of the fruit's physical parameters which was done in triplicate. Analysis of variance (ANOVA) and comparison of means by Tukey's test 5% were statistically evaluated by Statistica for Windows 7.0 Statsoft.

Results and discussion

The fruit's physical parameters

Table 1 shows results of parameters which caused the physical changes that characterize the start and final maturity (by a totally randomized experimental design, factor 2×2 , that is, two sizes and two color states of the fruit, with three repetitions), of ten fruits for each repetition. Whereas diameter and mass of the small-size fruit were approximately 16 mm and 3.0 g, the measurements of the big-sized fruit were 21 mm and 5.0 g. Fruit chromaticity at the start of maturity revealed averages with more pronounced trends towards light colors for luminosity 'L', while coordinate 'a' had lower rates with a trend toward green, the predominant color in the initial phase of maturity. There was no significant difference among samples at the start and end of maturity with regard to coordinate 'b' since both tended towards a yellowish color (+b). The above characteristics may foreground the establishment of objective parameters for the classification of the maturity degree of the fruit produced in Brazil as other countries have done, such as the 1999

Technical Norms of Colombia NTC 4580 with regard to the same fruit (ICONTEC, 1999; AGUILAR et al., 2006).

Physical and chemical characteristics of the fruit

Samples' ratio rates (Table 2) show that results increased in proportion to the fruit's maturity. Behavior was caused by increase in total sugars and by the degradation of organic acids due to the fact that ratio expressed the fruit's maturity/quality index.

Ascorbic acid (vitamin C) rates in *Physalis* decreased in mature fruits (Table 2) since the acid's contents naturally decreased according to maturing and were affected by climatic conditions such as temperature, soil humidity, culture and variety (N'DRI et al., 2010).

Moreover, pH rates between 3.64 and 3.88 were somewhat above average when compared with pH 3.43 by Carrasco and Zelada (2008) for the same fruit.

Samples' rates for total soluble solids (in °Brix) ranged between 13.2 and 14.1 for the start of maturity and between 14.8 and 15.1 for the end of maturity. Fruits analyzed within this band may be classified at color 2 (green-yellow) and at color 5 (orange) respectively (start and end), according to the seven distinct class by the 1999 Technical Norms of Colombia NTC 4580 (ICONTEC, 1999; AGUILAR et al., 2006) which is used for standard classification of the quality of fresh *Physalis* for consumers.

The fruit had an approximate 82.16% humidity and water activity with significant difference ($p < 0.05$) for the same fruit in their different sizes, with rates higher than 0.90. This rate may provide the fruit with a high probability for chemical and microbiological changes (KHOURYEH et al., 2005; OLIVEIRA et al., 2009) and may undergo oxy-reduction reactions due to low pH. Parameters are directly related to the quality and conservation of food and contribute towards the definition of strategies for harvest and adequate storing (GURJÃO et al., 2006; SOUZA et al., 2008; MANTOVANI; CLEMENTE, 2010).

Table 1. Physical characteristics of *Physalis peruviana* L. start (green-yellow) and end (orange) of maturity.

<i>Physalis</i>	Start of maturity		End of maturity	
	small	big	small	big
Weight (g)	2.91 ± 0.52^C	4.26 ± 0.57^B	3.52 ± 0.76^{BC}	5.82 ± 0.40^A
Diameter (mm)	16.47 ± 0.30^C	20.91 ± 0.49^B	16.72 ± 0.17^C	22.01 ± 0.54^A
Color	L*	42.83 ± 0.63^{AB}	40.91 ± 0.81^C	41.50 ± 0.53^{BC}
	a*	14.68 ± 0.57^C	17.88 ± 0.63^A	18.67 ± 0.61^A
	b*	19.71 ± 0.32^A	19.74 ± 0.40^A	20.30 ± 0.47^A

*Means and standard deviation analyzed in triplicate. Different capital letters on the same line indicate significant difference at 5% by Tukey's test.

Table 2. Chemical composition of the fruit *Physalis peruviana* L. at the start (green-yellow) and end (orange) of maturity.

<i>Physalis</i>	Start of maturity		End of maturity	
	small	big	Small	Big
Humidity (%)	81.44 ± 0.39 ^B	82.89 ± 0.39 ^A	82.66 ± 0.27 ^A	82.02 ± 0.50 ^{AB}
Aw	0.99 ± 0.01 ^{AB}	0.99 ± 0.01 ^A	0.98 ± 0.01 ^B	0.99 ± 0.01 ^{AB}
pH	3.64 ± 0.02 ^D	3.69 ± 0.01 ^C	3.79 ± 0.01 ^B	3.88 ± 0.01 ^A
Total titrated acidity TTA (g citric acid 100 g ⁻¹)	1.51 ± 0.07 ^B	1.75 ± 0.01 ^A	1.54 ± 0.02 ^B	1.83 ± 0.30 ^A
Vitamin C (mg 100 g ⁻¹)	219.92 ± 8.11 ^A	208.11 ± 5.06 ^B	162.76 ± 5.34 ^C	151.33 ± 8.74 ^D
Total Soluble solids, TSS (°Brix)	12.88 ± 0.25 ^B	13.25 ± 0.29 ^B	14.28 ± 0.10 ^A	14.50 ± 0.14 ^A
Ratio (TSS/ATT)	8.51	7.55	9.29	7.93

*Means and standard deviation analyzed in triplicate. Different capital letters on the same row show significant difference by Tukey's test 5%.

Phenolic compound rates in *Physalis peruviana*

When rates of phenolic compounds in *Physalis* (Table 3) were taken into account in current research, fruit's rutin at the start of maturity (green-yellow-big) had the lowest rate when compared to the fruits at different maturity stages and size. Rutin in the other samples varied from 5.89 µg g⁻¹ for the small fruit at the start of maturity to 6.90 µg g⁻¹ at the end and for the same size, with no statistical difference. At the start of maturity the big fruit had the highest rate of myricetin (1.31 µg g⁻¹) and differed statistically from the other fruits by a mean rate of approximately 1.11 µg g⁻¹.

Chirinos et al. (2010) analyzed the phenolic compounds in the camu-camu fruit (of the same family) at different maturity stages and reported that total phenolic rates increased according to the fruit's maturity. This fact occurred in *Physalis*'s rutin rate only. These results were presumably related to the process of fruit maturity according to harvest time which has a direct influence from climate, among other factors (BOWER et al., 2002; GIEHL et al., 2008).

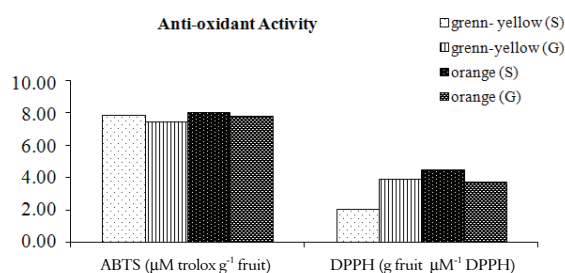
Quercetin, flavonol aglycon, was not registered in the fruit under analysis, possibly due to the fact that it is found in nature as glycosidic bond (BEHLING et al., 2004; SILVA et al., 2003).

The Trolox method (ABTS) may assess equivalent anti-oxidant activity in hydrophilic and lipophilic compounds which, in the samples, provided the highest rate for the small fruit at the end of maturity (Table 4). Besides the effect of phenolic compounds, it also included vitamin C and carotenoids of the fruits in current evaluation. Quantification by ABTS showed correlations with the quantity rutin, following equations in

Table 5, even though there was a decrease in myricetin and vitamin C contents in the samples (Tables 2 and 3).

Anti-oxidant activity

Figure 3 shows that anti-oxidant activity expressed by ABTS and DPPH was more intense for the small fruit at the end of maturity. Data indicated that the maturity stage affected the fruits' anti-oxidant activity.

**Figure 3.** Mean distribution of anti-oxidant activity by ABTS and DPPH methods in *Physalis* at the start and end of maturity.

When anti-oxidant activity was analyzed by DPPH, the lowest rate was reported for the small fruit at the start of maturity with no difference for the other samples which had anti-oxidant activity between IC₅₀ = 3.76 and 3.95. A direct regression (Table 5) with flavonol contents rutin and myricetin was reported for fruits at the end of maturity.

Table 5 demonstrates equations of the effect of codified rates of M (maturity), T (size), MT (interactivity among the factors), separately, on each bioactive compound, and the different expressions of anti-oxidant activity, with linear regression and r² (coefficient of determination of equations) ranging between 0.821 and 0.996.

Table 3. Phenolic compounds rates in *Physalis* at the start (green-yellow) and at the end (orange) of maturity

<i>Physalis</i>	Start of maturity		End of maturity	
	small	big	small	big
Rutin (µg g ⁻¹)	5.89 ± 0.75 ^A	4.46 ± 0.17 ^B	6.90 ± 0.40 ^A	6.76 ± 0.07 ^A
Myricetin (µg g ⁻¹)	1.11 ± 0.05 ^B	1.31 ± 0.04 ^A	1.08 ± 0.01 ^B	1.17 ± 0.05 ^B
Quercetin (µg g ⁻¹)	nd**	nd**	nd**	nd**

*Means and standard deviation analyzed in triplicate; **nd = not detected. Different capital letters on the same row show significant difference by Tukey's test 5%.

Table 4. Anti-oxidant activity rates in *Physalis* at the start and end of maturity.

<i>Physalis</i>	Start of maturity		End of maturity	
	small	big	small	big
ABTS (μM trolox g^{-1} fruit)	$7.88 \pm 0.01^{\text{AB}}$	$7.49 \pm 0.01^{\text{B}}$	$8.07 \pm 0.01^{\text{A}}$	$7.81 \pm 0.01^{\text{AB}}$
DPPH (IC_{50})**	$2.07 \pm 0.01^{\text{B}}$	$3.95 \pm 0.01^{\text{A}}$	$3.80 \pm 0.01^{\text{A}}$	$3.76 \pm 0.01^{\text{A}}$

*Means and standard deviation analyzed in triplicate; ** IC_{50} in g fruit μM^{-1} DPPH. Different capital letters on the same row indicate significant difference by Tukey's test 5%.

Table 5. Regression equations of the factors maturity and size on rutin, myricetin, vitamin C, ABTS and DPPH rates.

Rates	Regression equations	r^2
Rutin (μg g^{-1})	$Y_R = 6.00 + 1.65 \cdot M - 0.78 \cdot T + 0.64 \cdot M \cdot T$	0.833
Myricetin (μg g^{-1})	$Y_M = 1.16 - 0.08 \cdot M + 0.14 \cdot T - 0.05 \cdot M \cdot T$	0.821
Vitamin C (mg 100g^{-1})	$Y_{\text{VitC}} = 185.53 - 56.97 \cdot M - 11.61 \cdot T + 0.18 \cdot M \cdot T$	0.978
ABTS (μM Trolox g^{-1} fruit)	$Y_{\text{ABTS}} = 8.66 - 1.65 \cdot M + 1.49 \cdot T - 1.79 \cdot M \cdot T$	0.996
DPPH (IC_{50})	$Y_{\text{DPPH}} = 3.36 + 0.69 \cdot M + 0.16 \cdot T - 1.72 \cdot M \cdot T$	0.835

Y_R = Rutin rate; Y_M = Myricetin rate; Y_{VitC} = Vitamin C rate; Y_{ABTS} = ABTS rate; Y_{DPPH} = DPPH rate; M = maturity (start of maturity = green-yellow; end of maturity = orange); T = size (small between 16.17 and 16.89 mm; big between 20.42 and 22.55 mm diameter); r^2 = coefficient of determination.

Conclusion

Since the highest rutin rate in the samples was $6.90 \mu\text{g}$ g^{-1} in mature small-sized fruits, and the highest myricetin rate in big-sized fruits was $1.31 \mu\text{g}$ g^{-1} at the start of maturity, fruit size and maturity degree actually influenced the rate of the phenolic compounds in *Physalis*.

Anti-oxidant activity by ABTS had a positive correlation with the rate of the flavonol rutin: high for the small fruit at the end of maturity. When the DPPH method was applied, the Green-yellow fruit showed the highest anti-oxidant activity, without any statistical difference when compared to the fruit at the end of maturity in both sizes.

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