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Effect of different ripening stages on bioactive compounds and antioxidant capacity of wild *Rosa laevigata Michx*

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Abstract

Rosa Laevigata Michx., contents of bioactive components contribute to the health benefits of consuming fresh, snacks and medicine, is widely used as the Chinese medicinal herbs. The effect of fruit ripening on the bioactive compounds and antioxidant capacity of *Rosa laevigata Michx* fruits was investigated. The results showed that, the bioactive compounds, including titratable acidity, reducing sugar, total ascorbic acid, total polyphenol and total flavonoids, significant changes occurred at different ripening stages. The contents of reducing sugar, total polyphenol and total flavonoids were directly proportional to the maturity. However, the content of titration acid and ascorbic acid is inversely proportional to the maturity. Antioxidant capacity and ROS inhibition are proportional to the maturity, and the antioxidant capacity of *Rosa laevigata Michx* significantly increase during maturity. The *Rosa laevigata Michx* fruits at fully ripe stage had higher healthcare and medicinal value.

Keywords: *Rosa laevigata Michx.*; ripening stages; bioactive compounds; antioxidant capacity.

Practical Application: Optimizing harvesting and postharvest management of *Rosa laevigata Michx*.

1 Introduction

Rosa laevigata Michx. (Jinyinzi) is an evergreen climbing shrub prevalently distributed throughout southern regions of China, including Guizhou, Hulan and Zhejiang (Liu et al., 2013a). Several studies have reported that contents of nutrition and antioxidant components such as rutin, flavone, flavonols, polysaccharose, saponins and vitamin C contribute to the health benefits of consuming fresh, snacks and medicine (Zhao et al., 2003; Chen & Zhang, 2005; Xiao et al., 2011; Li et al., 2012; Liu et al., 2013b), and its fruit is widely used as the Chinese medicinal herbs. Numerous studies have demonstrated that *Rosa laevigata Michx* fruit has powerful antioxidant, Acetaminophen, hypolipidemic, hepato-protective and antithrombosis activities (Liu et al., 2010; Zhang et al., 2013a; Dong et al., 2014;), has the capability to treat chronic cough, frequent micturition, hyperpiesia and dermatologic diseases (Jia et al., 2012), the protective effects against paracetamol, high-fat diet, CCl₄ and hydrogen peroxide -induced liver injury (Zhang et al., 2013b; Liu et al., 2014). A number of preharvest and postharvest factors affect the physico-chemical properties, nutritional value and antioxidant components in fruit and vegetables (Opara et al., 2012). Literature evidence showed that the polysaccharose, vitamin C and evaporability composition of *Rosa laevigata Michx.* were affected by ripening stages (Yao & She, 1991; Cai et al., 2011). Studies have also shown that *Rosa laevigata Michx* harvested at fully ripe stage exhibited the highest level of flavonoid, saponins and antioxidant activity (Liu et al., 2010; Zhang et al., 2013c; Dong et al., 2014).

Although the chemical composition of *Rosa laevigata Michx* fruits is relatively known, as far as we know no work was developed on how ripening stages influences fruit composition and their

antioxidant activity. Understanding fruit physico-chemical properties and nutritional properties is important in the design of cost-effective and efficient postharvest handling equipment, optimization of bioprocesses in functional food manufacture, and drugs. There is currently a dearth of scientific information on the key nutrition changes that occur during ripeness of *Rosa laevigata Michx* that are widely grown in the Guizhou province of China to assist in optimizing harvesting and postharvest management. These fruits do not attain maturity simultaneously, coexisting in the same tree from green/yellow to red mature fruits. The most frequent situation during picking is to collect all fruits together when the majority is fully ripe. Hence, the aim of this work is to access the antioxidant ability of *Rosa laevigata Michx* fruits along ripening, by evaluation of bioactive compounds and antioxidant capacity.

2 Materials and methods

2.1 Samples

Fruit samples were collected in of January 2015, in the forestry Sinan County (Guizhou Province, China)(Location:N27°43', E108°09', Altitude: 756 m). A wild *Rosa laevigata Michx* fruit population constituted by different shrubs that grow in same agroclimatic condition and without any cultural intervention was selected. Immediately after harvest, the fruits were transported in to guizhou engineering research center for fruit processing and were divided into three batches based on visual observation of fruit colour, representing three ripening stages (unripe, intermediate and ripe, Figure 1a-c), ranging from green / golden to red, stored

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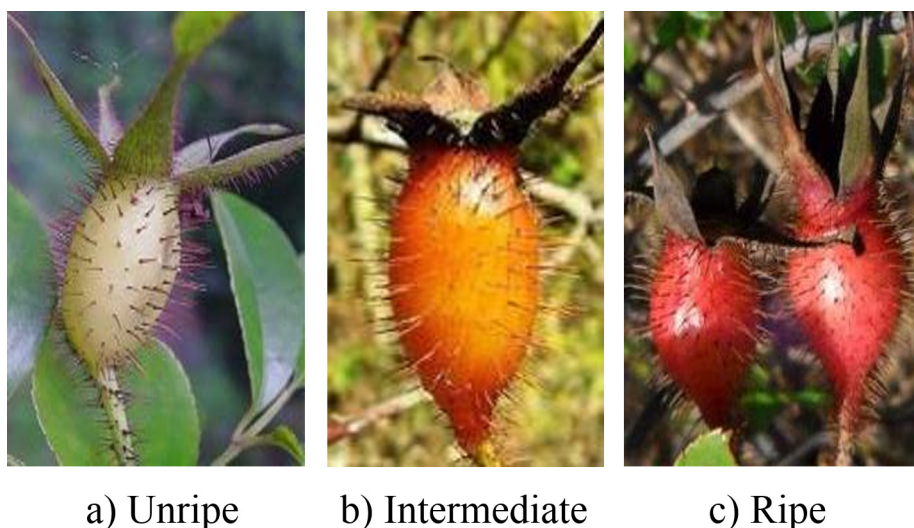


Figure 1. *Rosa laevigata Michx* ripening stages considered in this work.

in refrigerator. A sample of 90 fruits was randomly selected (100 g from each maturity stage), respectively. The fruit were washed with distilled water and then homogenized using a lapping machine (Model A11, IKA, Germany) for analysis of bioactive compounds and antioxidant activity.

2.2 Extraction conditions

A fresh fruits (5.0 g) was extracted using 10 mL of 60% ethanol for 60 min with microwave- (200 kW, 20 °C) in a ultrasonic wave cleaner (model KQ5200DB; Kun Shan Ultrasonic Instruments Co., Ltd, Kunshan, Jiangsu, China) (EM60m). After filtration to remove the residual particles, the supernatants were combined in a flask and filled up to obtain 100 mL of extraction. The extractions were performed in triplicate. All the extracts were analyzed for total phenolic, and total flavonoids content effect on antioxidant activities (DPPH, FRAP, and total reducing power) and reactive oxygen species (ROS) (H_2O_2 , OH^- and O_2^-) scavenging capacity assay.

2.3 Bioactive compounds content

The titratable acidity (TA) were determined by pH Meter (model PHS-3C; INESA Scientific Instrument Co., Ltd, Shanghai, China), using 0.1 N NaOH up to pH 8.1, results were expressed as g citric acid kg^{-1} (fw). The soluble protein (SP) content was measured according to coomassie brilliant blue G250 method (Xie et al., 2014), Bovine albumin was used as the standard and the results were expressed as mg Bovine Albumin Equivalents (BAE) / 100 g (fw). The reducing sugar (RS) content was measured according to 3,5-Dichlorosalicylic acid (DNS) method (Teixeira et al., 2012), results were reported as % (fw). Total ascorbic acid (TAA) content was determined according to the method of Cao et al. (2007), results were reported as mg Ascorbic Acid Equivalents (AAE)/100 g (fw). Total polyphenol content (TPC) was quantified using Folin-Ciocalteu reagent (Nuncio-Jáuregui et al., 2015), Gallic acid was used as the standard and the results were expressed as mg Gallic Acid Equivalents (GAE) / 100 g (fw). Total flavonoids (TFs) were determined

using the method developed by Dragović-Uzelac et al. (2010), rutin was used as the standard and the results were expressed as mg Rutin Equivalents (RE) / 100 g (fw).

2.4 Antioxidant activity

The DPPH • scavenging activity of extracts was assessed according to the method described by Tauchen et al. (2015), the results are expressed as μ mol Trolox Equivalents (TE)/100 g (fw). The ferric ion reducing antioxidant power (FRAP) assay was done according to Todorovic et al. (2015), results were expressed as mean \pm standard error and units in μ mol Trolox Equivalents (TE)/100 g (fw). The total reducing power assay (TRPA) was determined according to a previously described procedure (Oliveira et al., 2011), results were expressed as mean \pm standard error and units in Trolox Equivalents (TE) / 100 g (fw).

The superoxide radical scavenging activity was determined by the Autoxidation of Pyrogallol method (Xu et al., 2013). The hydroxyl radical scavenging activity (HRSA) and hydrogen peroxide scavenging activity (HPSA) of the samples was measured according to the method of Xie et al. (2011).

2.5 Statistical analysis

All sample characterization results were reported as mean \pm standard error. Statistical tests were performed using the SPSS® computer program, version 19.0 (SPSS Statistical Software, Inc., Chicago, IL, USA) to determine differences at the 5% level between different maturity stage using a one-way analysis of variance (ANOVA).

3 Results and discussion

3.1 Bioactive compounds

The extraction method (EM60m) was applied to the samples with different ripening stages (Figure 1a-c). The bioactive compounds of the three *Rosa laevigata Michx* varieties are presented in Table 1. The titratable acidity content showed a

Table 1. Bioactive compounds of fresh fruit of three ripe stage.

	TA (g CAE /kg)	SP (mg BAE/100 g)	RS (%)	TAA (mg AAE/100 g)	TPC (mg GAE/100 g)	TFs (mg RE /100 g)
Unripe	9.33 ± 0.19a	11.50 ± 0.00c	29.80 ± 0.06c	737.73 ± 0.88a	13.81 ± 0.09c	58.17 ± 1.29c
Intermediate	6.69 ± 0.10b	19.36 ± 0.53a	33.66 ± 0.75b	129.89 ± 0.73b	17.70 ± 0.03b	121.71 ± 2.20a
Ripe	1.95 ± 0.02c	12.62 ± 0.53b	40.13 ± 0.26a	37.54 ± 0.26c	23.83 ± 0.09a	137.05 ± 0.72b
P	< 0.001	0.002	< 0.001	< 0.001	< 0.001	< 0.001
R ²	0.998	0.876	0.977	0.999	0.995	0.983

Different letters in each column represent significant differences between the ripe stage ($P < 0.05$). All measures were performed in three independent samples.

different evolution pattern: while both unripe and intermediate fruits displayed higher values (9.33 ± 0.19 and 6.69 ± 0.10 g citric acid /kg of extract, respectively), the ripe stage of ripe stage presented significant lower amounts of titratable acidity content (1.95 ± 0.02 g citric acid /kg). Review of the literature showed that the total acid varied from 0.79% at 20 days after flowering to 0.46% at 220 days after flowering (Mou et al., 1991). The mean total acid of 0.39% at yellow stage and 0.45% at the red stage of reported by She et al. (1988).

So far there has been no detailed report on the soluble protein content in *Rosa laevigata Michx*. Maturity stage significantly affected the soluble protein, soluble protein content was higher in intermediate stage in comparison with other two stages, ranging from 11.50 to 19.36 mg BAE/100g. The reducing sugar content changed significantly during fruit ripening. Reducing sugar content of *Rosa laevigata Michx* increased and ranged from 29.80 to 40.13% during from unripe to ripe stage. This result is in agreement with that of Mou et al. (1991) who found that the reducing sugar content of *Rosa laevigata Michx* changed significantly during maturation and ripening.

The total ascorbic acid changed significantly during fruit ripening. Total ascorbic acid content ranged from 37.54 to 737.73 mg AAE/100g fw from unripe to ripe stage. In comparison with the results obtained in the present study with total ascorbic acid content of harvest at 1986 and 1987 ranging from 69.27 to 1164.36 and 55.60 to 1210.22 mg/ 100g fw during its growth and development, respectively, other researchers have reported lower or higher values for the different harvest time investigated. For instance, Mou et al. (1991) reported that vitamin C varied from 55.60 at 20 days after flowering to 1210.22 mg/ 100 g at 220 days after flowering. Yao & She (1991) analyzed the vitamin C contents at different ripening stages from July to November and concluded that vitamin C contents changed significantly during ripening. The authors found that the vitamin contents reach the peak in the middle of October, which is the suitable time for harvest.

The total polyphenol content and total flavonoids content showed a different evolution pattern: while both unripe ($13.81 \text{ mg} \pm 0.09 \text{ mg GAE/100 g}$ and $58.17 \text{ mg} \pm 1.29 \text{ mg RE /100 g}$ of extract, respectively) and intermediate fruits displayed lower values ($17.70 \text{ mg} \pm 0.03 \text{ mg GAE/100 g}$ and $121.71 \text{ mg} \pm 2.20 \text{ mg RE /100 g}$ of extract, respectively), the ripe stage of ripening presented significant higher amounts of total polyphenol ($23.83 \text{ mg} \pm 0.09 \text{ mg GAE/100 g}$ and $137.05 \text{ mg} \pm 0.72 \text{ mg RE/100 g}$). Statistically, it was observed

that total polyphenol content changed during ripening of *Rosa laevigata Michx*. Interestingly, total flavonoids content decreased significantly at the final stage of maturity. In comparison with literature, the total flavonoids content was lower than those reported in the literature (0.48% to 5.3%) by Xu et al. (2012) and Liu et al. (2013).

Table 1 also shows the correlation between ripening stage and the bioactive compounds. Higher titratable acidity content was found on the unripe fruits, and the content decrease as the maturation increases, a negative correlation was established. The very significant correlations were established between ripeness and bioactive compounds. For total ascorbic acid, a P value of < 0.001 was achieved, and for total flavonoids and total polyphenol content, the P values are < 0.001, proving the extreme influence of the maturation on the bioactive compounds of *Rosa laevigata Michx*. For the first one, a decrease of its content is noticeable, as the ripeness increase, also proved by the obtained negative linear correlation. For the total flavonoids and total polyphenol content, a linear correlation was achieved, proving that their content increase with the ripening of fruit.

3.2 Antioxidant activity

Rosa laevigata Michx was analyzed for expression of the antioxidant activity, measured in EM60m extracts of different ripen stage by DPPH, FRAP, and TRPA. The antioxidant activity of EM60m extracts of the *Rosa laevigata Michx* fruit is described in Table 2. *Rosa laevigata Michx* in ripe stage showed the highest antioxidant activity compared to the other maturity stage when measured by DPPH ($47.58 \text{ } \mu\text{M TE / g}$; $P < 0.001$), FRAP ($11.85 \text{ } \mu\text{M TE/ g}$; $P < 0.001$), and TRPA ($20.85 \text{ } \mu\text{g AAE/ g}$; $P < 0.001$) assays. By contrast, *Rosa laevigata Michx* in unripe stage exerted the lowest antioxidant activity when measured by DPPH, TRPA and FARP assays. *Rosa laevigata Michx* in ripe stage showed the highest ROS compared to the other maturity stage when measured by O_2^- ($20.30 \pm 0.03\%$; $P < 0.001$), OH^- ($70.95 \pm 0.39\%$; $P < 0.001$), and H_2O_2 ($137.36 \pm 3.48\%$; $P < 0.001$) scavenging ratio assays (Table 2), and the lowest ROS at unripe stage. By contrast, *Rosa laevigata Michx* in intermediate stage exerted the lowest scavenging ratio when measured by H_2O_2 , OH^- and O_2^- assays.

3.3 Correlation coefficient evaluate

Table 3 shows the correlation between antioxidant capacity and the bioactive compounds during different ripening stage. These findings mirrored the trend reported for all the studied bioactive

Table 2. Evolution of antioxidant activity and ROS during different ripe of *Rosa laevigata Michx*.

	DPPH(μ M TE/g)	FRAP(μ M TE/g)	TRPA(μ g AAE/g)	SRSA(%)	HRSA(%)	HPSA(%)
Unripe	25.70 \pm 0.03c	10.42 \pm 0.07c	9.05 \pm 0.03c	17.42 \pm 0.13c	42.50 \pm 0.89c	95.67 \pm 0.36c
Intermediate	28.50 \pm 0.06b	11.50 \pm 0.15a	19.85 \pm 0.03b	19.27 \pm 0.05b	48.56 \pm 0.59b	108.67 \pm 0.72b
Ripe	47.58 \pm 0.17a	11.85 \pm 0.08b	20.85 \pm 0.08a	20.30 \pm 0.03ac	70.95 \pm 0.39a	137.36 \pm 3.48a
P	< 0.001	0.002	< 0.001	< 0.001	< 0.001	< 0.001

Different letters in each column represent significant differences between the ripe stage ($P < 0.05$). All measures were performed in three independent samples.

Table 3. Correlation coefficient of 13 indices.

Indices	1	2	3	4	5	6	7	8	9	10	11	12
1 TAA	1											
2 TPC	-0.752	1										
3 TFs	-0.785	0.987**	1									
4 SP	-0.665	0.910**	0.930**	1								
5 TA	-0.972**	0.885**	0.901**	0.759*	1							
6 RS	0.032	0.664	0.616	0.717**	0.261	1						
7 TRPA	-0.999**	0.776*	0.809**	0.645	0.979**	0.067	1					
8 FRAP	0.578	0.932**	0.905**	-0.928**	0.750*	-0.809**	-0.608	1				
9 SRSA	-0.960**	0.558	0.611	0.441	0.878**	-0.198	0.954**	-0.388	1			
10 DPPH	-0.680	0.037	0.102	-0.078	0.495	-0.698*	0.658	0.165	0.840**	1		
11 HRSA	-0.417	-0.279	-0.205	-0.355	0.192	-0.884**	0.385	0.468	0.625	0.946**	1	
12 HPSA	-0.307	-0.365	-0.316	-0.506	0.093	-0.920**	0.285	0.562	0.528	0.897**	0.974**	1

*($P < 0.05$), **($P < 0.01$).

molecules (ascorbic acid, titratable acidity, total polyphenols, total flavonoids, and soluble protein, reducing sugar), which were constantly found in the highest amount in the fruit in ripe stage. A positive correlation between the antioxidant activity and the bioactive molecules was observed with TRPA and DPPH assays. The TRPA was strongly correlated with titratable acidity and total flavonoids ($R^2 = 0.979$ and $R^2 = 0.809$, respectively), weaker correlation with total polyphenols content ($R^2 = 0.776$), while strongly negative correlated with total ascorbic acid ($R^2 = -0.999$). The FRAP was strongly correlated with total polyphenols content and total flavonoids ($R^2 = 0.932$ and $R^2 = 0.905$, respectively), weaker correlation with titratable acidity ($R^2 = 0.750$), while strongly negative correlated with soluble protein and reducing sugar ($R^2 = -0.928$ and $R^2 = -0.809$, respectively). Previous study has indicated that total flavonoids, total saponins from *Rosa laevigata Michx* against DPPH, APAP-induced liver injury, attenuating injury caused by H_2O_2 , and hypolipidemic activity (Liu et al., 2010; Jia et al., 2012; Dong et al., 2014).

This study showed that the bioactive compounds of *Rosa laevigata Michx* changed significantly during on-vine ripening, with dramatic changes occurring during the transition to the last stage of ripe stage. The results achieved in this work may open the possibility of the use of *Rosa laevigata Michx* fruits as source of bioactive compounds, particularly those of ripe stage, and proper management of fruit ripening and harvesting are critical towards the optimization of bioactive compounds. They contain a considerable amount of bioactive compounds, as well as important against free-radicals and ROS, which combined with the fact that these fruits are a good source of ascorbic acid, flavonoids, polyphenol and reducing sugar, makes it as a promising sources of bioactive compounds. Furthermore, the production of *Rosa laevigata Michx* fruits derived products may

also be of application for the pharmaceutical and food industries. The detected influence that maturation stage has on bioactive compounds content is of major importance, when considering this fruits as a possible industrial source of bioactive compounds, in order to achieve higher and viable yields.

4 Conclusion

Thereby, it was found that the content of titration acid and ascorbic acid is inversely proportional to the maturity. The contents of reducing sugar, total polyphenol and total flavonoids were directly proportional to the maturity. Antioxidant capacity and ROS inhibition are proportional to the degree of maturity, and the antioxidant capacity of *Rosa laevigata Michx* significantly increase during maturity. The *Rosa laevigata Michx* fruits at fully ripe stage had higher healthcare and medicinal value.

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