

莲雾花色苷组分鉴定及其稳定性和抗氧化性

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摘要:【目的】鉴定莲雾花色苷类物质种类及其组成, 并研究其呈色稳定性和抗氧化性, 为莲雾果实色泽形成机制研究和花色苷的开发利用提供科学依据。【方法】以‘黑珍珠’和‘紫红’莲雾为材料, 利用液质联用技术(HPLC-ESI-MSⁿ)测定其果皮中的花色苷组分, 探讨pH值和温度对其稳定性的影响, 采用羟自由基和DPPH自由基清除法探讨其抗氧化能力。【结果】鉴定出莲雾中4种花色苷, 分别为矢车菊-3, 5-O-葡萄糖苷、矢车菊-3-O-葡萄糖苷、芍药素-3, 5-O-葡萄糖苷和芍药素-3-O-葡萄糖苷。2个品种所含花色苷种类、含量和比例不同, ‘黑珍珠’主要含芍药素-3-O-葡萄糖苷和矢车菊-3-O-葡萄糖苷, ‘紫红’莲雾以矢车菊-3-O-葡萄糖苷含量最高。莲雾花色苷稳定性随pH值升高而下降, 在酸性条件下较稳定; 对高温敏感, 在4~50 °C的环境中较为稳定。在试验范围内, 对羟自由基和DPPH自由基的清除能力随质量浓度增加而增大。【结论】莲雾所含花色苷以矢车菊素和芍药素为主, 不同品种所含组分和含量有差异, 同时莲雾花色苷具有较高的稳定性和较强的抗氧化性, 具有开发价值。

关键词:莲雾; 花色苷; 稳定性; 抗氧化性

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Identification of anthocyanins and their stability and antioxidant activity in wax apple

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Abstract:【Objective】Although the coloration of wax apple fruit is determined by the composition and contents of anthocyanins, the mechanism is still unclear. Therefore, the analysis of content and composition of the anthocyanins from wax apple, and investigation on their stability and antioxidant activities, would pave a way to further understanding the colors formation of wax apple fruit, and finally applying in food industry.【Methods】‘Black pearl’ fruits with pink peel and ‘Tub Ting Jiang’ fruits with dark red peel were chosen in this study. Sixty ripe fruit samples were harvested from three trees (twenty samples from each plant) and were used for chromatism analysis and anthocyanin extraction. The pericarp separated from forty fruits was immediately frozen in the liquid nitrogen and kept at -80 °C for anthocyanin extraction. Pericarp color measurement was performed by a HP-200 precision colorimeter. The RGB values were then converted to CIELAB parameter (*L*, *a*, *b*, *C*, *h*). Anthocyanins Extraction: 5 g of tissue were incubated with 50mL of MeOH-HCl (pH=3), kept in dark for 12h, and then centrifuged at 4 000 r·min⁻¹ (RT). Supernatant was collected and dried in a vacuum (40 °C) and then dissolved in 10 mL 0.01% (v/v) HCl. The product was washed with 10 mL ethyl acetate for three times, and then the aqueous phase was collected. After using AB-8 macroporous resin adsorbed, the product was washed with 0.01% (v/v) HCl, and target components were eluted with 0.01% (v/v) MeOH-HCl. Finally, the product was concentrated to 5 mL for HPLC-ESI-MS/MS analysis as well as testing for stability and antioxidant ac-

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tivities. The HPLC-MS analysis was carried out using an Agilent 1100 LC/MSD Trap VL detector. The chromatographic separation was performed on a C₁₈ column (Luna, 5 μm, 4.6 mm×250 mm). The injection sling was 10 μL. Elution was performed using mobile phase A (aqueous 10% formic acid solution) and mobile B (methanol). The detection was at 520 nm, and the column oven temperature was set at 35 °C. The flow rate was 1 mL· min⁻¹. The gradient program is described as follows: 0-20 min, 5%-60% B; 20-25 min, 60%-100% B; 25-30 min, 100% B. The mass spectrometry conditions were as follows: electrospray ion source; positive ion mode; capillary voltage, 3 500 V; nebulizer, 45 psi; dry gas: nitrogen; cone gas flow, 12 L· min⁻¹; dry temperature, 300 °C; ion trap, scan from m/z 200 to 1300. The anthocyanins were quantified by external calibration using Peonidin-3-O-glucoside standard (Extrasynthese, France). Stability Determination: (1) 1 mL of extract was diluted with a set of solutions (9 mL) with different pH (1-8), kept at room temperature for 2 h, and then measured absorption spectrum by a UV spectrophotometer (PerkinElmer Lambda 25) at 300-700 nm. (2) 1 mL of extract was diluted with 4 mL of ddH₂O, kept at 4 °C, 20 °C, 30 °C and 50 °C for 1.5 h, 3 h, 5 h, 7 h and 9 h. The absorbance at 530 nm and 600 nm was measured to calculate the residual rate. 【Results】Four kinds of anthocyanins were identified: cyanidin-3,5-glucoside(Cy3Gu5Gu), peonidin-3,5-glucoside(Pe3Gu5Gu), cyanidin-3-glucoside(Cy3Gu) and peonidin-3-O-glucoside(Pe3Gu). The anthocyanin types, contents and proportion were different between two wax apple varieties. Pe3Gu and Cy3Gu were the major components of 'Black pearl', and the contents of Pe3Gu and Cy3Gu were 15.94±1.90 mg· mL⁻¹ and 2.42±0.79 mg· mL⁻¹. The content of Cy3Gu (97.40±11.22) mg· mL⁻¹ was the highest in 'Tub Ting Jiang'. The differences in the colorimetric parameters and the Cy3Gu contents between two varieties were extremely significant. Correlation analysis shown that the content of Cy3Gu was significantly positively correlated with colorimetric parameter a and b, significantly negatively correlated with L and h°. Therefore, the fruits of two varieties with different colors was related to the content of Cy3Gu. The anthocyanins of wax apple were stable under acidic conditions, and its stability decreased when the pH increased. The radical rate of anthocyanins decreased significantly with the time at 70°C, but not so at 4-50°C. The residual rate of anthocyanins decreased with increasing temperature. Except for 1.5h treatment, the residual rate of anthocyanins at 70 °C was significantly lower than other temperature treatments. The scavenging ability of hydroxyl radicals and DPPH radicals increased with the concentration. 【Conclusion】The main anthocyanins in wax apple fruits were cyanidin and peonidin, and different varieties contained various components and contents. The anthocyanins of wax apple were highly stable under acidic conditions and at 4-50 °C. The strong antioxidant activity would be a new and worthy resource to be developed into functional ingredients and applied products with anthocyanin pigments.

Key words: Wax apple; Anthocyanins; Stability; Antioxidant Activity

果实色泽是影响果品商品价值的重要因素之一,是叶绿素、类胡萝卜素、类黄酮及酚类等物质的综合表现^[1-2]。花色苷来自苯丙烷类通路类黄酮代谢分支^[3],是由花色素和各种单糖以糖苷键结合而成的类黄酮物质^[4],广泛存在于葡萄^[5]、越橘^[6]、西红柿^[7-8]和马铃薯^[9]等果蔬中。花色苷组分及含量的不同使果实呈现红、蓝、紫等不同色泽。莲雾[*Syzygium samarangense* (Bl.) Merr. et Perry]又称洋蒲桃,为桃金娘科(Myrtaceae)蒲桃属(*Syzygium*)常绿果

树,果实具深红、大红、粉红、绿、白等色泽^[10],与苹果^[11]、梨^[12-13]等相似,富含花色苷使其果皮呈红色^[14-16]。但目前人们对莲雾花色苷的组分及其色泽形成缺乏详细了解,相关研究较少,Kurt等^[15]指出台湾粉红种(*S. samarangense* var. *Taiwan pink*)只含有矢车菊-3-O-葡萄糖苷,不含其他组分,而薛振晖^[16]研究结果表明,莲雾花色苷以芍药素和矢车菊素为主,但未获得其具体组分,此外未见其他报道。

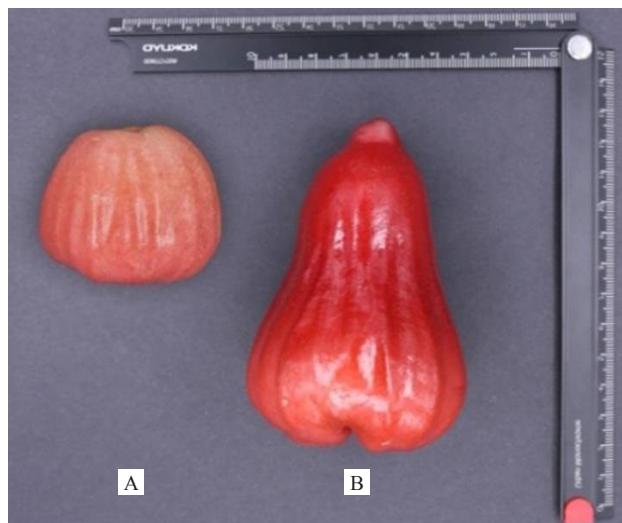
花色苷的组分和含量不仅影响果实商品特性,

也影响其营养价值。花色苷具高抗氧化性,B环上羟基越多抗氧化能力越强^[17],飞燕草素B环的3'、4'和5'位置均有羟基,其抗氧化活性最强,在蓝莓等浆果^[18]、桃金娘科植物^[15]等中均是如此。花色苷还具抗癌、抗炎、诱导凋亡和抑制细胞增殖等多种生理功能^[19-20],被广泛应用于食品、化妆品和医药等领域。但花色苷性质不稳定,其颜色和生物活性除受自身结构影响外,还受外界环境如pH值、温度、光照等的影响^[21-22],在食品加工和贮藏过程中易通过不同途径降解^[23],进而影响其加工制品的品质。因此,研究花色苷组分及其抗氧化性和稳定性具有重要意义。笔者以粉红色和深红色莲雾为研究对象,在提取纯化莲雾果实花色苷的基础上,利用HPLC-ESI-MSⁿ液质联用技术对其花色苷种类进行结构鉴定,并进一步研究其稳定性和抗氧化性,为研究莲雾果实色泽形成机制及其花色苷的开发利用提供理论依据和参考。

1 材料和方法

1.1 植物材料和试剂

试验材料‘黑珍珠’和‘紫红’莲雾成熟果(图1)分别采自漳州市东山县南埔莲雾种植场与东山圆发果蔬专业合作社。2017年7月,每品种均从3株树势较一致的树上各采20个果,分为3个重复。每处理取10个果进行果实色差检测,另10个果立刻用刮刀刮取果皮,置冻存管中,液氮速冻,后置于-80℃



A. 黑珍珠;B. 紫红。

A. Black pearl; B. Tub Ting Jiang.

图1 莲雾果实

Fig. 1 Fruit of wax apple

冰箱保存备用。花色苷标准品芍药素-3-O-葡萄糖苷(Peonidin-3-O-glucoside, Pe3Gu)购自法国 Extrasynthese(Genay, France),纯度≥95%。

1.2 方法

1.2.1 果实色差检测 用HP-200型精密色差仪(深圳汉谱光彩有限公司)测定果实果顶色度中的L值(亮度)、a值(红绿参数)、b值(黄蓝参数)、C值(饱和度)和h°值(综合色度)。

1.2.2 花色苷的提取 取5.0 g莲雾果皮,4℃加入50 mL盐酸-甲醇溶液(pH=3),匀浆后避光浸提12 h,4 000 r·min⁻¹离心10 min,上清液在40℃真空浓缩干燥,浓缩后用0.01% HCl溶液定容至10 mL,用10 mL乙酸乙酯洗提3次。取下层水相,经AB-8大孔树脂吸附,用0.01%盐酸溶液洗掉杂质,用0.01%盐酸-甲醇溶液洗脱,40℃浓缩干燥,用0.01% HCl溶液定容至5 mL用于液相色谱及质谱、稳定性和抗氧化性分析。

1.2.3 花色苷定性分析及结构鉴定 液相色谱条件:采用安捷伦(Agilent 1100 LC/MSD Trap VL)液质联用系统。色谱柱:Luna, 5 μm, C18柱, 4.6 mm×250 mm;柱温:35℃;进样量:10 μL;流动相:A 10% 甲酸,B 甲醇;梯度洗脱:0~20 min B 相 5%~60%;20~25 min B 相 60%~100%;25~30 min 100% B;流速:1 mL·min⁻¹;DAD扫描范围200~600 nm;检测波长:520 nm。

质谱分析条件:电喷雾离子源(ESI),离子阱分析器,正离子模式,全离子扫描,扫描范围200~1 300 m/z。毛细管电压3 500 V,喷雾器压力45 psi,干燥气为氮气(N₂),流速12 L·min⁻¹,干燥气温度300℃,毛细管出口电压为500 V,毛细管偏移电压为77.2 V。用LC/MSD Trap软件(5.2版)分析质谱结果。

1.2.4 稳定性测定 用磷酸氢二钠-柠檬酸缓冲液配制不同pH值(1~8)的溶液,取1 mL提取液,用不同pH的溶液稀释到10 mL,室温避光放置2 h,紫外分光光度计(PerkinElmer Lambda 25)测吸收光谱,每处理3次重复。

取1 mL提取液,用双蒸水定容至5 mL,分别置于4℃、20℃、30℃、50℃和70℃中1.5 h、3 h、5 h、7 h和9 h,冷却至室温后,测定530 nm与600 nm的吸光值,计算其残存率,每处理3次重复。

1.2.5 花色苷抗氧化指标测定 羟自由基清除能力

测定: 将提取液用双蒸水稀释成梯度质量浓度($\mu\text{g}\cdot\text{mL}^{-1}$)进行试验。反应体系中依次加入样品液1 mL, FeSO_4 (6 mmol· L^{-1})溶液2 mL, H_2O_2 (6 mmol· L^{-1})溶液2 mL, 混匀后放置10 min, 加水杨酸钠溶液(6 mmol· L^{-1})2 mL, 静置30 min后, 蒸馏水调零, 测510 nm吸光值 A_1 ; 将水杨酸钠溶液用相同体积的双蒸水代替, 测定吸光值 A_2 ; 将样品溶液用相同体积的蒸馏水代替, 测定吸光值 A_0 。羟自由基清除率(%)=[1-(A_1-A_2)/ A_0]×100, 每处理3次重复。

DPPH自由基清除能力测定: 参考Brand-Williams等^[24]的方法。12.5 mg DPPH溶解到甲醇溶液中, 定容到100 mL, 使用时再稀释5倍到25 mg· L^{-1} , 现配现用。25 μL 样品提取液加到2 mL DPPH甲醇溶液中, 避光反应20 min后在517 nm处测定吸光值 A_1 , 将DPPH溶液用相同体积的双蒸

水代替, 测定吸光值 A_2 ; 将样品溶液用相同体积的蒸馏水代替, 测定吸光值 A_0 。DPPH自由基清除率(%)=[1-(A_1-A_2)/ A_0]×100, 每处理3次重复。

2 结果与分析

2.1 莲雾果皮花色苷组分及含量

在520 nm波长下检测到‘黑珍珠’和‘紫红’莲雾的花色苷色谱峰见图2, 各花色苷紫外光谱特征及质谱数据总结见表1。共检测出莲雾果皮花色苷组分5个, 其中‘黑珍珠’5个组分均有, ‘紫红’仅有3个。二级碎片离子m/z 287和m/z 301分别是矢车菊素苷元和芍药素苷元的特征质荷比, 检测出的5个组分在紫外290~340 nm波长范围内无特征吸收峰, 表明这些组分结构中无酰基^[25]。根据花青苷 $A_{440}/A_{\text{vis}-\text{max}}$ 的值来判断糖苷的位置, 值约30%为花色苷-3-

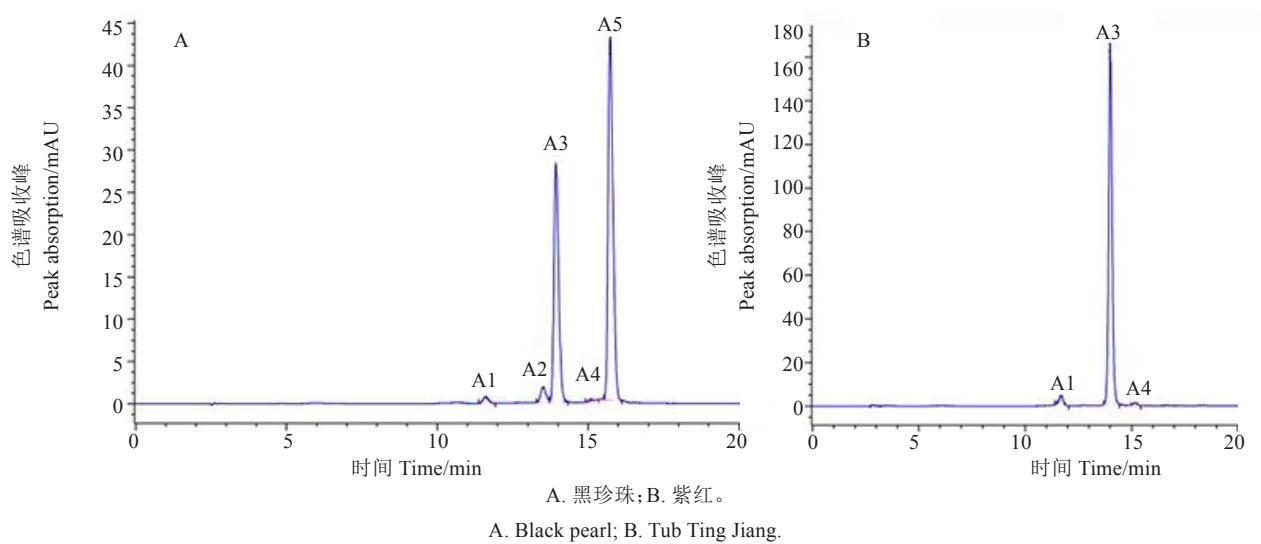


图2 莲雾果皮花色苷液相色谱分析
Fig. 2 HPLC chromatogram of anthocyanins from extract of wax apple peel

表1 莲雾果皮花色苷组分的紫外-可见吸收光谱和质谱数据

Table 1 Chromatographic, UV-vis and mass spectroscopy data of anthocyanins from wax apple peel

| 组分 Peak | 保留时间 Retention time/min | 最大吸收 $\lambda_{\text{max}}/\text{nm}$ | $A_{440}/A_{\text{vis}-\text{max}}/\%$ | 分子离子 Molecular ions/(m· z^{-1}) | 碎片离子 Fragmentsions/(m· z^{-1}) | 化合物 Compound | 参考文献 Reference |
|------------|----------------------------|--|--|--|---|--------------------------|-------------------|
| A1 | 11.598 | 268 516 | 18.0 | 611.0 | 449.1/286.9 | 矢车菊素-3,5-O-葡萄糖苷 Cy3Gu5Gu | [26-27] |
| A2 | 13.507 | 278 516 | 16.5 | 625.0 | 463.0/301.0 | 芍药素-3,5-O-葡萄糖苷 Pe3Gu5Gu | [15,28] |
| A3 | 13.930 | 280 518 | 30.4 | 449.0 | 287.0 | 矢车菊素-3-O-葡萄糖苷 Cy3Gu | [15,28-29] |
| A4 | 15.083 | 282 504 | 42.4 | 417.0 | - | 未知 Unknown | - |
| A5 | 15.726 | 280 518 | 31.0 | 463.0 | 301.0 | 芍药素-3-O-葡萄糖苷 Pe3Gu | [27-28,30] |

糖苷, 值约15%为花色苷-3,5-二糖苷^[31]。

A1: 质谱数据给出分子离子峰m/z 611.0[M+H]⁺, 裂解产生碎片m/z 449.1[M+H-162]⁺和m/z 286.9[M+H-(162+162)]⁺, 因此A1为矢车菊素, 其中参加糖苷化的是2个己糖(m/z 162u), 葡萄糖或半乳糖^[32-33],

由于 $A_{440}/A_{\text{vis}-\text{max}}$ 为18.0%, 根据Sarkar等^[26]对乌墨(*Syzygium cumini*)果实花色苷组分研究的报道, 将A1推断为矢车菊素-3,5-O-葡萄糖苷(Cyanidin-3,5-O-Glucoside, Cy3Gu5Gu)。

A2: 分子离子峰为m/z 625.0[M+H]⁺, 裂解产生

碎片 m/z 463.0[M+H-162]⁺ 和 m/z 301.0 [M+H-(162+162)]⁺,失去 2 个己糖得到 m/z 301.0, 其 A₄₄₀/A_{vis-max} 为 16.5%, 鉴于莲雾已有报道^[15],且本研究中 A1、A3 和 A5 组分中均为葡萄糖苷,将 A2 推定为芍药素-3,5-O-葡萄糖苷(Peonidin-3,5-O-Glucoside,Pe3Gu5Gu)。

A3: 分子离子峰为 m/z 449.0[M+H]⁺,丢失 1 个 m/z 162u 得到碎片离子 m/z 287.0 [M+H-162]⁺,由于 A₄₄₀/A_{vis-max} 为 30.4%,结合 Kurt 等^[15]和 Zanatta 等^[29]的报道,推断 A3 为矢车菊素-3-O-葡萄糖苷(Cyanidin-3-O-Glucoside,Cy3Gu)。

A4: 因其含量极微,多次试验均无法获得二级碎片信息,故未进行结构推定。

A5: 分子离子峰 m/z 463.0[M+H]⁺,碎片离子 m/z 301.0[M+H-162]⁺,丢失 1 个 m/z 162u,其 A₄₄₀/A_{vis-max} 为 31.0%,根据 A5 与标准品 Pe3Gu 的共洗脱特性,A5 为 Pe3Gu。

‘黑珍珠’果实呈粉红色,‘紫红’呈深红色,如表 2 所示,5 个色泽参数、Cy3Gu 含量在 2 个品种间差异均极显著,与目测结果一致。色泽参数与花色苷组分间的相关性分析表明二者间存在极显著的相关性(表 3),Cy3Gu 含量与 a 值和 C 值极显著正相关,与 L 值和 h° 值极显著负相关。a 值正的方向反映果实的红色程度,即 a 值越大果实越红,而 h° 值从 0 到 180 依次为紫红、红、橙、黄、黄绿、绿和蓝绿,h° 值越接近 0 果色越接近紫红,因此,Cy3Gu 含量的积累可降低果色的明度,增加果色的饱和度,有利于果色的红色

表 2 莲雾果皮色泽指标及花色苷组分含量
Table 2 Color parameters and anthocyanins content of wax apple peel

| 色泽参数/组分 Color parameters /Composition | 黑珍珠 Black pearl | 紫红 Tub Ting Jiang |
|---|--------------------|----------------------|
| L | 47.12±2.63 | 36.69±1.23** |
| a | 8.75±1.85 | 16.55±2.24** |
| b | 6.82±2.09 | 3.51±0.65** |
| C | 11.53±1.08 | 16.94±2.14** |
| h° | 42.022±9.15 | 11.238±2.33** |
| ρ(Cy3Gu5Gu) (mg·mL ⁻¹) | TA | 0.43±0.11 |
| ρ(Cy3Gu) (mg·mL ⁻¹) | 2.42±0.79 | 97.40±11.22** |
| ρ(Pe3Gu) (mg·mL ⁻¹) | 15.94±1.90 | ND |

注: TA. 微量; ND. 未检测到; ** 表示 2 个莲雾品种间差异达极显著($\alpha=0.01$)水平。下同。

Note: TA. Trace Amounts; ND. Not Detected; ** indicated that the difference between the two wax apple varieties was extremely significant ($\alpha=0.01$). The same below.

表 3 莲雾果皮花色苷组分含量和色泽指标的相关性
Table 3 Correlation between the anthocyanin content and the color index of wax apple peel

| 组分 composition | L | a | b | C | h° |
|-------------------|----------|---------|--------|---------|----------|
| Cy3Gu | -0.092** | 0.991** | -0.428 | 0.951** | -0.986** |
| Pe3Gu | 0.853 | -0.216 | -0.916 | -0.891 | -0.094 |
| Cy3G5Gu | 0.779 | -0.445 | 0.960 | -0.330 | 0.350 |

注:** 表示在 $\alpha=0.01$ 水平(双侧)上显著相关。

Note: ** indicates a significant correlation at the $\alpha=0.01$ level (both sides).

化,是造成 2 个莲雾品种果色差异的主要组分。

2.2 莲雾果皮花色苷的稳定性

莲雾果皮花色苷吸光度及吸收波长随 pH 的变化情况见图 3。2 个品种吸光值均在 pH 为 1 时最大,在 pH 为 1~3 的范围内,随 pH 的增加而减小,花色苷的最大吸收波长一致,均为 510 nm,颜色由鲜红色变为浅红色,‘紫红’吸光值大于‘黑珍珠’;‘紫红’花色苷在 pH 为 5 和 6 时,无明显最大吸收波长,当 pH 上升到 7 和 8 时,最大吸收波长向右偏移,分别是 560 nm 和 562 nm;‘黑珍珠’花色苷在 pH 为 4~8 范围内,都无明显最大吸收波长。

‘黑珍珠’和‘紫红’果皮花色苷在 4 °C、20 °C、30 °C、50 °C 和 70 °C 的环境中放置相同时间时,紫红莲雾果皮花色苷的残存率均高于‘黑珍珠’(图 4)。4~50 °C 时,二者花色苷残存率随时间延长下降不显著,而 70 °C 时随时间延长,下降呈显著或极显著变化。随温度升高,二者花色苷残存率下降,70 °C 时,除了 1.5 h 处理外,其他时间处理二者花色苷残存率均显著低于其他温度处理。由此可知,莲雾花色苷对高温敏感,在 4~50 °C 的环境中较为稳定。

2.3 莲雾果皮花色苷的抗氧化性

从图 5 可看出,在试验范围内,莲雾花色苷对·OH 的清除率随浓度的增加而增大,呈明显的量效关系,当质量浓度为 400 μg·mL⁻¹ 时,‘黑珍珠’和‘紫红’的清除率分别为 84.16% 和 84.64%。‘黑珍珠’和‘紫红’花色苷清除·OH 的 IC₅₀ 分别为 16.02 μg·mL⁻¹ 和 23.21 μg·mL⁻¹,可见其对·OH 有较强的清除能力。差异性分析表明,2 个品种花色苷对·OH 的清除能力在质量浓度为 5 μg·mL⁻¹、25 μg·mL⁻¹ 时存在极显著差异(图 5-A)。

莲雾花色苷在一定浓度范围内对 DPPH⁺ 的清除率与其质量浓度呈明显的量效关系,随质量浓度

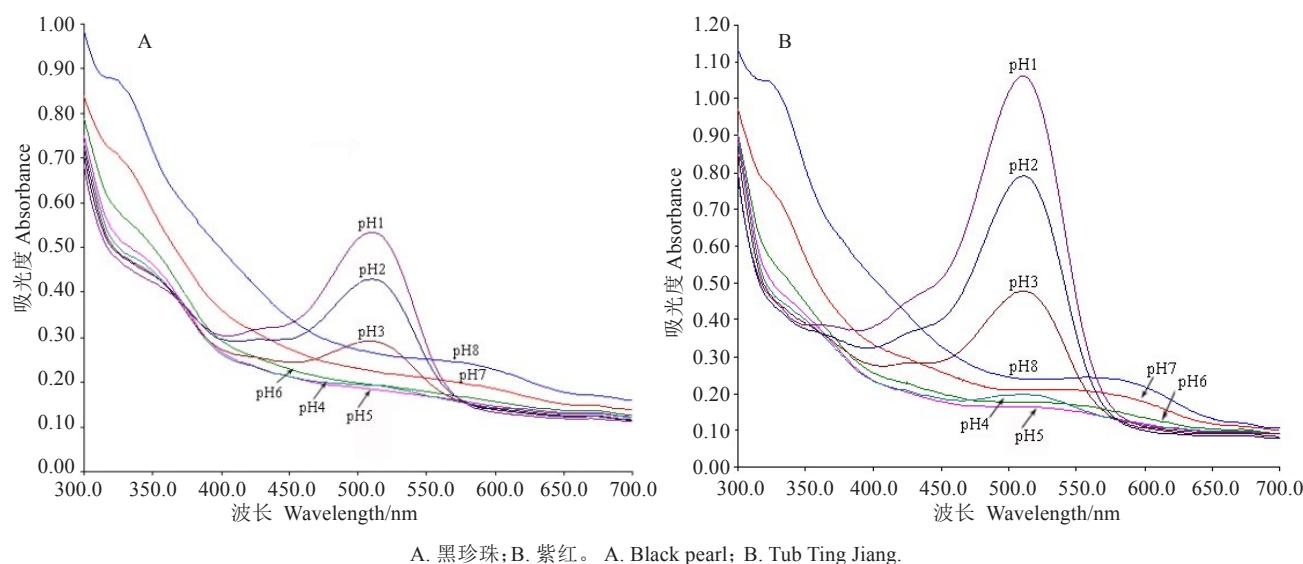
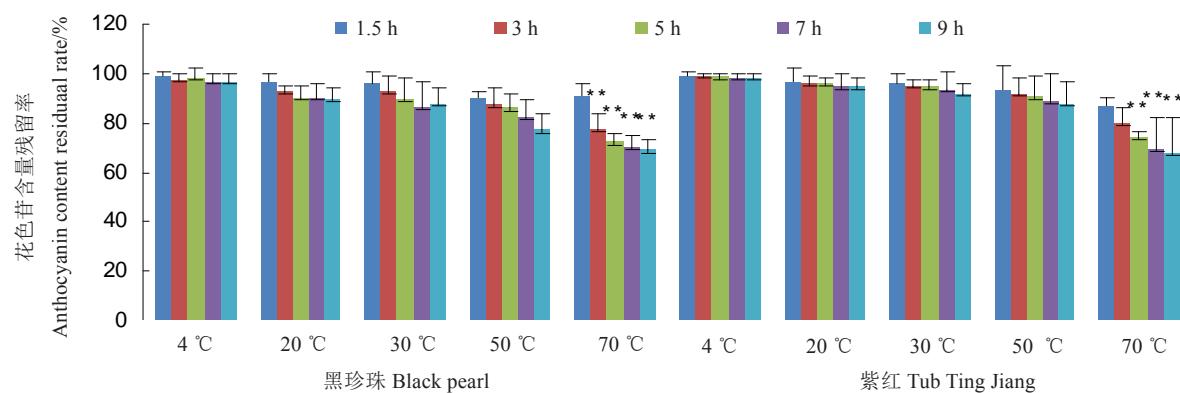


图3 莲雾果皮花色苷溶液在不同pH的紫外-可见吸收光谱

Fig. 3 Chromatographic, UV-vis of anthocyanins from extract of wax apple peel in buffer solutions with different pH

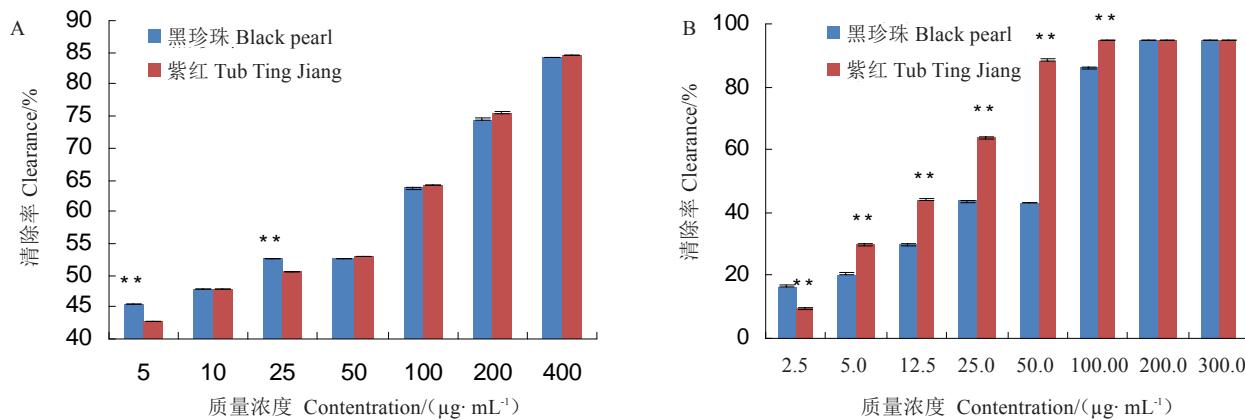


**表示同品种相同温度下其他时间处理与1.5 h处理的差异达极显著($\alpha=0.01$)水平。

** indicates that the difference among other time treatments and 1.5 h treatment at the same temperature of the same variety is highly significant ($\alpha=0.01$).

图4 温度对莲雾果皮花色苷稳定性的影响

Fig. 4 Temperature effect on stability of anthocyanins in wax apple peel



A. 羟自由基清除能力；B. DPPH 自由基清除能力。

A. Hydroxyl radical scavenging activity; B. DPPH free radical scavenging activity.

图5 莲雾果皮花色苷的抗氧化性

Fig. 5 Oxidation resistance of anthocyanins in wax apple peel

增加而增大(图5-B)。在质量浓度为2.5~100 μg·mL⁻¹时,‘紫红’花色苷对DPPH·清除率极显著($\alpha=0.01$)高于‘黑珍珠’。当质量浓度为300 μg·mL⁻¹时,二者的清除率分别为94.94%和94.67%。‘黑珍珠’和‘紫红’花色苷清除DPPH·的IC₅₀分别为55.24 μg·mL⁻¹和23.8 μg·mL⁻¹,说明莲雾花色苷对DHHP·自由基具有强清除能力,且‘紫红’花色苷清除能力强于‘黑珍珠’。

3 讨 论

许多植物叶、花、果呈色与花色苷组分及含量密切相关^[11,34-35],同一物种不同品种花色苷的组分和含量也不尽相同^[36-38],由此产生了颜色各异的叶、花、果。莲雾果实根据色泽可分为5类,深红、大红和粉红果实中含有花色苷^[14-16],绿色和白色果中则不含(数据未发表),Kurt等^[15]指出台湾粉红种仅含Cy3Gu,而薛振晖^[16]的研究表明,莲雾特征性花色苷成分为Cy和Pe。本研究中粉红种‘黑珍珠’含5个花色苷组分,主要组分为Cy3Gu和Pe3Gu,深红种‘紫红’含3个组分,主要组分为Cy3Gu,这与薛振晖^[16]的研究结果相似,不同于Kurt等^[15],这可能与品种不同有关。在花色苷合成途径上,Cy的B环C3'位置甲基化形成Pe,B环的甲基化导致花色苷λ_{vis-max}向长波移动,红色色调增加^[39],因此,Pe较Cy红。薛振晖^[16]指出Cy/Pe比值越小莲雾果实颜色越深。但在本研究中,单从Cy/Pe比值上看,‘黑珍珠’Cy/Pe为0.15,‘紫红’未检测到Pe,但其a值极显著高于黑珍珠,且与Cy3Gu呈极显著负相关,说明‘紫红’果色深于‘黑珍珠’,结果与薛振晖^[16]的研究相反,笔者认为这与‘紫红’所含花色苷总量远高于‘黑珍珠’有关(其总量是‘黑珍珠’的5倍),因此,花色苷总量相近时Cy/Pe比值越小莲雾果色才越深。

在不同pH值条件下,花色苷结构发生变化从而引起颜色变化,研究表明花色苷的苯并吡喃氧鎓离子结构在酸性条件下以氧鎓离子形式存在,呈红色;碱性条件下以醌型结构存在,呈蓝色^[11,34]。莲雾果皮花色苷吸光值(A_{max})随溶液的pH值增大而减小,且最大吸收波长随pH值增大向长波方向移动,当pH值为8时,颜色发生褐变,这说明在碱性环境下其稳定性遭到了破坏^[40]。因此,偏酸性的环境有利于莲雾花色苷色泽的保持。本研究中随加热温度升高,加热时间延长,莲雾花色苷降解加速。这与前人对

樱桃^[41]和草莓^[40]花色苷稳定性研究一致。

花色苷具有很强的抗氧化活性,而其抗氧化作用的主要活性基团是分子中的酚羟基^[42]。Garcia等^[43]对葡萄酒中花色苷的研究结果表明,飞燕草-3-O-葡萄糖苷B环上具有3个羟基,具有最强的抗氧化活性。矢车菊素B环上具有2个羟基(3'和4'位),芍药素B环3'位上羟基被甲基取代,仅具一个羟基,因此,矢车菊素抗氧化活性强于芍药素。本试验结果与此相符,‘紫红’花色苷以矢车菊素为主,而‘黑珍珠’则以芍药素为主,前者的DPPH·清除活性在质量浓度2.5~100 μg·mL⁻¹时均极显著高于后者。莲雾花色苷的抗氧化能力随着质量浓度的增加而增加,清除·OH的IC₅₀远低于蓝莓^[44],清除DPPH·的IC₅₀远低于蓝莓^[44]、樱桃^[45]和杨梅^[46],说明其可作为一种天然的抗氧化剂,具有较好的开发前景。

4 结 论

莲雾果皮含5种花色苷(2种矢车菊素、2种芍药素),不同品种所含组分及含量不同。莲雾花色苷pH稳定性随pH值升高而下降,酸性条件下较稳定;对高温敏感,在4~50 °C的环境中较稳定;对·OH和DPPH·的清除能力随质量浓度增加而增大,具强抗氧化活性,是一种值得开发的新型花色苷色素资源。

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