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Enzymatic Acylation of Anthocyanin Isolated from Black Rice with Methyl Aromatic Acid Ester as Donor: Stability of the Acylated Derivatives

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ABSTRACT

The enzymatic acylation of anthocyanin from black rice with aromatic acid methyl esters as acyl donors and Candida antarctica lipase B was carried out under a reduced pressure. The highest conversion of 91% was obtained with benzoic acid methyl ester as acyl donor, cyanidin 3-(6″-benzoyl)-glucoside, cyanidin 3-(6″-salicyloyl)-glucoside and cyanidin 3-(6″-cinnamoyl)-glucoside were successfully synthesized. This is the first report on the enzymatic acylation of anthocyanin form black rice with methyl aromatic esters as acyl donors and lipase as biocatalyst. Furthermore, the acylation with aromatic carboxylic acids enhanced both the thermostability and light-resistivity of anthocyanin. In particular, cyanidin 3-(6″-cinnamoyl)-glucoside was the most stable among the three acylated anthocyanins synthesized.

Keywords: Black rice; Anthocyanin; Cyanidin-3-glucoside; Candida antarctica lipase B; Enzymatic acylation
INTRODUCTION

Anthocyanins, the largest and most important group of water-soluble natural pigments, represent one of the most widely distributed classes of flavonoids in plants.\(^1\) They are responsible for a variety of bright colors, e.g., blue, purple, red and intermediate colorations of various plant tissues. As a group of flavonoids, they have been reported to have pronounced beneficial health effects associated with anti-oxidant, anti-viral, anti-microbial, anti-inflammatory, anti-tumor and chemo-preventive activities.\(^2\)\(^-\)\(^12\) Furthermore, epidemiological studies suggest that the consumption of anthocyanins prevents the risk of cardiovascular disease, oxidative stress and diabetes.\(^13\)

Due to their attractive colors, broad spectrum safety and beneficial health effects, there has been a growing industrial and academic interest in the use of anthocyanins as natural food colorants for replacing synthetic dyes.\(^14\)\(^-\)\(^16\) Unfortunately, anthocyanins are highly unstable, and their low stability is therefore the primary obstacle to the commercial application as colorants in food industry.\(^17\)\(^,\)\(^18\) However, it has been reported that rare forms of natural acylated anthocyanins exhibited high color stability under different conditions.\(^15\)\(^,\)\(^19\) In nature, the anthocyanin molecules may be acylated through the esterification of sugar residues with a broad range of organic acids such as \(p\)-coumaric, caffeic, ferulic, gallic, malonic, malic and succinic acids. The acylation plays a significant role in the improvement of anthocyanin stability through hydrophobic and \(\pi\)-\(\pi\)-interactions.\(^19\)\(^-\)\(^22\) Therefore, acylated anthocyanins may provide the desirable stability for food applications. However,
most commercial natural anthocyanin colorants are the mixtures of non-acylated and
acylated forms.

Enzymatic acylation of anthocyanins and other flavonoid glycosides in vitro can
increase their stability. The acylation of flavonoid glycosides can be achieved by
using lipases in organic solvents. Two reactions can be catalyzed by lipases to
acylate flavonoid glycosides: direct esterification and transesterification. In the first
reaction, fatty acids or phenolic acids are used as acyl donors in organic solvents at
low water activity, and the by-product water is removed by molecular sieve. In
the second reaction, fatty acids or aromatic carboxylic acid vinyl esters are used as
acyl donors, but acyl donors need be synthesized for the reaction in advance.

Methyl or ethyl esters can also be used as both acyl donor and reaction medium in
transesterification, in conjunction with a system for the online removal of the water
or alcohol under reduced pressure. Compared with flavonoid glycosides such as
isoquercitrin, rutin and naringin, information on acylation of anthocyanin by lipases
is limited. In one study, the crude anthocyanin extract of jaboticaba (Myrciaria
cauliflora) fruits was acylated enzymatically by Novozym 435 with palmitic acid as
acyl donor, and palmitic monoesters of delphinidin-3-glucoside and cyaniding
3-glucoside (1, Figure 1) were synthesized, but the conversion yields and acylation
position were not determined. In another study, Candida antarctica lipase B
(Novozym 435) was used to acylate directly the anthocyanins from blueberry with
phenolic acids, and several new acylated anthocyanins were synthesized, but the
product of reaction was not separated and purified for further study.
Black rice (*Oryza sativa* L.) is widely cultivated and consumed in China and other eastern Asia countries since ancient times. It has been regarded as a health-promoting food since it contains high levels of anthocyanin pigments. The anthocyanin composition of black rice is simpler compared with those of other materials such as black currant, purple corn and purple sweet potato. 1, the best known and most investigated non-acylated anthocyanin, comprises more than 90% of the total anthocyanins of black rice. Thus, black rice is a more outstanding source for the preparation of 1. In the present study, therefore, the anthocyanin from black rice was acylated by using aromatic carboxylic acid methyl ester as acyl donor and lipase (Novozym 435) as biocatalyst, aiming to investigate the effects of acyl donors on the regioselectivity and conversion yield of enzymatic acylation of anthocyanin. Furthermore, the stability of the acylated anthocyanin was investigated.

**MATERIALS AND METHODS**

**Materials and Reagents.** Black rice (Longjin No.1, *Oryza sativa* L. subsp. *Japonica*) was purchased from a local market in Nanjing, China. Novozym 435 (lipase B from *C. antarctica* immobilized on acrylic resin) was purchased from Novozymes (Copenhagen, Denmark). All chemicals, esters and organic solvent used were of the highest available purity and were purchased from Aldrich, Merck or Sigma.

**Isolation of Anthocyanin from Black Rice.** The anthocyanin substrate of reaction was prepared as follows. Black rice (1000 g) was extracted with 4000 mL aqueous ethanol solution (ethanol/water/HCl, 80:20:0.5) at room temperature for 24 h. The
extraction filtrate was concentrated to 50 mL by using a RE-5250 rotary evaporator and loaded onto an Amberlite XAD-7 column (800 × 40 mm i.d.). Then, the column was washed with 1.25 L of water and 500 mL of ethanol/water solution (70:30, v/v), respectively. The eluate of ethanol/water was concentrated to 50 mL under reduced pressure at 55 °C and applied onto a polyamide resin column (800 × 40 mm i.d.). The column was washed first with 1.25 L of water, then with 500 mL of a mixture of ethanol/water (30:70, v/v). The resulting eluate was concentrated under reduced pressure at 55 °C and lyophilized, affording the substrate for enzymatic reaction.

**Procedure of Enzymatic Acylation.** The substrate and lipase for reaction were dried over silica gel under vacuum for at least 1 week before use, pyridine and acyl donor (methyl benzoate, methyl salicylate and methyl cinnamate) were dried for at least 5 d with 4 Å molecular sieves. The acylation reaction was performed in the 250 mL evaporation flask of a rotary evaporator. The pressure was reduced by using a vacuum pump at the desired set point. For all reactions, 0.5 g substrate was dissolved with 5 mL pyridine, and 10 mL acyl donor and 1 g lipase (Novozym 435) were then added. The reaction was maintained at 40 °C and stirred at 30 rpm under vacuum of 900 mbar. After 48 h incubation, the reaction was stopped by filtration to remove the enzyme.

**LC-MS Analysis.** An 1100 series HPLC system with diode array detector (DAD) (Agilent Technologies) was used in this study. The chromatographic separation was conducted by using a TSKgel ODS-100Z column (4.6 × 150 mm, 5 µm, Tosoh Corp., Tokyo, Japan). The mobile phase consisted of eluent A (6% acetic acid in water) and
eluent B (6% acetic acid in acetonitrile), and the linear gradient at a flow rate of 0.6 mL/min was programmed as follows: 0-25 min, 5% to 40% B; 25-35 min, 40% to 80% B; 35-45 min, 8% to 5% B; 45-50 min, 5% B. The temperature of the column oven was set at 40 °C, and the injection volume was 10 µL. Two wavelengths were used, 520 nm for the detection of anthocyanins and 280 nm for the detection of all other phenolic compounds. A T-split was used to reduce the flow before the sample was introduced into the mass spectrometer (MS) (Agilent Technologies). The mass analysis was performed with an electro-spray ionization ion-trap MS (ESI-MS). Full mass spectra (m/z 100-2000) were recorded in a positive mode was using a capillary voltage of 3.5 kV. The pressure of nebulizer gas (N\textsubscript{2}) and flow rate of dry gas (N\textsubscript{2}) were set at 25 psi and 10 L/min, respectively. The capillary temperature was controlled at 350 °C.

**Purification of Acylated Anthocyanin.** The reaction mixture was dissolved and loaded onto a Toyopearl HW-40S column (30 × 1.6 cm) pre-equilibrated with solution of acetonitrile/water/acetic acid (15:79:6) at a flow rate of 2 mL/min. The elution process was programmed as follows: acetonitrile/water/acetic acid (15:79:6) for 30 min, acetonitrile/water/acetic acid (30:64:6) for 30 min, and 90% ethanol for 30 min. The elution was monitored by measuring the absorbance (Abs) at 280 nm and auto-collected (3 mL/tube). The fractions were analyzed by HPLC, and the desired fractions were collected, concentrated and dried, affording the desired products.

**NMR Analysis.** The structures of acylated anthocyanins were characterized by $^1$H NMR.
nuclear magnetic resonance (NMR) and $^{13}$C NMR spectrometry with CD$_3$OD as solvent, and the NMR spectra were recorded by using a Bruker high-resolution AVANCE III 500NMR spectrometer. Data for 2: $^{13}$C NMR (CD$_3$OD) $\delta$ 67.0 (C"-6), 73.1 (C"-4), 75.5 (C"-2), 76.6 (C"-3), 78.7 (C"-5), 96.2 (C-8), 102.6 (C"-1), 102.9 (C-6), 116.4 (C-10), 116.9 (C'-5), 121.2 (C'-2), 121.7 (C'-1), 130.4 (C'''-5), 130.6 (C'-6), 131.9 (C'''-4), 132.2 (C'''-3), 134.0 (C'''-2), 135.3 (C-4), 145.8 (C-3), 146.4 (C'-3), 154.3 (C'-4), 155.9 (C-9), 159.5 (C-5), 169.3 (C'''-1), 169.4 (C-2, C-7).

Data for 3: $^{13}$C NMR (CD$_3$OD with 1% CF$_3$COOH) $\delta$ 63.7 (C"-6), 70.2 (C"-4), 73.3 (C''-2), 74.3 (C"-3), 76.4 (C''-5), 94.0 (C-8), 101.9 (C"-1), 102.0 (C-6), 111.2 (C'''-7), 111.7 (C-10), 114.0 (C'-5), 115.9 (C'''-6), 117.5 (C'-2), 119.6 (C'-1), 126.7 (C'''-4), 129.4 (C'-6), 135.4 (C-4), 143.2 (C-3), 146.0 (C'-3), 154.3 (C'-4), 155.9 (C-9), 159.5 (C-5), 169.7 (C'''-1), 164.0 (C-2), 172.5 (C-7).

Data for 4: $^{13}$C NMR (CD$_3$OD) $\delta$ 63.4 (C"-6), 73.1 (C"-4), 75.5 (C"-2), 76.5 (C"-3), 78.8 (C''-5), 96.3 (C-8), 102.9 (C'-1), 102.9 (C-6), 115.0 (C-10), 116.4 (C'-5), 116.6 (C'-2), 116.8 (C'''-7), 121.1 (C'-1), 130.4 (C''-5), 130.5 (C'-6), 130.6 (C'''-6), 131.0 (C''-4), 132.4 (C''-3), 132.4 (C-4), 137.0 (C'-4), 146.4 (C''-2), 147.5 (C-3), 147.8 (C'-3), 154.3 (C'-4), 155.9 (C-9), 159.5 (C-5), 168.5 (C'''-1), 169.3 (C-7), 170.0 (C-2).

**Determination of Anthocyanin Thermostability.** The stability of acylated anthocyanin was evaluated with 0.01% (w/v) of pure anthocyanin dissolved in 0.01% aqueous HCl solution (pH 2.5). Effect of temperature on color stability of anthocyanin solution was investigated in a water bath at 65, 80 and 90 °C. After 2, 4, 6, 8, 10, 12, 16, 20 and 24 h of each treatment, change in color intensity was
determined with a DU730 Life Science UV/Vis spectrophotometer (Beckman Coulter Inc.) by measuring the Abs at 520 nm.

**Determination of Anthocyanin Light-resistivity.** To evaluate the light stability, the solutions of anthocyanin (5 mL) in glass open cuvettes were placed under the fluorescent light (5000 lx) and ultraviolet illumination (253.7 nm, 2.1 mW/cm²) (Suzhou Purification Equipment Co., Ltd, Suzhou, China) at 20 °C. After 3, 6, 12, 24, 48, 72, 120 and 168 h of each treatment, change in color intensity was determined by using the DU730 UV/Vis spectrophotometer by measuring the Abs at 520 nm.

**Statistical Analysis.** All the analyses in the present study were repeated 3 times. The data were analyzed by one way analysis of variance (ANOVA, version 8.15), and the least significant difference (LSD) post hoc test was conducted. *P* value of less than 0.05 was considered to be statistically significant.

**RESULTS AND DISCUSSION**

In this work, we firstly prepared an anthocyanin extract by use of black rice as starting material. According to HPLC analysis, the content of 1 in the extract was 3.21%. Thus, the extract was purified by column chromatography of macroporous resin (Amberlite XAD-7) and polyamide resin to afford 1 with a purity of 22.4% and 80.8%, respectively. Then, the partially purified anthocyanin was acylated by lipase B from *C. antarctica* and aromatic esters as acyl donors. The reaction was monitored by LC-MS, and the acylated derivatives of anthocyanin were purified from the reaction mixture. Finally, the stability of acylated anthocyanin derivatives was
Enzymatic Acylation of Anthocyanin. The reaction mixture was submitted to LC-MS analysis, and two wavelengths were used: 520 nm for the detection of anthocyanins and 280 nm for the detection of all other phenolic compounds. Figure 2A shows the LC-MS profile of substrate for acylation reaction, and a main peak at 11.8 min, accounting for 95.4% (520 nm) of the total peaks area, was observed. The molecular ion (m/z) for [M+H]⁺ was 449.2, consistent with that of 1. It has been reported that 1 is the major anthocyanin present in black rice. Thus, the major substrate from black rice for present acylation reaction was confirmed as 1.

Figure 2B-D shows the full scan spectra and MS data for the products of the acylation of 1 with methyl benzoate, methyl salicylate or methyl cinnamate as donor. Notably, one major product with similar retention time at 25-30 min was observed for the reaction of each acyl donor. With methyl benzoate as donor, the [M+H]⁺ ion for the reaction product was m/z 553.4 (Figure 2B), which is consistent with that of monoacylated cyanidin 3-glucoside with benzoyl (cyanidin 3-(6"-benzoyl)-glucoside, 2). Furthermore, the MS² data showed a characteristic base peak at m/z 287 for cyanidin from the parent ion (m/z 553.4), indicating that the acylation reaction was regioselective to the glycoside moiety and 2 was synthesized in the reaction system. In addition, the structure of 2 was confirmed by NMR. In the ¹³C NMR spectrum of 2, the signal for C"-6 of the glucose moiety shifted 4.6 ppm (from 62.4 to 67.0 ppm) compared with that of 1. In a similar manner, the corresponding reaction products (3 and 4) were obtained and characterized by LC-MS (Figure 2C and D) and NMR.
when methyl salicylate and methyl cinnamate were used as the acyl donors.

**Effect of Acyl Donor on Acylation Reaction Efficiency.** The effect of the acyl donor on the reaction efficiency was studied by using several available aromatic acid methyl esters as acyl donors. As shown in Figure 3, all reactions reached their reaction equilibrium between 30 and 40 h when methyl benzoate, methyl salicylate and methyl cinnamate were used as acyl donors. The total conversion yield decreased from 91% for methyl benzoate to 68% for methyl cinnamate. The structure of acyl donor used slightly affected the reaction rate as well as the conversion yield of 1. The conversion yield decreased from 91% for methyl benzoate as donor to 84% for methyl salicylate (methyl ortho-hydroxybenzoate) as donor. The low conversion yield for methyl salicylate as donor might be caused by the steric hindrance of the substrate. Conversion was also significantly affected by the structure of carbon chain of aromatic acid methyl ester. When methyl cinnamate was used as acyl donor under the same condition, the conversion yield of the reaction was only 68%, decreasing significantly (23%) relative to methyl benzoate used as acyl donor.

In this work, we obtained 1 from black rice by extraction and purification through column chromatography and synthesized its acyl derivatives by use of *C. antarctica* lipase B. The results demonstrated that Novozym 435 was a good catalyst for the acylation of 1 when several available aromatic acid methyl esters were used as acyl donors. The reaction was regioselective to the glycoside moiety and monoacylated products of 1 were synthesized. With respect to the position of
acylation mediated by immobilized lipase B from *C. antarctica* for glycosylated flavonoids, most studies show that the acylation always take place on the primary hydroxyl group present on the glycoside moiety of the molecule, more precisely on the C-6 carbon atom. As reported by Salem et al., the acylation of isoquercitrin (quercetin 3-*O*-glucoside) with fatty acid esters of various carbon chain lengths and Novozym 435 carried out in 2-methyl-2-butanol led to the synthesis of the sole isoquercitrin 6"-ester. Nakajima et al. and Stevenson et al. used vinyl cinnamate or 2-hydroxyphenylpropionic acid as acyl donors, leading to the same results. Therefore, it is reasonable to believe that it is the 6"-OH group of I being acylated in present study. In fact, the NMR data of the acylated anthocyanins confirmed the hypothesis.

Compared with most enzymatic direct acylation of flavonoids with aromatic acids as acyl donors, the conversion yield of the present reaction was quite high. Similar results were described in the literature for the synthesis of aromatic esters of phloridzin under reduced pressure in the presence of a large excess of acyl donor which also acted as a solvent for the acyl acceptor. These results may be explained by the nature of the reaction. The equilibrium of the reaction shifted towards synthesis when the methanol was evaporated; thereby the conversion yield increased. For acyl donor, the conversion yields were in the order of methyl benzoate > methyl salicylate > methyl cinnamate. The differences in the conversion yields may be due to the differences in structures of acyl donors, particularly the distribution of hydroxyl groups on the aromatic ring. Such results are in agreement with the
conclusion of previous reports.\textsuperscript{25,28} For example, naringin and isoquercetin are both acylated by Novozym 435 with phenylpropionic acid (PPA) and its hydroxylated derivatives as acyl donors, the conversion yields are in the following order: 2-hydroxy PPA > 3-hydroxy PPA > 3,4-dihydroxy PPA > PPA.\textsuperscript{25} It has also been reported that acylated isoquercitrins are synthesized by lipase-catalyzed transesterification with vinyl cinnamate, vinyl cinnamate derivatives and other carboxylic acid vinyl esters as acyl donors, but the conversion rates for vinyl cinnamate derivatives are lower than that for vinyl cinnamate or vinyl phenylpropionic acid ester.\textsuperscript{28}

\textbf{Thermostability of Acylated Anthocyanins.} In order to investigate the effect of acylation with aromatic acid methyl ester as donor on the thermostability of anthocyanin, the monoacylated products (2, 3 and 4) were synthesized and their thermostability at 65, 80 and 95 °C were examined. The logarithm of monomeric anthocyanin content (\(\ln(C/C_0)\)) was plotted versus time (t) (\textbf{Figure 4}), and it was found that the thermal degradation of 1 and its three monoacylated products (2, 3 and 4) followed first order reaction kinetics with respect to temperature. The results are in agreement with those of previous studies, that is the degradation of monomeric anthocyanins from various sources followed a first-order reaction model.\textsuperscript{41,47,48} The first order reaction rate constant (k) and half life time (t\(_{1/2}\)) were calculated by the following equations:

\begin{align*}
\ln(C/C_0) &= -k \times t \quad (1) \\
t_{1/2} &= -\ln(1/2) \times k^{-1} \quad (2)
\end{align*}
Dependence of the degradation rate constant on temperature is represented by the Arrhenius equation:

\[ \ln k = \ln k_0 - \frac{E_a}{RT} \]  

where \( C_0 \) is the initial anthocyanin content and \( C_t \) is the anthocyanin content after \( t \) minute heating at a given temperature, \( k_0 \) is the frequency factor \( (\text{min}^{-1}) \), \( E_a \) is the activation energy \( (\text{kJ/mol}) \), \( R \) is the universal gas constant \( (8.314 \text{ J/mol K}) \) and \( T \) is the absolute temperature (Kelvin).

The kinetic parameters are summarized as shown in Table 1. The \( t_{1/2} \) values showed that the thermostability of the four anthocyanins (1-4) decreased with increasing temperature. As expected, all \( t_{1/2} \) values of the three monoacylated products (2, 3 and 4) were higher than that of 1 at the selected temperature. The thermostability of the three synthesized products was in the following descending order: 4 > 3 > 2. The dependence of the degradation of 2, 3 and 4 on temperature was determined by calculating the value of \( E_a \). High \( E_a \) reaction, normally, is more susceptible to temperature change. Thus, as temperature increased, the increase in the degradation rate of these anthocyanins was in the following order: 3 > 2 > 4.

**Light-resistivity of Acylated Anthocyanins.** The \( t_{1/2} \) values of 1-4 illuminated by white fluorescent light and UV light at 20 °C were measured to examine the effects of the acyl moieties on the light-resistivity. The results are summarized as shown in Table 2. It is obvious that the decomposition rate of the anthocyanin under the UV exposure was greater than the rate under the fluorescent light. 2, 3 and 4 displayed relatively higher stability than 1 in the response to both fluorescent and UV light.
The results implied that the acylation on 1 significantly improved its stability not only under illumination but also under UV condition. Furthermore, 4 displayed the greatest residual color intensity for fluorescent light treatment, while 3 exhibited longer $t_{1/2}$ for UV treatment (Table 2). The results implied that the light-resistivity of acylated anthocyanin was dependent on the structure of acyl moiety.

Natural acylated anthocyanins from plants such as purple sweet potato exhibit an outstanding stability compared to non-acyl anthocyanins in different conditions. Furthermore, the stability of acylated anthocyanins synthesized with anthocyanin extract of black rice and octenyl succinate anhydride has been evaluated, and it was found that the acylated anthocyanins showed higher stability than non-acylated anthocyanins. Thus, for the first time, we explored the relative stability of 1 and its acylated derivatives synthesized by lipase as catalyst. As for thermostability, all $t_{1/2}$ values of synthesized products were higher than that of 1 from black rice. These results indicated that acylated substitution and the new ring formed through transesterification between 1 and methyl aromatic acid esters were beneficial to stabilize the anthocyanin molecules. The increasing color stability of anthocyanins is associated with the higher steric-hindrance, which probably protects the anthocyanin from hydration, and water is incapable of attacking the aglycone. In addition, it has been reported that the acylation with aromatic carboxylic acids improved the thermostability of isoquercitrin and isoquercitrin cinnamate, the stability of acylated product being higher than that of isoquercitrin benzoate. These results suggest that thermostability of acylated molecules is dependent on the aromatic ring in the acyl
In conclusion, the acylation offers an attractive strategy to improve the stability of natural non-acyl anthocyanins. This is the first report on the enzymatic acylation of anthocyanins from black rice with methyl aromatic acid esters as acyl donors and lipase as biocatalyst. The acylation reaction, mediated by lipase under a pressure of 900 mbar, was demonstrated to be very efficient and regioselective, 2, 3 and 4 were successfully synthesized. The highest conversion of 1 was obtained when methyl benzoic acid ester was used as acyl donor, indicating that the efficiency of the synthesis depended on the acyl donor. The acylation with aromatic carboxylic acids improved both the thermostability and light-resistivity of the anthocyanin. Among the three acylated anthocyanins synthesized, 4 was the most stable under the investigated temperature. Detailed studies including the bioactivity of acylated anthocyanins are in progress.

ACKNOWLEDGEMENTS

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**Figure Captions**

**Figure 1.** Structures of cyanidin 3-glucoside (1) and its three acylated derivatives (2, 3 and 4) synthesized with lipase B from *C. antarctica* as biocatalyst.

**Figure 2.** LC-ESI-MS chromatograms showing the reaction substrate (1) from black rice (A) and its reaction products (B, 2; C, 3; D, 4).

**Figure 3.** Time-course acylation of 1 with methyl benzoate (△), methyl salicylate (□) and methyl cinnamate (○) as acyl donors catalyzed by lipase B from *C. antarctica* at 40 °C under a reduced pressure of 900 mbar.

**Figure 4.** Degradation of acylated anthocyanins during thermal treatment at different temperatures.
Table 1

Thermal degradation parameters of anthocyanins

<table>
<thead>
<tr>
<th>Anthocyanin</th>
<th>Temperature (°C)</th>
<th>k (h⁻¹)</th>
<th>t₁/₂ (h)</th>
<th>Ea (kJ mol⁻¹)</th>
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<tr>
<td>1</td>
<td>65</td>
<td>0.0278 (0.9938)</td>
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<tr>
<td></td>
<td>80</td>
<td>0.0864 (0.9982)</td>
<td>8.02</td>
<td>59.31 (0.9746)</td>
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<td></td>
<td>95</td>
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<td>4.49</td>
<td></td>
</tr>
<tr>
<td></td>
<td>65</td>
<td>0.0107 (0.9980)</td>
<td>64.78</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>80</td>
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<td>18.58</td>
<td>61.37 (0.9574)</td>
</tr>
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<tr>
<td></td>
<td>65</td>
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<td>77.01</td>
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</tr>
<tr>
<td>3</td>
<td>80</td>
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<td>62.23 (0.9528)</td>
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<tr>
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<td>65</td>
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</tbody>
</table>

1, cyanidin 3-glucoside; 2, cyanidin 3-(6″-benzoate)-glucoside; 3, cyanidin 3-(6″-salicylate)-glucoside; 4, cyanidin 3-(6″-cinnamate)-glucoside.
Table 2

Effects of light treatments on the color stability of anthocyanins

<table>
<thead>
<tr>
<th>light</th>
<th>Half life time (t_{1/2}, h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Dark</td>
<td>248.34 (0.9833)</td>
</tr>
<tr>
<td>Fluorescent</td>
<td>113.32 (0.9750)</td>
</tr>
<tr>
<td>UV</td>
<td>24.12 (0.9645)</td>
</tr>
</tbody>
</table>

Numbers in parentheses are the determination coefficient. 1, cyanidin 3-glucoside; 2, cyanidin 3-(6″-benzoate)-glucoside; 3, cyanidin 3-(6″-salicylate)-glucoside; 4, cyanidin 3-(6″-cinnamate)-glucoside.
Figure 1
Figure 2
Figure 3
Figure 4
Graphic Abstract