Investigation of acylated anthocyanins from black rice by lipase-catalyzed: Improvement of lipid-soluble antioxidant activity and stability

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Abstract

In this study, a series of acylated anthocyanins from black rice with different weight ratio of caffeic acid has been successfully prepared by lipase-catalyzed in non-aqueous phase. The effects of acylation on the antioxidant capacity and degradation kinetics were also conducted. The ability of scavenging liposoluble radical and total antioxidant capacity for anthocyanins were improved by acylation reaction. The total antioxidant capacity reached maximum when the weight ratio of anthocyanins to caffeic acid was 1:1. Moreover, the decomposition stability of acylated anthocyanins was enhanced and the degradation followed first order reaction kinetics with respect to temperature, pH and light, respectively. The decomposition rate at relevant pH value for acylated anthocyanins with the weight ratio of 2:1 was the highest. However, the rate constant of anthocyanins against heat and light were decreased with the increasing content of caffeic acid, which illustrated that the thermal and light stability was increased with the content of caffeic acid increasing. The exploration of this study would extend the application of anthocyanin in organic phase and lay the groundwork for further exploring the potential application of anthocyanins in food industry.

1. Introduction

Anthocyanins as one class of the polyphenol pigments are widely distributed in fruits and vegetables. An important source of anthocyanins is black rice (*Oryza sativa L.*), which has been widely cultivated in Southeastern Asian countries for a long time (Loypimai et al., 2015). The total anthocyanin contents of black rice range from 1231 to 5101 mg (Kamiya et al., 2014). More than 600 anthocyanins are found in nature, which are mainly derivated from cyanidin, delphinidin, pelargonidin, peonidin, petunidin, and malvidin (Hou et al., 2013; Tatsuzawa et al., 2008). The main anthocyanins in black rice are cyanidin and paeoniflorin, as well as their glycosidyl structure (Hao et al., 2015).

Anthocyanins are responsible for a variety of bright colors, ranging from red, blue to purple and intermediate colorations of various plant tissues. Anthocyanins not only impart color to food but also will strengthen the antioxidant defense system of body. It has been widely reported that anthocyanins have an effect in reducing risk of oxidative damage and are a kind of the potential drug candidates to treat cancer and cardiovascular diseases (He et al., 2010; Koosha et al., 2019; Prasain et al., 2020; Pahlke et al., 2021).

Despite anthocyanins possess many outstanding bioactive properties, this type of pigments is water-soluble, which make it impossible to use in oil-soluble condition. Furthermore, Anthocyanins are unstable and easily destroyed by different factors, such as pH, light, thermal treatment, enzymes, oxygen, and copigments, which restrict its application severely (Moura et al., 2012; Swer et al., 2019; Zhu et al., 2020). Thus, many researchers have sought to improve the lipophilic property and stability of anthocyanins. A series of studies revealed that the glycosyl in anthocyanins could react with organic acid to form acylated anthocyanins, which exhibited excellent stability through the formation of hydrophobic and “π-π”-interactions (Castro et al., 2014; Fei et al., 2021). The acylation reaction of anthocyanins is mainly located at the C3, or C6, or the unite of C3 and C5 in glycosyl group (Shao et al., 2014). The increasing color stability of acylated anthocyanins is associated with the higher steric-hindrance, which probably protects the anthocyanin from hydration, and water is incapable of attacking the aglycone to prevent the formation of chalcone. Furthermore, acylated anthocyanins exhibited outstanding stability in a wide range of pH, especially in acidic and neutral environment (Zheng et al., 2022).

Recently, many researchers have been concentrated on the acylation of anthocyanins. Lu et al. have reported the stability properties of acylated anthocyanins from black rice, but the antioxidant capacity and degradation kinetics have not been studied yet (Lu et al., 2011). Despite Yan et al. have revealed the degradation kinetic parameters of acylated black rice anthocyanins, the antioxidant capacity and degradation kinetics at different pH have not been yielded (Yan et al., 2016). Liu et al. have explored the color stability and antioxidant activity of acylated blueberry anthocyanins, however, the ability to capture radicals of different polarity for anthocyanins and the stability in different conditions have not been investigated yet (Liu et al. 2020). At present, chemical and enzymatic strategies are the main acylation methods for anthocyanins. Although the stable structure can be formed through chemical strategy, those anthocyanins exbibit low selectivity and weaken antioxidant activity (Howell et al., 2020). Compared with chemical strategy, enzymatic strategy is conducted with high selectivity but poor stability. Consequently, obtaining acylated anthocyanins with high stability has drawn many attentions of scholars. Caffeic acid is a kind of aromatic acid, which is wished to provide both stable C = C bonds in the benzene ring but also phenol structure with antioxidant activity (Chebil et al., 2006).

In consequence, caffeic acid was selected as acyl donor to obtain acylated anthocyanins from black rice with excellent antioxidant activity lipo-solubility, and stability by lipase-catalyzed in this study. Ultraviolet spectrum (UV) and Fourier infrared spectrometer (FTIR) was used to characterize the structure of anthocyanins, Thermogravimetric analysis (TGA) was conducted to investigate the thermal property. The antioxidant capacity of genuine and acylated anthocyanins was evaluated by measuring the 2 - diphenyl − 1 - picrylhydrazyl (DPPH) radical scavenging activity, 2,2' - azino - bis (3 - ethylbenzothiazoline − 6 - sulfonic acid) diammoniumsalt (ABTS) radical scavenging activity and Power reduction antioxidant capacity (FRAP). Consequently, the degradation kinetics of acylated anthocyanins at different variates (temperature, pH, light) were investigated. This study will provide theoretical basis for the feasibility of anthocyanins in food processing and the improvement of health care value.

2. Materials And Methods

2.1 Materials

Black rice (*Oryza sativa* L.) was purchased from Heilongjiang Xinhe Ecological Agriculture Development Co., Ltd (China). Caffeic acid (assay > 98%), DPPH and ABTS were supplied by Shanghai Yuanye Biotechnology Co., Ltd (China). Lipase Novozyme 435 was obtained from the Zhejiang Novocata Biotechnology Co., Ltd (China). 4A zeolite was purchased from the Langfang Nake Biotechnology Co., Ltd (China). AB − 8 macroporous adsorption resin was procured from Tianjin Haiguang Chemical Co., Ltd (China). Other solvents and materials were analytical grade.

2.2 Preparation of the anthocyanin extracts from black rice

Anthocyanin extracts from black rice were obtained basing on the modified method reported by Hao et al (Hao et al., 2015). The black rice flour was extracted twice with ethanol/water/1% hydrochloric acid (50:50:0.5, v/v/v) by solid - liquid ratio (1:10) at 30 ^{o}C for 2 h. All extracts were centrifuged at 4000 r/min for 10 min and the supernatants were collected. The collected filtrates were subjected to a vacuum evaporator to remove the remaining ethanol. And then the concentrated extracts were loaded on an AB − 8 macroporous adsorption resin to remove major impurities according to the following manner: 10 g AB − 8 resin was added into 50 mL crude

anthocyanin extracts with stirring at room temperature for 24 h. The anthocyanin extracts contained with AB − 8 resin were filtered and subsequently recovered with 70% ethanol containing 1% hydrochloric acid. The ethanol eluent was evaporated under vacuum to yield the anthocyanin extracts. Finally, the anthocyanin extracts were freeze-dried in a freeze dryer at − 50 ^{o}C for 24 h to obtain anthocyanin powders.

2.3 Acylation of anthocyanin extracts

Anthocyanins from black rice were acylated according to the modified method described by Yan et al. (2016) (Yan et al. 2016). The appropriate masses of anthocyanins and caffeic acid basing on different weight ratio were added into a 250 mL three-neck flask. The black rice anthocyanins and caffeic acid were thoroughly mixed at the weight ratio of 1:0, 2:1, 1:1 and 1:2, respectively. And then 5 mL distilled water, 60 mL tert-butyl alcohol, 15 g 4A zeolite, and lipase Novozyme 435 (20 wt% black rice anthocyanins) were added in the reactant mixture and stirred at room temperature for 24 h. After the reaction finished, the organic reagents were evaporated and freeze-dried in a freeze dryer at − 50 ^{o}C for 24 h. The acylated anthocyanins prepared at the weight ratio of 1:0, 2:1, 1:1 and 1:2 labeled as AN/CA 1:0, AN/CA 2:1, AN/CA 1:1 and AN/CA 1:2, respectively. Figure 1 **(a)** represents the synthesis routine of acylated anthocyanins from black rice see.

2.4 UV analysis

The UV analysis of the anthocyanins from black rice were investigated by Puruisi UV − 18 model instrument (λ = 200–400 nm). The measurements were repeated intriplicates. In the text, the results of UV ranges will be given in terms of wave length (nm).

2.5 *FTIR analysis*

The chemical structure of the anthocyanins from black rice were measured by Perkin - Elmer Spectrum 100 FT - IR spectrometer with the prepared monomers at 4 cm^{-1} resolution after 32 scans by casting a thin film with KBr.

2.6 Thermal properties

The thermal weight loss value of acylated anthocyanins during heat was investigated by thermal gravimetric analysis (TGA), which was carried out by TA Instruments Q50 TGA from 25 to 400 ^{o}C at a heating rate of 20 ^{o}C/min, in a nitrogen atmosphere.

2.7 Assays for antioxidant

The antioxidant capacity was determined by three assays, including DPPH radical scavenging assay, ABTS radical scavenging assay, and ferric reducing antioxidant power (FRAP) assay, respectively. DPPH and ABTS scavenging assay were performed according to the method reported by Sahreen et al. with slight modification (Sahreen et al., 2010). The absorbance was taken at 517 nm and 734 nm by UV-visible spectrophotometer for DPPH and ABTS radical scavenging activity, respectively. And the scavenging rate was calculated by Eq. (1):

$$\text{Scavenging rate}\,(\%) = (1 - A / A_0) \times 100 \qquad (1)$$

where A is the absorbance of samples, A_0 is the absorbance of standard solution prepared under the same conditions but without sample added.

The FRAP of acylated anthocyanins concentration (AAC) was measured basing on the method reported by Thaipong et al. with slight modification (Thaipong et al., 2006). The FRAP stock solution was prepared from sodium acetate buffer (0.3 mol/L, pH 3.6), $FeCl_3$ solution (0.02 mol/L), and TPTZ solution (0.01 mol/L with 0.04 mol/L HCl) with the volume ratio of 10:1:1, respectively. 0.02 mL sample was mixed with 3.98 mL FRAP stock solution and stirred 30 min at room temperature. The absorbance of the samples was investigated at 593 nm. The standard curve was linear between 0 and 25 mM Trolox. Results are expressed in mM TE/mg AAC.

2.8 Stability studies

2.8.1 pH stability

The pH stability of acylated anthocyanins was investigated at pH 3.0, 4.0, 5.0, 6.0 and 7.0, respectively. 75 mg anthocyanin extracts were mixed with 75 mL citrate phosphate buffer at 25 ^{o}C and was divided into the test tube. And then evaluated their absorbance using UV spectrometer at 520 nm. Further, the anthocyanin contents were calculated per hour based on the percentage of the absorbance at each pH value until 5 hours.

2.8.2 Thermal stability

The effect of temperature on acylated anthocyanins from black rice was evaluated basing on the methodology reported by Swer with some modification (Swer et al., 2019). 75 mg acylated anthocyanins were dissolved in 75 mL citrate phosphate buffer. The mixed solution was divided into test tube with stopper and were subjected to heating in water bath at 50 ^{o}C, 60 ^{o}C, 70 ^{o}C, 80 ^{o}C and 90 ^{o}C, respectively. And then, the mixture was read immediately at 520 nm in UV spectrophotometer after cooled to room temperature, in which the same solvent was used as blank. The anthocyanin contents were tested per hour based on the percentage of the absorbance before heating until heated for 5 hours.

2.8.3 Light stability

75 mg acylated anthocyanins were mixed with 75 mL citrate phosphate buffer and divided into two parts. One part was put into an amber colored bottle whereas the other one was put into a transparent bottle and exposed continuously to ultraviolet light (UV intensity ≥ 90 uw/cm^2). Changes in color intensity were determined by UV-visible spectrophotometer by measuring the Abs at 520 nm after 0, 1, 2, 3, 4 and 5 h of each treatment.

2.8.4 Theoretical section

The degradation kinetics of the acylation of anthocyanins from black rice is basing on Eq. (2).

$$\frac{C}{C_0} = e^{-kt} \qquad (2)$$

where, C is the anthocyanin content at a specific time, C_0 is the initial value of anthocyanin content, t is time (in hours), and k is the rate constant (per unit time).

The k values could be obtained through the slope of the straight line basing on Eq. (2).

The relationship between k and its halftime ($t_{1/2}$) is used by Eq. (3).

$$t_{1/2} = \ln 2 / k \quad (3)$$

The temperature dependence on the rate constant is typically represented through the Arrhenius equation:

The Arrhenius model was used to describe the dependence of degradation rate constants on temperature:

$$\ln k = \ln k_0 - E_0 / RT \quad (4)$$

where, E_0 is the activation energy (kJ/mol), R is the universal gas constant, k_0 is the initial value of the rate constant for degradation (1/min), and T is the absolute temperature (K). The E_0 can be calculated from the slope of ln(k) vs. 1/T plot.

2.9 Statistical analysis

All the experiments were performed in triplicates with duplicate samples. Data was expressed in terms of average values and evaluated by one-way analysis of variance (ANOVA) using SPSS 22.0 (SPSS Inc., USA) and Origin 8.5 (Origin Institute Inc, USA). And p values of less than 0.05 were considered as statistically significant.

3. Results And Discussion

3.1 Acylation of anthocyanin extracts

The UV spectra of acylated anthocyanins are represented in Fig. 1**(b)**. The absorption maxima located at 269 nm for genuine anthocyanin extracts belonged to the characteristic absorbance of benzene. However, the acylation leaded to a small bathochromic shift changing the absorption maxima (λ_{max}) from 269 nm to 282 nm. λ_{max} = 278 nm to 282 nm were corresponded to the characteristic absorption for the ester group resulted by the acylation (Liu et al., 2020).

The FT-IR spectra of acylated anthocyanins are represented in Fig. 1**(c)**. As shown in this Figure, the band at 1214 cm^{-1} was attributed to the stretching vibrations of C – O–C for glucosidic bond in anthocyanins and acylated anthocyanins, which implied that anthocyanin kept its structure stability during acylation reaction (Zhao et al., 2015). The broad bands appear around 3219 cm^{-1} was the overlapped intramolecular hydrogen bonding in anthocyanins from black rice. After the acylation, the – OH vibration became sharp and dispersed. Moreover, the absorption bands of C = O – appeared at 1726 cm^{-1}, which confirmed the success of acylation reaction (Fei et al., 2011).

From those UV and FT-IR results, we could conclude that the acylated anthocyanins has been fabricated successfully.

Figure 1

3.2 Thermal resistant property

Figure 2**(a)** and Fig. 2**(b)** demonstrate the TGA and DTG curves for acylated anthocyanins, respectively. As can be seen in Fig. 2**(b)**, the decomposition for anthocyanins can be divided into three stages. The first decomposition stage appeared up to 200 ^{o}C was corresponded to the loss of adsorbed and bounded water. The second (175–240 ^{o}C) and third stages (243–385 ^{o}C) were belonged to the decomposition of anthocyanins main chain (Ben et al., 2020). However, acylated products exhibited different heat decomposition behavior. The decomposition rate at 140–200 ^{o}C increased and a new degradation appeared around 250–280 ^{o}C with the raising of caffeic acid, which was corresponded to the degradation of caffeic acid (Damasceno et al. 2019). Interestingly, the degradation of anthocyanins for AN/CA 2:1 and AN/CA 1:1 shifted to higher region compared with genuine anthocyanins, which demonstrated higher thermal stability. However, the thermal resistant tended to decrease for AN/CA 1:2, which might be attributed to the decomposition of excess caffeic acid. Furthermore, as can be seen from Fig. 2**(a)**, the char yield of all acylated anthocyanins at 400 ^{o}C was higher than that of genuine anthocyanins, which revealed better flame retardant for acylated anthocyanins (Wang et al., 2021). This result might be owing to the acylated structure raising the steric-hindrance, which hindered the decomposition of carbon chain (Alberts et al., 2012). This phenomenon implied that the acylation reaction improved the thermal decomposition stability of anthocyanins when the mass ratio of anthocyanins to caffeic acid lied in 2:1 and 1:1.

3.3 Antioxidant capacity

The antioxidant capacity (DPPH, ABTS and FRAP) of acylated anthocyanins is shown in Fig. 2**(c)**. As can be observed in Fig. 2**(c)**, the DPPH radical scavenging assay of acylated anthocyanins was higher than that of genuine anthocyanins. Nevertheless, the ABTS radical scavenging assay of acylated anthocyanins was slightly inferior to that of genuine anthocyanins. This might be attributed that the liposolubility of anthocyanins was increased by the acylation reaction, which improved the antioxidant capacity of the sample in organic solvents (Cai et al., 2020). Meanwhile, the DPPH radical was stable in organic solvents, but the ABTS radical was soluble in water (Wang et al., 2015). In addition, caffeic acid as acyl donor possessed excellent antioxidant activity, which made it more active than that of other acylated anthocyanins (Damasceno et al., 2019). Noticeably, the total antioxidant capacity obtained by FRAP method for acylated anthocyanins was roughly twice as that of genuine anthocyanins, which proved that the total antioxidant capacity for anthocyanins has been improved by acylation. In general, acylation reaction increased the antioxidant activity, especially the lipid-soluble antioxidant capacity.

Figure 2

3.4 Stability studies

3.4.1 pH stability

The effect of pH on the stability of acylated anthocyanins from black rice has been tested at varied pH from 3.0 to 7.0. Figure 3 illustrates the degradation of acylated anthocyanin treated at different pH values, and the kinetic parameter is listed in Table 1. As represented in Table 1, higher k value and lower $t_{1/2}$ value of anthocyanins and its acylated compounds were observed when the extracts were subjected to higher processing pH condition, which confirmed lower stability at higher pH value and were in close agreement with other literatures (Lu et al., 2011). Compared with anthocyanins, all acylated products exhibited higher pH stability. However, the accessed caffeic acid might be react with anthocyanins forming furfural compound, which accelerated the degradation of anthocyanins (Krifi et al., 2000). This condition resulted that the degradation rate for acylated anthocyanins

decreased with the caffeic acid content increasing, which still higher than that of non-acylated anthocyanins. Thus, the pH stability of anthocyanins has been enhanced by acylation, and AN/CA 2:1 was most effective in stabilizing anthocyanins against pH.

Table 1
Degradation kinetics data of acylated anthocyanins at different pH values

pH value	$k \times 10^{-2}$ (h^{-1})				$t_{1/2}$ (h)			
	A[a]	B	C	D	A	B	C	D
3	2.04 ± 0.37	1.50 ± 0.28	1.88 ± 0.37	1.93 ± 0.30	34.00 ± 2.48	46.10 ± 0.81	36.67 ± 0.77	35.84 ± 2.42
4	2.65 ± 0.45	1.88 ± 0.40	2.10 ± 0.69	2.24 ± 0.03	26.15 ± 0.45	36.97 ± 0.99	32.96 ± 0.32	31.00 ± 0.32
5	3.33 ± 0.34	1.91 ± 0.25	2.30 ± 0.37	2.42 ± 0.07	20.84 ± 1.98	36.23 ± 1.23	30.18 ± 2.56	28.66 ± 0.66
6	3.64 ± 0.54	2.20 ± 0.11	2.41 ± 0.37	2.56 ± 0.67	19.08 ± 2.54	31.59 ± 1.20	28.77 ± 3.01	27.09 ± 2.26
7	4.48 ± 0.50	3.32 ± 0.84	3.81 ± 0.52	4.40 ± 0.83	15.49 ± 1.61	23.39 ± 3.84	18.20 ± 2.53	15.77 ± 1.68

[a] Sample Code (A: AN/CA 1:0; B: AN/CA 2:1; C: AN/CA 1:1; D: AN/CA 1:2)

Figure 3

Table 1

3.4.2 Thermal stability

In order to revealed the influence of temperature on the stability of acylated anthocyanins, 50 ^{o}C, 60 ^{o}C, 70 ^{o}C, 80 ^{o}C and 90 ^{o}C were carried out, respectively. The variation of $\ln(C/C_0)$ as a function of heating time is shown in Fig. 4, and the kinetics data is listed in Table 2. As expected, k value decreased and $t_{1/2}$ increased gradually as the heating temperature increasing, which illustrated that heating could accelerate the degradation of anthocyanins (Baublis et al., 2010). Moreover, anthocyanins' stability has been enhanced after acylated through intramolecular, intermolecular copigmentation, and self-association reactions (Giusti et al., 2003). Acyl donor was arranged on one side of the pyrylium ring, which can be protected against the nucleophillic attack of water and therefore only a weak intermolecular effect might occur (Shi et al., 2010). Interestingly, k values and E_o values were raised with the increasing of acyl donor content, which implied that the more caffeic acid content the greater energy barrier to overcome duringdegradation. This phenomenon might be explained that the excessed caffeic acid reduced the pH values of the extracts, which was in accordance with the pH stability results above.

Table 2
Thermal degradation kinetics data of acylated anthocyanins

T (°C)	$k\times10^{-2}$ (h^{-1})				$t_{1/2}$ (h)				E_0 (kJ/mol)			
	A[a]	B	C	D	A	B	C	D	A	B	C	D
50	2.47 ± 0.18	1.61 ± 0.43	1.36 ± 0.61	1.12 ± 0.41	28.04 ± 2.12	43.02 ± 0.25	50.93 ± 0.72	61.95 ± 8.26	23.69 ± 1.22	25.52 ± 1.31	34.47 ± 1.53	39.27 ± 1.82
60	2.91 ± 0.41	2.65 ± 0.35	1.92 ± 0.76	1.46 ± 0.28	23.80 ± 3.16	26.18 ± 3.65	36.16 ± 1.17	47.56 ± 0.75				
70	4.39 ± 0.73	3.37 ± 0.57	2.18 ± 0.89	1.50 ± 0.32	15.80 ± 2.64	20.58 ± 3.77	31.74 ± 1.94	46.14 ± 2.26				
80	5.47 ± 0.92	4.29 ± 0.74	3.83 ± 0.29	3.43 ± 0.46	12.67 ± 2.10	16.16 ± 3.10	18.11 ± 1.40	20.23 ± 3.91				
90	7.51 ± 0.76	5.87 ± 0.15	5.69 ± 0.98	5.58 ± 0.18	9.23 ± 1.44	11.81 ± 0.31	12.17 ± 2.25	12.43 ± 1.75				

[a] Sample Code (A: AN/CA 1:0; B: AN/CA 2:1; C: AN/CA 1:1; D: AN/CA 1:2)

Figure 4

Table 2

3.4.3 Light stability

The influence of light on the stability of acylated anthocyanins was investigated by exposing the extracts under UV light at room temperature. Figure 5 and Table 3 represent the results for stability of acylated anthocyanins towards light. As expected, the decomposition rate of natural non-acyl anthocyanin extracts was greater than that of acylated derivatives with the exposure in UV light. This improvement in light-resistivity of acylated anthocyanins was not only due to the structural rearrangement after acylation but also attributed to the stable acryl ring in caffeic acid absorb light energy (Yan et al., 2016; Oliveira et al., 2020). Moreover, the light stability of acylated anthocyanins increased with the increasing of caffeic acid content. This phenomenon might be attributed to the extra caffeic acid provided an acid condition, which improved the stability of anthocyanins.

Table 3
Degradation kinetics data of acylated anthocyanins under UV light

Samples	$k \times 10^{-2}$ (h^{-1})	$t_{1/2}$ (h)
AN/AC 1:0	6.42 ± 0.44	10.43 ± 0.06
AN/AC 2:1	4.73 ± 0.33	14.65 ± 0.07
AN/AC 1:1	3.11 ± 0.24	22.28 ± 0.07
AN/AC 1:2	2.90 ± 0.43	23.90 ± 0.15

Figure 5

Table 3

4. Conclusion

The present study investigated the effect of acylation reaction on the antioxidant capacity and degradation kinetics for anthocyanins from black rice. The results indicated that acylation reaction increased the solubility of anthocyanins in organic solvent, which improved the ability of scavenging lipo-soluble DPPH radical for acylated anthocyanins but decreased the water-soluble ABTS radical scavenging activity. Nevertheless, the total antioxidant capacity of acylated anthocyanins was the double than that of genuine anthocyanins. Moreover, the degradation kinetics analysis revealed that the stability at relevant pH value for AN/CA 2:1 was prominent. However, the thermal and light stabilities of anthocyanins were increased with the increasing content of caffeic acid, which might be ascribed that the extra caffeic acid provided an acid condition to hinder the degradation of anthocyanins.

Declarations

Author's contribution Hui Wang and Lina Sun wrote the original draft and processed data. Yanhui Li Yue Kong and Zimeng Kang synthesized and characterized the raw material. Zenan Wu, Wenbiao Lv and Ruiwei Xie prepared figure 2~5. Fengying Xie provided supervision, administration and funding support. All authors reviewed the manuscript.

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Data Availability The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Code Availability Some or all data, models, or code generated or used during the study are available from the corresponding author by request.

Conflict of Interest The authors declare no competing interests.

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Figures

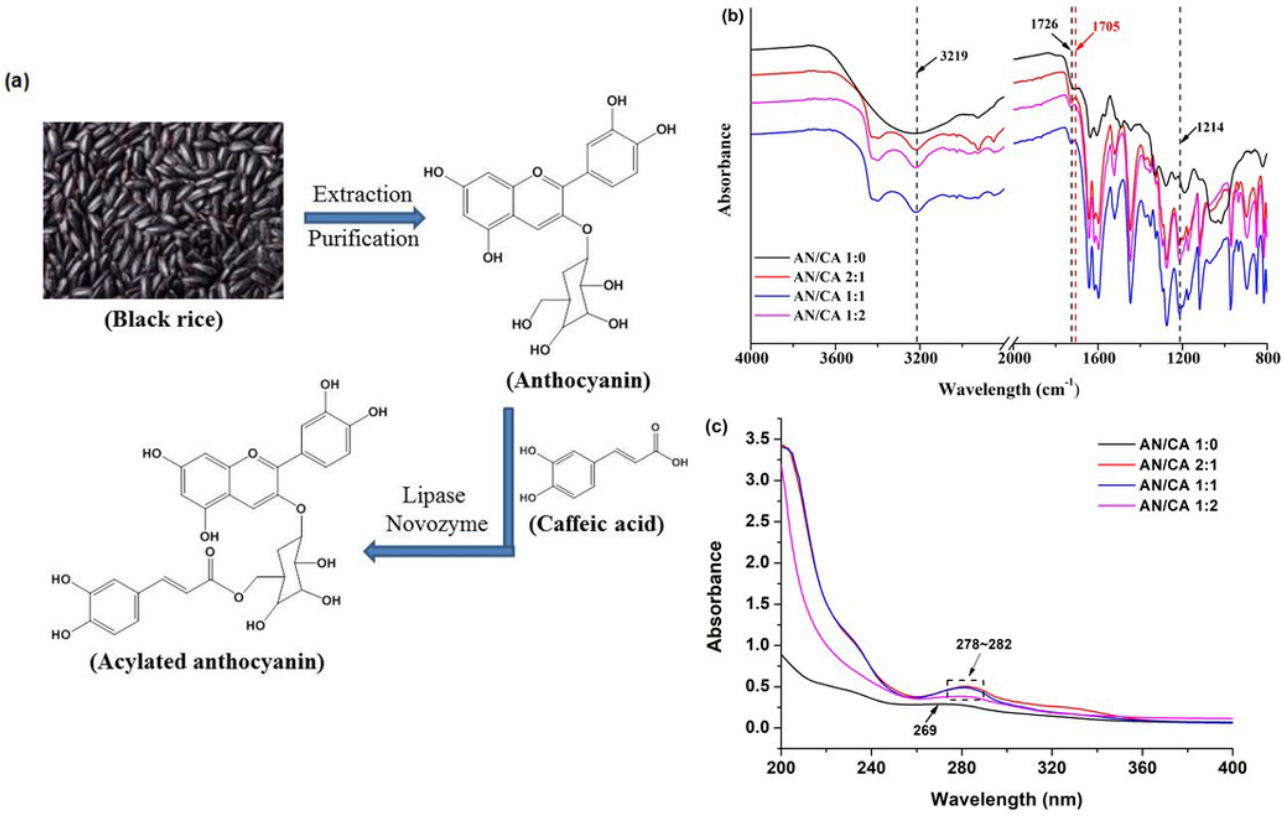

Figure 1

(a) Synthesis routine of acylated anthocyanins from black rice, (b) UV (A) spectrum of acylated anthocyanins and, (c) FT - IR spectrum of acylated anthocyanins.

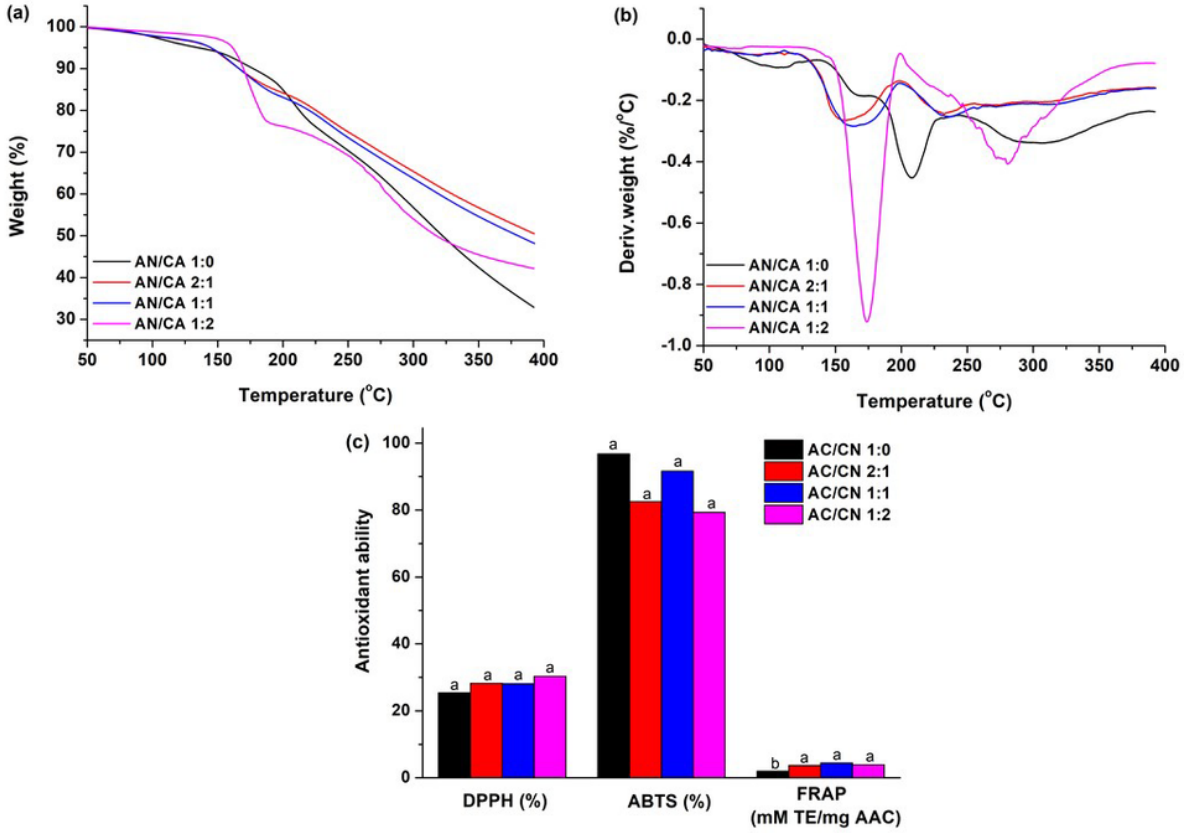

Figure 2

(a) TGA curves of acylated anthocyanins, (b) DTG curves of acylated anthocyanins, (c) Comparison of antioxidative activity (DPPH, ABTS and FRAP) for acylated anthocyanins.

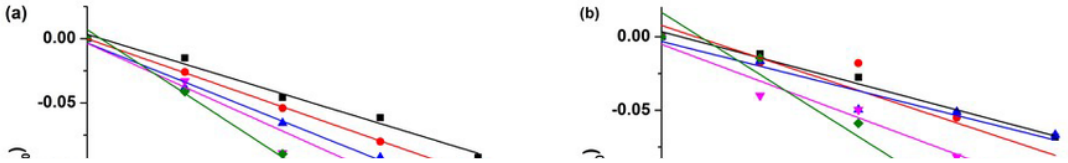

Figure 3

Degradation of acylated anthocyanin treated at different pH values (■ pH 3, ● pH 4, ▲ pH 5, ▼ pH 6, ◆ pH 7): (a) AN/CA 1:0, (b) AN/CA 2:1, (c) AN/CA 1:1, (d) AN/CA 1:2

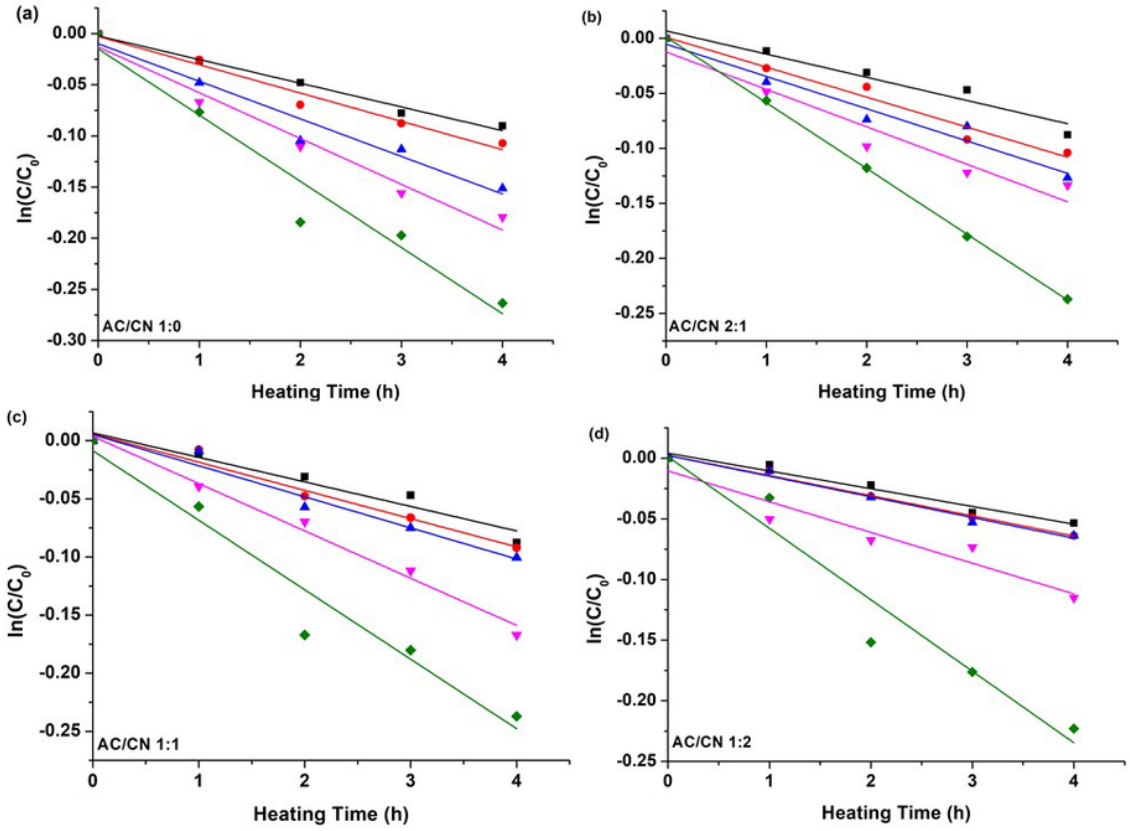

Figure 4

Degradation of acylated anthocyanin treated at different temperatures (■ 50 °C, ● 60 °C, ▲ 70 °C, ▼ 80 °C, ◻ 90 °C): (a) AN/CA 1:0, (b) AN/CA 2:1, (c) AN/CA 1:1, (d) AN/CA 1:2

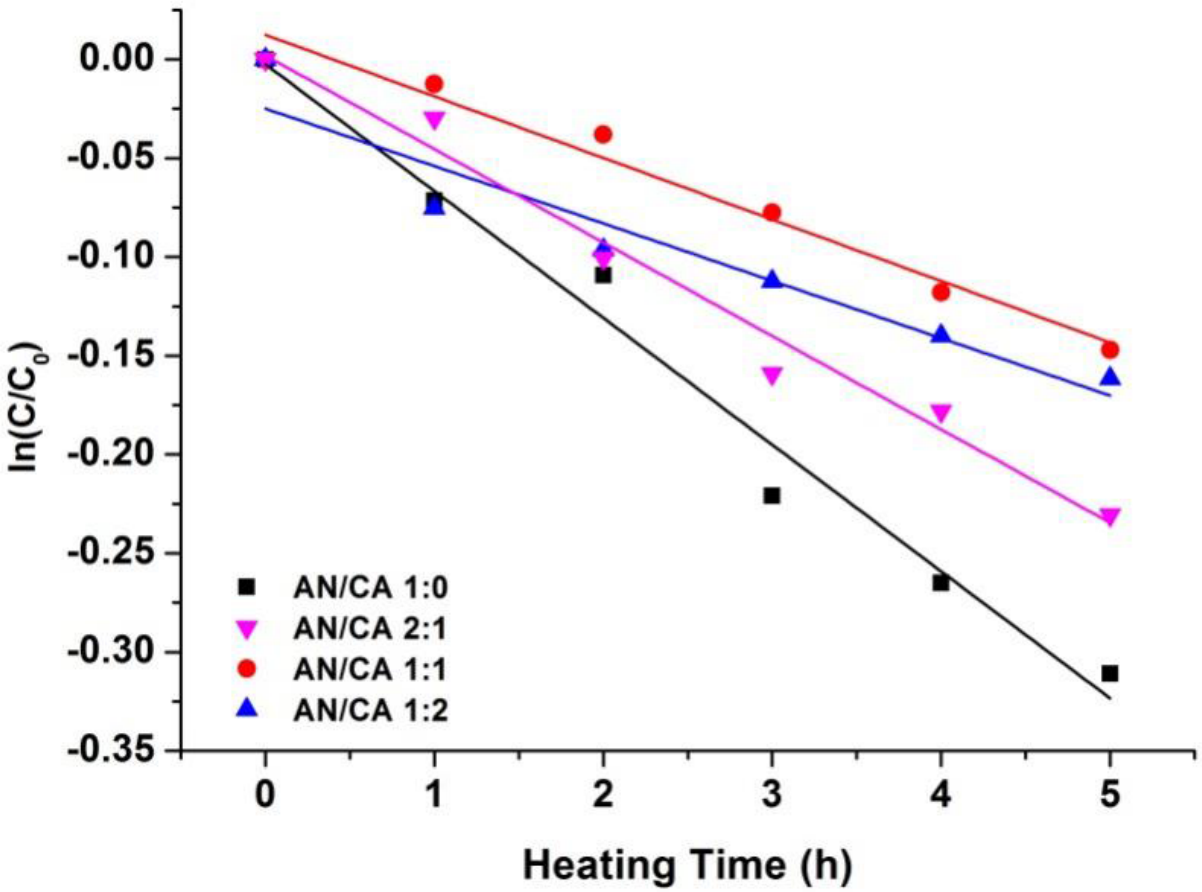

Figure 5

Degradation of acylated anthocyanin under UV light