Acylation and Stability Analysis of Blueberry Anthocyanins

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Keywords: Blueberry anthocyanins, Aliphatic carbonyl acids, Acylation, Stability.

Abstract. The instability of blueberry anthocyanins was a big obstacle for its usage. In order to improve the stability of anthocyanins, molecular modification was used. In this paper, the molecular modification of blueberry anthocyanins was acylated by using aliphatic carbonyl acids. The effects of the acylated agents with different length of carbon chain and different number of carbonyl group on stability of blueberry anthocyanins were investigated. The UV-Vis absorption spectrum showed that the blueberry anthocyanins had been acylated. The stabilities (light and heat) were significantly increased being compared with unacylated anthocyanins. Results showed that the acylation method could increase the stability of original anthocyanin extracts.

Introduction

Bluebery (or bilberry), also known as Vaccinium vitisidaea L., is rich in anthocyanidins, and blueberry anthocyanins own a variety of biological activities, such as antioxidant activity, anticancer activity, protection cardiovascular, improving vision, and so on [1, 2]. However, blueberry anthocyanin as a natural pigment exists some defects, especially its poor stability, which is affected by pH, oxidation, light, temperature, etc., and is a big obstacle for its application. In order to improve the stability of anthocyanins, molecular modification was used. Some studies reported that the acylation of natural pigment has high stability. For example, Fossen et al. found that petanin with one aromatic acyl group from blue potatoes (Solanum tuberosum) showed both higher colour intensity and higher stability than cyanidin 3-O-β-D-glucopyranoside from natural rice (Oriza sativa) at high pH [3]. Sadilova et al. demonstrated that the acylated anthocyanins of black carrot were more stable, and exhibited higher half-life value than the nonacylated anthocyanins at high temperature [4]. Yawadio and Morita proved that the color of black rice anthocyanin-rich fraction enhanced by adding carboxylic acids into the anthocyanin-glucosides medium [5]. Zhao et al. acylated directly cyanidin-3-glucoside with lauric acid, and found that the stability of acylated cyanidin-3-glucoside was obviously higher than that of the unacylated cyanidin-3-glucoside [6]. At present, the acylation of anthocyanins is mainly from the separation of natural products, and the use of chemical methods to synthesize acylated anthocyanins is rarely reported. In this article, by the method of chemical synthesis to introduce the aliphatic carbonyl acids to blueberry anthocyanin was carried out, and the effects of the acylated agents with different length of carbon chain and different number of carbonyl group on stability of blueberry anthocyanins were studied. The results would help further understand the relationship between the stability of anthocyanin and structure, and promote the development and application of blueberry anthocyanins.

Materials and Methods

Materials and Reagents

Fresh blueberries (Vaccinium ashei) were harvested from blueberry planting base in Majiang County, Guizhou Province, China, in August 2016. Blueberry anthocyanins were obtained according to a
method used by our group, and all chemicals used were of the highest grades available. Deionized water was obtained from a Milli-Q Element water purification system (Milli-pore Co., Billerica, MA, USA).

**Chemical Acylation Procedure**

Blueberry anthocyanin was dissolved in 30% methanol aqueous solution, and the solution was adjusted to pH 6 by dilute NaOH aqueous solution. Then, carboxylic acid (acetic acid, oxalic acid, propionic acid, or succinic acid) was added into the solution. The reaction was protected by nitrogen and continuously stirred from 1 h to 4 h at 60°C. Samples were obtained every 1 h, diluted with methanol, and analyzed by thin-layer chromatography (TLC) to monitor the extent of reaction. When the reaction was completed, it was stopped and cooled to room temperature. The reaction solution was filtrated. The filtrate was concentrated by vacuum and dried by sublimation.

**UV–Vis Analysis**

Absorption spectra before and after acylation of blueberry anthocyanin were recorded using a Lambda 35 UV–Vis spectrophotometer (PerkinElmer, US). The scanning wavelength range was 200 to 700 nm and the scanning time was 3. Blueberry anthocyanin and acylated blueberry anthocyanin were separately dissolved into citric acid–sodium citrate solution (pH 3.0) and filtrated by organic membrane.

**Stability Analysis**

Dual-wavelength pH differential method was used to figure out the retention rate, using UV detector to detect. Take 1mL diluted sample, add 9 mL buffer solution which pH is 1.0 and 4.5 respectively, and then detect the absorbance in 510 and 700 nm after a water bath for 40 min. The retention rate of blueberry anthocyanin was calculated by the following formula:

\[
\text{Retention rate} \ (\%) = \frac{A_2}{A_1} \times 100
\]

**Statistical Analysis**

Experiments were performed at repeating three times, and the results were expressed as means standard deviation. Statistical analysis was performed using SPSS 16.0. Differences between groups were considered significant at P<0.05.

**Results and Discussion**

**UV–Vis Analysis**

![UV–Vis spectrum of blueberry anthocyanin and acylated blueberry anthocyanin.](image)

Fig. 1 showed the UV–Vis spectrograms of blueberry anthocyanin and acylated blueberry anthocyanin. As shown in Fig.1, compared with the control group (blueberry anthocyanin), the
stronger absorption peak at about 330 nm corresponded to the absorption of acylation in the structure of acylated blueberry anthocyanin. According to the literature, the blueberry anthocyanin was acylated by carboxylic acids \[8\].

**Stability Analysis**

![Graph](image1)

A: temperature at 100°C; B: temperature at 120°C.

1: blueberry anthocyanin; 2: acetic acid acylated blueberry anthocyanin; 3: oxalic acid acylated blueberry anthocyanin; 4: propionic acid acylated blueberry anthocyanin; 5: succinic acid acylated blueberry anthocyanin

Figure 2. The effect of different temperatures on stability of blueberry anthocyanin and acylated blueberry anthocyanin.

Fig. 2 showed the retention rates of blueberry anthocyanin and acylated blueberry anthocyanin with increasing temperature and extension of heating time. All of the acylated blueberry anthocyanins (2, 3, 4, and 5) were found to possess higher heat resistance than the blueberry anthocyanin (1). When the heating time was longer than 4 h, the retention rate of acylated blueberry anthocyanin was obviously higher than that of blueberry anthocyanin. With increasing temperature, the effect of heat resistance became more obvious. When heated for 10 h at 100 and 120 °C, the retention rate of blueberry anthocyanin were 22.27 and 15.60%, respectively, and the acylated blueberry anthocyanins could be 54.04 and 47.00%, respectively. Blueberry anthocyanin has free hydroxyl groups in the cyclo-chromene and in the 6’glucoside. Thus, the hydroxyl groups make blueberry anthocyanin unstable. Acylation of the ester can improve blueberry anthocyanin’s stability. The ester group was more stable than hydroxide radicals, which is in agreement with the results reported by Sadilova \[4\].

![Graph](image2)

A: indoor natural light; B: indoor avoiding light

1-5 are the same as Figure 2

Figure 3. The effect of different lighting on stability of blueberry anthocyanin and acylated blueberry anthocyanin.

Fig. 3 showed the stability of blueberry anthocyanin and acylated blueberry anthocyanin on different lights with extension of lighting time. Compared with the control substance placed in indoor avoiding light place (Fig. 3B), blueberry anthocyanin and acylated blueberry anthocyanin were
exposed to indoor sunlight for a period of time (Fig. 3A), and the retention rate decreased rapidly. This indicated that blueberry anthocyanin had poor stability to light and need to be protected from light. As shown in Fig. 3A, with prolonged lighting time, the stability of blueberry anthocyanin and acylated blueberry anthocyanin decreased. The stability of acylated blueberry anthocyanin was higher than that of blueberry anthocyanin. When lighting was extended for 10 d, the retention rate of acylated blueberry anthocyanins remained higher than 65%, whereas, that of blueberry anthocyanin was 60%.

As can be seen from Fig. 2 and Fig. 3, the stability of acylated blueberry anthocyanins (2, 3, 4, and 5) on temperature and light increased with the increase of carbon chain of aliphatic carbonyl acid. When the number of carbon was the same, binary carboxylic acids were more stable on temperature and light than monocarboxylic acids (3 vs 2). 3 and 5 were both binary carboxylic acids, but 5 was more stable on temperature and light than 3.

Summary
The acylated blueberry anthocyanin had higher stability than blueberry anthocyanin at different temperatures and lighting times. The stability of acylated blueberry anthocyanin on temperature and light increased with the increase of carbon chain of aliphatic carbonyl acid. When the number of carbon was the same, binary carboxylic acids were more stable on temperature and light than monocarboxylic acids. Acylated blueberry anthocyanins with high stability can expand their application in food, nutraceutical, and pharmaceutical industry.

Acknowledgement
This work was financially supported by the Youth Fund Project of Guizhou Academy of Sciences, Guizhou province, China (J [2016]20), Science and Technology Project of Guizhou Province, China (JZ [2015]2006, Z [2015]6013, [2016]1139), and National Natural Science Foundation (81560603).

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