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Effects of low power ultrasonic treatment on the transformation of cyanidin-3-*O*-glucoside to methylpyranocyanidin-3-*O*-glucoside and its stability evaluation

Jianxia Sun[a,1], Haixia Luo[a,1], Xinghua Li[b], Xusheng Li[c], Yeyu Lu[a], Weibin Bai[c,*]

[a] Faculty of Chemical Engineering and Light Industry, Guangdong University of Technology, Guangzhou 510006, PR China
[b] Guangzhou Kingmed Diagnostics, Guangzhou 510006, PR China
[c] Department of Food Science and Technology, Jinan University, Guangzhou 510632, PR China

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ABSTRACT

Pyranoanthocyanins are anthocyanin derivatives that are formed during fermentation and oxygenation processes in aged wine. They play a critical role in assessing the aging of wine based on its colour quality. Naturally, the formation process of pyranoanthocyanins is fairly slow. In this respect, the present work studies the role and efficiency of ultrasonic treatment in accelerating the formation of methylpyranocyanidin-3-*O*-glucoside and investigates the spectral characteristics as well as the stability of this derivative toward heat, bisulfite bleaching and pH variation in a simulation system. Our results showed that the yield of methylpyranocyanidin-3-*O*-glucoside can be increased by 32.5% by ultrasonic treatment at 100 W for 40 min, and excessive treatment (≥60 min) does not have a positive effect on the yield. Moreover, this derivative exhibits a better stability toward heat, pH variation and bisulfite bleaching than its precursor cyanidin-3-*O*-glucoside.

1. Introduction

The colour of red wine is attributed to the presence of a significant group of natural phenolic pigments called anthocyanins. Among these pigments identified thus far, the pyranoanthocyanins are widely recognized as one of the most important classes of anthocyanin derivatives. Pyranoanthocyanin (Fig. 1a) is a type of pyranoflavonoids, and they form during fermentation and oxygenation processes in aged wine (Atanasova, Fulcrand, Cheynier, & Moutounet, 2002). Their basic skeleton possesses an additional dihydropyran ring (formed between the OH group at C-5 and C-4 of the anthocyanin) that differs from the original anthocyanin (de Freitas & Mateus, 2011; Rentzsch, Schwarz, & Winterhalter, 2007; Jiang et al., 2018). Pyranoanthocyanins consist of a series of natural pigments accomplished by various cycloaddition reactions between berry anthocyanins and intermediates generated from glucose metabolized during fermentation and aging, such as pyruvate (or pyruvic acid) (Morata, González, & Suárezlepe, 2007), acetoacetic acid, and acetaldehyde (Bakker & Timberlake, 1997; Fulcrand, Benabdeljalil, Rigaud, Cheynier, & Moutounet, 1998). Depending on their different formation pathways from natural sources, the pyranoanthocyanins display varying chemical structures. Their chemical transformations have also been detected in red wine during the aging process, in which the original pigments are replaced by other derivatives (Mateus & de Freitas, 2001; Mateus, de Pascual-Teresa, Rivas-Gonzalo, Santos-Buelga, & de Freitas, 2002). In recent years, a large number of pyranomalvidins from red wine have been identified (Fig. 1a) (Lu & Foo, 2001; Oliveira et al., 2011) [Fulcrand, 1998 #9]. However, studies on pyranocyanidins, a type of pyranoanthocyanins derived from cyanidins, are rare.

Lu and others reported the first isolation and identification of a unique class of methylated pyranocyanins from blackcurrants (*Ribes nigrum*) (Lu, Sun, & Foo, 2000). These new blackcurrant pigments were formed via an oxidative cycloaddition on blackcurrant anthocyanins with acetone, which was used as the extraction solvent. Therefore, the methylated pyranocyanins have been treated as an isolation artefact, and interestingly, the methylpyranoanthocyanins from port wine that underwent a similar process between malvidin-3-*O*-glucoside and acetoacetic acid were isolated later on (Rentzsch, et al., 2007). However, this transformation is fairly slow in natural aging. Since the specific colour of aged wine has always been regarded as one of its main sensorial attributes and is normally one of the first perceived by the consumer, developing methods to accelerate the formation of

* Corresponding author at: Department of Food Science and Technology, Jinan University, 601 Huangpu Road, Guangzhou 510632, PR China.
E-mail address: baiweibin@163.com (W. Bai).
[1] Authors Jianxia Sun and Haixia Luo have contributed equally.

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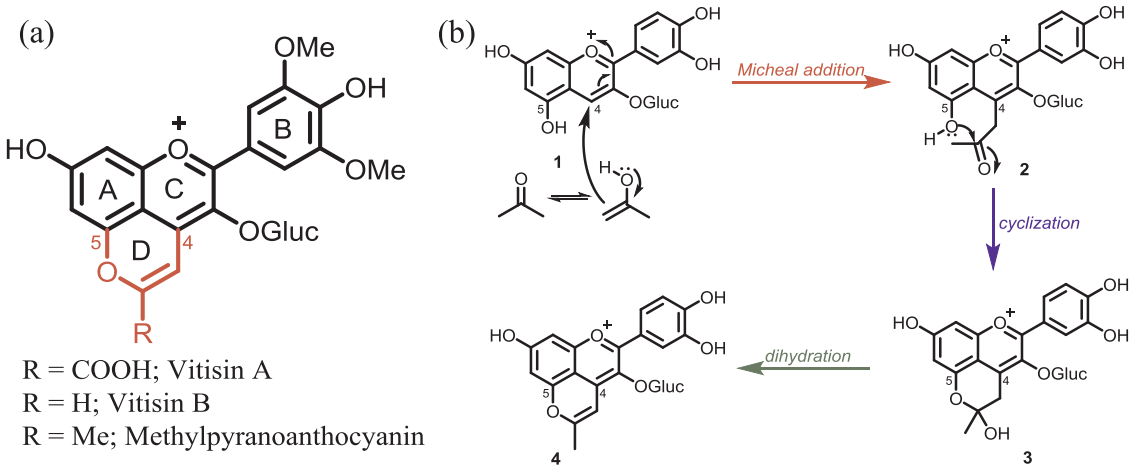

Fig. 1. Representative structures of pyranoanthocyanins (a) and a possible pathway for the formation of Me-Pycy-3-G (b).

pyranoanthocyanins and promote the colour maturity of wine is increasingly interesting for both academia and industry.

Ultrasonic treatment, a non-thermal processing technology, is capable of shortening the time-consuming aging process and improving wine quality as well as reducing the production costs in wine aging (Chang, 2005; Leonhardt & Morabito, 2007). When ultrasonic waves pass through a liquid, small bubbles form and collapse. This process is termed cavitation (Saterlay & Compton, 2000) which creates localized areas of high temperature and pressure, and it results in the formation of hydroxyl radicals (Hemwimol, Pavasant, & Shotipruk, 2006; Rokhina, Lens, & Virkutyte, 2009). These areas of localized high energy and hydroxyl radicals can then accelerate some chemical transformations, especially cycloaddition and oxidation reactions (Hemwimol, et al., 2006). Therefore, ultrasonic treatment is likely to accelerate the oxidative cycloadditions that frequently occur during wine aging. By this means the formation of anthocyanin adducts with some important intermediates in the alcoholic fermentation, such as pyruvate, acetone, acetaldehyde or hydroxycinnamic acid could be sped up, resulting in better colour quality in aged wines.

In our study, we evaluated the efficiency of ultrasonic treatment on accelerating the formation of methylpyranocyanidin-3-*O*-glucoside (Me-Pycy-3-G) and the stabilities of the derivatives towards heat, pH variation and bisulfite bleaching in the simulation system.

2. Materials and methods

2.1. Material and reagents

Cyanidin-3-*O*-glucoside (Cy-3-G) (≥98%) was from the Chengdu Institute of Biology, Chinese Academy of Sciences. Acetone (HPLC grade) was purchased from Aladdin (Shanghai, China). Water was of Milli-Q quality. Polyamide resin column chromatography (60–100 mesh), acetonitrile (HPLC grade), and formic acid (HPLC grade) were purchased from Aladdin (Shanghai, China). Absolute ethanol was purchased from the Tianjin Damao Chemical Industry (Tianjin, China).

2.2. Reaction under ultrasonic irradiation

Five milligrams of cyanidin-3-*O*-glucoside and 5 mL of acetone were incubated in 10 mL of HCO_2H–H_2O–MeOH (2:88:10, v/v) in a screw-cap vial. Then, the reaction solution was equally divided into four tapered tubes. The four samples were irradiated under an ultrasonic JY92-IIDN cell grinder (Ultrasonic Instrument, Ningbo, China) (100 W) for 10, 20, 40, and 60 min, respectively, and then stored in sealed glass containers to prevent evaporation. The disappearance of Cy-3-G and the formation of Me-Pycy-3-G after different treatments were monitored by HPLC-DAD chromatograms and UV–vis spectroscopy during the 9 week storage period in the dark and at room temperature. Spectral absorbance curves were recorded for all solutions from 350 to 700 nm, with a 1 nm sampling interval, using a 10 mm × 10 mm cell in a UV-1800PC MAPADA spectrophotometer (MAPADA Instruments, Shanghai, China).

2.3. Purification and isolation of Me-Pycy-3-G

The purification and isolation of Me-Pycy-3-G were performed according to the method described by previous reports with modification (He, Santos-Buelga, Silva, Mateus, & De Freitas, 2006; Oliveira et al., 2006). The reaction mixture was subjected directly to chromatography with a 300 × 35 mm i.d. polyamide resin column (60–100 mesh) eluted with 20% ethanol in water. The isolated pigments were collected and then purified by semi-preparative HPLC using a 250 × 4.6 mm i.d. reversed-phase C_{18} column (Agilent, US) with an injection volume of 500 μL and the same gradient program. The collected samples were finally concentrated under vacuum.

2.4. HPLC analysis

The Me-Pycy-3-G pigment was applied to a 250 × 4.6 mm i.d. reversed-phase C18 column and then analysed by a Waters-e2695 HPLC (Waters, US) with a photo-diode-array-detector (DAD) at 520 nm and 478 nm. The elution solvents were as follows: A, H_2O/HCOOH (9:1, v/v) and B, H_2O/CH_3CN/HCOOH (1.95:8:0.05, v/v). A linear gradient from 20% to 85% B for 70 min was used with a flow rate of 1.0 mL/min. The column was washed with 100% B for 20 min and then stabilized with the initial conditions for another 20 min.

2.5. UPLC-DAD/ESI-MS analysis

UPLC separation and identification of Me-Pycy-3-G were performed on a Waters Acquity TM Ultra-Performance LC system (Waters, Milford MA, US) equipped with a DAD and an LC/MSD Trap VL electrospray ionization mass spectrometry (ESI-MS/MS) system coupled to a Waters data processing station. The mass spectra data were processed with MassLynx software (version 4.1, Micro mass, Manchester, UK). The sample was injected (7.5 μL) after filtration (0.22 μm, polyester membrane, Hydrophilic PTFE SCAA-114, ANPEL, Shanghai, China) on a reversed-phase Agilent Eclipse Plus C_{18} column (2.1 × 100 mm, 1.8 μm) thermostated at 35 °C. The chromatographic conditions were adapted from the OIV method for the analysis of Me-Pycy-3-G in the reaction solution (Zaffalon et al., 2014), and the detection wavelengths were 520 nm and 478 nm. The solvents were as follows: A, 0.1% formic acid water and B, acetonitrile; the flow rate was 0.5 mL/min. The linear