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Anthocyanins in blackcurrant effectively prevent the formation of advanced glycation end products by trapping methylglyoxal

Xiu-Yu Chen [a], *I-Min Huang* [b], *Lucy Sun Hwang* [b], *Chi-Tang Ho* [b,c], *Shiming Li* [c], *Chih-Yu Lo* [a,*]

[a] *Department of Food Science, National Chiayi University, no. 300 Syuefu Road, Chiayi 600, Taiwan*
[b] *Graduate Institute of Food Science and Technology, National Taiwan University, Taipei 106, Taiwan*
[c] *Department of Food Science, Rutgers University, 65 Dudley Road, New Brunswick, NJ 08901, USA*

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ABSTRACT

Carbonyl stress is the major consequence for advanced glycation end products (AGEs). Polyphenols have been shown to inhibit the formation of AGEs. However, little is known about whether anthocyanins contribute to trapping methylglyoxal (MGO) and reducing carbonyl stress. Blackcurrant (*Ribes nigrum*) extract (BE) is known to have high content of anthocyanins. When an aliquot of 7.5 mg/mL BE sample was incubated with 2.9 mM MGO in pH 7.4 phosphate buffer solution (PBS) for an hour, 36.75% MGO decrease was observed. The BE extract sample was separated and purified by Sephadex LH-20 and reverse phase C18 column chromatography. Delphinidin-3-rutinoside (D3R) and cyanidin-3-rutinoside (C3R) were identified as major pigments in BE sample. Some 71.45 and 78.72% of D3R and C3R, respectively, remained when they were incubated alone in a phosphate buffer solution at 37 °C for 1 h, but only 2.48 and 1.83% of D3R and C3R, respectively, remained when individually incubated with MGO. D3R- and C3R-mono-MGO adducts were characterized by HPLC-MS^n.

1. Introduction

Blackcurrant (*Ribes nigrum*) is a temperate fruit crop widely cultivated for its abundant berries. It is a high value raw food material. In addition to its high content of ascorbic acid, blackcurrant (BCT) is rich in polyphenols such as flavonoids, hydroxycinnamic acids and hydroxybenzoic acids (Kahu, Jänes, Luik, & Klaas, 2008; Szajdek & Borowska, 2008). Anthocyanins are water-soluble phytochemicals with red to blue color. They are the major group of flavonoids in BCT, with reported concentrations of 560–4450 μg/g FW (fresh weight) (Anttonen & Karjalainen, 2006; Bordonaba, Crespo, & Terry, 2011; Bordonaba & Terry, 2008; Khoo, Clausen, Pedersen, & Larsen, 2012; Koponen, Happonen, Mattila, & Törrönen, 2007; Rubinskiene, Jasutiene, Venskutonis, & Viskelis, 2005) and 10,649–16,126 μg/g DW (dry weight) (Bordonaba et al., 2011). The main BCT anthocyanins accounting for 80% of total polyphenols are delphinidin-3-*O*-glucoside, delphinidin-3-*O*-rutinoside, cyanidin-3-*O*-glucoside and cyanidin-3-*O*-rutinoside.

Advanced glycation end products (AGEs) have been recognized as the result of non-enzymatic glycation of protein. Higher concentrations of various reactive carbonyl species (RCS), glyoxal, methylglyoxal (MGO), and 3-deoxyglucosone, are the major source of AGEs (Brownlee, 2001; Khuhawar, Kandhro, & Khand, 2006; Nemet, Varga-Defterdarović, & Turk, 2006). The

* *Corresponding author.* Tel.: +886 5271 7621; fax: +886 5271 7596.
E-mail address: chihyulo@mail.ncyu.edu.tw (C.-Y. Lo).
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adverse role of these AGE precursors is seen in a wide spectrum of pathogenic conditions including microvascular and macrovascular diseases such as nephropathy, retinopathy, peripheral neuropathy, and arteriosclerosis in diabetes mellitus (Ahmed, 2005; Calcutt, Cooper, Kern, & Schmidt, 2009; Kikuchi et al., 2003; Thomas, Baynes, Thorpe, & Cooper, 2005).

The reduction of carbonyl stress and AGEs can be an effective strategy for direct alleviation of diabetic complications. Recent data indicate that some phytochemicals are able to react with dicarbonyls (Hipkiss & Chana, 1998; Ho & Wang, 2013; Hu, Liu, Chen, & Hu, 2013; Hu, Liu, Chyau, & Hu, 2012; Lo et al., 2006; Lv, Shao, Chen, Ho, & Sang, 2011; Lv et al., 2010; Peng et al., 2008; Shao et al., 2008). Several studies indicate that anthocyanins in berries display a wide range of biological activities, including antioxidant, anti-inflammatory, anticarcinogenic, antimicrobial activities (Kivimäki, Siltari, Ehlers, Korpela, & Vapaatalo, 2013; Li et al., 2013; Lyall et al., 2009; Norberto et al., 2013; Prior et al., 1998; Satué-Gracia, Heinonen, & Frankel, 1997; Tabart et al., 2012; Wang & Lin, 2000) and prevent eye fatigue (Nakamura, Matsumoto, & Todoki, 2002). In addition, anthocyanin extracts from berries have been reported to have preventive effect on diabetes, and have the ability to ameliorate hyperglycaemia and insulin sensitivity through AMP-activated protein kinase (AMPK) activation in rats (Sarikaphuti et al., 2013; Takikawa, Inoue, Horio, & Tsuda, 2010). To the best of our knowledge, anthocyanins although existing in many berries, their potential health benefits for the reduction of RCS or trapping dicarbonyls have not been reported.

Therefore, the objective of this work was to evaluate the MGO trapping capacity of BCT anthocyanins under simulated physiological conditions. To conduct this study, the anthocyanins in blackcurrant extract were first analyzed. In order to obtain more precise information on anthocyanin–MGO trapping, the formation of adducts was confirmed by high performance liquid chromatography (HPLC) and electrospray tandem mass spectrometry (MS^n).

2. Materials and methods

2.1. Materials and reagents

Blackcurrant extract (BE, 20% anthocyanin content) was provided by the Mivalied Rohstoffhandel Company (Buchhols, Germany). Methylglyoxal (MGO, 40%), *o*-phenylenediamine (*o*-PDA) and phosphate buffered saline, pH 7.4 were purchased from Sigma (St. Louis, MO, USA). 2-Methylquinoxaline (2-MQ, 97%) and 5-methylquinoxaline (5-MQ, 98%; internal standard) were purchased from Aldrich Chemical Co. (St. Louis, MO, USA). Acetic acid (99.7%), ethanol and sodium acetate trihydrate were purchased from Mallinckrodt Baker (Center Valley, PA, USA). Reverse phase (C18; 25–40 μm) sorbent, potassium chloride (99.5%), HPLC grade acetonitrile and analytical grade methanol were purchased from Merck Chemical Co. (Damstadt, Germany). Lipophilic Sephadex (LH-20) resin and trifluoroacetic acid (98%) were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA).

2.2. Determination of total anthocyanin content

Total anthocyanin content was determined by a spectrophotometric pH differential method (Lee, Durst, & Wrolstad, 2005), modified in our laboratory. To obtain the maximum absorption ($A_{\lambda\ \text{vis-max}}$) at a specific wavelength, the 50 mg BE was dissolved in 10 mL solvent (1.5 M HCl: 95% ethanol = 15:85 [v/v]). The BE solution was scanned within 20–50 min of preparation over 230–800 nm wavelength. Maximum absorption was obtained at 520 nm. Absorbance of blackcurrant sample diluted with pH 1.0 potassium chloride buffer (0.025 M) and pH 4.5 sodium acetate buffer (0.4 M) was determined at both 520 and 700 nm, respectively. The diluted samples were read versus a blank cell filled with distilled water. Anthocyanin content was calculated as cyanidin-3-glucoside equivalents based on

$$\text{Anthocyanin content (mg/mg)} = (A \times MW \times DF \times 1000)/\varepsilon \times l \times c$$

where $A = (A_{520\ nm} - A_{700\ nm})_{pH\ 1} - (A_{520} - A_{700})_{pH\ 4.5}$, MW is the molecular weight of cyanidin-3-glucoside (449.2 g/mol), DF is the dilution factor, ε = 26,900 molar extinction coefficient in $L \times mol^{-1} \times cm^{-1}$ for cyanidin-3-glucoside, l in the path length in centimeters, and *c* is the concentration of the sample (mg/L). Mean value and standard deviation were calculated.

2.3. Blackcurrant extract/MGO trapping test

The potency of MGO scavenging ability by anthocyanidins or anthocyanins was evaluated first according to a previous method with minor modification (Lo et al., 2006). BE (7.5 mg/mL) was dissolved in pH 7.4 phosphate buffer solution (0.01 M). MGO (2.9 mM) was used in the experiment. The *o*-PDA (derivatization reagent, 30 mM) was dissolved in 100 mL, pH 7.4 phosphate buffer solution with an appropriate amount of 5-MQ (internal standard). All solutions were freshly prepared before each experiment. For BE sample, after 1 mL BE was mixed with 1 mL MGO in a sample vial, the MGO trapping reaction was performed in 37 °C, 50 rpm water bath for 1 h. Following the trapping reaction, 1 mL *o*-PDA derivatization reagent was added to BE sample vial and incubated in a 60 °C, 50 rpm water bath for 30 min. In the control sample, MGO derivatization only took place after 1 mL BE was mixed with 1 mL MGO in the sample vial. PBS (2 mL) was added after the derivatization reaction and the samples were diluted for HPLC analysis. The MGO decrease percentage was based on the reduction of 2-MQ between the control and BE samples and was calculated using the following equation:

$$\text{MGO decrease percentage (\%)} = [1 - (\text{Area ratio of (2-MQ/5-MQ) in BE sample})/(\text{Area ratio of (2-MQ/5-MQ) in the control sample})] \times 100\%$$

2.4. Sephadex LH-20 liquid chromatography for blackcurrant extract

After MGO trapping ability was determined based on previous test, BE (20% anthocyanidins/anthocyanins) was subjected to further investigation. In the first liquid chromatography (LC), 0.5 g BE was dissolved in a 50% methanol solution. After filtering with a 0.45 μm filter, the solution was loaded on a LH-