Isolation, purification and identification of etiolation substrate from fresh-cut Chinese water-chestnut (Eleocharis tuberosa)

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Fresh cut Chinese water-chestnut is a popular ready-to-eat fresh-cut fruit in China. However, it is prone to etiolation and the chemicals responsible for this process are not yet known. To address this problem, we extracted phytochemicals from etiolated Chinese water-chestnut and separated them using MPLC and column chromatography. Four compounds were obtained and their structures were determined by interpretation of UV, TLC, HPLC and NMR spectral data and by comparison with reported data. We identified these compounds as eriodictyol, naringenin, sucrose and ethyl-D-glucoside. Among those, eriodictyol and naringenin were both isolated for the first time in fresh-cut Chinese water-chestnut and are responsible for the yellowing of this fruit cutting.

1. Introduction

Because of their convenience as ready-to-eat products and the health benefits derived from their consumption, fresh-cut fruits and vegetables have rapidly gained economic importance (Pradas-Baena, Moreno-Rojas, & Luque de Castro, 2015). The Chinese water-chestnut (CWC, Eleocharis tuberosa) is one of the most popular hydrophytic vegetables in China because of its unique taste (Peng & Jiang, 2003) and it is usually peeled before being eaten. Sometimes, the CWC is also washed, peeled, sliced and packaged before being marketed as a ready-to-eat product. However, most of fruit and vegetables turn brown after being peeled (Sagar & Kumar, 2010), such as apples (Luo, Lu, Zhou, & Peng, 2011), pears (Gomes et al., 2014) and potatoes (Zvitov-Ya’ari & Nussinovitch, 2014). In contrast with other fruit and vegetables, fresh-cut CWC is prone to etiolation, reducing its shelf life and its commercial value (Ma, Wang, Hong, & Cantwell, 2010; Oms-Oliu et al., 2010; You et al., 2012). More that, the safety for human consumption of the etiolated component is also worth considering. Most studies report on the CWC yellowing is due to enzymatic browning and its inhibition (Peng, Yang, Li, Jiang, & Joyce, 2008; You et al., 2012; Zvitov-Ya’ari & Nussinovitch, 2014, Zhou, Li, Wu, Fan, & Ouyang, 2015), however, to date, there are no reports specifically on the chemicals controlling CWC yellowing. In general, the enzymatic browning reaction is mediated by polyphenol oxidases (PPO) and requires oxygen and phenolic compounds and is triggered by the enzymatic oxidation of monophenols into o-diphenols and quinones, which further undergo further non-enzymatic polymerization leading to the formation of pigments (Gao, Zhao, Duan, & Tao, 2014; Zhou et al., 2015). Cutting fruit and vegetables not only makes oxygen available, but also disrupts the subcellular departmentalization of the enzyme and substrates. In the plant tissues, only compounds such as catecholase, chlorogenic acid, dihydroxyphenylalanine and tyrosine act as substrates for PPO (Walker & Ferrar, 1998). Fresh-cut CWC contains tyrosine, gallic acid, chlorogenic acid and 2,4-dihydroxy-cinnamic acid during the early days of storage (Tong, 2005). In addition, fresh-cut CWC contains phenolic compounds, mainly dopa and epicate (Pan & Chen, 2008), but only at low concentration (You et al., 2007). However, neither of them can be detected in the subsequent storage period. PPO and Peroxidase (POD) are also closely associated with browning of fresh-cut fruit and vegetables (He & Luo, 2007) and they are also present in fresh-cut CWC. With the extension of storage time, PPO shows a downward trend while the POD showed a rising trend, even tough the extend of this change is very small (Pan & Chen, 2007). By contrast in potatoes, the activity of POD and PPO is much lower (Pang & Zhang, 2002). Exogenous salicylic acid inhibits the browning of fresh-cut CWC (Peng & Jiang, 2006), but it has no significant effect on the activity of PPO and POD. 4-hexyl benzodiazepines can inhibits the activity of PPO, but cannot prevent effectively CWC yellowing (Tong, 2005). All previous research implies that CWC yellowing is different from enzymatic browning.
Therefore, in order to clarify and control the mechanism of fresh-cut CWC etiolation and to control as well as to evaluate the safety of the etiolation components, we had to identify the chemicals involved in CWC etiolation. In this study we extracted, purified, and identified the chemicals involved in etiolation from flesh of fresh-cut CWC by mean of NMR analysis.

2. Materials and methods

2.1. Plant material

Fresh CWCs were purchased in Haikou, China and stored in our laboratory at 17 °C. Fruits without physical damage or diseases and with uniform size were selected for analysis. CWCs were treated as reporter by You et al. (2012) and Peng and Jiang (2003). 100 kg of fresh CWC were washed, peeled using a sharp stainless steel knife and chopped into small thick slices. Afterward, the slices were disinfected with 0.1% NaClO for 15 min before airing. The CWC were packed with plastic packaging machines and preserved in 17 °C for 3 days until the surface of the fruits became yellow.

2.2. Extraction and isolation of the compounds

The etiolation parts were extracted at room temperature with ethyl alcohol (1:5, m/v) for 2 h. The alcohol extract was concentrated to give a residue (280 g), which was dissolved in H2O and partitioned with ethyl acetate (EtOAc) to yield the EtOAc fraction (320 mg) and water fraction (277 g). The EtOAc extract was subjected to medium pressure liquid chromatography (MPLC), eluting with a solvent system of MeOH/H2O (0:100→25:75, v/v) to give four fractions (named fractions 1–4). After repeated CC on silica gel (CHCl3/MeOH, 6:1) and Sephadex LH-20 (MeOH, 100%), fraction 2 (30 mg, MeOH/H2O = 20:80→25:75, v/v) gave compound 1 (6 mg). Fraction 3 (19 mg, MeOH/H2O = 75:25, v/v) was further isolated and purified using silica gel (CHCl3/MeOH, 10:1→6:1, v/v) and Sephadex LH-20 (MeOH, 100%) columns to yield compound 2 (6 mg). The retention times (tR) of compound 1 and 2 on an analytical high-performance liquid chromatography (HPLC) Extend-C18 column (20%→100% MeOH in H2O over 8.0 min followed by 100% MeOH to 13 min, 1.0 ml/min, 20 °C) were 6.59, and 7.20 min, respectively. The water soluble fraction (277 g) was separated by reversed-phase column chromatography with a solvent system of MeOH/H2O (0:100→100:0, v/v) to yield fractions 5–8. Fraction 5 (230 g, MeOH/H2O = 0:100, v/v) was subjected to RP-18 CC eluted with a linear gradient of H2O to yield two fractions (fraction 5.1 and 5.2). Fraction 5.1 (180 g) and fraction 5.2 (45 g) were purified using Sephadex LH-20 using MeOH as eluents to obtain compound 3 (87 mg) and glucose (identified by thin layer chromatography), respectively. Fraction 6 was further purified over Sephadex LH-20 using MeOH as eluents to ultimately isolate compound 4 (10 mg). Structures of compounds 1–4 were characterized based on extensive spectroscopic analysis and their NMR data.

2.3. Laboratory equipment

NMR analysis was performed using Bruker DRX-500 with deuterated solvent signals as internal standards. Column chromatography (CC) was carried out with silica gel (200–300 mesh, Qingdao Marine Chemical Ltd., Qingdao, PR China), MPLC (5 μm, 21.2 × 150 mm; Buchi Labortecnik, Switzerland) with Chromatorex C-18 column (40–75 μm, Fuji Silysia Chemical Ltd., Japan) and Sephadex LH-20 column (Amersham Biosciences, Sweden). Fractions were analyzed by TLC (Qingdao Marine Chemical Ltd., Qingdao, PR China) and reversed-phase HPLC (Agilent 1200, Agilent Zorbax Extend-C18 column, 5 μm, 4.6 × 150 mm).

3. Results and discussion

Chemical form etiolated CWCs were extracted with absolute ethyl alcohol absolute, and the concentrated extract was partitioned with EtOAc and H2O. From the EtOAc and H2O extracts, four compounds were isolated through repeated silica gel column chromatography and characterized with 1H-NMR experiment. Our protocol led to the isolation of four purified, reported in Fig. 1.

3.1. Eriodictyol (compound 1), C15H12O6

We isolated 1 was a light yellow amorphous powder; UV (MeOH) λmax: 225, 283, 326; 1H-NMR (DMSO-d6, 500 MHz) δ: 12.13 (1H, s), 9.11 (1H, br.s), 9.07 (1H, br.s), 6.86 (1H, s), 6.73 (2H, s), 5.86 (2H, s), 5.35 (1H, dd, J = 12.5, 2.5 Hz), 3.17 (1H, dd, J = 17.0, 12.5 Hz), 2.64 (1H, dd, J = 17.0, 2.5 Hz). Polarity size: CHCl3/MeOH = 6:1, Rf = 0.5. The vanillin sulfuric acid solution TLC staining yielded a red coloration. We identified compound 1 as eriodictyol (Fig. 2A) by comparison of the spectral data available in the literature (Encarnacion et al., 1999). Eriodictyol is present in several fruit and vegetables (Jiménez-Atiénzar, Escribano, Cabanes, Gandía-Herrero, & García-Carmona, 2005), and it is particularly abundant in lemon (Minato et al., 2003). However, it has never been isolated in fresh-cut CWC before. As a flavonoids, eriodictyol has multiple chemical and biological properties, including antioxidant (Rossato et al., 2011), anti-inflammatory (Lee, 2011), antibacterial (Martins et al., 2015) and anticancer (Doostdar, Burke, & Mayer, 2000) activities, and thus it is a good candidate for a variety of applications for the food and pharmaceutical industry (Liu et al., 2013).

3.2. Naringenin (compound 2), C15H10O7

Compound 2 was isolated as a light yellow amorphous powder; UV (MeOH) λmax: 225, 283, 326; 1H-NMR (CD3OD, 500 MHz) δ: 7.29 (2H, d, J = 8.0 Hz), 6.81 (2H, d, J = 8.0 Hz), 5.88 (1H, d, J = 1.5 Hz), 5.87 (1H, d, J = 1.5 Hz), 5.31 (1H, dd, J = 12.5, 2.5 Hz), 3.09 (1H, dd, J = 17.0, 12.5 Hz), 2.67 (1H, dd, J = 17.0, 2.5 Hz). Polarity size: CHCl3/MeOH = 7:1, Rf = 0.5. The vanillin sulfuric acid solution TLC staining yielded a red coloration. We identified compound 2 as naringenin (Fig. 2B) by comparison of the spectral data with the literature (Olsen, Stafford, van Staden, Christensen, & Jäger, 2008). Naringenin is one of the most abundant flavonoids in fruit (Lee et al., 2001; Mulvihill et al., 2009). Like other flavonoids, naringenin has been reported to have as anti-oxidative, anti-atherogenic, and anti-cancer activities (Knowles, Zigrossi, Tauber, Gundas, & Winter, 2009; Hetzel, Hightower, & Milner, 2000; Mulvihill & Huff, 2010; Shi et al., 2010).

3.3. Sucrose (compound 3), C12H22O11

We isolated compound 3 as a white amorphous powder; 1H-NMR (D2O, 500 MHz) δ: 5.34 (1H, d, J = 4.0 Hz), 4.15 (1H, d, J = 9.0 Hz), 3.99 (1H, t, J = 8.5 Hz), 3.82 (1H, m), 3.79 (1H, m), 3.75 (4H, m), 3.69 (1H, t, J = 10 Hz), 3.61 (2H, s), 3.49 (1H, dd, J = 10.0, 4.0 Hz), 3.40 (1H, t, J = 10.0 Hz). Polarity size: EtOAc/MeOH = 1:2, Rf = 0.5. The vanillin sulfuric acid solution TLC staining yielded a black coloration. We identified compound 3 to be sucrose (Fig. 2C) by comparison of the spectra data available on line at the Spectral Database for Organic Compounds (http://sdfsdb.aist.go.jp/sdfs/cgi-bin/direct_frame disp.cgi?sdbsno=1188, accessed 11.03.2015).
3.4. Ethyl glucoside (compound 4), C_{8}H_{16}O_{6}

We isolated compound 4 as a colorless oil; ¹H-NMR (CD_{3}OD, 500 MHz) δ: 4.25 (1H, d, J = 8.0 Hz), 3.95 (1H, q, J = 7.0 Hz), 3.85 (1H, dd, J = 11.5, 5.5 Hz), 3.65 (1H, dd, J = 11.5, 1.5 Hz), 3.60 (1H, q, J = 7.0 Hz), 3.34 (1H, d, J = 8.5 Hz), 3.26 (1H, m), 3.16 (1H, dd, J = 8.5, 8.0 Hz), 1.21 (3H, t, J = 7.0 Hz). Polarity size: CHCl_{3}/MeOH = 2:1, Rf = 0.5. The vanillin sulfuric acid TLC staining yielded a black coloration. We identified this compound as ethyl glucoside (Fig. 2D) by comparison with previously reported spectral data (Teague et al., 2004).

4. Conclusions

In the present study we isolated and identified four compounds, eriodictyol, naringenin, sucrose and ethyl glucoside from the etiolated CWC. In particular, we identified the presence of the flavonoids eriodictyol and naringenin in etiolated fresh-cut CWC for the first time. Moreover, the yellow coloration of eriodictyol and naringenin may be associated with CWC yellowing after cutting. Previous works have reported that the eriodictyol and naringenin have antioxidant activity: hence, this implies that the etiolation of CWC after cutting do not pose treat to human consumption. Therefore, our investigation is a good starting point for providing a better theoretical understanding on how to efficiently inhibit the CWC. Due to the identification of phytochemicals with antioxidant, anti-atherogenic and anti-cancer activities phenols, CWC might have applications in the pharmaceutical or food industry. However, further studies are needed for a deeper understanding of the metabolic pathways of behind these components.

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References


