Food Chemistry 230 (2017) 306–315

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

# Protective property of mulberry digest against oxidative stress – A potential approach to ameliorate dietary acrylamide-induced cytotoxicity

Linxia Zhang [1], Yang Xu [1], Yuting Li, Tao Bao, Vemana Gowd, Wei Chen *

*Department of Food Science and Nutrition, Zhejiang Key Laboratory for Agro-Food Processing, Key Laboratory of Agro-Products Postharvest Handling, Ministry of Agriculture, National & Local Joint Engineering Laboratory of Intelligent Food Technology and Equipment, Zhejiang University, Hangzhou 310058, China*

ARTICLE INFO

*Article history:*
Received 8 October 2016
Received in revised form 8 March 2017
Accepted 8 March 2017
Available online 9 March 2017

*Keywords:*
Mulberry digest
Acrylamide
Cytotoxicity
Oxidative stress
Phenolic compounds

ABSTRACT

The aim of this study was investigating the protective effect of mulberry digest (MBD) on acrylamide-induced oxidative stress. Composition analysis of MBD revealed that it contained six major phenolic compounds (quercetin-3-*O*-rutinoside, quercetin hexoside, quercetin rhamnosylhexoside hexoside, kaempferol rhamnosylhexoside, cyanidin-3-*O*-glucoside and cyanidin-3-*O*-rutinoside). After *in vitro* digestion, the contents of two anthocyanins were both decreased significantly, while the contents of four flavonoid glycosides were all increased. In addition, MBD was found to successfully suppress acrylamide-induced ROS overproduction, restore the mitochondrial membrane potential, and inhibit the mitochondrial membrane lipid peroxidation and glutathione depletion. More interestingly, the protective effect of MBD against acrylamide-induced oxidative damage was enhanced compared with mulberry fruits without digestion (MBE). Further study revealed that MBD enhanced the cell resistance capacity to acrylamide-induced oxidative stress, rather than its direct reaction with acrylamide. Overall, our results indicate that MBD provides a potent protection against acrylamide-induced oxidative stress.



## 1. Introduction

Acrylamide (ACR) is colorless and odorless crystalline powder and it was classified as Group 2A carcinogen by the International Agency for Research on Cancer (IARC) in 1994. In 2002, ACR was widely detected in carbohydrate-rich foods like biscuits, fried potatoes and coffee. Maillard reaction between asparagine and reducing sugars during bakery food processing leads to formation of ACR. Statistically it was proved that human exposure to bakery products increased globally and due to presence of toxic substances such as ACR, in a long run exposure to bakery food products could have a negative impact on human health such as genotoxicity, cytotoxicity and neurotoxicity (Prasad & Muralidhara, 2012). Therefore, exposure of humans to ACR through their diet should not be neglected.

Recent studies on ACR revealed that the mitochondrial dysfunction and oxidative stress are the major mechanisms involved in its cytotoxicity and genotoxicity (Halliwell, 2006). Overproduction of reactive oxygen species (ROS) or an abrogation of antioxidant defense system could disturb cellular redox balance, resulting in cell injury or apoptosis. Studies from Chen et al. demonstrated that incubation of Caco-2 cells with acrylamide for 24 h accelerated the level of ROS and decreased the cell viability (Chen, Shen, Su, & Zheng, 2014; Chen, Su, Xu, Bao & Zheng, 2016). Therefore, the viable strategies to ameliorate ACR-induced toxicity are urgently needed. Among these strategies, natural antioxidants derived from vegetables and fruits that are capable of scavenging ROS and maintaining cellular redox balance have received more attention (Chen et al., 2011; Rodriguez-Ramiro, Ramos, Bravo, Goya, & Angeles Martin, 2011). Distribution of bioactive compounds in berry family fruits were extensively studied so far. For instance, raspberry, bayberry and mulberry fruits are found to be rich in phytochemicals such as anthocyanins, flavonoids, and phenolic acids, with extended biological and pharmacological importance (Chen, Su, Huang, Feng, & Nie, 2012; Natic et al., 2015; Wu, Tang, et al.,

*Abbreviations:* MBD, mulberry digest; MBE, mulberry extract; ACR, acrylamide; C3G, cyanidin-3-*O*-glucoside; C3R, cyanidin-3-*O*-rutinoside; ROS, reactive oxygen species; MMP, mitochondrial membrane potential; GSH, glutathione; CAT, catalase; SOD, superoxide dismutase.

* Corresponding author.
*E-mail address:* zjuchenwei@zju.edu.cn (W. Chen).

[1] The two authors contributed equally to this work.

http://dx.doi.org/10.1016/j.foodchem.2017.03.045
0308-8146/

2013). ROS inhibition studies revealed that myricitrin which prepared from bayberry was effectively inhibited the ROS generation and reduced its associated oxidative damage (Chen, Feng, et al., 2013; Chen, Zhuang, Li, Shen, & Zheng, 2013). Anthocyanins were the major components involved in the amelioration of oxidative stress *via* diminution of ROS production (Wu, Qi, et al., 2013). Moreover mulberry fruits extracts were demonstrated with potent hypolipidemic (Liu et al., 2009), hypoglycemic (Yan, Zhang, Zhang, & Zheng, 2016) and antioxidant (Yang, Yang, & Zheng, 2010) activities. However, mulberry fruits are supposed to go through the extreme gastrointestinal digestive process before it admits actual biological activity. During the process there are chances of trapping of bioactive compounds in mulberry matrix, further leading to formation of inactive compounds and other unusual bioactive compounds. Hence, consideration of simulated gastrointestinal digestion prior to assessment of biological activity of mulberry fruits is recommended. Therefore, in this study, we aimed to produce mulberry digest by *in vitro* simulated gastrointestinal digestion, and further investigate the protective effect of mulberry digest against ACR-induced oxidative damage in HepG2 cells.

## 2. Materials and methods

### 2.1. Materials and reagents

Mulberry fruits (*Morus alba L.*) were purchased from a local market in Hangzhou, China. Fresh fruits were washed with sterile distilled water and then dried at room temperature to remove the surface water. After that, fresh fruits of same size and integrity were screened and then stored at −80 °C prior to use.

Hoechst 33342, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT), Rhodamine 123 (RH123), 2′,7′-dichlor ofluorescin diacetate (DCFH-DA), Nonyl Acridine Orange (NAO), Naphthalene-2, 3-dicarboxal-dehyde (NDA), cyanidin-*3-O*-glucoside, cyanidin-*3-O*-ruitnoside, quercetin-*3-O*-rutinoside (rutin), acrylamide, Folin & Ciocalteu's phenol reagent, pepsin, pancreatin and bile salts were purchased from Sigma-Aldrich (St. Louis, MO, USA). BCA protein assay kit, cell lysis buffer, total superoxide dismutase assay kit and catalase assay kit were purchased from Beyotime Institute of Biotechnology., Ltd (Shanghai, China). All other reagents were of analytical grade.

### 2.2. In vitro simulated gastrointestinal digestion

The simulated gastrointestinal digestion was performed as detailed in earlier reports with slight modification (Kremer Faller, Fialho, & Liu, 2012). In brief, 10 g of mulberry fruits were homogenized with 5 mL of distilled water and then diluted to 20 mL for gastric digestion. Prior to gastric digestion, the solution was acidified to pH 2 using 5 M HCl, and porcine pepsin (6000 units) was added subsequently. After that, the mixture was kept for incubation in a shaking water bath (100 rpm) at 37 °C for about 90 min. After incubation the pH of the mixture was adjusted to pH 6.5 by adding 1 M sodium bicarbonate and further 5 mL of pancreatin was added (consisting of 4 mg/mL trypsin and 25 mg/mL porcine bile salts), followed by adjusting pH to 7.4 using 1 M sodium hydroxide before subjecting to intestinal digestion. Thereafter, the mixture was incubated in a shaking water bath (100 rpm) at 37 °C for 2 h. At the end of intestinal digestion, the mixture was diluted to a final volume of 30 mL with distilled water and then centrifuged for 10 min at 5000 rpm and supernatant was collected. The supernatant was referred to as MBD and was stored at −80 °C until use. As for preparation of mulberry fruits without digestion, 10 g of mulberry fruits were homogenized with 5 mL of distilled water and then diluted to a final volume of 30 mL with distilled water, followed by centrifuging for 10 min at 5000 rpm. The supernatant was collected and stored at −80 °C prior to use.

### 2.3. Assessment of anthocyanins in mulberry digest (MBD) by HPLC

Determination of anthocyanins in mulberry digest (MBD) was performed by HPLC-DAD (Dionex ultimate 3000, ThermoFisher Scientific, USA) and relevant standard (cyanidin-*3-O*-glucoside and cyanidin-*3-O*-rutinoside) were used. Briefly mobile phase A consists of formic acid and water (8.5:91.5, v/v) and mobile phase B consists of formic acid, methanol, acetonitrile and water (8.5:22.5:22.5:41.5, v/v/v/v). The elution was carried out at 30 °C on a Promosil C18 column (4.6 × 250 mm, 5 μm, Bonna-Agela Technologies, Tianjin, China), with flow rate of 1 mL/min, in the following linear gradient: from 93% to 75 % A for 35 min, from 75% to 35% A for 10 min, from 35% to 0% A for 1 min, 100% B for 4 min. The detection wavelength was set to 520 nm and the injection volume was 10 μL.

### 2.4. Identification of phenolic compounds by UPLC-Triple-TOF-MS

Identification of phenolic compounds in mulberry digest (MBD) was performed using an UPLC system (Acquity™, Waters, USA) connected to Triple-TOF Mass Spectrometry System (AB SCIEX, Triple-TOF 5600plus Framingham, USA). The elution was carried out at 30 °C on a Promosil C18 column (4.6 × 250 mm, 5 μm). The mobile phase consists of acetonitrile (A) and 0.1% aqueous formic acid solution (B), with flow rate of 0.8 mL/min, in the following linear gradient: 5% A for 5 min, from 5% to 16% A for 20 min, from 16% to 30% A for 8 min, from 30% to 90% A for 2 min, 90% A for 5 min, from 90% to 5% A for 5 min and then isocratic elution for 5 min. The detection wavelength was set to 360 nm, with injection volume 10 μL. MS spectra was obtained by full range acquisition covering the *m*/*z* range 50–2000 in negative ion mode, with the voltage and source temperature reached to 4.5 kV and 550 °C, respectively.

### 2.5. Quantitative analysis

Quantitative analysis of major components was performed using commercial available analytical standards. For anthocyanin quantification, cyanidin-*3-O*-glucoside and cyanidin-*3-O*-rutinoside were used as standards. For quercetin or quercetin derivatives quantification, quercetin-*3-O*-rutinoside (rutin) was used as a standard. The contents of other quercetin derivatives were expressed as quercetin-*3-O*-rutinoside equivalent.

### 2.6. Cell culture

Human HepG2 cells were obtained from Cell Bank of Type Culture Collection of Chinese Academy of Sciences. Cells were cultured in RPMI 1640 medium (Gibco) consisting of 100 units/mL streptomycin, 100 units/mL penicillin and 10% of the new calf serum and incubated in a humidified atmosphere with 5% $CO_2$ at 37 °C.

### 2.7. Cell viability assay

Cell viability was evaluated by the MTT method as detailed in earlier reports (Chen, Zhao, & Li, 2011). Briefly, HepG2 cells were seeded into 96-well cell culture plates at a density of $4 \times 10^3$ cells/well and then incubated for 24 h. After incubation HepG2 cells were pretreated with MBD solution (0.25 mg/mL, 0.5 mg/mL and 1 mg/mL) for 24 h and then treated with ACR (5 mM) for another 24 h. Subsequently, MTT (0.5 mg/mL) was added to cell culture plate and incubated for 4 h. Then the generated formazan precipitate was dissolved in 150 μL of DMSO