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Ultrasound extraction of polysaccharides from mulberry leaves and their effect on enhancing antioxidant activity

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ABSTRACT

A Box–Behnken design (BBD) was applied to optimize the ultrasound-assisted extraction of polysaccharides from mulberry leaves. Under the optimum conditions of an extraction temperature of 57 °C, an extraction time of 80 min and a liquid/solid ratio of 53 mL/g, the mulberry leaf polysaccharide (MLP) yield was 6.92 ± 0.29%. Then, three fractions of MLPs were obtained by deproteinization, dialysis and decolorization. The carbohydrate content, FT-IR spectrum and monosaccharide composition of the MLPs were also investigated. The antioxidant activities of the three fractions were compared, and the results indicated that the antioxidant activities decreased with the increasing MLP purity. Therefore, highly concentrated MLPs were shown to have very little antioxidant activity. After quercetin (10 μg/mL) was added, the antioxidant activities were improved significantly. This result showed that MLPs and quercetin have a synergistic effect on the antioxidant activity. Although the MLPs have very little antioxidant activity alone, they greatly enhance the antioxidant activity of flavonoids. Thus, MLPs can be used as an antioxidant activity enhancer in the food industry.

1. Introduction

Mulberry, belonging to the family *Moraceae*, is an important plant with a high economic value. In most Asian countries, mulberry leaves are used to feed silkworms (*Bombyx mori* L.), and the fruits are eaten raw or processed as juice, fruit wine and fruit jam (Gerasopoulos & Stavroulakis, 1997; Yildiz, 2013). Due to their significant bioactivities, mulberry leaves have been widely used to produce various functional foods, such as mulberry-leaf-carbonated beverages, health beverages and mulberry-leaf tea (Thirugnanasambandham, Sivakumar, & Maran, 2015). It has been reported that mulberry leaves or their extracts exhibit multiple therapeutic effects, including anti-diabetic, anti-inflammation and anti-cancer effects (Andallu, Suryakantham, Lakshmi, & Reddy, 2001; Andallu & Varadacharyulu, 2007; Naowaboot et al., 2009). Phytochemical investigation has indicated that there are many active constituents, such as flavonoids, alkaloids, polysaccharides, phenolics and steroids, in mulberry leaves (Doi, Kojima, Makino, Kimura, & Fujimoto, 2001; Thabti, Elfalleh, Hannachi, Ferchichi, & Campos, 2012). Most previous studies were focused on alkaloids isolated from mulberry leaves, including 1-deoxynojirimycin (DNJ), 1,4-dideoxy-1,4-imino-D-arabinitol (DAB) and 1,4-dideoxy-1,4-imino-D-ribitol (DRB) (Kimura & Nakagawa, 2004; Li et al., 2011; Li, Ji, Zhong, Lv, & Lin, 2013; Sharma, Gupta, Singh, Rajpoot, & Shukla, 2010), which are α-glycosidase inhibitors with hypoglycemic activities. However, researches on mulberry leaf polysaccharides (MLPs) are limited, especially for antioxidant activity of MLPs. Oxygen is essential to many living organisms for producing energy in biological processes. However, about 2–3% of the oxygen taken into the human body is converted to reactive oxygen species (ROS) and free radicals, which enhance oxidative damage to various biomolecules, including DNA, proteins, small cellular molecules and membrane lipids (Lu, Lin, Yao, & Chen, 2010).

The extraction method is an important factor for the use of plant active components. Traditional extraction methods, such as Soxhlet extraction, heating reflux extraction and maceration, have some disadvantages: they are time-consuming and inefficient. Especially for soxhlet extraction and heating reflux extraction, the energy consumption of these methods is high and thus not environmentally friendly (Kimbaris et al., 2006; Zhang et al., 2013). Ultrasound-assisted extraction (UAE) is an effective extraction method that has been used in the extraction of many kinds of polysaccharides (Liu et al., 2014; Xu, Zhang, Yang, Song, & Yu, 2015). The acoustic cavitation in UAE can destroy cell walls, reduce particle sizes and enhance the contact between solvents and targeted compounds (Rostagno, Palma, & Barroso, 2003). Moreover, the UAE method also has some advantages, such as lower energy consumption, lower

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solvent consumption, higher extraction efficiency and a higher level of automation (Ying, Han, & Li, 2011; Zhao, Zhang, Li, Dong, & Liu, 2015).

In the present study, the UAE method was applied to the extraction of MLPs. The effects of main operating parameters on the extraction yields were investigated. The polysaccharides were preliminarily characterized by FT-IR, and the monosaccharide components were also determined by GC-MS. Furthermore, the antioxidant activity of the polysaccharides was investigated by the ferric reducing antioxidant power test and the ABTS test.

2. Materials and methods

2.1. Plant material

Mulberry leaves were collected in autumn from Sericultural Research Institute, Chinese Academy of Agricultural Sciences, China, and dried at 60 °C. Then, the samples were ground and sieved (20–80 mesh). Finally, they were kept away from light in a desiccator at room temperature until they were used.

2.2. Chemicals and reagents

Ethanol of analytical grade was bought from Tianjin Kermel Chemical Reagent Co. (Tianjin, China). Deionized water was purified by a Milli-Q water-purification system from Millipore (Bedford, MA, USA).

2.3. Extraction procedure

One gram of mulberry leaves was extracted with deionized water in a KQ-2200DB ultrasonic cleaning bath (Kunshan Co., Ltd., China; frequency range 40−100 Hz, ultrasonic power 100 W, heating power 600 W, temperature setting range 20−80 °C). The extraction temperature is controlled by the ultrasonic bath. Before extraction process start, the temperature is preset. Then the ultrasonic bath start to heat, when the temperature reaches the set value, the sample is placed in the ultrasonic bath and ultrasound start. During the extraction process, the temperature is maintained by the ultrasonic bath. After extraction, the extraction solutions were separated by membrane filtration. Then, the filtrates were precipitated with three volumes of absolute ethanol for 24 h at 4 °C. The precipitates were obtained by centrifugation (6000 rpm, 15 min), washed with acetone and then dried to obtain crude polysaccharides. The yield of polysaccharide was calculated as follows:

$$\text{Polysaccharide yield}(\%) = \frac{\text{Content of crude polysaccharedes(g)}}{\text{Weight of mulberry leaves(g)}} \times 100$$

2.4. Experimental design

A three-level (1, 0, +1) three-factor Box–Behnken design (BBD) was applied to evaluate the effects of the main experimental factors—temperature (X_1), time (X_2) and liquid–solid ratio (X_3)—on the extraction yields of the MLPs (Y). A 2^3 factorial portion Box–Behnken design combined with a response surface methodology (RSM) was used. In the BBD test, 12 experiments and five replicates at the centre were employed to fit the full quadratic equation model. The process variables and their ranges are shown in Table 1. The general equation is

$$Y = \beta_0 + \sum_{j=1}^{k} \beta_j X_j + \sum_{j=1}^{k} \beta_{jj} X_j^2 + \sum \sum_{i<j} \beta_{ij} X_i X_j (k = 3)$$

Table 1
Results of Box–Behnken design (BBD) on the extraction yields of MLP.

No.	Temperature (X_1,°C)	Time (X_2,min)	Liquid/solid ratio (X_3, mL/g)	Y[a] (%)
1	−1 (40)	−1 (30)	0 (40)	0.328
2	1 (80)	−1 (30)	0 (40)	0.382
3	−1 (40)	1 (90)	0 (40)	0.369
4	1 (80)	1 (90)	0 (40)	0.456
5	−1 (40)	0 (60)	−1 (20)	0.352
6	1 (80)	0 (60)	−1 (20)	0.407
7	−1 (40)	0 (60)	1 (60)	0.365
8	1 (80)	0 (60)	1 (60)	0.418
9	0 (60)	−1 (30)	−1 (20)	0.321
10	0 (60)	1 (90)	−1 (20)	0.445
11	0 (60)	−1 (30)	1 (60)	0.403
12	0 (60)	1 (90)	−1 (20)	0.449
13	0 (60)	0 (60)	0 (40)	0.481
14	0 (60)	0 (60)	0 (40)	0.475
15	0 (60)	0 (60)	0 (40)	0.47
16	0 (60)	0 (60)	0 (40)	0.466
17	0 (60)	0 (60)	0 (40)	0.496

where Y is the predicted response; β_0, β_j, β_{jj} and β_{ij} are the regression coefficients for the intercept, linear terms, quadratic terms and interaction terms, respectively; and X_i and X_j are the independent coded variables.

The experimental data in this test were analyzed by software (Design-Expert 7.0 Trial, State-Ease, Inc., Minneapolis MN, USA). Analysis of variance (ANOVA) was performed for calculating and modeling the optimal conditions for the MLP yields. A significance level of $p < 0.05$ was considered for each influential factor.

2.5. Purification of polysaccharides

The crude MLP precipitates were re-dissolved in deionized water, and the solutions were deproteinized by the Savage method (Sevage, 1934) and dialyzed against tap and deionized water. Then, macroporous resin ADS-17 (functional group: ester group, surface area: 90–120 m^2/g, average pore diameter: 25.0–30.0 nm, particle diameter: 0.3–1.25 mm, polarity: middle-polar) was used to decolour the solutions and remove flavonoids and phenols. Finally, the solutions from each step were lyophilized, and three polysaccharide samples were obtained, coded as MLP1 (deproteinized), MLP2 (dialyzed) and MLP3 (treated by ADS-17).

2.6. Analytical methods

2.6.1. Total sugar content

The total sugar content was determined by phenol–sulphuric acid analysis using D-glucose as a standard (DuBois, Gilles, Hamilton, Rebers, & Smith, 1956).

2.6.2. FT-IR analysis

The MLPs were ground with KBr powder and then pressed into pellets for FT-IR (Affinity -1, Shimadzu, Japan; resolution, 4 cm) measurement in the wavenumber range of 4000–400 cm^{-1} to detect functional groups. The scan number was 20.

2.6.3. Monosaccharide composition

The MLPs (10 mg) were dissolved in 4 M trifluoroacetic acid (TFA, 2 ml) and hydrolyzed at 105 °C for 12 h. Then, the TFA was removed from the samples by a rotary evaporator. A trimethylsilylation method was used to derivatize the hydrolyzed products, according to Chan, Chan and Tang (2006), with some modifications. The hydrolyzed products were dissolved in 2 mL pyridine, and then 0.4 mL of hexamethyldisilane (HMDS) and 0.8 mL of trimethylchlorosilane (TMCS) were added. The mixtures reacted