International Journal of Biological Macromolecules xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Microwave-assisted extraction of polysaccharides from mulberry leaves

Q1 K. Thirugnanasambandham*, V. Sivakumar*, J. Prakash Maran

Department of Food Technology, Kongu Engineering College, Perundurai, Erode 638052, Tamil Nadu, India

ARTICLE INFO

Article history:
Received 9 May 2014
Received in revised form 16 July 2014
Accepted 16 July 2014
Available online xxx

Keywords:
Polysaccharide
Mulberry leaves
Extraction
Microwave irradiation
Box–Behnken design
Optimization

ABSTRACT

In this study, microwave-assisted extraction (MAE) of polysaccharides from mulberry leaves was investigated using response surface methodology (RSM). The effects of three extraction factors on the yield of polysaccharides was examined. The results showed that optimum extraction conditions were determined as follows: weight of the sample of 20 g, microwave power of 170 W, extraction time of 10 min. Under these optimal extraction conditions, polysaccharide yield was found to be 9.41%. Three factors-three level Box–Behnken response surface design (BBD) coupled with RSM was used to model the extraction process. ANOVA was used to examine the statistical significance of the developed model. Extracted polysaccharide was analyzed using Fourier transform infrared (FT-IR) spectroscopy.

1. Introduction

Mulberry is a traditional herbal medicine and grows well all over the year, especially in India. In most mulberry-growing countries, mulberry foliage is used to feed silkworms [1]. As mulberry and its various parts has significant bioactivities, it is widely used to produce various functional foods, such as mulberry leaf-carbonated beverage and healthy beverage. Particularly, polysaccharides from the mulberry leaves have significant antihyperglycemic and antihyperlipidemia activities. Hence, in the last few decades, various technologies such as soxhlet, solvent, and ultrasound extraction were used to extract the bioactive polysaccharides from mulberry leaves [2]. But, these extraction techniques show disadvantages including lower extraction efficiency, increased operating cost and abnormal extract quality. Therefore, there is a critical need to design a favorable technique to extract polysaccharides from mulberry leaves [3].

Nowadays, microwave-assisted extraction (MAE) is widely used to extract polysaccharides from various kinds of plant materials due to its enhanced extraction efficiency, when compared to other conventional methods [4]. When microwave-assisted extraction is used to extract the polysaccharide, two principal mechanisms are simultaneously functioning. One of these is a rapid increase in temperature, which reduces emulsion viscosity and breaks the outer film of plant material, thus improving the extraction rate. The other is molecular rotation, which neutralizes the Zeta potential [5]. This phenomenon rearrange the electrical charges surrounding the molecules, resulting in enhanced movement of ions which increase the efficiency of extraction process. Moreover, microwave-assisted extraction requires less time and the yield and quality of polysaccharide produced using MAE is the same as that produced by conventional extraction. In addition, process variables in MAE such as weight of the sample, microwave power and extraction time were significantly affect the process efficiency, and the optimization of these parameters will increase the yield of polysaccharide significantly [6].

Response surface methodology (RSM) is a collection of statistical techniques commonly used to understand the performance of complex systems and optimize any kind of complex extraction process [7]. This technique can also be used to evaluate the relative significance of several affecting factors even in the presence of complex interactions between the independent variables. However, to the best of our knowledge, MAE to perform the extraction of polysaccharide from mulberry leaves using response surface methodology (RSM) has not yet described in literature. Hence, in this present study, response surface methodology (RSM) coupled with Box–Behnken response surface design (BBD) was used to optimize and investigate the process variables such as weight of the sample, microwave power and extraction time in MAE of

* Corresponding authors. Tel.: +91 4294 226606; fax: +91 4294 220087.
E-mail addresses: thirusambath5@gmail.com, pthirusambath5@gmail.com (K. Thirugnanasambandham), drvsivakumar@yahoo.com (V. Sivakumar).

http://dx.doi.org/10.1016/j.ijbiomac.2014.07.031
0141-8130/© 2014 Published by Elsevier B.V.

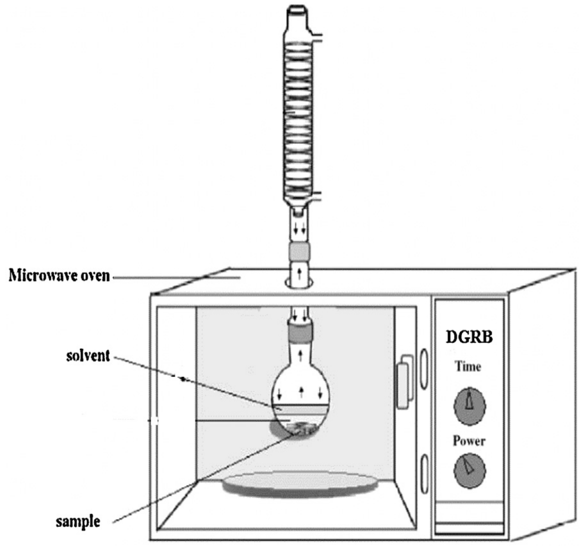

Fig. 1. Microwave-assisted extraction unit.

polysaccharide from mulberry leaves. Finally, the extract has been analyzed using Fourier transform infrared (FT-IR) spectroscopy analysis.

2. Materials and methods

2.1. Materials and chemicals

The mulberry leaves used were collected from Pungamuthur, Udumalpet, Tamil Nadu, and dried in the room temperature. Potassium bromide (KBr) and all other chemicals reagents used were of analytical grade and were purchased from Sigma-Aldrich, Chennai.

2.2. Extraction of polysaccharides

The mulberry leaves were ground to make fine powder, then it was defatted with petroleum ether. Then it was pretreated with 80% ethanol for two times prior to experiments. Desired amount of the ground powder was mixed with distilled water in a plastic bag and then placed in a microwave extraction apparatus (Fig. 1). Extractions were carried out with various microwave power and extraction time. After extraction, debris fragments of polysaccharide extracts were removed by centrifugation. Then the aqueous solution of polysaccharides was precipitated with four volumes of 95% (v/v) ethanol for 48 h at 4 °C. The precipitates were obtained by centrifugation (6000 rpm, 30 min), washed with acetone and then dried to obtain the crude polysaccharides. The yield of polysaccharide was calculated as follows [8]:

$$Y = \frac{X}{Z} * 100 \quad (1)$$

where X is the weight of polysaccharide and Z is the weight of mulberry leave powder.

2.3. Experimental design

In this present study, response surface methodology coupled with three factors-three level Box–Behnken response surface experimental design (BBD) was employed to investigate the individual and interactive effects of process variables to extract the polysaccharide from mulberry leaves using MAE. Weight of mulberry leave powder (A), microwave power (B) and extraction time (C) are selected as independent variables, whereas extraction yield of polysaccharide (Y) is selected as response. Process variables and their ranges are shown in Table 1. The obtained BBD results were fitted into the empirical second-order polynomial model, as follows [9]:

Table 1
Process variables and their ranges.

Level	−1	0	1
A	6	15	24
B	50	150	250
C	5	10	15

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

where Y is the response, X_i and X_j are variables (i and j range from 1 to k), β_0 is the model intercept coefficient, β_j, β_{jj} and β_{ij} are interaction coefficients of linear, quadratic and the second-order terms, respectively, k is the number of independent parameters ($k = 3$ in this study) and e_i is the error. Three-dimensional (3D) response surface plots were used to study the interactive effect of extraction variables on the yield of polysaccharide. Finally, optimization of extraction process variables was obtained by using Derringer's desired function methodology. All the statistical analyses were done using Design-Expert 8.0.7.1 (State-Ease Inc., Minneapolis, MN, USA) package [10].

2.4. Fourier transform infrared (FT-IR) measurement

An FT-IR spectrum of the extracted polysaccharide was determined using FT-IR spectrometer in the frequency range of 4000–400 cm^{-1} with potassium bromide (KBr) as a reference [11].

3. Results and discussions

3.1. Mathematical modelling

In order to select the suitable mathematical equation among various models such as linear, interactive, quadratic and cubic, to represent the extraction of polysaccharide from mulberry leaves using MAE, BBD experimental data (Table 2) were analyzed by sequential model sum of squares test (Table 3). The obtained results indicate that linear and interactive (2FI) models are exhibited high p-values and lower F-values, when compared with the quadratic model. Cubic model is found to be aliased. Therefore, the quadratic

Table 2
BBD experimental design with results.

S. No.	Weight of sample (A)	Microwave power (W)	Extraction time (C)	Extraction yield of polysaccharide (%)
1	15	150	10	9.22
2	15	150	10	9.22
3	15	150	10	9.22
4	24	150	5	6.54
5	24	50	10	5.24
6	6	150	15	2.54
7	15	250	15	7.34
8	24	150	15	4.24
9	15	50	5	6.84
10	15	150	10	9.22
11	6	150	5	1.24
12	15	150	10	9.22
13	15	50	15	8.86
14	6	50	10	1.02
15	6	250	10	6.35
16	24	250	10	9.16
17	15	250	5	7.54

Please cite this article in press as: K. Thirugnanasambandham, et al., Int. J. Biol. Macromol. (2014), http://dx.doi.org/10.1016/j.ijbiomac.2014.07.031