ISSN : 0974 - 7435 Volume 10 Issue 21

2014

BioTechnology

An Indian Journal

FULL PAPER

BTAIJ, 10(21), 2014 [13132-13137]

Optimization of high hydrostatic pressure extract 1-deoxynojirimycin from mulberry leaves with response surface methodology

Zhang Pei-Qi, Zhang Li-Hua, Zong Wei*

College of Food and Biological Engineering, Zhengzhou University of Light Industry, Zhengzhou, 450002,China

E-mail : zongwei1965@126.com

ABSTRACT

In order to optimum ultra high pressure extraction (UHPE) parameter of 1-deoxynojirimycin (DNJ) from mulberry leaves. On the basis of single-factor test, response surface methodology was used to optimum extraction parameter. The mathematical regression model is established about the dependent variable (extraction yield of DNJ) and independent variables (alcohol concentration, extraction time and extraction pressure) through Box-Benhnken center composite design and response surface methodology. The results indicated that the optimal extraction conditions were alcohol concentration of 73%, extraction time of 3min, extraction pressure of 400 MPa. Under these conditions, the maximum yield of DNJ was 0.0931%. It’s proved that UHPE was a suitable method for extraction of DNJ as its higher extraction yield and shorter time.

KEYWORDS

Ultra high pressure; Extraction; Optimum; 1-Deoxynojirimycin; Mulberry leaves.

INTRODUCTION

Mulberry leaves (*Morus alba* L.) is a traditional herbal medicine and have been cultivated in many Asian countries such as China, India Korea and Japan[1]. Scientific and clinical studies have confirmed that mulberry leaves have significant bioactivities such as hypoglycemic effect, anti-diabetic activity, antioxidant activity, antimicrobial activity and anti-inflammatory[2-6]. Recently, research has shown 1-deoxynojirimycin (DNJ) is the main constituents of mulberry leaves[7-8]. Hence, in the last few decades, various technologies such as soxhlet, microwave extraction and ultrasound extraction were used to extract the DNJ from mulberry leaves[9-10]. But, these extraction techniques show disadvantages including lower extraction efficiency and long extract time. Therefore, there is a need to design a suitable technique to extract DNJ from mulberry leaves. Ultra high pressure extraction (UHPE) is a new technology used solvent for the extract of plant components from natural products for it has short extraction time and higher yield[11-13]. But extraction of DNJ by UHPE from mulberry leaves has fewer reports. The objective of this study was to identifying optimal range of UHPE using response surface methodology.

EXPERIMENT

Materials and reagents

Mulberry leaves was obtained from Xinzheng (Henan, China). Leaves was collected and were dried to final moisture of 6% (w/w). The dry leaves was ground to 60 mesh. DNJ, ethanol and others reagentwere purchased from Sigma.

Ultra high pressure extraction of DNJ

UHPE was conducted in an Ultra high pressure extractor (HPP600MPa/2L, Baotou Kefa High Technology Co., Ltd. China). One hundred milligramof dried and ground leaves and solvent weremixed and vacuum sealed in packing bag. The bag placed into the extractor cavity. Leaves was extracted at set pressur, set extract time and set ratio of solid to liquid. After extractin, the mixture was centrifuged at 5000g for 20 min. After centrifugation, the supernatant was filtered through a 0.45 μm filter. The extraction yield of was calculated as follow:

$$Y\% = \frac{W_2}{W_1} \times 100\%$$

Y%: the extraction yield (%);
W2: the weight of DNJ in extracts (g);
W1: the weight of leaves (g).

Determination of DNJ

The concentration of total phenols in extracts was measured by HPLC[14].

20 μL of the extract was injected intoan HPLC system (Waters, New York, USA). A Waters XTerra RP-18(150 mm×4.6 mm 5μm) column with a Waters 2996 photodiodearray detector was used to measure DNJ. The conditions were as follows: column temperaturewas 25℃, detection wavelength 254 nm, mobile phase of acetonitrile–0.1% aqueousacetic acid (1:1, v/v) at 1.0 mL/minfor 10 min.

Optimization of UHPE with response surface methodology

According single-factor test, pressure (X1), extract time (X2) and r thanol concentration ((X3) were used critical various processing parameters. Three levels (low, medium and high) denoted as-1, 0, and +1 respectively. The variables were coded according to follow equation: xi=(Xi- X0)/ΔXi.(where xi is the coded value of an independent variable. X_i is the real value of an independent variable. X_0 is the real value of an independent variable at the center point and ΔX_i is the step change value. The code and level of factors chosen for the trials in this study are given in TABLE 1.

TABLE 1 : Code and level of factors

Various parameters	Code	Code values	Levels
Pressure (MPa)	x_1	-1	350
		0	400
		1	450
extract time(min)	x_2	-1	2.5
		0	3
		1	3.5
Ethanol concentration(%)	x_3	-1	70
		0	75
		1	80

The response function used was a quadratic polynomial equation as given below:

$$Y = A_0 + \sum_{i=1}^{n} A_i X_i + \sum_{i=1}^{n} A_{ij} X_i X_j$$

Where Y is DNJ yield. A_0 is constant; A_i is linear coefficients and A_{ij} is cross-product coefficients and quadratic coefficients. All data was analysis by SAS software.

RESULT AND DISSCUSS

Single-factor test of extraction parameters on yield

To study the effect of extraction parameters on yield. The experimental design was presented in Figure 1.

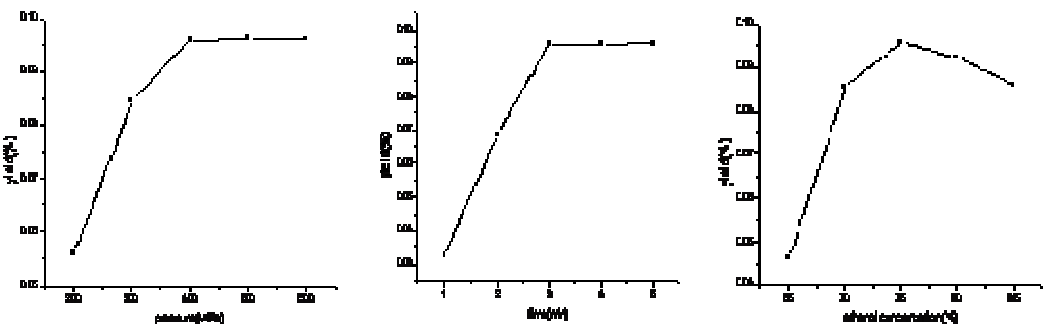

Figure 1 : Effect of extraction parameters on yield

It Indicates the influence of pressure (200MPa-600MPa), extraction time (1-5 min) and the ratio of solid to solvent (0.075-0.175 g/mL) on the extract yield.

In the effect of extraction parameters on yield, the yield was increase with pressure when pressure lower at 400Mpa. This is because the cell of leaves were destroyed with pressure increased. The DNJ easy to release from cell. When more than 400MPa, cell has been full destroyed.

The mechanism of the UHPE process here has two main stages. First, dissolution of DNJ was increased with time, this is theinxreased cell destroyed with time increase. Secondly, cell has been full destroyed. Theyield keep constant.

In the effect of ethanol on yield, While 75% EtOH as extraction solvent was highest level of extract yield. Accordingly, water is acting as the plant swelling agent, while ethanol is believed to disrupt the bonding between the solutes and plant matrices. So suitable EtOH concentration is important.

Optimization of inclusion conditions

According the design, the test results were presented in TABLE 2. A Box-Behnken design under RSM was used analysis interactive of pressure (X_1), extract time (X_2) and r thanol concentration ((X_3). The code and level of factors were selected from single factor test. he regression model of the effect of different variables including interaction between variables on the yield were also obtained. Regression equation as follows:

$$Y=0.092-1.612x_1-7.512x_2-1.950x_3-3.025x_1x_2-5.550x_1x_3-2.800x_2x_3-0.034x_1^2-7.448x_2^2-0.511x_3^2$$

TABLE 2 : Box-Behnken expertmental design and results

No.	X_1	X_2	X_3	Y/%
1	0	-1	-1	0.0789
2	0	-1	1	0.0812
3	0	1	-1	0.0712
4	0	1	1	0.0623
5	-1	0	-1	0.0465
6	-1	0	1	0.0531

7	1	0	-1	0.0512
8	1	0	1	0.0356
9	-1	-1	0	0.0554
10	-1	1	0	0.0447
11	1	-1	0	0.0614
12	1	1	0	0.0386
13	0	0	0	0.0910
14	0	0	0	0.0854
15	0	0	0	0.0956

Statistical analysis of quadratic regression model was tested by the Fisher's F-test for analysis of variance (ANOVA). The statistical software package Design-Expert 6.0 and the analysis of variance for regression equation was shown in TABLE 3.

In TABLE 3, the model F-value of 53.46 implies that model is highly significant. Values of "Prob > F "less than 0.00001indicate that model terms are very significant. Another evidence is the lack-of-fit F value. The lack-of-fit F value of 0.456 implies the lack of fit is not significant relative to the pure error.

TABLE 3 : Analysis of variance table

Source	Squares	df	Mean square	F Value	Prob>F	significant
Model	6.730	9	7.478	53.46	<0.0001	yes
x_1-pressure	2.080	1	2.080	1.49	0.2622	no
x_2-time	4.515	1	4.515	32.28	0.0007	no
x_3-ethanol	3.042	1	3.042	2.17	0.1838	no
x_1x_2	3.660	1	3.660	2.62	0.1498	no
x_1x_3	1.232	1	1.232	8.81	0.0209	no
x_2x_3	3.136	1	3.136	2.24	0.1780	no
x_1^2	4.938	1	4.938	353.04	<0.0001	no
x_2^2	2.335	1	2.335	16.69	0.0047	no
x_3^2	4.977	1	4.977	35.58	0.0006	no
Residual	9.792	7	1.399			no
Lack of Fit	2.683	3	8.944	0.50	0.7004	no
Pure Error	7.109	4	1.777			
Cor Total	6.828	16	R	0.9672	R2	0.9857

The effect of one factor solely depends on the value of the other. The fitted response surface plot was generated by statistically significant above model by Design Expert program to understand interaction of parameters required for optimum yield. Figure 2 represents that interaction of the pressure (x_1), time (x_2) and ethanol concentration (x_3) on yield.

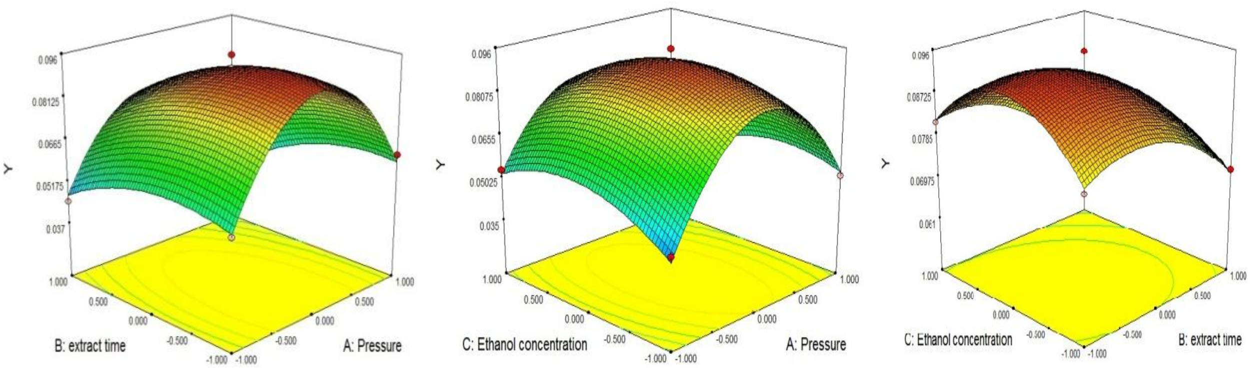

Figure 2 : Response surface plot and it's contour plot

By analysis plots of Figure 2, the predicated yield is as 0.0936% and lies in the following ranges of the examined variables: alcohol concentration of 73.125%, extraction time of 2.76min, extraction pressure of 400 MPa. Under these conditions, the trail experiments were conducted under optimized process condition. Ethanol concentration of 73%, extraction time of 3min, extraction pressure of 400 MPa were used for extract. The yield was 0.0931%.

CONCLUSIONS

Single factor experiment and response surface methodology were carried out to obtain the optimum extraction process of DNJ from leavesThe extraction by response surface methodology could accurately reflect the effects of factors on the extraction yield of DNJ and interaction among factors. The obtained regression model could agree well with practical situation, which could provide a certain theoretical direction for extraction of DNJ. Moreover, UHPE can increase the extract yield of in leaves, because it can destroy the cell of leaves.

ACKNOWLEDGEMENT

This study was financially supported by the national science and technology support of China (2012BAD36B07).

REFERENCES

[1] Chunjiu Ren, Yao Zhang, Weizheng Cui; A polysaccharide extract of mulberry leaf ameliorates hepatic glucose metabolism and insulin signaling in rats with type 2 diabetes induced by high fat-diet and streptozotocin. International Journal of Biological Macromolecules, **72(1)**, 951-959 **(2015)**.

[2] Chieh-Jung Liu, Jin-Yuarn Lin; Anti-inflammatory and anti-apoptotic effects of strawberry and mulberry fruit polysaccharides on lipopolysaccharide-stimulated macrophages through modulating pro-/anti-inflammatory cytokines secretion and Bcl-2/Bak protein ratio. Food and Chemical Toxicology, **50(9)**, 3032-3039 **(2012)**.

[3] Eunkyo Park, Seung-Min Lee, Jung Eun Lee; Anti-inflammatory activity of mulberry leaf extract through inhibition of NF-κB. Journal of Functional Foods, **5(1)**,178-186 **(2013)**.

[4] Yao Zhang, Chunjiu Ren, Guobing Lu; Anti-diabetic effect of mulberry leaf polysaccharide by inhibiting pancreatic islet cell apoptosis and ameliorating insulin secretory capacity in diabetic rats. International Immunopharmacology, **22(1)**, 248-257 **(2014)**.

[5] Hye In Chung, Joohee Kim, Ji Yeon Kim; Acute intake of mulberry leaf aqueous extract affects postprandial glucose response after maltose loading: Randomized double-blind placebo-controlled pilot study. Journal of Functional Foods, **5(3)**, 1502-1506 **(2013)**.

[6] Bondada Andallu, N.Ch.Varadacharyulu. Antioxidant role of mulberry (Morus indica L. cv. Anantha) leaves in streptozotocin-diabetic rats. Clinica Chimica Acta, **38(1)**, 3-10 **(2003)**.

[7] Chaluntorn Vichasilp, Kiyotaka Nakagawa, Phumon Sookwong; Development of high 1-deoxynojirimycin (DNJ) content mulberry tea and use of response surface methodology to optimize tea-making conditions for highest DNJ extraction.LWT - Food Science and Technology, **45(2)**, 226-232 **(2012)**.

[8] Chaluntorn Vichasilp, Kiyotaka Nakagawa, Phumon Sookwong; A novel gelatin crosslinking method retards release of mulberry 1-deoxynojirimycin providing a prolonged hypoglycaemic effect.Food Chemistry, **134(4)**, 1823-1830 **(2012)**.

[9] Hua Jun-Li, Liu Shu-xing, Chen Su-juan; Study on ultrasonic extraction of 1-Deoxynojirimycin(DNJ)from Mulberry leaves.Journal of Food Science and Biotechnology, **28(6)**, 845-849 **(2009)**.

[10] Hu Rui- Jun, Che Zhen- Ming, Xu Dan; Study on the microwave- assisted extraction method of DNJ from mulberry leaves.Food Science and Technology, **33(8)**, 139-142 **(2007)**.

[11] Chul Gue Joo, Keun Ha Lee, Chung Park; Antioxidative activities and composition analysis of *Pinus densiflora* root by ultra high pressure extraction.Journal of Industrial and Engineering Chemistry, **17(4)**, 712-716 **(2011)**.

[12] K.Nagendra Prasad, Bao Yang, Mouming Zhao; High pressure extraction of corilagin from longan (*Dimocarpus longan* Lour.) fruit pericarp.Separation and Purification Technology, **70(1)**, 41-45 **(2009)**.

[13] K.Nagendra Prasad, Bao Yang, John Shi; Enhanced antioxidant and antityrosinase activities of longan fruit pericarp by ultra-high-pressure-assisted extraction.Journal of Pharmaceutical and Biomedical Analysis, **51(2)**, 471-477 **(2010)**.

[14] Nitra Nuengchamnong, Kornkanok Ingkaninan, Wiroje Kaewruang; Quantitative determination of 1-deoxynojirimycin in mulberry leaves using liquid chromatographytandem mass spectrometry.Journal of Pharmaceutical and Biomedical Analysis, **44(4)**, 853-858 **(2007)**.