Food Chemistry 194 (2016) 650–658

Contents lists available at ScienceDirect

Food Chemistry

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

# Optimisation of pressurised water extraction of polysaccharides from blackcurrant and its antioxidant activity

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ARTICLE INFO

*Article history:*
Received 22 February 2015
Received in revised form 17 August 2015
Accepted 18 August 2015
Available online 21 August 2015

*Chemical compounds studied in this article:*
D-Glucose (PubChem CID: 5793)
D-Galacturonic acid (PubChem CID: 84740)
Trifluoroacetic acid (PubChem CID: 6422)
Phenanthroline (PubChem CID: 1318)
Pyrogallol (PubChem CID: 1057)
1,1-Diphenyl-1-picrylhydrazyl (PubChem CID: 2735032)
Ascorbic acid (PubChem CID: 54670067)

*Keywords:*
Blackcurrant
Polysaccharides
Pressurised water extraction
Response surface methodology
Chemical characterisation
Antioxidant activity

ABSTRACT

Pressurised water extraction (PWE) of polysaccharides from blackcurrant fruits was investigated using a response surface methodology (RSM). The optimal conditions for PWE were: time 51 min, pressure 1.6 MPa, and temperature 52 °C. Under these conditions, the experimental yield of *Ribes nigrum* L. polysaccharides (RNLP) was 11.68 ± 0.12%, which closely agreed with the predicted value (11.77%). After preliminary purification with D4006 macroporous resin, RNLP I was obtained and its chemical characterisation was undertaken by GC, HPLC, and IR spectroscopy. RNLP I was composed of rhamnose, arabinose, xylose, mannose, galactose, and glucose with a molar ratio of 2.89:14.82:1.02:1.00:2.53:6.39 and its molecular weight was $1.49 \times 10^4$ kDa. The antioxidant activity of RNLP I was evaluated by free radical scavenging assays and a reducing power assay *in vitro*. RNLP I showed strong DPPH and superoxide radical scavenging activities and reducing power.



## 1. Introduction

Blackcurrant (*Ribes nigrum* L.) is a deciduous shrub, native to Northern and Central Europe as well as to Asia. It is also widely cultivated in North America for berry production. Blackcurrant is rich in many health-beneficial substances, such as polysaccharides, unsaturated fatty acids, different vitamins, organic acids, anthocyanins, and flavonoids (Liu, Kallio, & Yang, 2014). The interest in blackcurrant for human consumption has increased because of its nutritional and health benefits, including antitumor, antioxidant, anti-inflammatory, anticoagulant, and antimicrobial effects (Bishayee et al., 2011; Miladinović et al., 2014). During recent years, the *Ribes nigrum* L. polysaccharides (RNLP) have been attracting attention for their potent immunostimulatory and antitumor activities (Takata, Yamamoto, Yanai, Konno, & Okubo, 2005). In addition, RNLP has been used in cosmetics and dermatology for skin regeneration and neurodermatitis (Zippel, Deters, Pappai, & Hensel, 2009). Recently RNLP has been described as having anti-adhesive activity against *Helicobacter pylori* (Messing, Niehues, Shevtsova, Borén, & Hensel, 2014). Thus, an efficient technology capable of obtaining large quantities of high-quality RNLP is needed to realise these promising applications.

In general, botanical polysaccharides are extracted using water or aqueous organic solvents (Chen, Zhang, Jiang, Mu, & Miao, 2012; Wang et al., 2012). However, as the cell wall consists of complex polymers, it is not easy to extract active polysaccharides using a solvent extraction process (Wijesinghe & Jeon, 2012). Thus, some assistant methods are also used to improve this extraction process, such as microwaving (Zeng, Zhang, Gao, Jia, & Chen, 2012), ultrasonication (Prakash Maran, Manikandan, Thirugnanasambandham, Vigna Nivetha, & Dinesh, 2013), enzymatic action (Zhu et al., 2014), the pressurised water extraction method (Lo, Tsao, Wang, & Chang, 2007), *etc*. The main advantages of these techniques are

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http://dx.doi.org/10.1016/j.foodchem.2015.08.061
0308-8146/

their reduced extraction time, lower solvent volume, and higher yield. Among current extraction procedures, pressurised water extraction (PWE) seems to be a relatively new and promising technique. PWE is an environmentally clean extraction process, which is based on the use of water at temperatures and pressures high enough to maintain the solvent in its liquid state throughout the extraction procedure (Beyer & Biziuk, 2008). High temperature and pressure both increase the solubility of target compounds and the solvent diffusion rate, and decrease the solvent viscosity and surface tension, allowing better penetration into the sample matrix (Lou, Janssen, & Cramers, 1997). PWE has been demonstrated to be useful in the extraction of biologically active substances (Lo et al., 2007; Truong, Hu, Thompson, Yencho, & Pecota, 2012; Xynos et al., 2014). To the best of our knowledge, there is no report available about the use of PWE technology in RNLP extraction.

Response surface methodology (RSM), as an effective statistical tool, can be used to evaluate interactions among various factors, as well as simultaneously estimate the effects of several process variables and their interactions on response variables (Prakash Maran et al., 2013). RSM has been applied successfully to improve and optimise the bioactive compound extraction process (Chen, Wang, Zhang, & Huang, 2012; Ye & Jiang, 2011; Zhu et al., 2014). However, to date no detailed investigation has been conducted on the optimisation of RNLP extraction with PWE using RSM.

In the current study, the RSM saw a Box–Behnken design (BBD) used to optimise the operational parameters (temperature, time, pressure) of PWE to obtain the maximum yield of RNLP. Furthermore, the basic characteristics of RNLP I obtained by the purification with D4006 macroporous resin were estimated using high-performance liquid chromatography (HPLC), gas chromatography (GC), and infrared spectroscopy (IR). In addition, the antioxidant properties of RNLP I, that is, the radical scavenging and reducing capacities, were evaluated through *in vitro* assays such as: DPPH, hydroxyl and superoxide radical scavenging, and reducing power.

## 2. Materials and methods

### 2.1. Materials

Blackcurrant (*Ribes nigrum* L., Heifeng) fruits were obtained from Harbin (Heilongjiang, China). The fruits were harvested at the fully mature stage, then were washed and stored at −20 °C until used for further analysis. 1,1-Diphenyl-1-picrylhydrazyl (DPPH), phenanthroline, pyrogallol, and L-(+)-ascorbic acid (Vc) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Other chemicals were of analytical grade. All aqueous solutions were prepared with deionised water purified by a Milli-Q Water Purification system (Millipore, MA, USA). D4006 macroporous resin was made in a chemical plant at Nankai University (Tianjin, China).

### 2.2. Extraction of RNLP

Before the extraction process, the berries were gently defrosted and crushed in a tissue homogenisation machine (JJ-2, Changzhou Guohua Electric Appliance Co., Ltd., Jiangsu, China). A portion of homogenate (20 g) and deionised water at different liquid to solid ratios (10:1–50:1 (expressed as *w*/*w* throughout)) were put into a 500 mL beaker, then the polysaccharides were extracted at different temperatures (40–90 °C) in a laboratory-scale high pressure reactor (WHF-0.25, Weihai Auto-Reactor Co., Ltd., Shandong, China) at various pressures (1.2–2.0 MPa) for various periods (20–60 min). A picture of PWE extraction equipment was provided as Supplementary Material. The extract was centrifuged at 3500 rpm for 20 min and the supernatant was gathered by membrane filtration (0.45 μm, Millipore, USA). The filtrate was diluted with deionised water to determine the yield of polysaccharide. The yield of polysaccharide $Y$ was calculated as $Y\ (\%) = c \times v / w \times 100\%$, where, $c$ is the concentration of polysaccharide in the sample solution (mg/mL), $v$ is the volume of sample solution (mL), and $w$ is the mass of the fresh sample (mg).

The polysaccharide content was calculated as a total sugar content minus the reducing sugar content (Xiao et al., 2009). Total sugar and reducing sugar contents were determined by phenol–sulphuric acid and 3,5-dinitrosalicylic acid tests, using D-glucose as a standard, respectively (Hu et al., 2005). Hot water extraction (HWE) was performed as a control experiment. The extraction method was as follows: temperature 80 °C, time 2 h, liquid to solid ratio 30:1 (*v*/*w*) (Luo, Ni, & Zhou, 2011).

### 2.3. Single-factor PWE experiments

A series of single factor experiments were carried out to examine the effects of each factor on the extraction and to identify the independent variables and appropriate ranges of the BBD. The effect of each factor was evaluated by determining the yield of RNLP.

### 2.4. Experimental design

#### 2.4.1. Optimisation of PWE by RSM

RSM was used to determine the optimal processing conditions of PWE for RNLP. Three processing variables including time, temperature, and pressure were chosen based on the results of single-factor experiments. Then, the effects of these key variables were investigated using BBD and the yield ($Y$) was taken as the response of the design experiments. The range of independent variables and their levels are presented in Table 1. The selected variables were coded according to the following equation:

$$x_i = \frac{X_i - X_0}{\Delta X} \quad i = 1,\ 2,\ 3 \tag{1}$$

where $x_i$ and $X_i$ are the dimensionless coded and actual values for the $i$th independent variable, respectively, $X_0$ is the actual value for the $i$th independent variable at the centre point, and $\Delta X$ is the step change value.

**Table 1**
Factors and levels for response surface methodology, Box–Behnken design matrix (in coded and uncoded levels of three variables), experimental data and predicted values for three-level-three-factor response surface analysis.

| Run | Variable levels | | | Yield of RNLP (%) | |
|---|---|---|---|---|---|
| | $X_1$ (extraction time, min) | $X_2$ (extraction pressure, MPa) | $X_3$ (extraction temperature, °C) | Observed | Predicted |
| 1 | 50(0) | 1.6(0) | 50(0) | 11.69 ± 0.05 | 11.73 |
| 2 | 50(0) | 1.6(0) | 50(0) | 11.81 ± 0.12 | 11.73 |
| 3 | 40(−1) | 1.8(1) | 50(0) | 10.48 ± 0.06 | 10.56 |
| 4 | 60(1) | 1.6(0) | 40(−1) | 9.86 ± 0.09 | 9.94 |
| 5 | 40(−1) | 1.6(0) | 60(1) | 10.43 ± 0.10 | 10.35 |
| 6 | 50(0) | 1.8(1) | 40(−1) | 10.58 ± 0.08 | 10.45 |
| 7 | 50(0) | 1.4(−1) | 40(−1) | 10.01 ± 0.03 | 10.01 |
| 8 | 50(0) | 1.6(0) | 50(0) | 11.65 ± 0.13 | 11.73 |
| 9 | 50(0) | 1.8(1) | 60(1) | 10.82 ± 0.11 | 10.82 |
| 10 | 40(−1) | 1.6(0) | 40(−1) | 10.17 ± 0.02 | 10.22 |
| 11 | 60(1) | 1.8(1) | 50(0) | 10.77 ± 0.05 | 10.82 |
| 12 | 50(0) | 1.4(−1) | 60(1) | 10.59 ± 0.07 | 10.72 |
| 13 | 60(1) | 1.6(0) | 60(1) | 10.93 ± 0.14 | 10.88 |
| 14 | 40(−1) | 1.4(−1) | 50(0) | 10.48 ± 0.02 | 10.43 |
| 15 | 60(1) | 1.4(−1) | 50(0) | 10.49 ± 0.09 | 10.41 |
| 16 | 50(0) | 1.6(0) | 50(0) | 11.87 ± 0.04 | 11.73 |
| 17 | 50(0) | 1.6(0) | 50(0) | 11.65 ± 0.05 | 11.73 |