Carbohydrate Polymers 83 (2011) 600–607

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Extraction of antioxidant pectic-polysaccharide from mangosteen (*Garcinia mangostana*) rind: Optimization using response surface methodology

Chee-Yuen Gan*, Aishah A. Latiff

Doping Control Centre, University Sains Malaysia, 11800 USM, Penang, Malaysia

ARTICLE INFO

Article history:
Received 6 August 2010
Accepted 11 August 2010
Available online 18 August 2010

Keywords:
Extraction
Mangosteen
Garcinia mangostana
Optimization
Pectic-polysaccharide
Response surface methodology

ABSTRACT

Box-Behnken design (BBD) was employed to optimize the incubator temperature (X_1: 50–80 °C), extraction time (X_2: 2–4 h) and pH (X_3: 2–4) to obtain a high antioxidant pectic-polysaccharide yield with high uronic acid content and antioxidant activity from mangosteen rind. Analysis of variance showed that the contribution of quadratic model was significant for the extraction yield and antioxidant activity whereas linear model was significant for pectin content. Optimization study using response surface methodology was performed and 3D response surfaces were plotted from the mathematical model. Two optimal conditions were given: condition (1) X_1 = 80.0 °C; X_2 = 3.93 h; X_3 = 2.45, and condition (2) X_1 = 67.7 °C; X_2 = 3.67 h; X_3 = 2.00. These optimum conditions yielded pectic-polysaccharide of ~12.0–12.4%, uronic acid content of ~20.2–21.1 mg/g, and %DPPHsc/g extract of 225–252, respectively. Close agreement between experimental and predicted values was found. This could therefore be applied in extraction of mangosteen-derived functional pectic-polysaccharide in industry.

1. Introduction

Lignocellulosic biomass is the most abundant plant-derived materials (e.g. agricultural residues, herbaceous crops, forestry wastes, woods) that have been left behind by the agro-food industries due to the modern agriculture practices that had increased food production yield tremendously and parallel to this is the amount of waste generated from this large scale cultivation. This occurrence has brought up a serious concern in environmental issues as well as the alternatives to solve the problems encountered. Globally, part of these waste materials have been used for animal feed and fertilizer (Mamma, Kourtoglou, & Christakopoulos, 2008), however, a large portion of these materials is still deposited annually. In recent decades, lignocellulosic biomass has attracted attention in biorefinery process. It was suggested that this material could be the largest potential feedstock for bioethanol production (Huang, Ramaswamy, Tschirner, & Ramarao, 2008; Kim et al., 2010; Kszos, 2006). Apart from bioethanol, natural products should also be considered. Therefore, food, nutraceutical and pharmaceutical industries have come into place where the industries are searching for new ingredients from natural sources (Guerrero, Torres, & Nuñez, 2008; Kasankala, Xue, Weilong, Hong, & He, 2007; Levigne, Ralet, & Thibault, 2002; Masmoudi et al., 2008; Wu, Cui, Tang, & Gu, 2007).

Apart from cellulose, all plant cell walls have a similar structure that consists of pectins (also known as pectic-polysaccharides) (Lerouxel, Cavalier, Liepman, & Keegstra, 2006). These plant-derived materials are widely used as gelling agents, thickeners, stabilisers, emulsifiers and fat-substitutes, and are listed as ingredients in numerous food products (Rolin, Nielsen, & Glahn, 1998; Willats, Knox, & Mikkelsen, 2006). Citrus peels and apple pomace are currently the important sources of pectin manufacturing, whilst other potentially valuable sources remain largely unexplored. Recent published work showed that *Parkia speciosa* pod could produce functional pectic-polysaccharide (Gan, Abdul Manaf, & Latiff, 2010a, 2010b). Wong, Alkarkhi, and Easa (in press, 2010) have also extracted this pectic-polysaccharides from durian rind and found that this extracted pectic-polysaccharide could act as biosorbent to remove heavy metal such as lead, nickel, and copper. Water-soluble pectin extracted from a durian rind was also found to have wound healing properties (Hokputsa et al., 2004). Other health effects of pectins, such as lowering cholesterol and serum glucose levels (Yamada, 1996; Behall & Reiser, 1986), inducing apoptosis in human colonic adenocarcinoma cells (Olano-Martin, Rimbach, Gibson, & Rastall, 2003) and anticancer activities (Yamada, Kiyohara, & Matsumoto, 2003), were also evident. Hence, this search for plant-derived biomaterials has therefore stimulated research interest in producing functional components from under-utilized bulk agro-waste, such as fruit peels.

In the current study, mangosteen (*Garcinia mangostana*) rind was used. Mangosteen, known as "queen of fruits", is cultivated in Southeastern Asia. The fruit is whitish colour with soft texture whereas the rind is firm and dark purple in colour. Traditionally,

* Corresponding author. Tel.: +60 4 6595606x2682; fax: +60 4 6569869.
E-mail address: mattgan81@yahoo.com (C.-Y. Gan).

0144-8617/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2010.08.025

Table 1
Experimental domain of Box-Behnken design (BBD).

Variables	X_j		Factor levels		
	Uncoded	Coded	−1	0	1
Temperature (°C)	X_1	x_1	50	65	80
Time (h)	X_2	x_2	2	3	4
pH	X_3	x_3	2	3	4

the latter is used for skin infection and wound treatments. It is also widely used to against inflammation, diarrhea, cholera and dysentery. Other medicinal properties of the rind were listed by Pedraza-Chaverri, Cárdenas-Rodríguez, Orozco-Ibarra, and Pérez-Rojas (2008). Up to date, several researches have demonstrated that mangosteen rind possessed antioxidant, antitumoral, antiallergy, antiinflammation, antibacterial and antiviral activities. In this matter, xanthones were claimed to be responsible for these biological activities (Suksamrarn et al., 2006; Pedraza-Chaverri et al., 2008). Preliminary study showed that the extract possessed high antioxidant pectic-polysaccharide. Following the discovery of medicinal phytochemicals, extraction of antioxidant pectic-polysaccharide from mangosteen rind should also be conducted. This material may suggest possible therapeutic applications that related to mangosteen in future.

The objectives of this study were to explore the potential of mangosteen rind in producing pectic-polysaccharide and to optimize the conditions for the extraction of pectic-polysaccharide that obtain high extraction yield, uronic acid content and antioxidant activity (i.e. %DPPHsc/g extract). Response surface methodology (RSM) was applied to fit and to exploit a mathematical model representing the relationship between the responses (i.e. extraction yield, uronic acid content and %DPPHsc/g extract) and variables (i.e. temperature, extraction time and pH).

2. Materials and methods

2.1. Materials

One batch of 20 kg of mangosteen was purchased from local market (Air Itam market) located in Penang, Malaysia. The raw samples were rinsed with distilled water to remove other impurities (such as dust and pesticide). The rinds were immediately separated from the fruits and the former was lyophilised and milled. The powder obtained was sieved (60-mesh size screen) and stored at 4 °C until use. All chemicals (ethanol, disodium phosphate and citric acid) used in the experiment were of analytical grade and were purchased from Sigma–Aldrich (Malaysia).

2.2. Extraction of pectic-polysaccharide

Extractions were carried out in a conical flask placed in an incubator shaker (IKA KS 4000i, Germany) as follows according to Masmoudi et al. (2008): 1.0 g of lyophilised mangosteen rind powder (MRP) was added to 50 ml of the citrate–phosphate buffer (solid–liquid ratio: 1:50, w/v) at different pH in each flask. The pH's of the mixtures were adjusted with 0.1 M HCl/NaOH and subsequently incubated in an incubator with constant agitation (250 rpm) at different incubation temperatures and times (Table 1). The resulting slurries were immediately filtered through a muslin cloth after incubation. The filtrates were then centrifuged at 20 °C for 30 min at 5000 × *g* to remove the remaining solid particles. Two volumes of 95% (v/v) ethanol were subsequently added to one volume of the extracts in order to precipitate the extracted pectic-polysaccharide (EPP). The obtained mixtures were kept for 2 h at 4 °C prior to filtration. The precipitates were washed three times with 50, 75 and 100% ethanol and filtered in order to remove the mono- and disaccharides. The EPP were then dried at 50 °C to a constant weight. The extraction yields (*Y*), subject of this study, were calculated as follows:

$$Y\ (\%,\ \text{w/w}) = \frac{W_{EPP}}{W_{MRP}} \times 100 \quad (1)$$

where W_{EPP} was defined as weight of EPP whereas W_{MRP} was defined as weight of MRP used.

2.3. Determination of uronic acid

Uronic acid was determined by m-hydroxydiphenyl method (Blumenkrantz & Asboe-Hansen, 1973). Samples (0.5 ml) were mixed thoroughly with 3.0 ml of 0.0125 M sodium tetraborate solution (in concentrated sulphuric acid) in an ice bath. The mixtures were heated in a boiling bath for 5 min and subsequently cooled in an ice bath. The mixtures were added with 0.05 ml of 0.15% *m*-phenylphenol (in 0.5% sodium hydroxide solution). The absorbances at 520 nm were recorded after standing for 5 min. Standard curve was obtained using galacturonic acid (0–100 μg/ml).

2.4. Antioxidant activity

The DPPH free radical scavenging activity of each sample was determined according to Liu et al. (2009). EPP was reconstituted with distilled water and pre-diluted (20×). Aliquots of each sample (0.1 ml) were added to 3 ml of methanolic DPPH solutions (0.1 mM). Discolorations were measured at 517 nm after incubation for 30 min at 30 °C in the dark. The %DPPH which was scavenged ($\%DPPH_{sc}$) was calculated using:

$$\%DPPH_{sc}/\text{g extract} = \left\{ \frac{A_{cont} - A_{sample}}{A_{cont}} \right\} \times \frac{100}{W_{EPP}} \quad (2)$$

where A_{cont} was defined as absorbance of the control, A_{sample} was defined as absorbance of the sample (the extracts) whereas W_{EPP} was defined as weight of EPP.

2.5. Experimental design

The extraction parameters were optimized using RSM. A Box-Behnken design (BBD) was employed in this regard. Incubator temperature (X_1), extraction time (X_2) and pH (X_3) were chosen for independent variables. The range and center point values of three independent variables presented in Table 1 were based on the results of preliminary experiments. The experimental design consists of twelve factorial points and five replicates of the central point (Table 2). Yields of EPP, uronic acid content and %DPPHsc/g extract were selected as the responses for the combination of the independent variables given in Table 2. Three experiments of each condition were carried out and the mean values were stated as observed responses. Experimental runs were randomized to minimize the effects of unexpected variability in the observed responses.

The variables were coded according to the equation:

$$x = \frac{(X_i - X_o)}{\Delta X} \quad (3)$$

where *x* is the coded value, X_i is the corresponding actual value, X_o is the actual value in the center of the domain, and ΔX is the increment of X_i corresponding to a variation of 1 unit of *x*.

The mathematical model corresponding to the composite design is:

$$Y = \beta_o + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=1+1}^{3} \beta_{ij} X_i X_j + \varepsilon \quad (4)$$