Carbohydrate Polymers 106 (2014) 179–189

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Ultrasound-assisted extraction of pectins from grape pomace using citric acid: A response surface methodology approach

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ARTICLE INFO

Article history:
Received 22 October 2013
Received in revised form 26 January 2014
Accepted 5 February 2014
Available online 14 February 2014

Keywords:
Pectin
Molecular weight
Degree of esterification
Grape pomace
Ultrasound-assisted extraction
Response surface methodology

ABSTRACT

An ultrasound-assisted procedure for the extraction of pectins from grape pomace with citric acid as the extracting agent was established. A Box–Behnken design (BBD) was employed to optimize the extraction temperature (X_1: 35–75 °C), extraction time (X_2: 20–60 min) and pH (X_3: 1.0–2.0) to obtain a high yield of pectins with high average molecular weight (MW) and degree of esterification (DE) from grape pomace. Analysis of variance showed that the contribution of a quadratic model was significant for the pectin extraction yield and for pectin MW whereas the DE of pectins was more influenced by a linear model. An optimization study using response surface methodology was performed and 3D response surfaces were plotted from the mathematical model. According to the RSM model, the highest pectin yield (~32.3%) can be achieved when the UAE process is carried out at 75 °C for 60 min using a citric acid solution of pH 2.0. These pectic polysaccharides, composed mainly by galacturonic acid units (<97% of total sugars), have an average MW of 163.9 kDa and a DE of 55.2%. Close agreement between experimental and predicted values was found. These results suggest that ultrasound-assisted extraction could be a good option for the extraction of functional pectins with citric acid from grape pomace at industrial level.

1. Introduction

Recently, there has been an increasing concern for the preservation of the environment and sustainability of resources. Thus, the utilization of natural resources is receiving renewed interest as an alternative to non-renewable resources in material technology (Galanakis, 2013; Liu, Cao, Huang, Cai, & Yao, 2010).

Fruit processing in the food industry gives rise to large amounts of by-products. Grapes (*Vitis vinifera* L.) belong to the world's largest fruit crops with a global production of around 68 million tons in 2009 (FAOSTAT, 2011). Since about 75–80% of the total amount is used in winemaking, around 12 million tons of grape pomace is produced within a few weeks of the harvest campaign. Grape pomace constitutes the main by-product, and is of great interest to the food industry, since the available carbohydrate fraction can be used to provide dietary fiber and other bioactive compounds (González-Centeno et al., 2010, 2012).

An emerging field of clinical importance is the growing interest in the role of dietary carbohydrates. Previous reports suggest that polysaccharides possess a wide range of pharmacological properties such as anti-tumor and antioxidant, as well as anti-diabetic activity and immunity-modulation. Thus, one of the most characterized bioactive roles for pectin is as an anti-cancer agent (Glinsky & Raz, 2009; Kwon, Qiu, Hashimoto, Yamamoto, & Kimura, 2009; Morris, Gromer, Kirby, Bongaerts, & Patrick Gunning, 2011; Yan et al., 2011). Pectin is also a high-value functional food ingredient widely used as gelling and stabilizing agent. It is also an abundant, ubiquitous and multifunctional component of the cell walls of all land plants (Willats, Knox, & Mikkelsen, 2006). The pectin is a group of polysaccharides in which the presence of partly methyl-esterified galacturonic acid and, to a lesser extent, rhamnose is a distinctive feature. The molecular weight (MW) and the degree of esterification (DE) of pectic polysaccharides affect the commercial use of pectin as gelling and thickening agents (Atmodjo, Hao, & Mohnen, 2013; Morris, Ralet, Bonnin, Thibault, & Harding, 2010; Morris, Foster & Harding, 2000; Prakash, Sivakumar, Thirugnanasambandham, & Sridhar, 2013).

Industrially, pectins are extracted from apple pomace, sugar beet pulp, and citrus peels using water acidified with a strong mineral acid, notably, nitric, hydrochloric or sulphuric acid (the so-called conventional acid extraction) under pH, temperature, and duration conditions, most often, in the order of 1.4–3, 60–100 °C,

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http://dx.doi.org/10.1016/j.carbpol.2014.02.013
0144-8617/

and 20–360 min, respectively (Min et al., 2011; Prakash Maran et al., 2013a, 2013b). Moreover, pectins produced using low pHs (<2) and high temperatures are, generally, enriched in galacturonic acid (or homogalacturonic building blocks) as a result of substantial hydrolysis of pectin neutral sugars present in rhamnogalacturonic regions. However, the well-known toxicity of these strong mineral acids, and the environmentally unfriendly (corrosive) effluents they generate, are their main drawback. Special treatments, able to remove potentially toxic elements from pectin extracts, are therefore needed for the extracted product to abide by the GRAS (generally recognized as safe) status and to be accepted for consumption (Yapo, 2009).

In connection with the emerging concept of 'Green Chemistry', recent emphasis has been given to nonconventional chemistry based on a combination of chemical and physical treatments. Thus, the processing for pectin isolation is no exception, thereby introducing environment- and human-friendly technology (Galanakis, 2013; Yapo, 2009). Recent studies have reported that citric acid is a good pectin extracting agent with regard to the pectin yield and the main intrinsic parameters (DE and average MW) governing gel formation (Canteri-Schemin, Fertonani, Waszczynskyj, & Wosiacki, 2005; Pinheiro et al., 2008; Yapo, 2009). Furthermore, power ultrasound has a great potential in a wide variety of technological processes. The technique has been used in commercial applications for many years, and over the past two decades, application of ultrasound in chemistry as well as the food and pharmaceutical industries has become an exciting new field of research. In fact, ultrasound has been recognized as an alternative approach to traditional extraction methods (Awad, Moharram, Shaltout, Asker, & Youssef, 2012; Ebringerová & Hromádková, 2010; Hromádková, Ebringerová, & Valachovic, 1999; Galanakis, 2013; Rastogi, 2011).

Ultrasound-assisted extraction (UAE) is a process that uses acoustic energy and solvents to extract target compounds from various plant matrices. The extraction mechanism involves two types of physical phenomena: diffusion through the cell walls and washing out (rinsing) the cell contents once the walls are broken both of which are significantly affected by ultrasound irradiation (Vinatoru, 2001). Although the application of UAE of different compounds from plant material has been widely published (Ebringerová & Hromádková, 2010; Esclapez, García-Pérez, Mulet, & Cárcel, 2011), few studies have focused on pectin extraction. Increased yields as well as an important reduction in extraction time have been reported as the main advantages of using ultrasounds (Bagherian, Ashtiani, Fouladitajar, & Mohtashamy, 2011; Panchev, Kirtchev, & Kratchanov, 1988). To the best of our knowledge, there are no reports of pectin extraction combining the use of ultrasound technology and citric acid.

At present the economical feasibility of an industrial process also requires working in such a way that high extraction efficiency is attained. Many factors have been established to improve the extraction efficacy, such as temperature, time and solvent type. When several factors and their potential interactions may affect a desired response, response surface methodology (RSM) is an effective tool to optimize the process (Nwabueze, 2010). RSM has been very popular for optimization studies in recent years. In fact, RSM has been used to carry out the optimization of pectin extraction process from apple pomace (Shin, Kim, Cho, & Hwang, 2005; Wang et al., 2007), banana peel (Qiu et al., 2010), passion fruit peels (Pinheiro et al., 2008), cacao pod husks (Vriesmann, Teófilo, & Petkowicza, 2011), mangosteen rind (Gan & Latiff, 2011), durian rind (Wai, Alkarkhi, & Easa, 2009), orange peels (Prakash Maran et al., 2013a, 2013b) and Pumpkin (Prakash Maran et al., 2013a, 2013b).

The aim of this study is to optimize the ultrasonic assisted extraction of pectins from grape pomace, using citric acid as the extracting agent. For this purpose, the RSM technique was applied considering the extraction temperature, extraction time and pH as variables.

2. Experimental

2.1. Sample

Pomace of the grape variety Cabernet Sauvignon was provided by a winery in Mallorca (Spain). The grape pomace of Cabernet Sauvignon, mainly composed of pressed skins and seeds, had a moisture content of ~62 g/100 g fresh weight. The grape pomace was milled to an average particle size of ~2 mm, with a commercial mill (Braun KSM 2, Mexico City, Mexico), and then vacuum-packed and stored at −20 °C until extractions.

2.2. Ultrasound-assisted extraction (UAE) of pectin from grape pomace

Pectin was extracted using constant ultrasonic power in an ultrasonic bath with internal dimensions of 24.0 cm × 13.7 cm × 15.0 cm and a capacity of 4.25 L (Elmasonic S 40 H, Singen, Germany). The variable conditions were extraction temperature (X_1), extraction time (X_2), and pH (X_3) of the citric acid solution.

To determine the power that was acting on the sample, a characterization of the ultrasonic bath was performed according to the equation (Eq. (1)) proposed by Raso et al. (1999). The calculated power was expressed as power density (Sivakumar and Pandit, 2001).

$$\text{Power Density} = \frac{dT}{dt} c_p m \quad (1)$$

where c_p is the heat capacity of the solvent (in J kg^{-1} K^{-1}) and m is the mass of solvent used (in kg). The solvent used in all experiments was citric acid. Thus, the ultrasonic bath was working at a frequency of 37 kHz, power output of 140 W and power density of 0.05 W mL^{-1}.

The extraction was carried out according to the methods proposed by Canteri-Schemin et al. (2005) and Panchev, Kirtchev, and Kratchanov (1994), with slight modifications. Ten grams of grape pomace were mixed with 100 mL of citric acid solution. The flask was placed in the center of the bath using the conditions of experimental design. The hot acid extract was filtered through a screen of 1 mm mesh, created by two layers of cheesecloth, then the filtrate was cooled down to 4 °C. Following that, the filtrate (containing pectin) was precipitated with ethanol 95% (1:2, v/v) and stirred for 10 min. Next, the mixture was centrifuged at 1750 × g for 30 min. The precipitated pectin was separated by filtration (#40 glass fiber) and rinsed twice with ethanol 95% and acetone. The pectin extracted ultrasonically was dried at room temperature for 12 h. Finally, the resulting material was milled to obtained powdered pectin. The yield was calculated as grams of product obtained per 100 g of grape pomace (fresh weight). Uronic acids were determined by colorimetry (Blumenkrantz & Asboe-Hansen, 1973), using samples hydrolyzed for 1 h at 100 °C in 1 M H_2SO_4. Results showed that uronic acid accounted for 96–99% of the pectin extracted in all samples.

2.3. Determination of the molecular weight

The average molecular weight (MW) of extracted pectins was determined by using high performance size exclusion chromatography connected with a refractive index detector (Agilent 1100 series, CA, USA). Pectin material (3 mg) was dissolved in 50 mM sodium nitrate (3 mL) and filtered through a 5 μm filter. Then,