Carbohydrate Polymers 140 (2016) 59–65

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Optimization of microwave assisted extraction of pectin from sour orange peel and its physicochemical properties

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ARTICLE INFO

Article history:
Received 9 October 2015
Received in revised form
17 November 2015
Accepted 20 December 2015
Available online 23 December 2015

Keywords:
Sour orange
Pectin
Optimization
Degree of esterification

ABSTRACT

Microwave assisted extraction technique was used to extract pectin from sour orange peel. Box–Behnken design was used to study the effect of irradiation time, microwave power and pH on the yield and degree of esterification (DE) of pectin. The results showed that the optimum conditions for the highest yield of pectin (29.1%) were obtained at pH of 1.50, microwave power of 700 W, and irradiation time of 3 min. DE values of pectin ranged from 1.7% to 37.5%, indicating that the obtained pectin was low in methoxyl. Under optimal conditions, the galacturonic acid content and emulsifying activity were 71.0 ± 0.8% and 40.7%, respectively. In addition, the emulsion stability value ranged from 72.1% to 83.4%. Viscosity measurement revealed that the solutions of pectin at low concentrations showed nearly Newtonian flow behavior, and as the concentration increased, pseudoplastic flow became dominant.

1. Introduction

Pectin is a complex mixture of polysaccharides in cell walls of all land plants, which is composed mainly of D-galacturonic acid and neutral sugars, such as L-rhamnose, L-arabinose, and D-galactose (Axelos, Thibault, & Lefebvre, 1989; Basanta, Ponce, Rojas, & Stortz, 2012; Pagan & Ibarz, 1999). The majority of the structure of pectin contains a backbone of $\alpha(1 \rightarrow 4)$ linked to D-galacturonic acid units that are partially esterified with methanol or acetic acid at the carboxylic acid (Xu et al., 2014; Yapo, Robert, Etienne, Wathelet, & Paquot, 2007).

The degree of esterification (DE; is defined as the percentage of carboxyl groups esterified with methanol) of the pectin is an important parameter for the definition of their application (Santos, Espeleta, Branco, & de Assis, 2013; Seixas et al., 2014). Depending on DE, pectin is divided into two major groups: high methoxyl pectin (HMP), with DE higher than 50% and low methoxyl pectin (LMP), with DE lower than 50% (Thakur, Singh, Handa, & Rao, 1997; Yapo et al., 2007). HMP form gels when heated in solutions with a low pH (2–3.5) and at high concentrations of sugar (55–75%); while LMP can gel in a wide pH range (2–6) with or without a little amount of sugar in the presence of divalent ions such as calcium (Ca^{2+}) (Lofgren & Hermansson, 2007; Lopes da Silva & Rao, 2006; Wai, Alkarkhi, & Easa, 2010). HMP and LMP have different physicochemical properties and thus different applications (Chan & Choo, 2013). Pectin is used as a gelling agent, stabilizer, emulsifier and thickener in the food industry for the production of jams and jellies (Liu, Cao, Huang, Cai, & Yao, 2010; Thakur et al., 1997). In addition, LMP may be used as a gelling agent in low calorie products for diabetics and overweight patients (Gardner, Schwartz, Krista, & Merimee, 1984; Wai et al., 2010).

The majority of sources for commercial pectin production are citrus peels, apple pomace and sugar beet pulp (May, 1990; Mesbahi, Jamalian, & Farahnaky, 2005). Also, pectin from non-commercial sources such as cocoa husks (Chan & Choo, 2013), mulberry branch bark (Liu et al., 2010), peach pomace (Pagan & Ibarz, 1999), sisal waste (Santos et al., 2013), watermelon rind (Maran, Sivakumar, Thirugnanasambandham, & Sridhar, 2014), soy hull (Kalapathy & Proctor, 2001), pumpkin (Ptichkina, Markina, & Rumyantseva, 2008) and banana peel (Qiu et al., 2010) have been studied.

Citrus aurantium is the scientific name for a plant that is commonly called sour orange, bitter orange or seville orange (Haaz et al., 2006). This fruit is one of the citrus fruits that exist in large quantity in northern and southern Iran (Khormaei, Nasernejad, Edrisi, & Eslamzadeh, 2007). Due to its sour and bitter taste, it is used less often as edible fruit, but the fruit is more widely used as a medicinal or dietary supplement (He, Lian, Lin, & Bernart, 1997). Sour orange peel is a by-product of fruit juice factories, and it is largely used as animal feed. The use of this waste as a new source for the production of high value-added products such as pectin can be valuable.

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http://dx.doi.org/10.1016/j.carbpol.2015.12.051

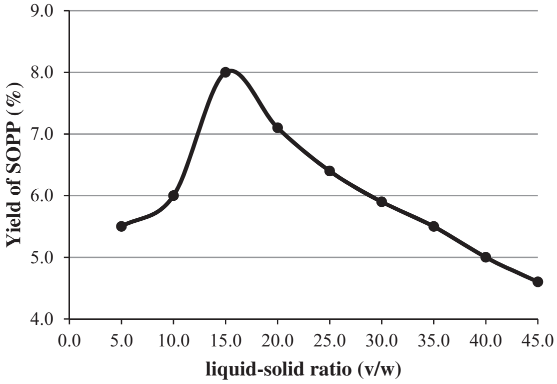

Fig. 1. Effects of different LSR on extraction yield of SOPP with irradiation time of 2 min, microwave power of 500 W, pH of 2.25.

In the traditional extraction process, pectin is extracted using hot water acidified with mineral acids (Xu et al., 2014). The use of such strong acids in the extraction step leads to corrosion of equipment and devices as well as damage to the environment. In addition, the process is time consuming and leads to pectin degradation (Seixas et al., 2014). The need for replacement of this extraction method with newer and better methods could be a potential means of minimizing the stated adverse effects. Microwave assisted extraction is an interesting alternative to traditional extraction methods, and it has many advantages such as shorter processing time, less solvent, higher extraction yield, better quality products with lower production cost (Bagherian, Ashtiani, Fouladitajar, & Mohtashamy, 2011; Kratchanova, Pavlova, & Panchev, 2004).

The aim of this paper is to develop a microwave assisted extraction of sour orange peel pectin (SOPP) as a new source of pectin and to obtain optimum conditions for maximum extraction yield using Box–Behnken experimental design, and to study the physicochemical properties of the pectin extracted from sour orange fruit peel.

2. Materials and methods

2.1. Raw materials and reagents

Sour orange (*C. aurantium* L.) peels were obtained from a fruit orchard in Fasa, Fars, Iran. The peels were removed, finely cut with a sharp knife. The cut fragments were placed on stainless trays, and dried in a hot air oven at 50 °C until it attained a constant weight. The dried peels were pulverized and passed through a 40-mesh sieve to obtain powdered sample. The powder was stored in dark bags and kept in a dry environment prior to the experimental analysis. Citric acid, sodium hydroxide, hydrochloric acid, phenolphthalein reagent, sulfuric acid, sodium tetraborate and sodium azide were purchased from Merck Chemical Co. (Darmstadt, Germany). Sigma Chemical Co. (St. Louis, MO, USA) supplied meta-hydroxydiphenyl reagent and standard citrus pectin (poly-D-galacturonic acid methyl ester).

2.2. Pectin extraction

Microwave assisted extraction of SOPP was performed according to the methods described by Li, Jia, Wei, and Liu (2012) with minor modifications. The dried peels powder (liquid–solid ratio (LSR) of 15 (v/w), Fig. 1) was poured into citric acid aqueous solution adjusted to the desired pH values (1.5, 2.25, 3), and thereafter stirred. The solution was then extracted with three powers of 300, 500, 700 W for three times, 1, 2, 3 min. After extraction, the mixture was allowed to cool down to room temperature (25 °C), filtered using filter paper, centrifuged (10 000 × g for 15 min) and the supernatant was precipitated with ethyl alcohol (96%). The coagulated pectin mass was washed with ethanol (96%) for three times to remove the monosaccharides and disaccharides (Minkov, Minchev, & Paev, 1996). In the next step, the wet pectin was dried at 40 °C in the hot air oven until a constant weight was obtained (approximately 7 h). The pectin extraction yield was calculated as follows:

$$\%\,\text{Yield} = \frac{\text{Weight of dried pectin}\ \ (\text{g})}{\text{Weight of dried powder}\ \ (\text{g})} \times 100 \qquad (1)$$

2.3. Determination of degree of esterification

The degree of esterification (DE) of the SOPP was determined by the titrimetric method (USP 26 NF 21, 2003) with a slight modification. Briefly, dried pectin sample (100 mg) was moistened with 2 ml ethanol and dissolved in 20 ml of distilled water free of carbon dioxide at 40 °C. After complete dissolution of the sample, five drops of phenolphthalein reagent were added and the sample was titrated with 0.1 M NaOH (V_1). Thereafter, 10 ml of 0.5 M NaOH was added and the sample was stirred, after which the sample was allowed to stand for 20 min for hydrolysis. In the next step, 10 ml of 0.5 M HCl was added and the sample was shaken until the pink color disappeared. Finally, five drops of phenolphthalein reagent were added and the mixture was titrated with 0.1 M NaOH until a pale pink color that persisted after vigorous shaking was obtained (V_2). The DE of the pectin (%) was calculated as follows:

$$\%\,\text{DE} = \frac{V_2}{V_1 + V_2} \times 100 \qquad (2)$$

2.4. Galacturonic acid content

The galacturonic acid (GA) content was determined by the meta-hydroxydiphenyl method using a UV–Vis spectrophotometer according to Blumenkrantz and Asboe-Hansen (1973) with minor modifications. To 1 ml of the sample (200 μg/ml), 6 ml of H_2SO_4 containing 0.0125 M sodium tetraborate was added. The tubes were cooled in a water-ice bath. The mixture was agitated with a vortex mixer and the tubes heated in a boiler for 5 min. After cooling in a water-ice bath, 20 μl of the meta-hydroxydiphenyl reagent was added. The tubes were shaken for 5 min and the absorbance was read at 520 nm. The galacturonic acid content was determined by comparison with a standard curve obtained from standard galacturonic acid solutions (0–250 μg/ml).

2.5. Emulsifying properties

Emulsifying activity (EA) and emulsion stability (ES) were assessed using the methods described by Dalev and Simeonova (1995) with minor modifications. In a nutshell, oil-in-water (O/W) emulsions were prepared by adding 5 ml of sunflower oil to 5 ml pectin solutions (0.5%, w/w) containing 0.02% sodium azide as a bactericide. The pre-emulsions were spun vigorously in a homogenizer at 10 000 × g for 3 min. In the next step, the emulsions were centrifuged in graduated tubes at 3000 × g for 5 min. The EA was calculated as follows:

$$\text{EA}\ \ (\%) = \frac{\text{EPV}}{T_v} \times 100 \qquad (3)$$

where T_v was the total volume of the system and EPV was the volume of the emulsion phase.