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Acoustic cavitation assisted extraction of pectin from waste grapefruit peels: A green two-stage approach and its general mechanism

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ABSTRACT

In the present study, acoustic cavitation assisted extraction (ACAE) which is a green approach was used for two-stage extraction of pectin from waste grapefruit peels regarding the specific two stages and different peel particle sizes. The yields, physicochemical properties and structure prediction of pectin from different peel particle sizes and different stages by two methods were compared. Highest pectin yield of 23.49% was achieved at the peel size of 0.9 mm and was slightly higher than the yield of 23.44% at finer particle size. ACAE achieved four times higher yield compared with conventional heating method (CHE) at the second stage. ACAE pectin which had lower molecular weight and degree of methoxylation was richer in rhamnogalacturonan-I (RG-I) region with long side chains compared with CHE pectin from chemical and FT-IR analysis. Based on the results, a potential general mechanism for ACAE of plant cell wall viscous polysaccharides was discussed in terms of the effects of cavitation on the swelling index and morphological study of residue to reveal the "barrier effect" during the process. Furthermore, the energy consumption for ACAE was significantly lower than the conventional method, indicating its promising application in industrial scale.

1. Introduction

Pectin is one of the ubiquitous natural polymers with high structural diversity that is found in the primary cell walls of terrestrial plants. It consists of the heteropolysaccharides family rich in D-galacturonic acid (GalA) and is capable of trapping water to form gels at low concentration. Pectin is widely used as a gelling agent, thickening agent or stabilizer in jams, confectionary, bakery and dairy products. Besides that, it can be complemented as dietary fiber, prebiotic and bioactive substances including fat replacer, functional ingredient and nutraceutical ingredient (Naqash, Masoodi, Rather, Wani, & Gani, 2017; Thakur, Singh, & Handa, 1997; Zouambia, Moulai-Mostefa, & Krea, 2009).

Nowadays, China is the largest citrus planting and harvesting country in the world contributing to nearly 70% production of canned citrus segments on the global market (Chen et al., 2017; Ministry of Agriculture, P. R. C., 2013). This process generates a tremendous amount of waste citrus peels which contain various beneficial compounds including pectin up to 20–30% dry weight. However, these peels are generally discarded or used as animal feeds in industry, which is a huge waste of valuable components and may cause environmental problems. Among them, pectin from grapefruit peel has more rhamnogalacturonan I backbones, potentially increasing the valuable bioactivities compared with other citrus fruits including lime, lemon and orange (Kaya, Sousa, Crepeau, Sorensen, & Ralet, 2014).

The conventional method for extracting pectin is soaking materials in hot water (60–100 °C) under acidic condition for several hours, which is obviously time consuming and limited yielding. Therefore, sustainable and quicker alternatives of extracting pectin from biological materials are in high demand (Adetunji, Adekunle, Orsat, & Raghavan, 2017). Fidalgo et al. (2016) has found an innovative eco-friendly process by employing only water as dispersing medium and microwave as energy source to extract pectin from waste orange and lemon peel. Matharu, Houghton, Lucas-Torres, and Moreno (2016) reported the microwave-assisted hydrothermal extraction of pectin and porous cellulose from mango peeling waste. Among these sustainable approaches,

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acoustic cavitation assisted extraction (ACAE) or ultrasound assisted extraction (UAE), has been proved a greener process with higher efficiency and less cost compared with conventional heating extraction (CHE) (Holkar et al., 2016; Jadhav, Holkar, Goswami, Pandit, & Pinjari, 2016). Our previous study has investigated the extraction kinetics, optimal extraction conditions of time, temperature and power density, along with the characteristics of the pectin processed by UAE (Wang et al., 2015; Wang et al., 2016; Xu et al., 2014). However, different extraction stages and related phenomena including swelling effect during the extraction process have not been well explored and explained.

Moreover, many researchers recently reported limited yields of pectin extracted using ultrasound technology (Bagherian, Ashtiani, Fouladitajar, & Mohtashamy, 2011; Grassino et al., 2016; Wang et al., 2016). Even though the mechanism of ACAE has been discussed since 1963 (Lott & Demaggio, 1963), most studies merely referenced theories for intracellular components and overlooked the differences from the cell wall constituents, therefore they are not suitable for pectin extraction analysis and the mechanism is still unclear.

The objective of this research was to compare the yield, physico-chemical properties and molecular structure of waste grapefruit peels pectin extracted by ACAE with those by CHE regarding two extraction stages and different peel sizes. A potential general mechanism for ACAE of plant cell wall viscous polysaccharides was also proposed.

2. Materials and methods

2.1. Materials and reagents

Fresh grapefruit (*Citrus paradisi* Macf. cv. Changshanhuyou) was collected from Taizhou, China. Waste peel was separated and immediately soaked in a water bath at 90 °C for 5 min for enzyme inactivation. Subsequently, it was dried in an oven with air circulation at 50 °C and cut into pieces with size of larger than 10 mm. Later it was gradually grounded by an electric grinder (BSY-200T, Boou Metal Factory, China) and a high speed ultra-centrifugal mill (ZM200, Retsch, Germany). Finally, the powder was separated into four groups by a series of sieves of 4 mm, 0.9 mm and 0.18 mm opening. The samples were vacuum-packed and stored in desiccators until use.

All chemical reagents used in the experiments were of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Methyl alcohol and acetonitrile used for high-performance liquid chromatography (HPLC) were HPLC-grade (Tedia, USA). HCl with pH of 1.5 was assigned as the extraction solvent. Monosaccharides standards were bought from Aladdin Reagents (Shanghai, China).

2.2. Two-stage extraction and purification of pectin using ACAE and CHE

Acoustic cavitation was achieved by a 20 kHz probe system (Sonics, VCX800, USA) from a probe with tip diameter of 25 mm. The experimental design regarding different peel particle sizes was shown in Table 1. Waste dried grapefruit peels ($m \approx 3$ g) were prepared by dissolving in 150 mL solvent. At the first stage, peels were kept at 80 °C plus stirring for 90 min without ultrasound irradiation for CHE. Whereas for ACAE, duty cycle of ultrasound pulse was set at 50% (2 s on: 2 s off). At the same time, periodical agitation (10 s on: 50 s off) was performed to keep the extraction temperature relatively uniform. Extracted conditions were optimized in our previous study (Wang et al., 2015), applying 58% amplitude of ultrasound, extraction temperature of 67 °C and sonication time of 28 min. A calorimetric procedure was used to determine the actual ultrasonic power (P, W) introduced in the liquid system (Boukroufa, Boutekedjiret, Petigny, Rakotomanomana, & Chemat, 2015; Raso, Manas, Pagan, & Sala, 1999). According to our previous study (Xu et al., 2014), 58% amplitude power could be transferred to actual ultrasonic power of 61.3 W (25 mm probe: y = 118.65x-7.4783, R^2 = 0.9961; y is the output power, x is the % of the maximal amplitude) and was equal to the calculated power density of 0.41 W/mL or power intensity of 12.49 W/cm^2. After the first stage extraction, the volume of mixture (V_m) was measured. Then the mixture was cooled, coagulated, washed, and lyophilized as previous study (Wang et al., 2016). The purified pectin, labeled as *Pectin I*, and its weight (m_1, g) weighed in an analytical balance (Mettler Toledo GB204, Switzerland).

Table 1
Experimental group for different particle size of materials.

NO.	Particle size of materials	Extraction methods	*Pectin I*	*Pectin II*	Total pectin
1	> 10 mm	ACAE[a]	U11	U12	U1
2	0.9–4 mm	ACAE	U21	U22	U2
3	0.18–0.9 mm	ACAE	U31	U32	U3
4	< 0.18 mm	ACAE	U41	U42	U4
5	< 0.18 mm	CHE[b]	C41	C42	C4

[a] ACAE: acoustic cavitation assisted extraction.
[b] CHE: conventional heating extraction.

The second stage extraction process was an add-on to the first one. Distilled water (150 mL) of 70 °C was mixed with the pellet and stirred without sonication for 30 min. Later on, the treated mixture was cooled and centrifuged (9000 × *g*, 20 min), the filtrate was also coagulated. After lyophilization, the second stage pectin was weighed as m_2 (g) and labeled as *Pectin II*. The yield (Y, %) of pectin was calculated as follows: $Y = (m_1 + m_2)/m$ (g) × 100%, where m is the dry peel weight, m_1 is the weight of *Pectin I* for the first stage and m_2 is the weight of *Pectin II* for the second stage.

To analyze the degree of swelling of peel particles during the extraction, the swelling index (SI) of tissue residue was measured. It defined as the volume in milliliters occupied by 1 g raw material according to Toma, Vinatoru, Paniwnyk, and Mason (2001) with minor modification. SI was calculated as SI = $(V_m - V_f)/m$, where m is the dry peel weight.

2.3. Physico-chemical properties of pectin

2.3.1. Determination of molecular weight

Weight-average molecular weight (M_w) of extracted pectin was determined by high performance size exclusion chromatography (HPSEC) system according to Zhang et al. (2013). Dextrans of M_w of 1.27, 11.60, 80.90, 147.60, 273.00, 409.80, and 667.80 kDa (Sigma-aldrich, USA) were used to establish a standard curve.

2.3.2. Determination of monosaccharide composition

The monosaccharide composition of the extracted pectin was determined by HPLC using the method described by Zhang et al. (2013) with some modifications as described clearly in our previous study (Wang et al., 2016).

2.3.3. Determination of galacturonic acid content

The galacturonic acid content of pectin was analyzed according to Dische (1947) with some modifications. The samples and standard galacturonic acid were hydrolyzed with sulfuric acid in boiled water for 15 min and cooled down in an ice bath. 0.15% carbazole ethanol solution was added and vortexed. After 30 min incubation at room temperature (25 °C), the absorbance of resultant mixture was measured at 530 nm using microplate reader spectrophotometer (Multiskan GO 1510, Thermo Fisher Scientific, Vantaa, Finland).

2.3.4. Determination of degree of methoxylation (DM) and acetylation (DA)

DM and DA were calculated as the molar percentage of methanol or acetic acid content from the galacturonic acid content. Methanol and

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