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Extraction of pectin from navel orange peel assisted by ultra-high pressure, microwave or traditional heating: A comparison

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ABSTRACT

Ultra-high pressure (UHP) was applied for pectin extraction from navel orange peel. The effects of pressure, temperature and pressure-holding time on the extraction yield and viscosity of pectin were investigated. A 2-factor 3-level design, following a single-factor experiment, was carried out to optimize the extraction parameters and the optimal conditions of UHP extraction were determined as pressure 500 MPa, temperature 55 °C, pressure-holding time 10 min. Under the optimal conditions, the yield of pectin (20.44% ± 0.64) was significantly higher than those extracted by traditional heating (15.47% ± 0.26) and microwave (18.13% ± 0.23). The physicochemical properties and rheological characteristics of pectin extracted by ultra-high pressure, traditional heating and microwave as well as commercial pectin were also compared. The results showed that the intrinsic viscosity and viscosity-average molecular weight of pectin extracted by UHP (0.7604 L/g and 3.063×10^5 Da) were much higher than those extracted by traditional heating (0.4276 L/g and 1.521×10^5 Da), microwave (0.3591 L/g and 1.230×10^5 Da), and the commercial pectin (0.2160 L/g and 0.663×10^5 Da). The same results were also obtained in the rheological characteristics, activation energy and gelling properties determination. These results clearly demonstrated that UHP is a more efficient, time saving, and eco-friendly alternative for pectin extraction from navel orange peel, especially for pectin with higher viscosity and stability.

1. Introduction

Pectin, a family of complex heteropolysaccharides consisting predominantly of partially methoxylated galacturonic acid residues, is extensively distributed in almost all of the fruits and vegetables as the structural unit of fresh cells and the junction between the cells (Ridley, O'Neill, & Mohnen, 2001; Thakur, Singh, & Handa, 1997). Its structure is based on 1, 4-linked α-D-galacturonic acid, interrupted by L-rhamnose residues with side-chains of neutral sugars (mainly D-galactose and L-arabinose) (Mohnen, 2008; Ridley et al., 2001). Pectin is widely used as gelling agent and stabilizer in a variety of food, pharmaceutical, cosmetic products (Thakur et al., 1997). Previous research on pectin showed that not only the intrinsic factors, such as the galacturonic acid (GalA), neutral sugar (NS) contents, degree of methyl-esterification (DM), degree of acetyl-esterification (DA) and average-molecular weight, but also extrinsic factors, such as the pH, ash content, and ionic strength of pectin solutions, can influence their functional properties (Baississe, Ghannem, Fahloul, & Lekbir, 2010; Fishman, Chau, Cooke, & Hotchkiss, 2008; Koubala et al., 2008; Singthong, Cui, Ningsanond, & Goff, 2004; Yapo, Robert, Etienne, Wathelet, & Paquot, 2007). Pectin has been extracted from several resources, such as orange peel, apple pomace, lemon and sugar beet pulp. Orange peel and apple pomace are particularly abundant and contain high levels of pectic polysaccharides (Fishman et al., 2008; Koubala et al., 2008; Kurita, Fujiwara, & Yamazaki, 2008; Wang et al., 2007; Yeoh, Shi, & Langrish, 2008). The utilization of a suitable method for pectin extraction is significant in order to maximize its extraction yield and improve the product quality. Numerous scientific publications have studied the influence of extraction conditions on the physicochemical characteristics and functional properties of the pectin extracted from various plant tissues (Koubala et al., 2008; Levigne, Ralet, & Thibault, 2002; Yapo, 2009; Yapo et al., 2007). The most commonly used methods for the extraction of pectin include direct boiling and microwave heating (Levigne et al., 2002; Liu, Shi, & Langrish, 2006; Yeoh et al., 2008). On the industrial scale, pectin is usually extracted using hot water acidified with a mineral acid (the so-called conventional acid extraction) under the pH, temperatures, and duration

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conditions generally in the range of 1.3–3, 60–100 °C, and 20–360 min, respectively (Koubala et al., 2008). Due to a relatively long period of direct heating, the extracted pectin undergoes thermal degradation, which could result in undesired changes in physicochemical and functional properties of pectin (Koubala et al., 2008). There is, therefore, a need to explore new methods of pectin extraction or modification of the existing methods to obtain pectin with the desired extraction yield and quality attributes.

Several kinds of new technologies, such as microwave heating at ambient and moderate pressures, ultrasonication, and super-high frequency electromagnetic field are currently used, either sequentially or independently, in the extraction of pectin from various resources to give increased extraction yield, higher quality, and environmental protection compared with conventional direct boiling acid extraction (Fishman, Chau, Hoagland, & Ayyad, 2000; Fishman, Chau, Hoagland, & Hotchkiss, 2006; Kratchanova, Panchev, Pavlova, & Shtereva, 1994; Manabe, Naohara, Sato, & Okada, 1988; Wang et al., 2007; Wu et al., 2009). Ultra-high pressure (UHP) or high hydrostatic pressure (HHP), usually in the range from 100 MPa to 800 MPa, is a novel technology that can be applied in the food industry relevant to the preservation and processing of food, including the destruction of micro-organisms, inactivation of enzyme activity, control of phase changes and altered conformation of biopolymers (Bang & Chung, 2010; Guerrero-Beltran, Estrada-Giron, Swanson, & Barbosa-Canovas, 2009; Penniston, 1971). UHP can also cause some structural changes in structurally fragile material, such as cell deformation, cell membrane damage, protein denaturation (Eisenmenger & Reyes-De-Corcuera, 2009; Guerrero-Beltran et al., 2009; Lima, Swansona, & Clark, 2008). Based on the phase behavior theory, the solubility is larger while the pressure increases (Richard, 1992). Hence, UHP was also used in the extraction of bioactive ingredients from natural products increasingly (Corrales, Garcia, Butz, & Tauscher, 2009; Xi, 2009; Xi et al., 2009). However, there is no report published on the utilization of UHP for the extraction of pectin.

The purpose of this study was to explore the potential of UHP extraction of pectin from navel orange peel (*Citrus sinensis* Osbeck) and optimize the extraction conditions, such as pressure, temperature, and pressure-holding time, using extraction yield and viscosity of pectin as indexes. In addition, comparative analysis of the extraction yield and physicochemical properties of pectin extracted by different methods, including ultra-high pressure extraction (UHPE), traditional heating extraction (THE), and microwave assisted extraction (MAE), were also investigated.

2. Material and methods

2.1. Material and chemical reagents

Fresh navel orange (*C. sinensis* Osbeck), cultivated in the south of Jiangxi province, was purchased from a local market (Beijing, China). The collected orange peel was first soaked in a water bath at 90 °C for 5 min to inactivate their enzymes, then drying was carried out at 40 °C in a vacuum drying oven (CIMO DZF-6050, Shanghai CIMO Medical Instrument manufacturing Co., Ltd., China) until the water content was reduced to 15%. It was then milled to a powder (ca. 60 mesh) in an electric grinder (HY-04A, Beijing Huanya Tianyuan Machine Technology Co., Ltd., Beijing, China). Finally, the vacuum-packed sample was stored for subsequent extraction. Commercial pectin (CP) of orange peel (P9135, Sigma–Aldrich, St. Louis, USA) and D-galacturonic acid (Sigma–Aldrich, St. Louis, USA) were purchased from the Beijing Biodee Biotechnology Co., Ltd. (Beijing, China).

All chemical reagents, including ethanol, hydrochloric acid, sodium hydroxide, galacturonic acid, etc., used in the experiments were analytical grade and purchased from the Lanyi reagent company (Beijing, China). Deionized water with pH of 1.5 adjusted by 0.5 M HCl was used as the extraction solvent.

2.2. Apparatus

UHP extraction was carried out using a pressure-assisted thermal high hydrostatic pressurization unit (CAU-HHP-700-6, Baotou Kefa High Pressure Food Processing Inc., Inner Mongolia, China) with a cylindrical pressure chamber capacity of 7 L. Distilled water was used as the pressure-transmitting medium. The rate of pressure increase was about 130 MPa/min and the pressure release was immediate. The treatment time reported in this study did not include the pressure increase time and pressure release time. The pressure levels and pressure-holding time were continuously recorded during the pressurization cycle.

2.3. Extraction and purification of pectin

2.3.1. Ultra-high pressure extraction

Fifty gram sample mixed with 2.5 L of extraction solvent was placed into a polyethylene bag. The bag was sealed using a vacuum sealing machine (DZQ 500 vacuum packer, Beijing Rishang Science & Trade Co., Ltd., Beijing, China) and placed into the ultra-high pressure vessel. The mixture was filtered by vacuum filtration for subsequent purification after processing in the ultra-high pressure apparatus using high pressure levels from 100 to 600 MPa, pressure-holding time from 5 to 30 min and temperatures from 10 to 30 °C, respectively. The filtrate was collected and stored in a refrigerator at 4 °C for subsequent purification.

2.3.2. Traditional heating extraction

Pectin extracted by traditional heating extraction method (THE) was based on the method of Kratchanova, Pavlova, and Panchev (2004) with slight modification. The sample (50 g) was mixed with 2.5 L of extraction solvent and then heated to 80–82 °C using a water bath oscillator, the extraction was carried out with continuous stirring for 1 h. The hot mixture was filtered by vacuum filtration and the filtrate was collected and stored in a refrigerator at 4 °C for subsequent purification.

2.3.3. Microwave assisted extraction

Pectin extraction using microwaves (MAE) was based on the method of Wang et al. (2007) with slight modification. 50 g sample was subjected to extraction by adding 2.5 L extraction solvent and then heated at 80 °C for 21 min in a microwave oven (MAS-II, Shanghai Sineo Microwave Chemistry Technology Co., Ltd., China) with an output power of 500 W. After processing the sample, the mixture was filtered by vacuum filtration and the filtrate was collected and stored in a refrigerator at 4 °C for subsequent purification.

2.3.4. Pectin purification procedure

One volume of crude pectin filtrate extracted by various technologies was precipitated using two volumes of 95% (v/v) ethanol and kept overnight without stirring at 4 °C. The precipitated pectin was separated by centrifugation and vacuum filtration, and then washed three times using 95% ethanol to remove the monosaccharides and disaccharides (Minkov, Minchev, & Paev, 1996). After purification, the wet pectin was dried at 40 °C in a vacuum drying oven until its weight was constant; and then the dried pectin was weighed with an analytical balance (AY120, Shimadzu, Japan). The extraction yields (Y) of diverse technologies were calculated as follows:

$$Y\ \ (\%, \mathrm{w/w}) = \frac{\text{pectin weight}}{\text{sample weight}} \times 100$$

2.4. Methods of analysis

2.4.1. Determination of anhydrouronic acid and degree of esterification

Total anhydrouronic acid (AUA) content of polysaccharide was determined by the m-hydroxybiphenyl method (Blumenkrantz & Asboe-Hansen, 1973). Galacturonic acid solutions (10–100 μg/mL) were used to construct the standard curve for the determination of the AUA content. The degree of esterification (DE) of pectin was determined by the titrimetric method of Food Chemical Codex (FCC, 1981) and USP 26 NF 21 (2003) with slight modification.

2.4.2. Intrinsic viscosity and viscosity-average molecular weight

To determine the intrinsic viscosity ($[\eta]$) of the extracted pectin, the viscosity of pectin solutions (0.1 M, pH = 7.0 phosphate buffer) in different concentrations range from 0.2 to 1.0% (w/w) were determined using a Controlled Stress Rheometer (AR 550, TA Instruments, New Castle, DE, USA) with attached computer software (Rheology Advantage Data Analysis Program, TA). The apparent viscosity was determined using a conical concentric cylinder at the angular velocity of 100 rpm at 25 °C using a temperature-controlled circulating water bath (Julabo F18 circulating waterbath, Germany). The intrinsic viscosity ($[\eta]$) was estimated by extrapolation of Martin curves to “zero” concentration (Arias, Yagüe, Rueda, & Garcia Blanco, 1998) by following equation:

$$\ln\left(\frac{\eta_{sp}}{c}\right) = \ln[\eta] + K[\eta]c$$

$$\eta_{sp} = \frac{\eta - \eta_0}{\eta}$$

where $[\eta]$ is the intrinsic viscosity (L/g), η_{sp} is specific viscosity, c is concentration of pectin solution (g/L), η is viscosity of pectin solution (Pa s), η_0 is viscosity of solvent (Pa s), K is Martin's constant.

The viscosity-average molecular weight (M_v) was calculated from the Mark–Houwink–Sakurada equation:

$$[\eta] = kM_\mathrm{v}^{\alpha}$$

where $[\eta]$ is the intrinsic viscosity (L/g), M_v is viscosity-average molecular weight, k and α are constants for given solute–solvent system and temperature. The constants k and α were take as 2.34×10^{-5} and 0.8224, respectively (Kar & Arslan, 1999).

2.4.3. Rheological measurement

Samples were dissolved in deionized water with the pH of 3.3 by adding 0.5 M citric acid. The viscosity of the samples was determined using a Controlled Stress Rheometer (AR 550, TA Instruments, New Castle, DE, USA) with attached computer software (Rheology Advantage Data Analysis Program, TA). A platinum resistance thermometer positioned in the center of the plate ensured proper temperature control and measurement. Flow curves with increasing shear rate (1–300 s^{-1}) were measured at 25 °C using a plate geometry (SST ST 40 mm diameter, cone angle 2°, 52 μm gap). Each sample was tested in triplicate. Shear stress was plotted against shear rate.

2.4.4. Activation energy measurement

The activation energy of pectin was determined by the method of Wang, Wang, Li, Xue, and Mao (2009) with slight modification. Samples were dissolved in deionized water adding 0.5 M citric acid to adjust the pH to 3.3. The apparent viscosity was determined over temperatures range from 10 to 50 °C using a Controlled Stress Rheometer (AR 550, TA Instruments, New Castle, DE, USA) at a constant shear rate of 10 s^{-1} with a plate geometry (SST ST 40 mm diameter, cone angle 2°, 52 μm gap). The influence of temperature on the apparent viscosity at a constant shear rate (10 s^{-1}) of pectin solution can be calculated using the Arrhenius equation:

$$\eta_\mathrm{a} = \eta_\infty \exp\left(\frac{E_\mathrm{a}}{RT}\right)$$

where η_a is the apparent viscosity at a specific shear rate, η_∞ is a constant for the specific sample termed frequency factor, E_a is the activation energy (kJ/mol), R is the gas constant (8.3145 J/mol K), and T is the absolute temperature (K).

2.4.5. Gelling properties measurement

To evaluate gelling properties of THE, WAE, UHPE and CP pectin, dynamic oscillation experiments were carried out using a Controlled Stress Rheometer (AR 550, TA Instruments, New Castle, DE, USA) with a parallel steel plate (40 mm diameter, 1.0 mm gap) based on the method of Singthong et al. (2004). Three pectin concentrations (0.33%, 0.5%, and 0.67%, w/w) with 60% (w/w) of sucrose at pH of 2.85 were chosen for the determination. The viscoelastic properties, including storage modulus (G'), loss modulus (G'') and delta values (δ), were determined through oscillation frequency sweep at frequencies from 0.1 to 10 Hz with the temperature of 25 °C. Prior to the dynamic experiments, oscillation strain sweep at a constant oscillation frequency of 0.1 Hz was carried out to determine the linear viscoelastic region. All oscillatory tests were performed at a strain value of 0.02 (within the linear viscoelastic region).

2.5. Statistical analysis

All experiments were performed in triplicate. The data was analyzed using analysis of variance (ANOVA) by OriginPro 7.5 (OriginLab Corporation, Northampton, USA), and expressed as mean value ± standard deviation ($n = 3$). The confidence level for statistical significance was set at a probability value of 0.05.

3. Results and discussion

3.1. Optimization of UHP extraction conditions

In the extraction process, many factors, including extraction solvent, pH of solvent, solid liquid ratio, extraction temperature and extraction time, can affect the efficiency of pectin extraction. According to the previous studies and the physicochemical properties of pectin, deionized water with pH of 1.5 adjusted by 0.5 M HCl was used as extraction solvent, and solid–liquid ratio was 1:50 (Kratchanova et al., 2004; Wang et al., 2007).

To determine the optimal extraction conditions of UHP, three factors, including pressure, temperature and pressure-holding time, that can affect the effectiveness of UHP extraction were considered as major factors (Corrales et al., 2009; Xi, 2009). The effect of each individual factor was investigated by single-factor experiments firstly. The experimental procedures and results were presented as follows.

In the study, high pressures from 100 to 600 MPa were applied with a constant temperature of 45 °C and pressure-holding time of 15 min. The extraction yield and viscosity of the pectin were considered as the indicators of extraction efficiency. As shown in Fig. 1(A), the extraction yield and viscosity of pectin were greatly influenced by pressure levels. With increasing the pressure from 100 to 600 MPa, the extraction yield of the pectin was significantly increased from 8.0% to 15.5% ($p < 0.05$). However, the viscosity of the pectin showed a different change. It was significantly increased with increasing the pressure from 100 MPa to 500 MPa whereas decreased at 600 MPa ($p < 0.05$). It was obvious that high pressure was useful for improving the extraction of pectin, and many other studies showed an improved extraction of bioactive constituents

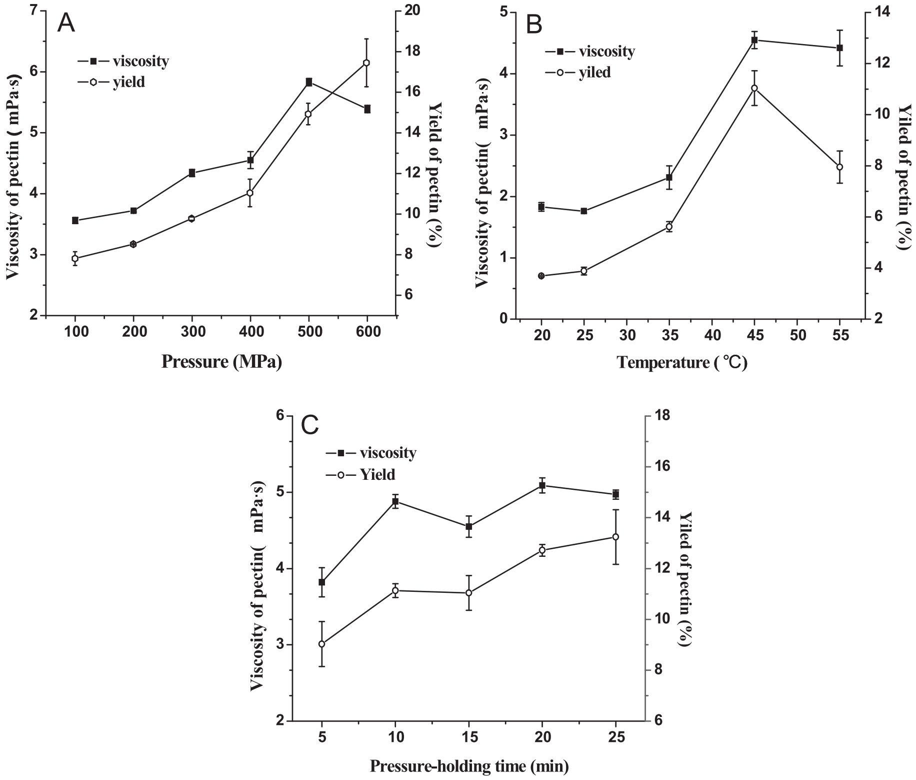

Fig. 1. Effect of pressure, temperature and pressure-holding time on extraction yield and viscosity of pectin extracted by UHP from orange peel. UHPE conditions: pressure from 100 to 600 MPa, temperature from 20 to 55 °C, pressure-holding time from 5 to 25 min, liquid–solid ratio of 50:1.

with pressure (Prasad et al., 2009; Xi et al., 2009; Xi, 2009). Due to high pressure can cause cell deformation, cell membrane damage, even cell rupture and improve the mass transfer rate, solvent permeability in cells as well as the secondary metabolite diffusion (Butz & Tauscher, 2002; Li et al., 2011; Prasad et al., 2009). Therefore, the pressure of 500 MPa was considered as the best extraction pressure level.

Fig. 1(B) shows the effect of extraction temperature on the extraction yield and viscosity of pectin using UHP extraction. The profile clearly demonstrated that both the extraction yield and viscosity of pectin were significantly increased with the extraction temperature from 20 to 45 °C. However, there was slightly decreased in the extraction yield and the viscosity of pectin when extraction temperature increased from 45 to 55 °C. This could be explained by the solubility of the extracted pectin increased with increasing the extraction temperature, giving a higher rate and yield of extraction. Moreover, the diffusion coefficient of extract solvent increased and so that the rate and yield of extraction improved (Treybal, 1980). However, the extraction at high temperatures caused the dissolved pectin denaturation and degradation (Masmoudi et al., 2008). Therefore, the extraction temperature of 45 °C was considered as the optimum when the extraction pressure and pressure-holding time were 400 MPa and 15 min, respectively.

The effect of pressure-holding time on the extraction yield and viscosity of pectin was investigated in the range from 5 to 25 min. Fig. 1(C) clearly indicates that the extraction yield and viscosity of pectin were significantly increased when pressure-holding time prolonged from 5 to 10 min, but there was no significant increase in the extraction yield and viscosity of pectin with increasing pressure-holding time from 10 to 25 min. It is well recognized

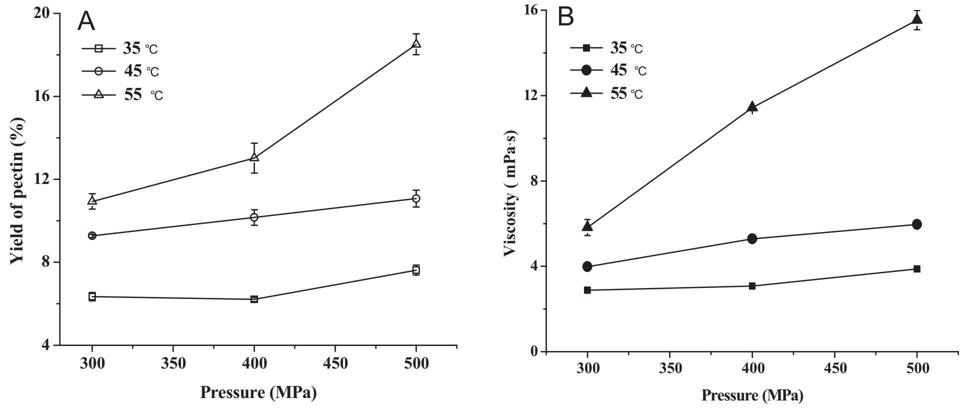

Fig. 2. Effect of pressure and temperature combination on the extraction yield and viscosity of pectin extracted by UHP from orange peel. UHPE conditions: pressure from 300 to 500 MPa, temperature from 35 to 55 °C, pressure-holding time of 10 min, liquid–solid ratio of 50:1.

Table 1
Comparison of extraction time and extraction yield of pectin extracted by traditional heating, microwave heating and ultra-high pressure.

Extraction method	Extraction time (min)	Extraction yield (%)
THE	60	15.47% ± 0.26
MAE	21	18.13% ± 0.23^b
UHPE	10	20.44% ± 0.64^a

For each treatment, values are means ± standard deviations of triplicate, the means within the column followed by different letters were significantly different at $p < 0.05$.

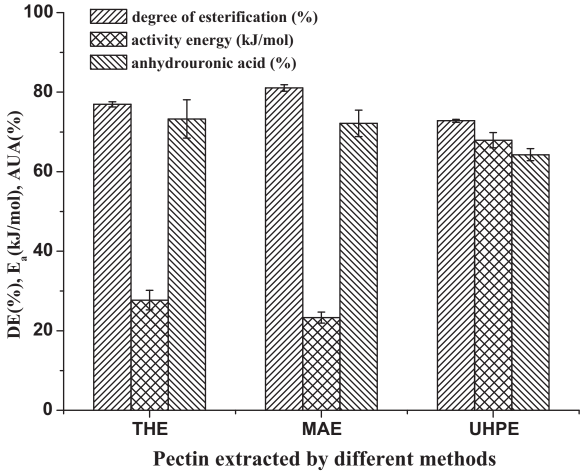

Fig. 3. Physicochemical properties of pectin extracted by UHPE, THE, WAE. UHPE conditions: pressure of 500 MPa, temperature of 55 °C, pressure-holding time of 10 min, liquid–solid ratio of 50:1; THE conditions: temperature of 80–82 °C, time of 60 min, liquid–solid ratio of 50:1; MAE conditions: temperature of 80 °C, time of 21 min, microwave power of 500 W, liquid–solid ratio of 50:1.

that in UHP extraction the pressure transfers to the whole material was a uniform and instant process and the extraction could finished in a shorter time (Butz & Tauscher, 2002; Li et al., 2011). Therefore, a pressure-holding time of 10 min was enough to attain the equilibrium of mass transfer during HPP extraction.

To optimize the extraction conditions based on the above-mentioned results of single factor experiment, a two factor three level design, including pressure and pressure-holding time, was carried out. Fig. 2(A) and (B) shows the extraction yield and viscosity of pectin under the different extract conditions, respectively. The extraction yield and viscosity of pectin both reached a significant summit at the pressure of 500 MPa and temperature of 55 °C with the pressure-holding time of 10 min. Therefore, we concluded that the pressure of 500 MPa and temperature of 55 °C with the pressure-holding time of 10 min was the optimal extraction conditions.

3.2. Comparison of extraction yield of UHPE, THE and MAE

To evaluate the extraction efficiency of UHP, a comparison between UHPE, THE and MAE was carried out. As shown in Table 1, among these three extraction methods, the extraction yield of UHPE (20.44% ± 0.64) was significantly higher than those of THE (15.47% ± 0.26) and MAE (18.13% ± 0.23) and the extraction time of UHPE (10 min) was much shorter than the extraction time of THE (60 min) and MAE (21 min) as well. The results of this study were in accordance with many other research on extraction of bioactive compounds by UHP, including polyphenols from green tea leaves, corilagin from longan fruit pericarp, caffeine extraction from green tea leaves, etc. (Prasad et al., 2009; Xi, 2009; Xi et al., 2009). These definitely demonstrated that UHP as a novel method can be utilized in the extraction of many bioactive compounds from various natural materials with less time consumption and high efficiency.

3.3. Properties of pectin extracted by UHPE, THE and MAE

The properties and characteristics of the pectin often varied in a large range according to the different extraction conditions (Koubala et al., 2008; Kurita et al., 2008; Yeoh et al., 2008). The content of anhydrouronic acid, degree of esterification and activation energy had great influence on the gelling, stabilizing, and thickening properties of pectin (Koubala et al., 2008; Levigne et al., 2002; Yapo et al., 2007). It was possible to obtain pectin with specific characteristics using different extraction conditions and thus to enlarge their potential uses.

In order to compare the physicochemical characteristics and functional properties of pectin extracted by UHP with those extracted by THE and MAE, anhydrouronic acid, degree of esterification, intrinsic viscosity, viscosity-average molecular weight, activation energy, rheological characteristics, and gelling properties of all of the extracted pectin were determined and analyzed. Fig. 3 shows that the degree of esterification and content of anhydrouronic acid of the pectin extracted by different methods had no significant difference, however, the activation energy of the pectin extracted by UHP was significantly greater than those extracted by THE and MAE. The high activation energy of pectin indicated more inter-interactions and intra-interactions between polysaccharide chains at the concentration studied (Wang et al., 2009). The result of activation energy in Fig. 3 also illustrated that the stability and the structure of pectin extracted by UHP was much better than those extracted by THE and MAE.

Intrinsic viscosity ([η]), as a measure of the hydrodynamic volume occupied by a macromolecule, is closely related to the size and conformation of the macromolecular chains in a particular solvent (Lai & Chiang, 2002). In the present study, the viscosity of pectin solutions in five different concentrations range from 0.2% to 1.0% (w/w) were determined, and their intrinsic viscosity were obtained by linear extrapolation at zero concentration of three equations developed by Huggin, Kraemer and Martin (Arias et al., 1998). From the result of liner regression, we found that Martin equation provided a better fit for the pectin ($R^2 > 0.9898$), so the intrinsic viscosity of pectin was fitted and calculated using Martin equation. According to the intrinsic viscosity, viscosity-average molecular weight (M_v) of pectin was also calculated from the Mark–Houwink–Sakurada equation, where the constants k

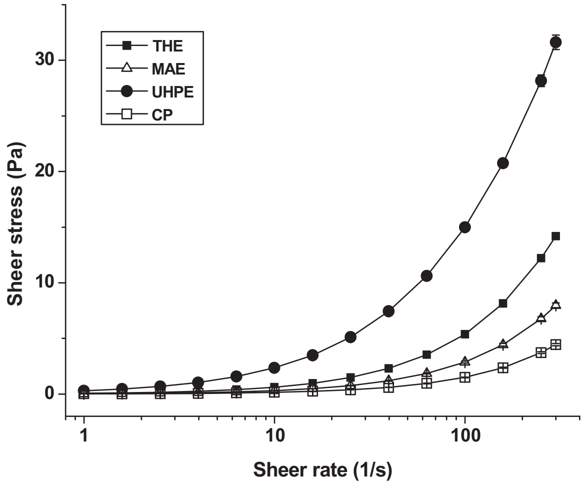

Fig. 4. Changes in shear stress as a function of shear rate of pectin extracted by UHPE, THE, MAE and CP. Analysis conditions: plate geometry: diameter of 40 mm, cone angle of 2°, gap of 52 μm, shear rate from 1 to 300 s^{-1}, temperature of 25 °C.

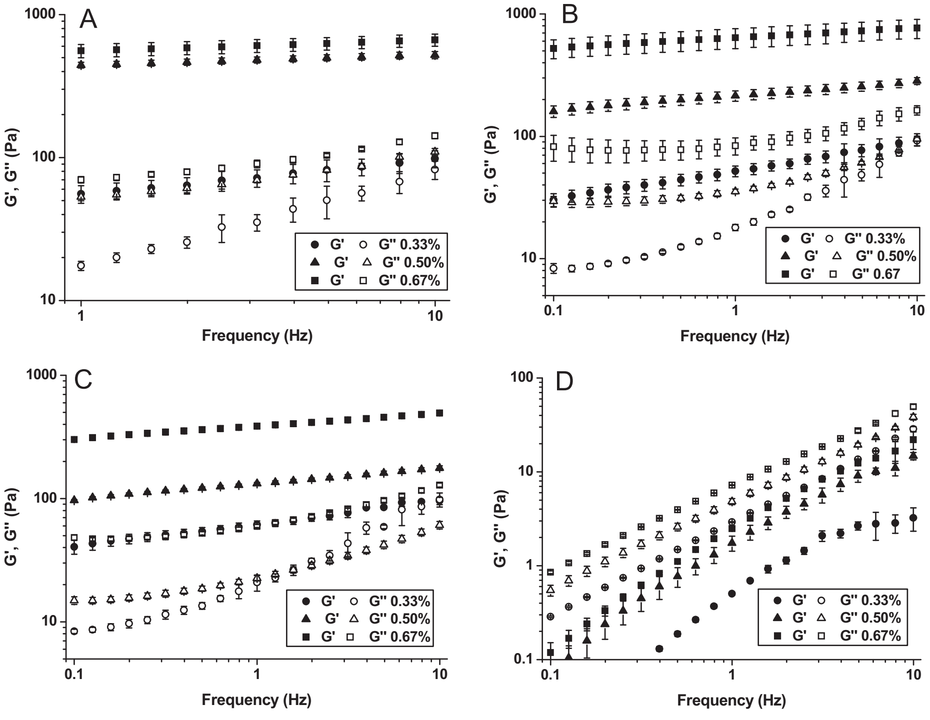

Fig. 5. Frequency dependence of storage (G') and loss (G'') modulus of different pectin gel in the different concentration (0.33%, 0.50%, 0.67%, w/w), (A) THE, (B) MAE, (C) UHPE, (D) CP. Analysis conditions: plate geometry: diameter of 40 mm, gap of 1.0 mm, frequency from 0.1 to 10 Hz, temperature of 25 °C.

and α were take as 2.34×10^{-5} and 0.8224, respectively (Kar & Arslan, 1999). As shown in Table 2, the intrinsic viscosity and viscosity-average molecular weight of pectin extracted by UHP (0.7604 L/g and 3.063×10^5 Da) were about or more than twice higher than those extracted by THE (0.4276 L/g and 1.521×10^5 Da) and MHE (0.3591 L/g and 1.230×10^5 Da), as well as CP (0.2160 L/g and 0.663×10^5 Da). This clearly proved that UHP has less degradation action than traditional heating and microwave heating.

Fig. 4 shows the changes in shear stress as a function of shear rate of different kinds of pectin, including UHPE, THE, MAE and CP.

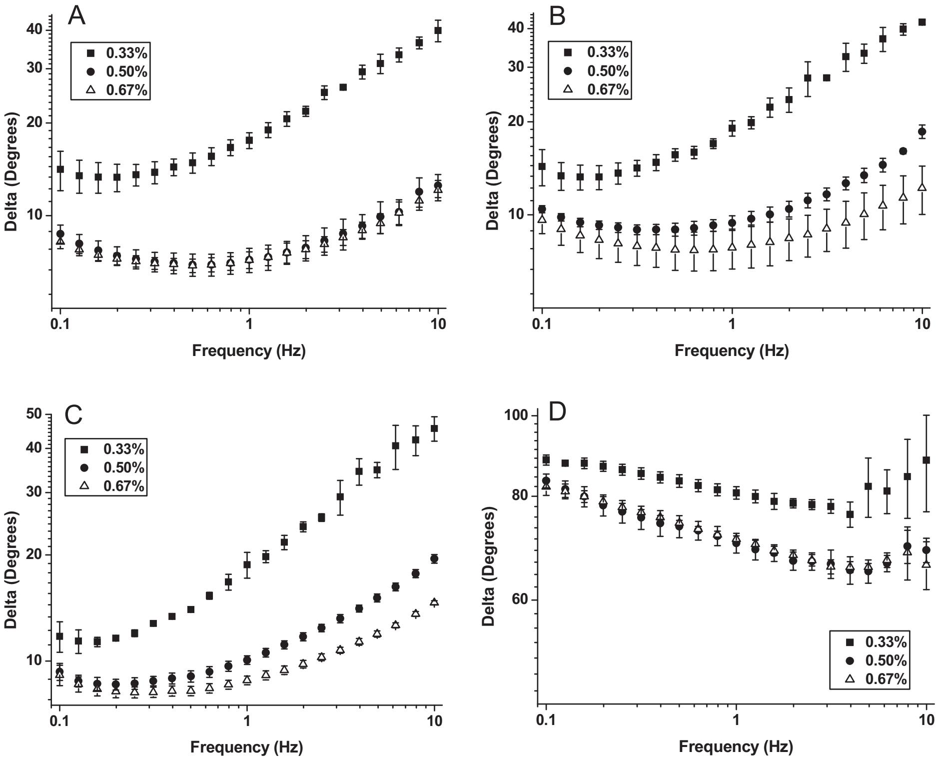

Fig. 6. Frequency dependence of delta values of different pectin gel in the different concentrations (0.33%, 0.50%, 0.67%, w/w), (A) THE, (B) MAE, (C) UHPE, (D) CP. Analysis conditions: plate geometry: diameter of 40 mm, gap of 1.0 mm, frequency from 0.1 to 10 Hz, temperature of 25 °C.

Table 2
Comparison of intrinsic viscosity ($[\eta]$) and viscosity-average molecular weight (M_v) of pectin extracted by different methods.

Extraction method	Martin equation	R^2	$[\eta]$ (L/g)	$M_v \times 10^{-5}$ (Da)
THE	$\ln(\eta_{sp}/c) = 0.2213c - 0.8496$	0.9912	0.4276	1.521
MAE	$\ln(\eta_{sp}/c) = 0.1852c - 1.0650$	0.9949	0.3591	1.230
UHP	$\ln(\eta_{sp}/c) = 0.2477c - 0.2739$	0.9898	0.7604	3.063
CP	$\ln(\eta_{sp}/c) = 0.1208c - 1.5324$	0.9984	0.2160	0.663

The profile illustrated that the viscosity of the pectin extracted by UHP was significantly greater than those extracted by THE, MAE and CP, and this was a positive change for the utilization of pectin as texturizer or stabilizer in a variety of food, pharmaceutical, cosmetic products (Thakur et al., 1997). The shear stress as a function of shear rate also indicated that the extraction methods had significant influence on the rheological behavior of pectin.

The dynamic elastic modulus (G') is the in-phase component of stress with an oscillating strain and the viscous modulus (G'') is the out-of-phase component of stress and is a measure of energy lost through viscous flow (Padmanabhan, Kim, Pak, & Sim, 2003). The loss tangent ($\tan\delta$) is defined as the ratio of the loss modulus (G'') to the storage modulus (G') and is dimensionless. It is a measure of the ratio of energy lost to energy stored in a cycle of deformation and provides a comparative parameter that combines both the elastic and the viscous contribution to the system (Padmanabhan et al., 2003).

The viscoelastic properties of pectin gels in different concentrations were depicted in Fig. 5 and Fig. 6. Fig. 5(A)–(C) shows that the THE, MAE and UHPE pectin can clumping a gel structure in the concentrations of 0.33%, 0.50% and 0.67% (w/w) as their storage modulus G' were much greater than the corresponding loss modulus G'' at frequencies from 0.1 to 10 Hz. In addition, the profile also illustrated that the gel strength enhanced with increasing sample concentration, because increasing the pectin concentration offers more facility to establish junctions between pectin molecules, thus explaining the increase in G' (Agoda-Tandjawa, Durand, Gaillard, Garnier, & Doublier, 2012). Fig. 6(A)–(C) illustrates more clearly that with increasing the oscillation frequency the δ increased but still less than 45°, which demonstrated the increase of oscillation frequency can result in the decrease of gel strength but the gel still dominated by the elastic component. Fig. 5(D) and Fig. 6(D) prove that CP cannot form a gel structure in the concentrations of 0.33–0.67% at 25 °C as its G' was less than the corresponding G'' and the δ is greater than 45°, which indicated CP solutions were predominantly viscous than elastic.

From the results of pectin gels viscoelastic properties, we can conclude that UHPE pectin has a similar gel properties with THE and MAE pectin, and better than CP. Considering the highest intrinsic viscosity and activation energy, it could be concluded that UHPE pectin significantly increased the steady state viscous properties, and thus it is more suitable as a thickener, stabilizer rather than gelatinizing agent.

4. Conclusions

Ultra-high pressure extraction (UHPE), as an emerging novel technology, was applied to extract pectin from navel orange peel for the first time and the extraction conditions were optimized using single factor experiment and two factor three level experiment. Under the optimal conditions, the extraction yield of UHPE was significantly higher than those of THE and MAE. The comparison of physicochemical, rheological and gelling properties of pectin among different extraction methods indicated that steady state viscous properties and stability of pectin extracted by UHP were much better than those extracted by THE, MAE and CP. The results of the studies clearly demonstrated that UHP was an efficient, time-saving, and eco-friendly method for extraction of pectin from navel orange peel, especially for the pectin with higher viscosity and stability.

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