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A new kinetic model of ultrasound-assisted extraction of polysaccharides from Chinese chive

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ABSTRACT

Chinese chive, a famous green vegetable, is widely cultivated in the Asia. In the present study, we found that ultrasound caused the degradation of Chinese chive polysaccharides (CCP) in the process of extraction. Since lacking the consideration of polysaccharide degradation, the traditional kinetic models can not reflect the real extraction process of CCP. Therefore, a modified kinetic model was thus established by introducing a parameter of degradation coefficient based on the Fick's second law, suggesting the diffusion and degradation of CCP is highly dependent on the ultrasonic power, extraction temperature and solid-liquid ratio. According to this modified model, the maximum CCP yield was obtained under an optimal extraction condition including extraction temperature 37 °C, ultrasonic power 458 w, extraction time 30 min and solid-liquid ratio 1:32. The objective polysaccharides responding to ultrasound were shown to be four different fractions, contributing to the increased diffusion and degradation of CCP by ultrasound treatment.

1. Introduction

Chinese chive (*Allium tuberosum* Rottl. Ex Spreng) is a well-known green vegetable in China, Japanese, Korea and some other Asian countries. As a commercial crop, it is widely cultivated in the East and Southeast Asia (Pandey, Pradheep, & Gupta, 2014). The leaves and seeds of this plant are often used in traditional folk medicines for the treatment of impotence and nocturnal emission in China (Hu et al., 2009). According to the records in traditional Chinese medicine, the leaves of Chinese chive can also treat abdominal pain, diarrhea, hematemesis, snakebite and asthma (Liu, Zhao, Su, Wang, & Zhang, 2006). These suggest that the Chinese chive has functional components being beneficial for human health. Up to date, some new steroidal saponins, alkaloids and amides have been isolated and characterized from the seeds of *Allium* genus, as wells as sulfur-containing compounds (Mnayer et al., 2014). Polysaccharides, an important type of natural biopolymers, have been suggested to possess various nutritional values and health functions (Shashidhar, Giridhar, & Manohar, 2015). In recent years, polysaccharides extracted from the plants of *Allium* genus have been reported to exhibit prominent benefits to human health, including antioxidant, antitumor and immunostimulating activity (Lee et al., 2009; Mladenovic et al., 2011; Nikolova et al., 2013). Recently, adenine-induced experimental mouse model was employed to screen the bioactive ingredients with protective effects on kidney in our research group. We found that the protective ability of Chinese chive against chronic renal failure is positively associated with the intake of polysaccharide dosage, indicating polysaccharide is one of the main bioactive compounds of Chinese chive to protect kidney.

Quantity and quality of extracts from raw material are powerfully related to the extraction method. With respect to the polysaccharide extraction from plants, the conventional extraction technology is hot-water extraction (HWE) involved in heating or boiling (Cheung & Wu, 2013). However, these methods exhibited inherent shortcomings of high-energy consumption, more extraction time consuming and limited polysaccharide production (Cheung & Wu, 2013). To overcome these drawbacks of hot-water extraction method, the application of new technologies in the extraction of polysaccharides has been concerned by the food, pharmacological and chemical scientists in recent years. Among different innovative extraction methods, ultrasound-assisted extraction (UAE) has been recognized as an efficient and environment-friendly extraction technique with strong advantages of high extraction yield, low energy input and short extraction time (Chen, You, Abbasi, Fu, & Liu, 2015), suggesting UAE is an ideal alternative to extract polysaccharides from raw materials. It is well

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accepted that the enhancement of extraction yield by ultrasound is attributed to the ultrasonic cavitation, as well as the accompanied physical and chemical effects (Hashemi, Michiels, Yousefabad, & Hosseini, 2015). Actually, an efficient extraction process should maximize the recovery of target compounds with minimal degradation. Literature survey revealed that the diffusion of polysaccharides is commonly accompanied by their degradation in the process of ultrasonic-assisted extraction (Yu et al., 2015; Zhao, Zhou, & Liu, 2012). Therefore, it is really needed to take some measures to control the degradation of polysaccharides in the extraction process. To solve this problem, some theoretical, empirical and semi-empirical models have been developed to describe the behavior of diffusion and degradation of polysaccharides in the extraction process. From an engineering point of view, the establishment of these kinetic models would be helpful to facilitate the design, optimization and control of the extraction process, as well as provide useful information for scaling up the industrial production (Imamoglu & Sukan, 2013; Wang & Sun, 2003). In recent years, several mathematical models like pseudo first-order model, diffusion model, Weibull-type model, saeman's model and two site kinetic model have been employed to simulate the extraction process of polysaccharides in the solid-liquid extraction system (Cheung, Siu, & Wu, 2013; Zhao, Morikawa, Qi, Zeng, & Liu, 2014). Unfortunately, they only considered a single process of diffusion or degradation of polysaccharides. As far as our best knowledge to be ascertained, the kinetic model for simultaneous consideration of diffusion and degradation has not been reported to simulate the real ultrasound assisted extraction process of polysaccharides.

Thus, to unravel the effects of ultrasonic power, extraction time, extraction temperature and solid-liquid ratio on the extraction yield of polysaccharides from Chinese chive, the present study was aimed to establish a suitable kinetic model considering both polysaccharide diffusion and degradation processes under ultrasound-assisted extraction. Based on this model, the extraction conditions were optimized for obtaining the maximum yield of polysaccharides from Chinese chive. Meanwhile, the ultrasound-acted target of polysaccharide was also determined through comparison with that extracted by the conventional technology.

2. Materials and methods

2.1. Material preparation

Chinese chive used in the present study was purchased from local supermarket in Hefei city of China. It was dried at 40 °C and smashed to fine powder. The particle size of the powder was determined to be the mean value of 114 μm by laser particle size analyzer (BT-9300HT, Beijing Hongxianglong Biotechnology Development Co. Ltd., Beijing, China).

2.2. Reagents and equipments

Standard dextran (T-2000, T-700, T-580, T-110, T-80, T-70, T-40 and T-11) were purchased from Sigma-Aldrich company (Louis, MO, USA). All other reagents were of analytical grade. The ultrasound-assisted extraction was carried out in a thermostatic ultrasonic processor (SY-1000E, Beijing Hongxianglong Biotechnology Development Co., Ltd., Beijing, China). The ultrasonic extractor was equipped with a digital controlled low-frequency sonotrode (25 kHz) and a powerful probe-shaped ultrasonic transducer. The sonotrode can change the ultrasonic power from 0 to 900 W. Moreover, this extractor was equipped an automatic temperature controlling system.

2.3. General methods for the extraction of polysaccharides from Chinese chive

The powder of Chinese chive (18.0 g) was mixed with distilled water in a 1000 mL reaction vessel and the ultrasonic probe was inserted into the mixture about 3 cm deep. The extraction temperature was controlled by an automatic temperature controlling system. For all ultrasound-assisted extraction, the ultrasonic processing was set 5 s followed by 1 s interval. At the end of each extraction, the crude extracts were centrifuged at 10,000 rpm for 5 min to separate the liquid extract from the solid residue. After the liquid extract was condensed to 40 mL, the concentrate was further mixed with ethanol at final concentration of 80% (v/v) to precipitate overnight at room temperature. The precipitates were further performed to remove proteins using Sevag method (Staub, 1965), dialyzed with 3500 Da dialysis bags and freeze-dried, giving the crude polysaccharide of Chinese chive (CCP).

2.4. Experimental design

The effects of ultrasonic power, extraction time, extraction temperature and solid-liquid ratio on the extraction yields of CCP were investigated in the present study. For the effect of solid-liquid ratio, it was performed at three different levels of 1:20, 1:30 and 1:50 (w/v) while the ultrasonic power was set at 160 and 320 W. To explore the effects of ultrasonic power and extraction temperature, sonication was carried out at four levels of 160, 240, 320 and 480 w, meanwhile the extraction temperature (Kelvin temperature, k) was set at 303, 313, 323, 333 and 343 k. For all experiments, the extraction time was set at seven levels of 0, 5, 10, 20, 40, 60 and 80 min.

2.5. Changes in the particle size of Chinese chive by ultrasonic treatment

After the powder of Chinese chive was extracted for 30 min under the treatment with ultrasound at 320 and 640 w, the particle size was determined by laser particle size analyzer and compared with that of original powder without ultrasound treatment.

2.6. Comparison of extraction performance

The performance of ultrasound-assisted extraction was compared with that of conventional hot-water extraction (HWE). For the extraction of CCP using UAE method, the Chinese chive powder was extracted under an optimal extraction condition obtained from the established kinetic model of present study. For the extraction of CCP using HWE method, the extraction condition was set as the same as that of UAE method except using ultrasonic power.

2.7. Analysis of polysaccharide content

The phenol-sulfuric acid method was employed to determine the polysaccharide content for each experiment (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956).

2.8. Analysis of target CCP fractions responding to ultrasound

After the crude CCP was prepared using UAE and HWE, fractional precipitation was employed to purify the crude polysaccharide using ethanol at final concentration of 40%, 60% and 80% (v/v) in turn, giving three different polysaccharide fractions. Subsequently, the molecular weight distribution were analyzed by High Performance Liquid Chromatography system (HPLC, 1260 Infinity, Agilent Technologies) equipped with TSKgel column G4000PWXL and G5000PWXL connected in series. The column temperature

was fixed at 25 °C. The response time of 1260 refractive index detector was set at 4 s. The eluent was double distilled water at a flow rate of 0.5 mL/min. To obtain the average molecular weight, T-series dextrans (T-2000, T-700, T-580, T-110, T-80, T-70, T-40 and T-11) were used as the standard.

2.9. Analysis of glycosidic bond composition

The methylation reaction was performed to analyze the glycosyl-linkages of polysaccharides according to our previous reports (Fang et al., 2015).

2.10. Data processing

The kinetic model and constants were regressed according to the experimental data by nonlinear fitting with Matlab 7.0 software and the Levenberg-Marquardt (LM) method was used for the numerical solution of dynamic equations. The experiment data used for the regression of model parameters were the averages of at least three duplicate experimental results.

3. Results and discussion

3.1. Development of kinetic model

In the present study, the unsteady state diffusion model based on the Fick's second law was employed to investigate the kinetic process of polysaccharide extraction from Chinese chive by UAE method. In order to analyze the extraction process using this model, some assumptions need to be made as follows:

(a) The geometry of the Chinese chive particles was considered as spherical. Since the size of Chinese chive particles was determined from about 0.068 mm–0.495 mm, the mean diameter of 0.114 mm was used in this model.
(b) Since ultrasound processing can cause the convection in the bulk liquid medium (Tao & Sun, 2015), the extraction solvent was assumed to be mixed very well in the extractor and the external resistance to mass transfer was negligible.

Based on the assumptions mentioned above, a first-order diffusion model derived from the Fick's second law was employed to depict the mass transfer of polysaccharides from spherical particles (Guerrero, Torres, & Nuñez, 2008):

$$\frac{\partial C}{\partial t} = D_s\left(\frac{1}{r^2}\frac{\partial}{\partial r}\left(r^2\frac{\delta C}{\delta r}\right)\right) \quad (1)$$

where C is the polysaccharide concentration (mg/mL), r is the distance to the centre of spherical particle (m), D_s is the effective diffusion coefficient of polysaccharides (m^2/s), and t is the extraction time (s).

The initial and boundary conditions were described as follows:

$$r = 0, C = C_0; \quad r = R_0, \quad \frac{\partial C}{\partial t}\cdot V = -D_s\cdot\cdot S\left(\frac{\partial C}{\partial r}\right)r = R_0$$

where C_0 is the initial polysaccharide concentration at the beginning of extraction (mg/mL), V is the solvent volume (mL), S is the contacting area between the particles and extracting solvent (m^2) and R_0 is the radius of Chinese chive particle (m).

Considering the initial and boundary conditions, the analytical solution of Eq. (1) is obtained (Jaganyi & Ndlovu, 2001; Spiro & Jago, 1982):

$$Ln\frac{C_\infty}{C_\infty - C} = kt + b, \quad k = \frac{\pi^2 D_s}{R_0^2} \quad (2)$$

where C_∞ is the equilibrium concentration (mg/mL) of polysaccharide extracted at infinite time without considering degradation, k is the observed rate constant (min^{-1}), and b is an empirical intercept. Since the initial polysaccharide concentration is C_0 when the extraction time is at t = 0, the value of b in Eq. (2) can be expressed as Eq. (3). It has been reported that the value of b is dependent on the distribution of the solute, as well as the size and shape of particles (Anekpankula, Gotob, Sasakib, Pavasanta, & Shotipruk, 2007).

$$b = Ln\frac{C_\infty}{C_\infty - C_0} \quad (3)$$

To integrate the Eq. (2) with Eq. (3), the Eq. (2) can be written as Eq. (4). The Eq. (4) is a first-order kinetic equation used to describing the extraction process of natural product (Yu et al., 2015). The observed rate constant can be determined from the slop of the straight line when plotting the logarithm of $(C_\infty - C_0)/(C_\infty - C)$ against time.

$$Ln\frac{(C_\infty - C_0)}{(C_\infty - C)} = kt \quad (4)$$

Since ultrasonic processing can result in the degradation of polysaccharides (Yu et al., 2015; Zhou, Yu, Zhang, He, & Ma, 2012), the polysaccharide degradation is needed to be considered in ultrasound-assisted extraction. Therefore, the Eq. (4) can not reflect the real process of polysaccharide extraction. Considering the degradation of Chinese chive polysaccharides in ultrasound-assisted extraction, the Eq. (4) was modified as Eq. (5). The Eq. (5) included the diffusion and degradation processes of polysaccharides. In this equation, k_D is the parameter of degraded rate constant (min^{-1}) which represents the degraded degree of polysaccharides in the process of extraction. The k_D is related to extraction temperature, ultrasonic power and ratio of material to solvent.

$$Ln\frac{(C_\infty - C_0)}{(C_\infty - (1 + k_D t)\cdot C)} = kt \quad (5)$$

The polysaccharide yield was given by the following equation:

$$y = \frac{CV}{M} \quad (6)$$

where y is the yield of Chinese chive polysaccharide (%) and M is the initial mass of Chinese chive powder. Thus, the Eq. (5) can be written as Eq. (7), which represents the real kinetic model of polysaccharide extraction including the process of diffusion and degradation.

$$y = \frac{y_\infty}{1 + k_D t}[1 - \exp(-kt)] + \frac{y_0}{1 + k_D t}\exp(-kt) \quad (7)$$

where y is the yield of polysaccharides at each reaction time and y_∞ is the equilibrium yield of polysaccharide extracted at infinite time without considering degradation. The parameters of y_∞, k and k_D are functions of the variables of extraction temperature (T, Kelvin temperature (k)), ultrasonic power (P, w) and solid-liquid ratio (L). Determining the relation of y_∞ and k, k_D with these variables is one of the objectives of this work.

3.2. Determination of kinetic constants

According to Eq. (7), to determine the parameters involved in the kinetic models, the value of y versus extraction time should be measured. Fig. 1 showed the effects of ultrasonic power and solid-liquid ratio on the extraction yield of Chinese chive polysaccharides when fixed the extraction temperature at 313 k. The yield of polysaccharides at each extraction time was determined by phenol-sulfuric acid method. As shown in Fig. 1, we found that both ultrasound power and solid-liquid ratio had a significant

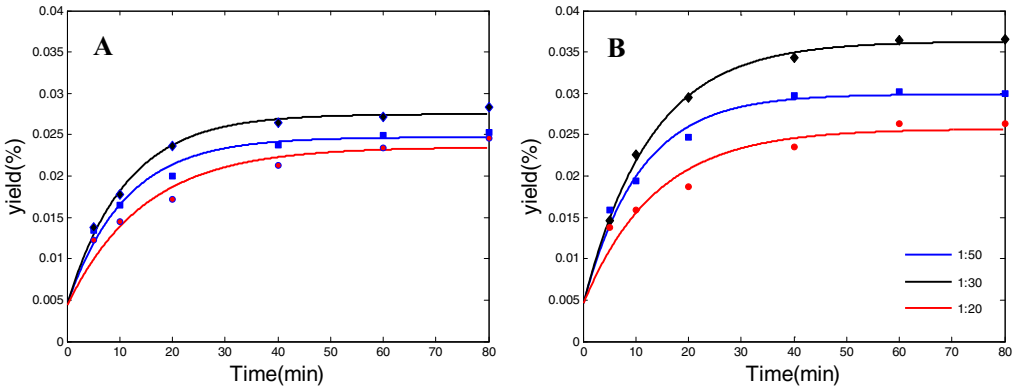

Fig. 1. Polysaccharides extracted from *A. tuberosum* using the method of ultrasound-assisted extraction with different solid-liquid ratio. A, 160 w; B, 320 w.

influence on the yield of polysaccharides, suggesting these two variables are the crucial factors for the extraction of Chinese chive polysaccharides. For the effects of ultrasound power, the polysaccharide yields obtained under 320 w was higher than those of 160 w (Fig. 1A and B). For the effects of solid-liquid ratio, the polysaccharide yield was not enhanced with the increase of extraction solvent (water) among the tested range, meanwhile, the 1:30 of solid-liquid ratio was suggested to be preferable for the extraction of polysaccharides. Results also showed that the process of polysaccharide diffusion could be divided into fast diffusion phase and slow diffusion phase under each solid-liquid ratio. For example, when the solid-liquid ratio was fixed at 1:50, the yield of polysaccharide rapidly increased from 0.005% to 0.02% within 20 min under 160 w of ultrasonic power, then a very slow extraction rate was observed from 20 min to 80 min (Fig. 1A). Fig. 2 showed the effects of extraction temperature and ultrasonic power on the extraction yields of Chinese chive polysaccharides when fixed the solid-liquid ratio at 1:30. Results indicated that the level of ultrasonic power-induced the decrease of polysaccharide yields was strongly associated with the extraction temperature. As shown in Fig. 2A and B, the ultrasonic power at 160 w, 240 w and 320 w could resulted in the increase of polysaccharide yield with the extension of extraction time. However, the ultrasonic power at 480 w caused a significant decrease of polysaccharide yield after extraction for 20 min. As shown in Fig. 2C to E, when the extraction temperature was increased from 323 k to 343 k, the ultrasonic power-induced the decrease of polysaccharide yields reduced from 320 w to 160 w.

Figs. 1 and 2 also indicated that the regression model curves fit the experimental data very well. According to the experimental data obtained under the extraction conditions with different solid-liquid ratio and different temperature when the ultrasonic power was fixed at 160 w, the corresponding regressed kinetic constants were obtained and listed in sTable 1 (Supporting information). These results indicated that both k and k_D increased with the solid-liquid ratio (L) and temperature (T). The relation between the rate constants (k or k_D) and these variables can be described by Eq. (8) that is an expanded Arrhenius equation (Zhao et al., 2014).

$$k = A\exp(-Ea/RT)L^m \tag{8}$$

where A, Ea, R and L represents pre-exponential factor, activation energy, molar gas constant (8.31 J/mol∗K) and solid-liquid ratio (mg/mL), respectively. The m is reaction order with respect to solid-liquid ratio. By taking logarithm, Eq. (8) can be expressed as Eq. (9).

$$\ln k = \ln A - Ea/RT + m\ln L \tag{9}$$

Therefore, the corresponding parameters can be fitted by plotting lnk versus 1/T and L with multivariate linear regression. According to the data in sTable 1 (Supporting information), the parameters in Eq. (9) were fitted and shown in sTable 2 (Supporting information). We found that the activation energy of polysaccharide diffusion is much lower than that of polysaccharide degradation, suggesting the degradation of Chinese chive polysaccharides is more sensitive to extraction temperature and solid-liquid ratio than those of polysaccharide diffusion in the extraction process. The high values of R^2 also suggested that this equation is significant and reliable to describe the relationship between rate constants (k, k_D) and reaction severity.

The Eq. (10) is an empirical equation to describe the relationship among D_s, acoustic energy density (AED) and temperature (Grcic, Sipic, Koprivanac, & Vrsaljko, 2012). Therefore, the diffusion rate constant k can be further expressed as Eq. (11) by comprehensive consideration of Eqs. (2) and (10). In the two equations, a_0, a_1 and a_2 are regression coefficients. The P and V represent ultrasonic power and solvent volume, respectively.

$$D_s = a_0(AED)^{a_1}(T)^{a_2}, \quad AED = P/V \tag{10}$$

$$k = \frac{\pi^2 a_0}{R_0^2}(P/V)^{a_1}(T)^{a_2} \tag{11}$$

Since ultrasonic treatment might cause the disruption of particle microstructures (Xu & Pan, 2013), it is essential to investigate the granularity change of *A. tuberosum* particles before and after ultrasonic treatment. As shown in sFig 1 (Supporting information), no changes were observed in the radius of Chinese chive particle (R_0) after extraction with ultrasound at 320 w and 640 w, suggesting the effect of R_0 on the kinetic model can be neglected in the present study. The mean diameter of Chinese chive particles was determined to be 114 μm in the present study. Thus, the Eq. (12) can be obtained from Eq. (11) with a minor modification.

$$k = a(P/V)^{a_1}(T)^{a_2} + a_3 \tag{12}$$

As the degradation rate constant k_D is similar with k, the Eq. (12) is reasonable to be applied for the expression of k_D:

$$k_D = b(P/V)^{b_1}(T)^{b_2} + b_3 \tag{13}$$

The same application is used for y_∞:

$$y_\infty = c(P/V)^{c_1}(T)^{c_2} + c_3 \tag{14}$$

where a-a_3, b-b_3, c-c_3 are regression coefficients. Therefore, the corresponding rate constants for the extraction of Chinese chive polysaccharides can be calculated from these equations above. The corresponding regressed kinetic constants are listed in sTable 2 and Table 1. Taken together, the extraction process of Chinese chive

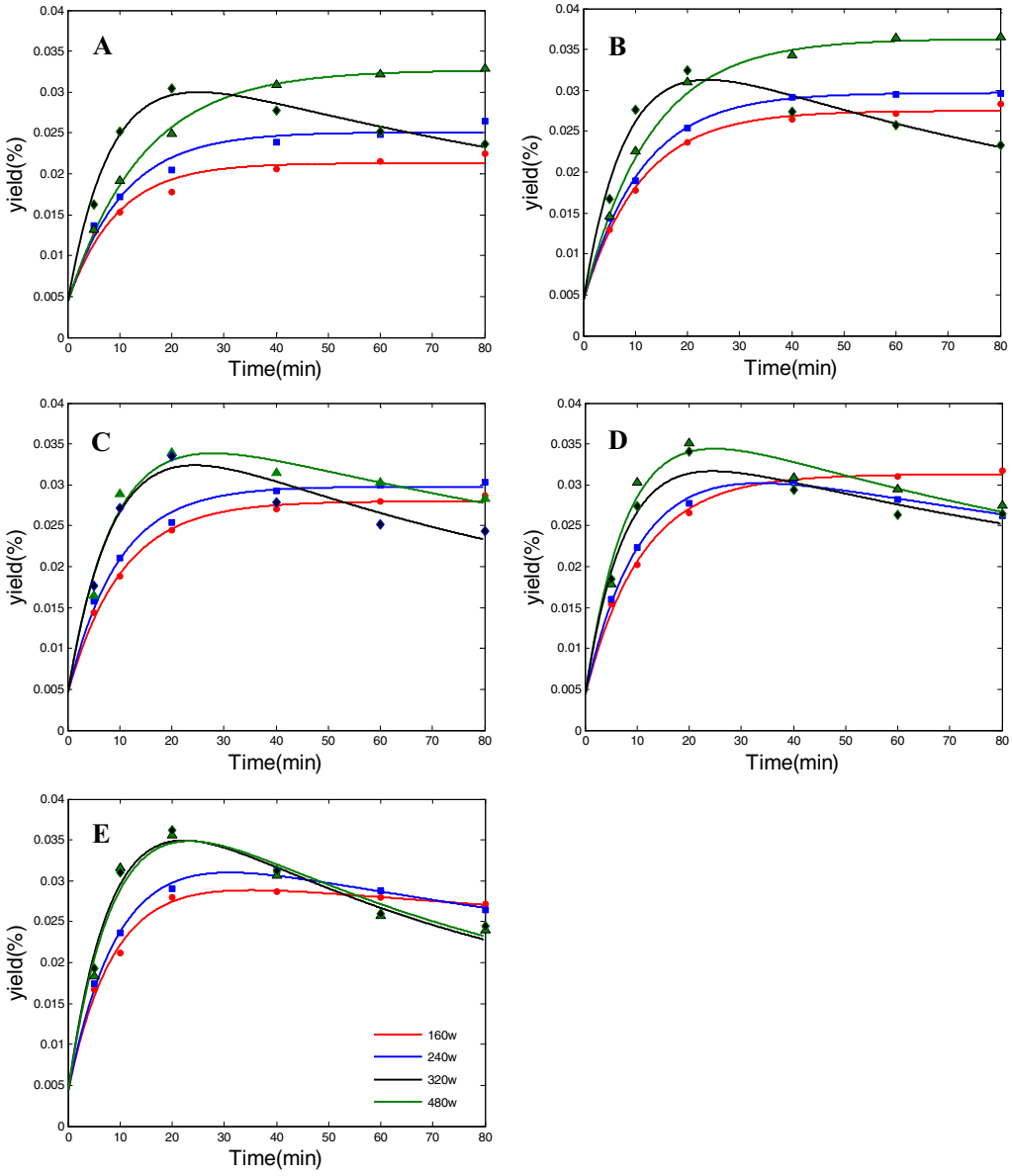

Fig. 2. Polysaccharides extracted from *A. tuberosum* using the method of ultrasound-assisted extraction with different extraction temperature (Kelvin temperature, k). A, 303 k; B, 313 k; C, 323 k; D, 333 k; E, 343 k.

polysaccharides by UAE method can be estimated by the following equations:

$$y = \frac{y_\infty}{1 + k_D t}[1 - \exp(-kt)] + \frac{y_0}{1 + k_D t}\exp(-kt) \quad (7)$$

$$\ln k = \ln 0.9362 - 3.995/T + 0.1575 \ln C \quad (15)$$

$$k_D = (1.712E - 4) * (T - 273)^{1.202} * (P/540)^{1.599} - 0.003 \quad (16)$$

$$y_\infty = (4.95E - 3) * (T - 273)^{0.578} * (P/540)^{0.597} + 0.0044 \quad (17)$$

To prove the effectiveness of the developed models, the predicted data were further compared with the experimental data. As shown in Fig. 3, most of the experimental data can be exactly predicted by the model, suggesting the model is reliable. Weibell's model is a widely used kinetic model applied in the extraction process of polysaccharides from botanic materials because of its simplicity and satisfactory experimental data (Cheung et al., 2013; Wu, Zhu, Diao, & Wang, 2014). To further compare this novel model with conventional Weibell's model, experimental kinetic data were predicted by model calculation and shown in Fig. 4. It is noticed that the predicted data by weibull's model exhibited significant deviations from experimental results, while the calculated data by the model of present work kept good pace with those of experiments. These results indicated that the kinetic model established in the present study can describe the real extraction process of Chinese chive polysaccharides using UAE method.

3.3. Optimization of extraction conditions and verification of the predictive model

According to the established mathematical model, an optimal extraction condition and the corresponding maximum yield of polysaccharide were obtained by Matlab 7.0 software. Results showed that the maximum polysaccharide yield was predicted to be 3.66% under an optimal extraction condition including 37 °C

Table 1
Kinetic constants for the extraction of polysaccharides from Chinese chive regressed by experimental data with different ultrasonic power and different temperatures.

T (k)	P (w)	y_∞	y_0	k_d (min^{-1})	k (min^{-1})	D_s (m^2/min)
303	160	0.0214	0.0046	1.25E−17	0.0693	4.00E−13
313	160	0.0256	0.0046	1.35E−17	0.0787	4.55E−13
323	160	0.0284	0.0046	1.39E−17	0.0941	5.45E−13
333	160	0.0313	0.0046	1.72E−17	0.1014	5.86E−13
343	160	0.0315	0.0046	0.002	0.1044	6.04E−13
303	240	0.0251	0.0046	3.74E−17	0.0881	5.09E−13
303	320	0.0327	0.0046	6.95E−17	0.0888	5.13E−13
303	480	0.0376	0.0046	0.0074	0.0896	5.18E−13
313	240	0.0271	0.0046	6.02E−17	0.0918	5.31E−13
313	320	0.0363	0.0046	8.31E−17	0.0932	5.39E−13
313	480	0.0415	0.0046	0.0093	0.0933	5.39E−13
323	240	0.0298	0.0046	7.4E−17	0.0951	5.49E−13
323	320	0.0420	0.0046	0.0064	0.0970	5.60E−13
323	480	0.0479	0.0046	0.0121	0.1032	5.96E−13
333	240	0.0372	0.0046	0.0022	0.1040	6.01E−13
333	320	0.0455	0.0046	0.0087	0.1044	6.04E−13
333	480	0.0537	0.0046	0.0162	0.1059	6.12E−13
343	240	0.0376	0.0046	0.0036	0.1054	6.09E−13
343	320	0.0480	0.0046	0.0116	0.1057	6.11E−13
343	480	0.0579	0.0046	0.0196	0.1067	6.17E−13

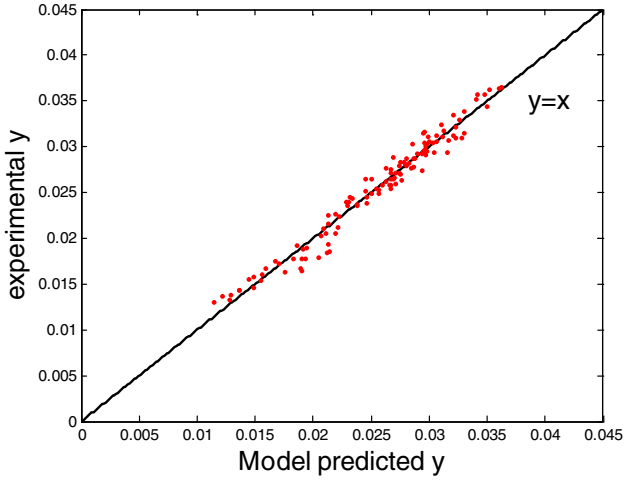

Fig. 3. Comparison of model-predicted data with experimental data for the extraction of *A. tuberosum* polysaccharides using the method of UAE. The red dot represents the experimental data; The line represents the model-predicted data. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

of extraction temperature, 458 w of ultrasonic power, 30 min of extraction time and 1:32 of solid-liquid ratio. To verify this prediction, verification experiments were further performed using this optimal condition for four times. Results exhibited that there is only 2.94% of relative tolerance between the predicted value and the experimental value, further confirming the response model is satisfactory and accurate for prediction of polysaccharide extraction from Chinese chive.

3.4. Determination of the main objective fraction of polysaccharides extracted by ultrasonic technology

In order to evaluate the performance of ultrasound technology, the extraction yield of polysaccharides by ultrasonic method was compared with that of conventional extraction technology (HWE). For conventional extraction technology, all parameters were the same as those of ultrasonic method except ultrasonic treatment. Results showed that the extraction yield of ultrasonic technology was enhanced by 1.31-fold than that of conventional extraction technology. After precipitation with ethanol at the final concentration of 40%, 60% and 80% (v/v) in turn, we found that the ultrasound mainly enhanced the extraction of polysaccharides precipitated with 40% and 60% ethanol (sTable 3 in Supporting information). HPLC was further employed to analyze the molecular weight distribution of different polysaccharide fractions precipitated with 40%, 60% and 80% (v/v) ethanol (Fig. 5). An interesting result was observed that the content of polysaccharides with different molecular weight in the three polysaccharide fractions extracted by ultrasound technology are significantly different from those of conventional extraction technology. As shown in Fig. 5A and B, the peak area of CCP1 at the retention time from 39.532 to 41.344 min, CCP2 at the retention time of 43.774 min, and CCP3 at the retention time of 20.35 min were significantly enhanced by ultrasound treatment, indicating the increased extraction of these three fractions might be the main reason for the enhancement of polysaccharide extraction by ultrasound. In contrast, the peak area of polysaccharide (CCP4) at the retention time from 42.695 to 43.712 min was drastically decreased in the fraction precipitated with 80% ethanol by ultrasound treatment when compared to that of conventional technology (Fig. 5C), attributing to the degradation of CCP during ultrasound-assisted extraction. These results also confirmed the findings by the determination of polysaccharide content in different fractions precipitated with ethanol (sTable 3 in Supporting information). Moreover, according to the calibration curve between the retention time and the logarithms of standard molecular weights, the average molecular weight of CCP1, CCP2, CCP3 and CCP4 were determined to be about 7.42×10^3–1.29×10^4 Da, 3.533×10^3 Da, 4.6×10^6 Da and 3.60×10^3–4.91×10^3 Da, respectively. Therefore, we can conclude that CCP1, CCP2, CCP3 and CCP4 are the main objective polysaccharides responding to ultrasound treatment.

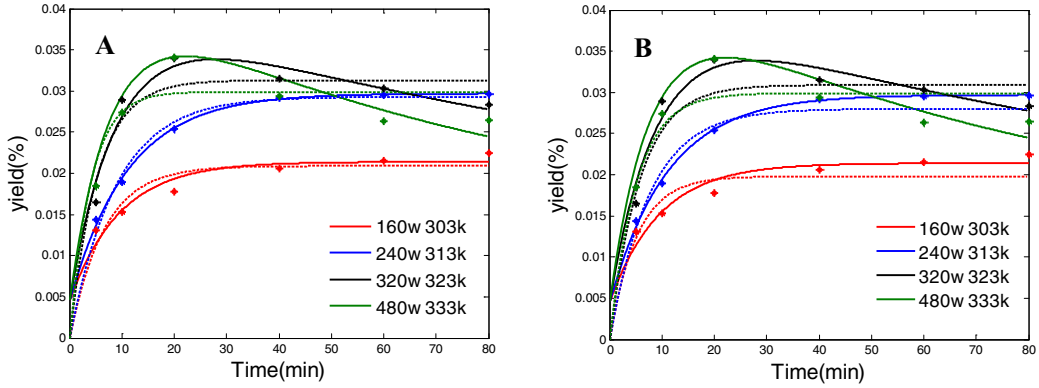

Fig. 4. The differences in the predicted polysaccharide extraction yields by the Weibell's model and the established model of the present study. A, Weibell's model n = 1; B, Weibell's model n = 2; symbol, experimental data; solid line, predicted data by the model developed in the present work; dash line, predicted data by Weibell's model.

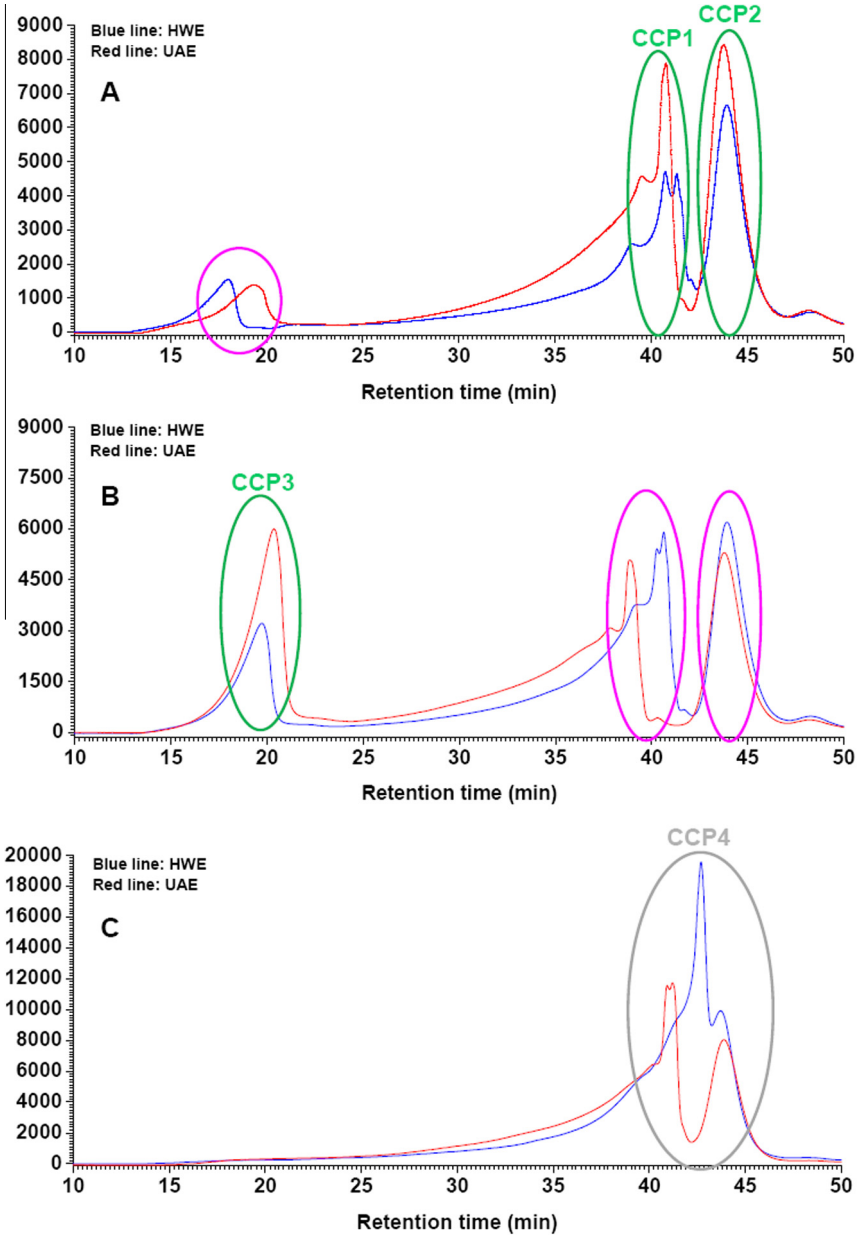

Fig. 5. HPLC chromatogram of different polysaccharide fractions extracted from *A. tuberosum* using the extraction methods of UAE and HWE. A, polysaccharide fraction precipitated with 40% ethanol (v/v); B, polysaccharide fraction precipitated with 60% ethanol (v/v); C, polysaccharide fraction precipitated with 80% ethanol (v/v).

3.5. Composition of glucosidic bonds in polysaccharides extracted by ultrasonic technology

According to the data summarized in sTable 4, it is concluded that the polysaccharides of Chinese chive were composed of mannose, galactose and glucose. Results showed that mannose, galactose and glucose were referred to be linked by (1 → 3,6), (1 → 6) and (1→) glucosidic bond, respectively. Although each polysaccharide fraction contained the same glycosyl-linkages, different contents of these glucosidic bonds were observed in each sample.

4. Conclusion

In the present work, a reliable kinetic model was established to describe the real extraction process of Chinese chive polysaccharides during ultrasound-assisted extraction. Using this model, an optimal extraction condition was obtained, which includes 37 °C of extraction temperature, 458 w of ultrasonic power, 30 min of extraction time and 1:32 of solid-liquid ratio. Results of HPLC showed that the polysaccharides with molecular weight at 8.83×10^3 Da, 3.533×10^3 Da, 4.6×10^6 Da and 4.91×10^3 Da were the main objective fractions responding to ultrasound.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2016.05.144.

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