# Optimization of pectin extraction and antioxidant activities from Jerusalem artichoke\*

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Received Nov. 15, 2014; accepted in principle Feb. 19, 2015; accepted for publication Apr. 15, 2015 © Chinese Society for Oceanology and Limnology, Science Press, and Springer-Verlag Berlin Heidelberg 2016

**Abstract** Jerusalem artichoke is an economic crop widely planted in saline-alkaline soil. The use of Jerusalem artichoke is of great significance. In this study, the response surface method was employed to optimize the effects of processing variables (extraction temperature, pH, extraction time, and liquid-to-solid ratio) on the yield of Jerusalem artichoke pectin. Under the optimal extraction conditions: pH 1.52, 63.62 min, 100°C and a liquid-to-solid ratio of 44.4 mL/g, the maximum pectin yield was predicted to be 18.76%. Experiments were conducted under these optimal conditions and a pectin yield of 18.52 $\pm$ 0.90% was obtained, which validated the model prediction. The effects of different drying methods (freeze drying, spray drying and vacuum drying) on the properties of Jerusalem artichoke pectin were evaluated and they were compared with apple pectin. FTIR spectral analysis showed no major structural differences in Jerusalem artichoke pectin samples produced by various drying treatments. The antioxidant activities of pectin dried by different methods were investigated using in vitro hydroxyl and DPPH radical scavenging systems. The results revealed that the activities of spray dried pectin (SDP) and apple pectin (AP) were stronger than those of vacuum oven dried pectin (ODP) and vacuum freeze dried pectin (FDP). Therefore compared with the other two drying methods, the spray drying method was the best.

Keyword: Jerusalem artichoke pectin; response surface; drying methods; antioxidant activities

# **1 INTRODUCTION**

Pectin is a polysaccharide, which is an abundant, ubiquitous and multifunctional component of plant cell walls and provides channels for the passage of nutrients and water (Willats et al., 2006). The dominant structural feature of pectin is a linear  $1 \rightarrow 4$ -linked chain of poly- $\alpha$ -D-galacturonic acid and neutral sugars, such as L-rhamnose, L-arabinose, and D-galactose. It is widely used as a stabilizing, thickening, or gelling agent in food, pharmaceutical and cosmetic industries (Axelos et al., 1989). Pectin also has important biological and physiological functions, such as the reduction of cholesterol levels and cancer incidences, and stimulation of immune response (Behall and Reiser, 1986; Yamada, 1996). Pectin is mostly produced from citrus peel and apple pomace (May, 1990). Agricultural by-products including sunflower heads (Iglesias and Lozano, 2004), sugar beet residues (Lü et al., 2013), passion fruit peel (Pinheiro et al., 2008) and soy hull (Monsoor and Proctor, 2001) have been investigated as sources of pectin. Pectin extraction is a multiple-stage physicochemical process, and extraction conditions affect the composition of pectin (Rolin, 1993). In the process of hydrolysis and extraction of pectin from plant tissue, many factors such as temperature (Wettasinghe and Shahidi, 1999), pH, time and liquidto-solid ratio (Cacace and Mazza, 2003) may significantly affect the extraction efficacy. After hydrolysis, the pectin solution is centrifuged, filtered, precipitated with alcohol and gently dried. Drying

<sup>\*</sup> Supported by the National High Technology Research and Development Program of China (863 Program) (No. 2012AA021205) and the National Key Technology R&D Program of China (No. 2011BAC02B04) \*\* Corresponding author: ytyi@yic.ac.cn

methods also influence pectin properties. There are mainly three drying methods: vacuum oven drying (Yapo et al., 2007), vacuum freeze drying (Kalapathy and Proctor, 2000) and spray drying (Monsoor and Proctor, 2001).

Many human diseases are associated with the activation of free radicals that negatively affect biological membranes (Henrotin et al., 2003). These harmful effects can be prevented by antioxidants, which scavenge free radicals and detoxify organisms. Epidemiological studies have shown that the consumption of fruits and vegetables is associated with a reduced risk of chronic diseases. It has been demonstrated that polysaccharides have strong antioxidant activities and this is because of the reduction power and free radical scavenging activities of these molecules (Yang et al., 2004; Mateos-Aparicio et al., 2010; Tomida et al., 2010). Studies suggest that pectin can interact directly with oxidants and free radicals (Khasina et al., 2003).

Jerusalem artichoke, widely cultivated in northern China, is a flowering plant of the helianthus. Compared with grain crops, Jerusalem artichoke can grow well in arid saline soils and resist many plant pests and diseases (Bajpai and Bajpai, 1991). In addition, planting Jerusalem artichoke can also benefit the ecological environment (Yuan et al., 2008). Because Jerusalem artichoke tubers are mainly used to produce inulin, a large amount of residues (plant by-products) are produced. These residues should be used properly to add value to the whole Jerusalem artichoke production process and reduce damage to the environment. Our preliminary experiments indicated that Jerusalem artichoke pulp contained about 15% pectin on a dry weight basis. However, to the best of our knowledge, there is no published work on the physicochemical properties and antioxidant activity in pectin from Jerusalem artichoke pulp.

In the present work, we examined the effects of extraction temperature, pH, time and liquid-to-solid ratio on pectin yield. A second-order polynomial model was developed to predict the yield of pectin as a function of extraction conditions (temperature, pH, time, and liquid-to-solid ratio) using the Box-Behnken design under response surface methodology (RSM). Pectin solutions were obtained under the optimized conditions and then dried by different methods (vacuum freeze drying, spray drying and vacuum oven drying). We also investigated the relationship between drying treatments and pectin properties (surface structures and antioxidant activities).

# 2 MATERIAL AND METHOD

#### 2.1 Materials and chemicals

Raw Jerusalem artichoke tubers were collected from Dongying, Shandong province. All the chemical reagents and solvents used were of analytical grade and used without further purification.  $\alpha,\alpha$ -diphenyl- $\beta$ picrylhydrazyl (DPPH) was purchased from TCI Development Co. Ltd., Japan. Phosphoric acid, safranine O, hydrogen peroxide and ammonia were purchased from Yongda Chemical Reagent Co. Ltd., Tianjin, China. Carbazole, disodium hydrogen phosphate and sodium dihydrogen phosphate were purchased from Sinopharm Chemical Reagent Co. Ltd., Beijing, China. Sulfuric acid was purchased from Sanhe Chemical Reagent Co. Ltd., Yantai, China. Ultra-pure water was used throughout the experiments.

#### 2.2 Residues pretreatment

Jerusalem artichoke residues were obtained after extracting inulin using hot water at 85°C for 1 h. The residues were then dehydrated in a cross flow hot air drier at 50°C for 24 h. Subsequently, the dried residues were milled to a 40-mesh size and packed in lowdensity polyethylene bags until analysis.

#### 2.3 Extraction of pectin

Pectin was extracted from coarsely ground, dehydrated Jerusalem artichoke residues (moisture 5% in weight). The residues were suspended in diluted acid solutions at specific conditions (extraction temperature, pH, the length of extraction time and liquid-to-solid ratio). After the reaction was completed, the suspension was further filtered using a Buchner funnel, and adjusted to pH 3.5 with NH<sub>3</sub>·H<sub>2</sub>O. The soluble fractions were then concentrated to 1/3 of the initial volume in a rotatory evaporator at 50°C. Ethanol (95% purity) was added to the solution at a ratio of 2:1 (v/v) and the solution was kept at room temperature for 4 h. The floating pectin was collected by centrifugation, washed twice with 75% ethanol and dried in a vacuum oven at 50°C for 24 h.

# 2.4 Selection of extractants

The extraction conditions were fixed as follows: extraction temperature 90°C, pH 2, extraction time 60 min and the liquid-to-solid ratio 30 mL/g. The effects of different extractants (hydrochloric acid, sulfuric acid, phosphoric acid, and sulfurous acid) on pectin yield were compared.

| Independent factor    | Unit | Symbol | Coded levels |     |     |
|-----------------------|------|--------|--------------|-----|-----|
|                       |      |        | -1           | 0   | 1   |
| Temperature           | °C   | $X_1$  | 80           | 90  | 100 |
| pH                    |      | $X_2$  | 1            | 1.5 | 2   |
| Time                  | Min  | $X_3$  | 50           | 70  | 90  |
| Liquid-to-solid ratio | mL/g | $X_4$  | 30           | 40  | 50  |

Table 1 Levels and codes of factors

#### 2.5 Response surface experiment design

According to the results of preliminary experiments, a response surface methodology (RSM) was applied to optimize the conditions and the integral effects of four independent variables (temperature, time, pH, and liquid-to-solid ratio) at three levels. The levels of the variables and their codings are presented in Table 1.

### 2.6 Determination of pectin yield

The yield of pectin was calculated using Eq.1:

Pectin yield/%=
$$(m_1/m_2) \times 100$$
, (1)

where  $m_1(g)$  is the weight of dried pectin,  $m_2(g)$  is the weight of dried material.

#### 2.7 Different drying treatments

Pectin was extracted from Jerusalem artichoke residues (300 g) under the optimized condition (pH 1.52, 63 min, 100°C and a liquid-to-solid ratio of 44 mL/g). The extracts were cooled to room temperature and centrifuged. The supernatants were collected and adjusted to pH 3.5 with NH<sub>3</sub>·H<sub>2</sub>O. The extracts were then concentrated by ultrafiltration through membranes with a 10 kDa molecular weight cut-off. The concentrated solution was divided into three parts for each drying treatment. The inlet/outlet air temperature for a spray drying process was 160±5°C/75±5°C. For vacuum oven drying and freeze drying, double the volume of ethanol was added to the solutions to precipitate pectin. Subsequently, the pectin was vacuum freeze dried at -50°C or vacuum oven dried at 45°C for 24 h, respectively. The dried pectin samples were subjected to the following analysis.

#### 2.8 Surface structure analysis

According to Mateos-Aparicio et al. (2010), pectin was incorporated into KBr and pressed into a 1 mm disk. Fourier Transform Infrared (FT-IR) spectra were recorded at the absorbance mode from 4 000 to 400/cm (mid infrared region) at a phase resolution of 4/cm, averaging 30 scans/min and using a FT-IR 4200 Jasco Fourier Transform spectrophotometer (Shanghai, China). At least triplicate spectra were recorded for each sample.

# 2.9 Antioxidant activities of Jerusalem artichoke pectin

### 2.9.1 Hydroxyl radical scavenging activities

The hydroxyl-radical scavenging activities of Jerusalem artichoke pectin dried by the three different methods were measured according to the method described by Guo et al. (2005, 2007) with slight modifications. The reaction solution (total volume 4.5 mL) containing pectin (1, 2, 3, 4, 5 mg/mL) was incubated with EDTA-Fe<sup>2+</sup> (0.945 mmol/L), safranine O (80 mg/L), H<sub>2</sub>O<sub>2</sub> (3%) and potassium phosphate buffer (0.15 mmol/L, pH 7.4) for 30 min at 37°C. Finally, the absorbance of the reaction solution was measured at 520 nm. The effect of scavenging hydroxyl radicals was calculated using the following equation:

Scavenging effect (%)=[
$$(A_{\text{sample520nm}}-A_{\text{blank520nm}})/$$
  
 $(A_{\text{control520nm}}-A_{\text{blank520nm}})$ ]×100, (2)

where  $A_{\text{blank520nm}}$  was the absorbance of the blank (distilled water instead of the samples),  $A_{\text{control520nm}}$  was the absorbance of the control (distilled water instead of the sample and EDTA-Fe<sup>2+</sup>).

#### 2.9.2 DPPH radical scavenging activities

The DPPH radical scavenging activities of Jerusalem artichoke pectin dried by the three different methods were determined in triplicate according to the method described by Yamaguchi et al. (1998) with slight modifications. An ethanolic solution of DPPH (2 mL, 0.2 mmol/L) was added to 2 mL of the saccharide solutions (2, 4, 6, 8, and 10 mg/mL). The mixture was left in the darkness at room temperature for 30 min, and then the absorbance of the mixture was measured at 517 nm. The effect of DPPH radical scavenging was calculated using the following equation:

Scavenging effect (%)= $[1-(A_i-A_j)/A_c)]\times 100$ , (3) where  $A_i$  was the absorbance of the sample,  $A_j$  was the absorbance of the blank (95% ethanol instead of the DPPH),  $A_c$  was the absorbance of the blank (95% ethanol instead of the sample).

# 2.10 Statistical analysis

All determinations were performed in triplicates

|              |                     | Fac                     | Pectin yield (%)          |  |              |           |
|--------------|---------------------|-------------------------|---------------------------|--|--------------|-----------|
| Run<br>order | Temperature $(X_1)$ | рН<br>(X <sub>2</sub> ) | Time<br>(X <sub>3</sub> ) | Liquid-<br>to-solid<br>ratio (X <sub>4</sub> ) | Experimental | Predicted |
| 1            | -1                  | 0                       | -1                        | 0  | 10.25        | 10.52     |
| 2            | 1                   | -1                      | 0                         | 0  | 11.69        | 11.6      |
| 3            | 0                   | 0                       | -1                        | -1   | 12.99        | 12.48     |
| 4            | 0                   | 1                       | 1                         | 0  | 8.20         | 7.61      |
| 5            | 0                   | -1                      | 1                         | 0  | 7.50         | 6.94      |
| 6            | -1                  | 1                       | 0                         | 0  | 4.82         | 5.36      |
| 7            | 1                   | 0                       | 0                         | -1   | 15.43        | 15.9      |
| 8            | 1                   | 0                       | 0                         | 1  | 18.39        | 18.31     |
| 9            | -1                  | -1                      | 0                         | 0  | 4.32         | 4.96      |
| 10           | 0                   | 0                       | 1                         | 1  | 13.32        | 13.98     |
| 11           | -1                  | 0                       | 0                         | -1   | 9.65         | 8.99      |
| 12           | -1                  | 0                       | 1                         | 0  | 9.93         | 10.31     |
| 13           | 0                   | 0                       | 1                         | -1   | 10.97        | 10.91     |
| 14           | 0                   | -1                      | 0                         | 1  | 8.3          | 8.38      |
| 15           | 1                   | 1                       | 0                         | 0  | 12.80        | 12.27     |
| 16           | 0                   | 0                       | -1                        | 1  | 14.03        | 14.23     |
| 17           | 0                   | 0                       | 0                         | 0  | 15.41        | 15.18     |
| 18           | 0                   | 1                       | 0                         | 1  | 9.00         | 9.05      |
| 19           | -1                  | 0                       | 0                         | 1  | 12.30        | 11.40     |
| 20           | 0                   | 0                       | 0                         | 0  | 14.73        | 15.18     |
| 21           | 0                   | 1                       | -1                        | 0  | 8.45         | 8.52      |
| 22           | 0                   | 0                       | 0                         | 0  | 15.12        | 15.18     |
| 23           | 0                   | 1                       | 0                         | -1   | 6.18         | 6.64      |
| 24           | 0                   | 0                       | 0                         | 0  | 15.32        | 15.18     |
| 25           | 0                   | 0                       | 0                         | 0  | 15.34        | 15.18     |
| 26           | 0                   | -1                      | 0                         | -1   | 5.68         | 5.97      |
| 27           | 1                   | 0                       | 1                         | 0  | 16.36        | 16.53     |
| 28           | 1                   | 0                       | -1                        | 0  | 18.07        | 18.13     |
| 29           | 0                   | -1                      | -1                        | 0  | 7.95         | 7.85      |

 Table 2 Box-Behnken Design and results

and values were expressed as means $\pm$ standard deviation. Design Expert 8.0.6 software was used for the experimental design, statistical analysis and data processing. The significances of the regression coefficients were tested by an *F*-test. Figures were plotted using Origin 8.

# 3 RESULT AND DISCUSSION

#### 3.1 Effects of different extractants on pectin yield

Figure 1 illustrates that extractants affected pectin

yield. When extracting with sulfuric acid, the highest pectin yield of  $5.81\pm0.21\%$  was achieved. Therefore, phosphoric acid was selected to be used as the extractant in the subsequent experiments.

#### 3.2 Response surface experiments

The experimental and predicted values of Box-Behnken experimental design are shown in Table 2.

#### 3.2.1 Model fitting

A second order polynomial equation was used to build a mathematical model aiming at an optimal region for the responses studied and to study the combined effects between the process variables and the response. The developed second-order model in term of coded values is given below:

$$Y=15.18+3.16X_{1}+0.33X_{2}-0.46X_{3}+1.2X_{4}+$$

$$0.15X_{1}X_{2}-0.35X_{1}X_{3}+0.33X_{3}X_{4}-0.28X_{1}^{2}-$$

$$6.42X_{2}^{2}-1.03X_{3}^{2}-1.25X_{4}^{2},$$
(4)

where *Y* is the pectin yield (%), and  $X_1$ ,  $X_2$ ,  $X_3$ ,  $X_4$  are the coded variables representing extracting temperature, pH, time, and liquid-to-solid ratio respectively.

3.2.2 Analysis of variance for regression model of pectin yield

Results from the analysis of variance (ANOVA) for the fitted quadratic polynomial model representing the pectin yield is shown in Table 3. The ANOVA shows that the quadratic regression model is highly significant as the Fisher F-test yields a very low probability value (P<0.000 1). This probability value means that there is only a 0.01% chance that a "Model F-Value" of this magnitude could occur because of noise. However, the F-value (4.11) of the Lack of Fit implies it is not significant, which means it is adequate to predict the pectin yield within the range of variables employed. The high value of  $R^2$  (0.990 2), adj- $R^2$ (0.983 8), and pre- $R^2$  (0.965 6) also states that, the predicted model seems to reasonably represent the observed values. Therefore, the response was sufficiently explained by the model. The low coefficient of variation (CV=4.42%) among the replicate experimental data indicates a high degree of precision and a good deal of reliability for experimental values. The significance of each coefficient is determined using the F-test and P-value. The test indicates that variables with the largest effect on pectin yield are  $X_1, X_4, X_2^2, X_3^2, X_4^2$ . Variables  $X_2, X_3$ affects the pectin yield significantly and  $X_1X_2$ ,  $X_1X_3$ ,



Fig.1 Effects of different extractants on pectin yield

 $X_3X_4$ ,  $X_1^2$  affects the pectin yield non-significantly. From the above analysis, it was concluded that the regression equation can be confidently used to navigate the design space.

# 3.2.3 Analysis of response surfaces

The tri-dimensional representation of the response surfaces and two-dimensional contour plots illustrates the relationship between responses and independent variables. The response surfaces and contour plots showing the effect of extraction temperature are shown in Figs.2 and 3. As can be seen from the figures, temperature had a linear increase on pectin yield, and the linear relationship held at all the temperature tested. This phenomenon could be because the increase of temperature loosened cell walls and epidermal tissues, disrupted the interactions between pectin with protein or polysaccharides and decreased the solvent viscosity, which contributed to the exchange of extractant and materials, thus giving a higher rate of extraction (Vriesmann et al., 2011).

Figure 2 also shows the effect of pH on pectin yield. The effect of pH on the surface displayed a quadratic effect when pH ranged from 2.0 to 1.0. When the pH of the solution decreased, pectin yield first increased and then decreased. This phenomenon could occur as acidic conditions contribute to hydrolyze the insoluble pectic constituents into soluble pectin, which then increased the pectin recovery (Masmoudi et al., 2008). However, when the acidity of the solution further reduced, the pectin yield decreased and this might be due to the aggregation of pectin, which then retarded the release of pectin.

The response surfaces contour plots showing the effect of extraction time are shown in Figs.3 and 4. Extraction time was one of the key factors affecting

| · ·         |                |                   | •                  |          |            |
|-------------|----------------|-------------------|--------------------|----------|------------|
| Source      | Sum of squares | Degree of freedom | Mean<br>square     | F value  | P-value    |
| Model       | 439.13         | 11                | 39.92              | 155.36   | <0.000 1** |
| $X_1$       | 143.31         | 1                 | 143.31             | 557.72   | <0.000 1** |
| $X_2$       | 1.34           | 1                 | 1.34               | 5.21     | 0.035 5*   |
| $X_3$       | 2.48           | 1                 | 2.48               | 9.67     | 0.006 4*   |
| $X_4$       | 17.38          | 1                 | 17.38              | 67.62    | <0.000 1** |
| $X_1X_2$    | 0.093          | 1                 | 0.093              | 0.36     | 0.555 3    |
| $X_1X_3$    | 0.48           | 1                 | 0.48               | 1.88     | 0.188 2    |
| $X_3X_4$    | 0.43           | 1                 | 0.43               | 1.67     | 0.213 6    |
| $X_{1}^{2}$ | 0.51           | 1                 | 0.51               | 2.00     | 0.175 2    |
| $X_{2}^{2}$ | 267.48         | 1                 | 267.48             | 1 040.94 | <0.000 1** |
| $X_{3}^{2}$ | 6.89           | 1                 | 6.89               | 26.80    | <0.000 1** |
| $X_{4}^{2}$ | 10.18          | 1                 | 10.18              | 39.62    | <0.000 1** |
| Residual    | 4.46           | 18                | 0.25               |          |            |
| Lack of fit | 4.46           | 14                | 0.30               | 4.11     | 0.091 3    |
| Pure error  | 0.30           | 4                 | 0.076              |          |            |
| Cor total   | 443.50         | 28                |                    |          |            |
| Std. dev    | 0.51           |                   | $\mathbb{R}^2$     | 0.990 2  |            |
| Mean        | 11.47          |                   | Adj-R <sup>2</sup> | 0.983 8  |            |
| C.V. (%)    | 4.42           |                   | Pre-R <sup>2</sup> | 0.965 6  |            |
|             |                |                   |                    |          |            |

Table 3 Analysis of variance (ANOVA) for response surface quadratic model for pectin yield

\* P<0.05, significant; \*\* P<0.01, highly significant.

the pectin yield. From the results, it was observed that pectin yield increase steadily with the extension of time. The absorption of energy in the extraction system promoted the hydrolysis of insoluble pectin. However, the excessive time exposure may lead to partial degradation of pectin (Zheng et al., 2011). Similar results were observed with pectin extracted from passion fruit peel by Kulkarni and Vijayanand (2010).

The response surface contour plot showing the effect of liquid-to-solid ratio is shown in Fig.4. Solvent quantity is also an important factor affecting pectin yield. It can be seen that the pectin yield increased with increasing liquid-to-solid ratio, which was probably because larger volumes of extraction solvent caused excessive swelling of the materials (Guo et al., 2001). Therefore, the cell walls ruptured, which resulted in easy release of pectin into the surrounding medium. However, when the liquid-to-solid ratio was exceeded beyond 44 mL/g, the solution was saturated with the solute, which hindered the mass transfer rate, barricaded the penetration of pectin into the solution and decreased the extraction yield.



Fig.2 Response surface and contour plots illustrating the effects of temperature and pH on pectin yield



Fig.3 Response surface and contour plots illustrating the effects of temperature and time on pectin yield

Monsoor and Proctor (2001) obtained similar results when pectin was extracted from soy hull.

#### 3.2.4 Validation of optimized conditions

The objective of optimization was to determine the conditions needed to produce the maximum extraction yield of pectin. Based on our regression model, the optimum extraction conditions were pH 1.52, 63.62 min, 100°C and a liquid-to-solid ratio of 44.4 mL/g. Under those conditions, the maximum pectin yield was up to 18.76%. Triplicate experiments were conducted under selected optimal conditions and the actual result was 18.52 $\pm$ 0.9%. This result was in agreement with the predicted value, which indicated that the established model in this study was feasible.

# **3.3** Surface structure analysis of pectin treated by different drying methods

The diffuse reflectance FTIR spectra (4 000–400/cm) of Jerusalem artichoke pectin produced by vacuum

oven drying, spray drying, vacuum freeze drying, and apple pectin are presented in Fig.5. The wave patterns of pectin treated by the different drying methods and that of apple pectin were almost the same, which illustrates that drying methods have no detrimental effects on the structure of Jerusalem artichoke pectin and that Jerusalem artichoke pectin produced by different methods are comparable to apple pectin. Monsoor (2005) who studied the effects of drying methods on soy hull pectin, reported similar results.

The major absorption between 3 600 and 2 500/cm were caused by hydroxyl groups and inter- and intramolecular hydrogen bonds. In pectin samples, absorption of this region is because of the inter- and intra-molecular hydrogen bonding of the galacturonic acid backbone (Singthong et al., 2004). The band around 2 900/cm corresponds to the C-H stretching vibration of CH<sub>2</sub>. Stronger bands occurring at 1 640 and 1 750/cm are derived from free and esterified carboxyl groups (Monsoor and Proctor, 2001). It was



Fig.4 Response surface and contour plots illustrating the effects of time and liquid-to-solid ratio on pectin yield



Fig.5 Diffuse reflectance Fourier transform infrared spectra of the 4 000–400/cm region of Jerusalem artichoke pectin treated by different drying methods a. vacuum freeze drying; b. vacuum oven drying; c. spray drying; d. apple pectin.

observed that the intensity of the absorbance or band area of the ester carbonyl groups (1 730-1 760/cm) increased with the increase in DE (Degree of Esterification). In a similar manner, the intensity of the absorbance or band area of the free carboxylate groups (1 630-1 600/cm) increased with the decrease in DE. Thus, FT-IR can be used for the quantitative analysis of DE of pectin samples (Singthong et al., 2004). Gnanasambandam and Proctor (1999) have successfully used this method to determine the degree of esterification of commercial pectin samples. Absorption bands below 1 500/cm are considered as the 'fingerprint' for a given compound and corresponds principally to coupled C-C, C-O-C and C-OH vibration modes of the carbohydrate ring and to the glycosidic linkage vibrations (Urias-Orona et al.,

2010). The absorption bands between 1 100 and 1 200/cm are from ether (R-O-R) and cyclic C-C bonds in the ring structure of pectin molecules (Kalapathy and Procter, 2000). The presence of FT-IR absorption bands at 1 000 and 1 140/cm corresponds to the stretching vibrations of (C-OH) side groups and the (C-O-C) glycosidic bond vibration.

#### 3.4 Antioxidant activity

3.4.1 Scavenging of hydroxyl radical by pectin treated by different drying methods

The hydroxyl radical is the strongest reactive free radical among reactive oxygen species, and it can react with all bio-macromolecules in living cells (Huang et al., 2005; Sun et al., 2009). However no enzymes found in organisms are known to degrade the hydroxyl radical (Akashi et al., 2004). Figure 6 exhibits positive correlations between concentrations of the samples and scavenging activity against the hydroxyl radical generated by the Fenton reaction. Obviously, drying methods did have effects on the hydroxyl radical-scavenging abilities and the hydroxyl radical-scavenging abilities of SDP and AP were higher than that of the ODP and FDP at all the same concentrations. At the concentration of 2.5 mg/mL, the scavenging activity of SDP was 91.04±1.8%, which was higher than the scavenging activities of pectin produced through the other drying methods. According to previous studies, polysaccharides scavenging hydroxyl radicals occur via two main mechanisms: one suppresses the generation of the hydroxyl radical, and the other scavenges the hydroxyl radical generated (Zhang et al., 2010). It has been



Fig.6 Scavenging of hydroxyl radical by pectin treated by different drying methods

suggested that pectin interacts directly with oxidants and free radicals. The mechanism of pectin treated by different drying methods on the hydroxyl radical needs to be further investigated.

3.4.2 Scavenging of DPPH radical by pectin treated by different drying methods

DPPH, a stable organic free radical that shows maximum absorbance at 517 nm, has been widely used for the determination of antioxidant capacity of different natural compounds. Jerusalem artichoke pectin exhibited a dose-dependent free radical scavenging activity, as shown in Fig.7. In concentrations ranging from 0.5-2.5 mg/mL, AP scavenged 20.90%-45.23% of DPPH radical, 16.09%-43.47% for SDP, 14.1%-42.10% for ODP, and 10.51%-39.31% for FDP. Obviously, drying methods do have effects on DPPH radical-scavenging abilities and the DPPH radical-scavenging abilities of AP and SDP were higher than those of the ODP and FDP at all the same concentrations. Studies revealed that there was no significant relationship between DPPH and pectin DE (Yang et al., 2004). The bioactivities of polysaccharides and their conjugates can be affected by many factors including chemical components, molecular mass, structure, conformation, and even the extraction and isolation methods (Xu et al., 2009). A relatively low molecular weight and a high uronic acid content in polysaccharides appeared to increase the antioxidant activity (Chen et al., 2004). Polyphenolic compounds react with DPPH radicals in two ways (Kishk and Al-Sayed, 2007): (i) direct abstraction of phenol hydrogen-atom, and (ii) electron



Fig.7 Scavenging of DPPH radical by pectin treated by different drying methods

transfer from ArOH or ArO- to DPPH. Therefore, scavenging of pectin against DPPH radicals could be due to their reduction activities, which may be related to the hydroxyl groups and electron transfer from pectin (ROH or RO-) to DPPH, thus the radical chain reaction is terminated. However, the mechanism of polysaccharides to scavenge free radicals of is still not fully understood. Our results suggest that Jerusalem artichoke pectin could be a valuable source of antioxidants from plant origin which could be used in food formulation.

# **4 CONCLUSION**

In this study, the response surface method was employed to optimize the effects of processing variables (extraction temperature, pH, the length of extraction time and liquid-to-solid ratio) on the yield of pectin from the Jerusalem artichoke. From the experimental results, a highly correlated quadratic mathematical model was developed to optimize the conditions for maximum recovery of pectin from Jerusalem artichoke. The optimal conditions determined by Derringer's desired function methodology were as follows: pH 1.52, 63.62 min, 100°C and a liquid-to-solid ratio of 44.4 mL/g, and the maximum pectin yield was up to 18.76%. Experiments were conducted under these optimal conditions and the actual result was 18.52±0.9%. The result between the predicted value and the actual value was consistent, indicating the established model in this study was feasible. The results of the present study indicate that drying methods had no effect on the structure of Jerusalem artichoke pectin.

Experiments on the antioxidant activity of pectin dried by different methods showed that activities of spray dried pectin (SDP) and apple pectin (AP) were stronger than those of vacuum oven dried pectin (ODP) and vacuum freeze dried pectin (FDP). The present findings present a basis for further structural analysis and evaluation of the bioactivities of the Jerusalem artichoke pectin for its future application in food and medicinal fields.

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