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Characterization of pectic polysaccharides extracted from apple pomace by hot-compressed water

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ABSTRACT

Response surface methodology (RSM) was used to optimize the extraction of pectic polysaccharides from apple pomace by hot-compressed water, by which the optimum levels of the parameters were obtained as follows: extraction temperature 140 °C, extraction time 5 min, S:W ratio 1:14. Compared with commercial pectin, the *Mw*, galacturonic acid content, DM and protein of the extracted pectic polysaccharides were lower while ash content and neutral sugars were higher. The endothermic transition temperature and fusion heat of the extracted pectic polysaccharides was lower than commercial one according to DSC analysis. For its rheological properties, it was found that the viscosity of the extracted pectic polysaccharides solution was slightly lower than commercial pectin at lower shear rate region while it decreased sharply when the shear rate increased. Besides, both G' and G'' moduli of the extracted pectic polysaccharides were lower than the commercial pectin's possibly because of weaker polymer chain interaction, which was also reflected in gel textural properties. However, the extracted pectic polysaccharides showed higher in vitro antioxidant capability and inhibitory effect on HT-29 colon adenocarcinoma cells than commercial pectin.

1. Introduction

Apple pomace, which is mainly composed of apple peel, core, seed and pulp remains, is the main by-product in cider and apple juice processing industries and accounts for about 25–30% of the original fruit mass (Shalini & Gupta, 2010; Vendruscolo, Albuquerque, Streit, Esposito, & Ninow, 2008). Apple pomace contains about 80% of moisture and is a rich source of carbohydrate, acids, vitamin C and minerals, which make it quite perishable and become a severe environmental problem because of it's vast amounts (Shalini & Gupta, 2010). China now is the largest apple exporter and leading apple juice concentrate (AJC) producer worldwide (Gale, Huang, & Gu, 2010), thus approximately one million tons of apple pomace are annually produced (Wang, Sun, et al., 2007). However, only small amounts of apple pomace are used for deep-processing, the majority of apple pomace are not efficiently utilized yet (Wang, Sun, et al., 2007). Actually, many attempts have been made to utilize apple pomace for several value-added products, such as polyphenol, enzymes, single cell protein, aroma compounds, ethanol, organic acids, polysaccharides, and mushroom culture (Cetkovic et al., 2008; Thakur, Singh, & Handa, 1997). However, pectin is still considered as one of the most reasonable way for apple pomace utilization both from an economical and from an ecological point of view (Schieber, Stintzing, & Carle, 2001).

Pectin is a family of complex polysaccharides that contain 1,4-linked α-D-galacturonic acid (Gal*p*A) residues, in which homogalacturonan, rhamnogalacturonan-I, and rhamnogalacturonan-II were isolated and characterized as major pectin (Willats, Knox, & Mikkelsen, 2006). Pectin has been widely used in food, cosmetic and pharmaceutical industries as stabilizer, gelling agent and thickener (Voragen, Coenen, Verhoef, & Schols, 2009; Willats, Knox, & Mikkelsen, 2006). Recently, pectin is used for cardiovascular disease theraphy, induction of prostate cancer cells apoptosis, colon-specific drug delivery, anti-inflammation, probiotic growth promotion and even a new raw material for porous materials production (Das & Ng, 2010; Jackson et al., 2007; Licht et al., 2010; Popov et al., 2011; Theuwissen & Mensink, 2008; White, Budarin, & Clark, 2010).

Commercially, pectin is usually extracted from citrus peel and apple pomace by hot acid method (Liu, Cao, Huang, Cai, & Yao, 2010). Besides, other extraction methods, such as enzymatic, microwave-assisted, ultrasound-assisted, extrusion and alkaline extraction, were reported (Kost'alova, Hromadkova, & Ebringerova, 2010; Ptichkina, Markina, & Runlyantseva, 2008; Shin, Kim, Cho, & Hwang, 2005; Wang, Chen, et al., 2007; Zykwinska, Rondeau-Mouro, Garnier, Thibault, & Ralet, 2006). Hot-compressed water, also called subcritical water, was proved to be effective for extraction of pectin from citrus peel (Hoshino, Tanaka, Terada, Sasaki, & Goto, 2009; Tanaka, Takamizu, Hoshino, Sasaki, & Goto, 2012;

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Ueno, Tanaka, Hosino, Sasaki, & Goto, 2008), however, the detailed chemical composition as well as the functional characteristics of the extracted citrus peel pectin were not completely investigated. Moreover, as one of the two main feedstocks for pectin production, apple pomace was not investigated for pectin extraction by hot-compressed water and the optimal conditions were unknown. Further, the physicochemical and functional properties of the extracted pectic polysaccharides from apple pomace were focused on and simultaneous comparison was carried out between the extracted pectic polysaccharides and commercial apple pomace pectin in this work.

2. Materials and methods

2.1. Feedstock

Apple pomace provided by Shaanxi Haisheng Fresh Fruit Juice Co. Ltd. was used as raw material in all experiments. Apple pomace was dried at 105 °C for 24 h thereafter it was comminuted and sieved (100 mesh pass) before experiments. Commercial pectin was bought in the market produced by Yantai Andre Pectin Co. Ltd. China (apple pomace as feedstock).

2.2. Extraction of pectic polysaccharides by hot-compressed water

An autoclave with 500 mL working volume was used for pectic polysaccharides extraction by hot-compressed water, which is water under subcritical temperature and pressure. A thermocouple and a pressure gauge were used to assay the temperature and pressure inside the reactor. Apple pomace (10 g) and distilled water were added into the reactor, whereafter the nitrogen gas was inputted to exclude oxygen for prevention of oxidation reaction. Then the reactor was tightly enclosed into an electric heater and the extraction time was counted after the temperature inside the reactor reached designed temperature. After the extraction, the reactor system was immediately quenched to room temperature by an inside cooling coil. All the experiments were performed in triplicate, with the average value reported.

After hot-compressed water extraction, water-soluble portion was retrieved by filtration and the filtrate was collected for precipitation by anhydrous ethanol (final ethanol ratio was approximately 75%). The precipitated pectic polysaccharides were washed several times by anhydrous ethanol by which small molecular weight compounds, such as phenolic compounds and degradation compounds (furaldehyde as example) were removed. The precipitated and washed pectic polysaccharides were dried at 105 °C for 24 h. The pectic polysaccharides yield and polygalacturonic acid yield (calculated according to galacturonic acid content in precipitated pectic polysaccharides) were calculated according to Eq. (1) and Eq. (2) as follow:

$$\text{Pectic polysaccharides yield}(\%) = \frac{\text{Pectic polysaccharides}(g)}{\text{Apple pomace}(g)} \times 100 \quad (1)$$

$$\text{Polygalacturonic acid yield}(\%) = \frac{\text{Polygalacturonic acid}(g)}{\text{Apple pomace}(g)} \times 100 \quad (2)$$

2.3. Experimental design

Response surface methodology (RSM) was widely used for various process optimization (Im & Zoh, 2013; Salari, Chayjan, Khazaei, & Parian, 2013; Wang, Li, et al., 2013). This method can deal with two or more factors at several levels, by which a wide range of experimental conditions can be comprehensively investigated in limited trials. The RSM was used in present study to optimize process parameters for pectic polysaccharides extraction by hot-compressed water, through which maximum pectic polysaccharides yield and maximum polygalacturonic acid yield could be obtained. The Box–Behnken experimental design as a rotatable second-order designs based on three-level incomplete factorial designs was adopted for the optimization process, in which three central points were used for estimation of the experimental error as well as to investigate the suitability of the proposed model. Coding of the independent variables was done according to Eq. (3),

$$x_i = \frac{(X_i - X_0)}{\Delta X_i} \quad (3)$$

where x_i and X_i were the dimensionless coded and the actual values of the independent variable i, X_0 was the actual value of the independent variable i at the central point and ΔX_i was the step change of X_i.

The temperature (X_1, °C), extraction time (X_2, min) and S:W ratio (X_3) were selected as the independent variables according to preliminary experiments. The range and the levels of both coded and actual values of the independent variables were listed in Table 1. The performance of the process could be described by the following quadratic polynomial Eq. (4).

$$Y = \beta_0 + \sum_{i=1}\beta_i x_i + \sum_{i=1}^{n}\beta_{ii}x_i^2 + \sum_{i=1}^{n}\sum_{j>i}^{n}\beta_{ij}x_i x_j \quad (4)$$

where Y represented response variable, β_0, β_i,β_{ii}, β_{ij}, were intercept coefficient, linear term, quadratic term and interaction term, respectively. x_i, x_j were coded levels of independent variables. Y represented pectic polysaccharides yield and polygalacturonic acid yield as dependent variables. The Design Expert 8.00 (trial) software was used for the regression analysis and numerical optimization.

2.4. Analytical methods

2.4.1. Molecular weight determination and chemical composition analysis

The molecular weight (abbreviated as *Mw*) of samples were determined by gel-permeation chromatography (GPC) as described in the references (Jia, Yang, & Sun, 2013; Ying, Han, & Li, 2011). Waters HPLC apparatus (Waters Co. Ltd., USA) equipped with three Ultrahydrogel linear columns (7.8 mm × 300 mm) in series and Waters 2414 refractive index detector were used to determine the *Mw*, in which various dextrans were used as standards.

After the protein was removed by sevag method (Staub, 1965), galacturonic acid was determined by modified carbazole method (Bitter & Muir, 1962). The degree of methylation was determined by the titration of free carboxyl groups before and after basic hydrolysis (Schultz, 1965). The protein content was determined via the Bradford method (Bradford, 1976). Ash content was determined by incinerating pectic polysaccharides samples at 575 °C for 8 h in a muffle furnace.

Neutral monosaccharides were released from pectic polysaccharides by acid hydrolysis with trifluoroacetic acid (2 M) at 120 °C for 1.5 h, whereafter trifluoroacetic acid was removed by rotary evaporation at 60 °C. Then the sodium borohydride was added into the solution at room temperature for 1.5 h, after which gas chromatography was used to determine alditol-acetate derivatization products of the monosaccharides (Blakeney, Harris, Henry, & Stone, 1983; Masmoudi, Besbes, Ben Thabet, Blecker, & Attia, 2010). Gas chromatography (Shimadzu 2014 C) with a high performance capillary column, DB-17 (30 m L × 0.25 mm ID, 0.25 μm film thickness, Agilent) was used to determine the neutral monosaccharides' derivatives.

Table 1
Experimental ranges and levels of both coded and actual values of the independent variables.

Variables	Independent variables		Factor levels		
	Uncoded	Coded	−1	0	1
Temperature (°C)	X_1	x_1	140	150	160
Time (min)	X_2	x_2	5	10	15
S:W ratio	X_3	x_3	1:4	1:9	1:14

2.4.2. Differential scanning calorimetric (DSC) analysis

Differential scanning calorimetry (DSC Q2000 TA system, USA) was used to investigate the thermal properties of samples according to the described method (Sharma & Ahuja, 2011). After finely ground and dried, sample (5 mg) was added into a standard aluminum crucible and immediately sealed. The crucible was heated from 40 °C to 300 °C at a heating rate of 10 °C per minute in dynamic nitrogen atmosphere (50 mL/min). Simultaneously, an empty standard aluminum crucible was used as reference.

2.4.3. Rheological characteristics of extracted pectic polysaccharides

The rheological properties of samples were determined by rheometer (AR1000, TA instruments, USA) with a 20 mm parallel plate. The solution for rheological tests was prepared by mixing sample with distilled water (2%, w/w). The sample solutions were subjected to steady-shearing at 25 °C with the shear rates ranged from 0.02 to 100 s^{-1}. Oscillatory measurements were used to determine the storage modulus (G') and loss modulus (G'') of sample solutions. Strain sweep (0.01–100% at 1 Hz) was applied to test the linear viscoelastic region of the samples. And the frequency dependence of G' and G'' was determined by a frequency sweep (0.1–10 Hz at 1% strain).

2.4.4. Preparation of pectic polysaccharides gel and its textural properties

Pectic polysaccharides sample (0.5 g) and sucrose (35 g) were dissolved in distilled water (final volume 50 mL), of which the pH was adjusted to 3 by 12.5% citric acid solution. Thereafter, the mixture was immediately placed in a refrigerator at 4 °C for 24 h. Before the texture analysis, the prepared gel was placed at room temperature for 0.5 h.

A Texture Analyser TA.XT Plus (Stable Micro Systems, UK) was used to determine the textural properties of the gels. The compression tests were performed using a cylindrical probe (5-mm radius, PC-0.5R). A standard program was used to compress the gels by probe with 1 g original force at 0.1 mm/s and the puncture test was stopped when probe was penetrated into the gel for 10 mm, after which the probe was withdrawn from the gel at 0.1 mm/s. Textural parameters of firmness, cohesiveness, consistency and viscosity index of gels were obtained (Angioloni & Collar, 2009). Maximum force for gel penetration was taken as gel firmness and the positive area was recorded as consistency. The maximum negative force was taken as cohesiveness and the negative area was taken as viscosity index.

2.4.5. In vitro antioxidant properties of extracted pectic polysaccharides

2.4.5.1. DPPH radical scavenging activity. The antioxidant activity of the sample was assayed by DPPH free radical scavenging capability described by Rha et al. (2011). Pectic polysaccharides solution (2 mL) was added to 4 mL 100 μM DPPH–ethanol solution and the reaction mixture was kept in dark at room temperature for 30 min, after which the absorbance of the mixture was determined at 517 nm. The DPPH scavenging activity was calculated by Eq. (5),

$$\text{Scavenging activity}(\%) = \frac{B - (S - SC)}{B} \times 100 \quad (5)$$

where B, S, and SC refer the absorbance of blank, sample, and sample control, respectively.

All experiments were done three times with average value reported. The quercetin was used as the standard for comparison of DPPH scavenging activity. EC50 values indicated the concentration of sample required to scavenge 50% DPPH free radicals, it can be use to evaluate the DPPH radical scavenging activity (Rha et al., 2011).

2.4.5.2. $ABTS^{\bullet+}$ radical scavenging activity. The $ABTS^{\bullet+}$ radical scavenging activity of the sample was assayed by the described method (Braca et al., 2001). Pectic polysaccharides solution (2 mL) was added to 4 mL diluted $ABTS^{\bullet+}$ solution and the reaction mixture was kept in dark at room temperature for 6 min, whereafter the absorbance of the solution was determined at 734 nm. The $ABTS^{\bullet+}$ scavenging activity was calculated by Eq. (6),

$$\text{Scavenging activity}(\%) = \frac{B - (S - SC)}{B} \times 100 \quad (6)$$

where B, S, SC refer the absorbance of blank, sample, and sample control, respectively. The experiments were repeated three times with average value reported, in which quercetin was also used as standard for comparison. EC50 values indicated the concentration of sample required to scavenge 50% ABTS free radicals, which can be use to evaluate the $ABTS^{\bullet+}$ radical scavenging activity.

2.4.6. Inhibitory effect on HT-29 colon adenocarcinoma cells of the extracted pectic polysaccharides

The activity of cell proliferation inhibitory was determined by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay method described by Wang Yangcai, et al. (Wang, Ma, et al., 2013). HT-29 cells (1×10^5) were separately added into 96-well cell plates with 200 μL culture medium and allowed to adhere for 24 h at 37 °C, 5% CO_2. Subsequently, the medium was removed and the cells were treated with 180 μL of medium with pectic polysaccharides at various concentrations (0.04, 0.2, 1, 5, and 10 mg/mL) for 24 h. Each concentration of pectic polysaccharides was repeated in five wells and control sample was treated without pectic polysacchrides addition. MTT (20 μL, 5 mg/mL in PBS) was added into each well and the supernatant was carefully removed after 4 h incubation. The resulting formazan was dissolved in 200 μL DMSO and measured by absorbance at 490 nm using a microplate reader (Bio-Rad, USA). Proliferation inhibitory by pectic polysaccarides was calculated as percentage of cell viability, in which control cells were considered as 100%. The IC50, which represented the concentration of sample that lowers MTT reduction by 50%, was calculated by regression model between sample doses and MTT reduction percentages. The IC50 was used to quantify the pectic polysaccharides inhibitory effect on colon adenocarcinoma cell proliferation, in which the lower value of IC50 indicated the

higher inhibitory effect on HT-29 colon adenocarcinoma cells. The cell proliferation inhibitory activity was calculated by Eq. (7),

$$\text{Cell proliferation inhibitory activity}(\%) = \left(\frac{1 - \text{ODs}}{\text{ODc}}\right) \times 100 \quad (7)$$

where ODs and ODc refer to the absorbance of sample and control, respectively.

3. Results and discussion

3.1. Optimization of extraction process by RSM

3.1.1. Pectic polysaccharides yield

The matrix of Box–Behnken experimental design and experimental results are shown in Table 2. Neglecting the statistically insignificant terms ($p > 0.05$), step-wise regression model of response surface for pectic polysaccharides yield was represented by the Eq. (8) in terms of coded values.

$$Y_1(\text{pectic polysaccharides yield}) = 9.21 - 4.13x_1 - 1.24x_2 + 2.63x_3 + 2.01x_2^2 \quad (8)$$

The results were analyzed by using analysis of variance (ANOVA) as shown in Table 3. The F-value (218.85) of model indicate the regression model is statistically significant ($p < 0.0001$). High proportion of variability ($R^2 = 0.9444$) in the response model can be explained successfully by the model. However, a large value of R^2 does not always imply that the regression model is good because R^2 will increase with variable addition regardless of whether the additional variable is statistically significant or not. Thus, it is preferred to use an Adj-R^2 to evaluate the model adequacy and it should be over 90%. Table 3 shows that R^2 and Adj-R^2 values for the model do not differ dramatically which indicate that insignificant terms have not been included in the model. The significance of each term after step-wise removal of insignificant terms ($p > 0.05$) is also shown in Table 3 based on p-value. The smaller the magnitude of p-value, the more significant is the corresponding term. According to the p-value of all significant terms in the model, temperature (x_1) and S:W ratio (x_3) produce largest effect on pectic polysaccharides yield, followed by terms of extraction time (x_2) and quadratic extraction time (x_2^2).

To visualize the relationship between variables and responses, the tri-dimensional response surface plots were generated for the models in function of two variables. Fig. 1 shows the three dimensional graph of the effects of temperature, time and S:W ratio on the pectic polysaccharides yield.

It is shown that increase of temperature and time leads to decrease of pectic polysaccharides yield. That is, higher temperature and longer reaction time will lower the pectic polysaccharides yield because the polysaccharides will decompose to monosaccharides or other small moleculars when temperature and reaction time increase (Lu & Saka, 2010, 2012). Increase of S:W ratio leading to increase of pectic polysaccharides yield indicates that the lower solid loading can possibly improve the accessibility of hot-compressed water to solid apple pomace and make the extracted polysaccharides more easier diffuse from apple pomace. Moreover, lower extracted polysaccharide concentration avoid possible inter-reaction between extracted polysaacharides themselves or other conjugate constituents.

3.1.2. Polygalacturonic acid yield

According to the experimental results of Box–Behnken experimental design (Table 2), step-wise regression model of response surface for polygalacturonic acid yield is represented by the Eq. (9) after removing the statistically insignificant terms ($p > 0.05$).

$$Y_2(\text{Polygalacturonic acid yield}) = 3.00 - 2.14x_1 - 0.65x_2 + 1.01x_3 - 0.73x_1 * x_3 + 1.07x_2^2 \quad (9)$$

The results of one-way analysis of variance (ANOVA) are shown in Table 4. The F-value (54.49) of the model means that the regression model is statistically significant ($p < 0.0001$). High R^2 (0.9396) and Adj-R^2 (0.9060) indicate that the model is adequate and insignificant terms have not been included in the model. According to p-value of each term in Table 4, temperature produce largest effect on polygalacturonic acid yield, followed by terms of quadratic extraction time, S:W ratio, extraction time and interaction term of temperature and S:W ratio.

It is shown in Fig. 2 that the effects of temperature, time and S:W ratio on polygalacturonic acid yield by three dimensional response surface plots. It is shown that polygalacturonic acid yield decrease when temperature and time increase, which indicate that polygalacturonic acid will be degraded when temperature increase and treatment time is prolonged. It is also shown that polygalacturonic acid yield increase when S:W ratio increase, which indicate that lower solid loading improve the polygalacturonic acid yield.

3.2. Numerical multiobjective optimization

Optimum conditions for the extraction process were intended to obtain maximum pectic polysaccharides yield as well as maximum polygalacturonic acid yield, in which quadratic polynomial models for each response obtained in this study (Eqs. (8) and (9)) were utilized to obtain the optimal conditions by desirability function method widely used in process optimization (Mohajeri, Aziz, Isa, & Zahed, 2010; Pinzi, Lopez-Gimenez, Ruiz, & Dorado, 2010; Pourfarzad, Mandavian-Mehr, & Sedaghat, 2013; Salmasnia, Kazemzadeh, & Tabrizi, 2012). It is shown in Fig. 3 that desirability decrease when temperature and time increase, however, it increase when S:W ratio increase. That is, optimal desirability can be obtained at lower temperature, shorter extraction time and higher S:W ratio. Generally the highest desirability corresponds the optimal solution, thus numerical multi-objective optimization was carried out for maximum desirability, by which optimal conditions for maximum predicted pectic polysaccharides yield and polygalacturonic acid yield could be obtained. The obtained optimal conditions were 140 °C, 5 min, 1:14 (S:W ratio).

Triple validating experiments at optimal conditions were done to confirm the prediction. The average pectic polysaccharides yield and polygalacturonic acid were 17.55% and 8.46% respectively, which were approximately equal to calculated 18.64% pectic polysaccharides yield and 8.29% polygalacturonic acid yield according to the regression models.

3.3. Physicochemical and functional properties of pectic polysaccharides extracted by hot-compressed water at optimal conditions

3.3.1. Chemical composition of extracted pectic polysaccharides at optimal conditions

The *Mw* and chemical composition of the extracted pectic polysaccharides is shown in Table 5. The *Mw* of the pectic polysaccharides is extremely lower than commercial one's. Because pectinase is generally used in apple juice concentrate process to improve juice yield, thus pectin in apple pomace used in this work was actually degraded before our extraction, which made its *Mw* significantly lower than commercial one. Moreover, the pectin was possibly hydrolyzed and decomposed in hot-compressed water just as other polysaccharides (Lu, Yamauchi, Phaiboonsilpa, & Saka, 2009),

Table 2
Box–Behnken experimental design and results of pectic polysaccharide extraction by hot compressed water.

Run	Temperature	Time	S:W ratio	Actual pectic polysaccharide yield	Predicted pectic polysaccharide yield	Actual polygalacturonic acid yield	Predicted polygalacturonic acid yield
1	−1.00	−1.00	0.00	16.45	16.59	6.34	6.85
2	1.00	−1.00	0.00	7.24	8.34	2.02	2.57
3	−1.00	1.00	0.00	14.03	14.11	6.05	5.56
4	1.00	1.00	0.00	5.50	5.86	1.13	1.28
5	−1.00	0.00	−1.00	10.78	10.71	3.33	3.40
6	1.00	0.00	−1.00	4.47	2.46	0.86	0.58
7	−1.00	0.00	1.00	16.12	15.97	6.40	6.88
8	1.00	0.00	1.00	7.17	7.72	1.00	1.14
9	0.00	−1.00	−1.00	9.44	9.84	3.47	3.71
10	0.00	1.00	−1.00	7.38	7.36	2.61	2.42
11	0.00	−1.00	1.00	16.75	15.10	7.01	5.72
12	0.00	1.00	1.00	13.05	12.62	3.91	4.43
13	0.00	0.00	0.00	8.89	9.21	2.72	3.00
14	0.00	0.00	0.00	7.30	9.21	3.41	3.00
15	0.00	0.00	0.00	9.76	9.21	3.28	3.00

Table 3
Results of ANOVA of pectic polysaccharide yield.

Source	Sum of squares	DF	Mean square	*F* value	*p*-value
Model	218.85	4	54.71	42.50	<0.0001
x_1-Temperature	136.13	1	136.13	105.74	<0.0001
x_2-Time	12.30	1	12.30	9.55	0.0114
x_3-S:W ratio	55.27	1	55.27	42.93	<0.0001
x_2^2	15.16	1	15.16	11.78	0.0064
Residual	12.87	10	1.29		
Lack of fit	9.76	8	1.22	0.78	0.6691
Pure error	3.11	2	1.55		
R^2	0.9444				
Adj-R^2	0.9222				

which would also lead to lower *Mw*. The galacturonic acid content, degree of methylation, and protein content of the extracted pectic polysaccharides were lower than commercial one's while ash content of the extracted pectic polysaccharides was higher than commercial one's. Though qualitative profile of monosaccharides of the pectic polysaccharides and commercial pectin is similar as other researches (Garna et al., 2007; Schols, Bakx, Schipper, & Voragen, 1995; Schols, Vierhuis, Bakx, & Voragen, 1995), the quantities of the monosaccharides are different possibly because hot-compressed water has significant effect on pectic polysaccharides structure, especially neutral monosaccharides composition. For example, the amount of arabinose is lower than previously reported value (Aspinall & Fanous, 1984; Schols, Posthumus, & Voragen, 1990) because arabinose existing in the hairy region of pectin will be

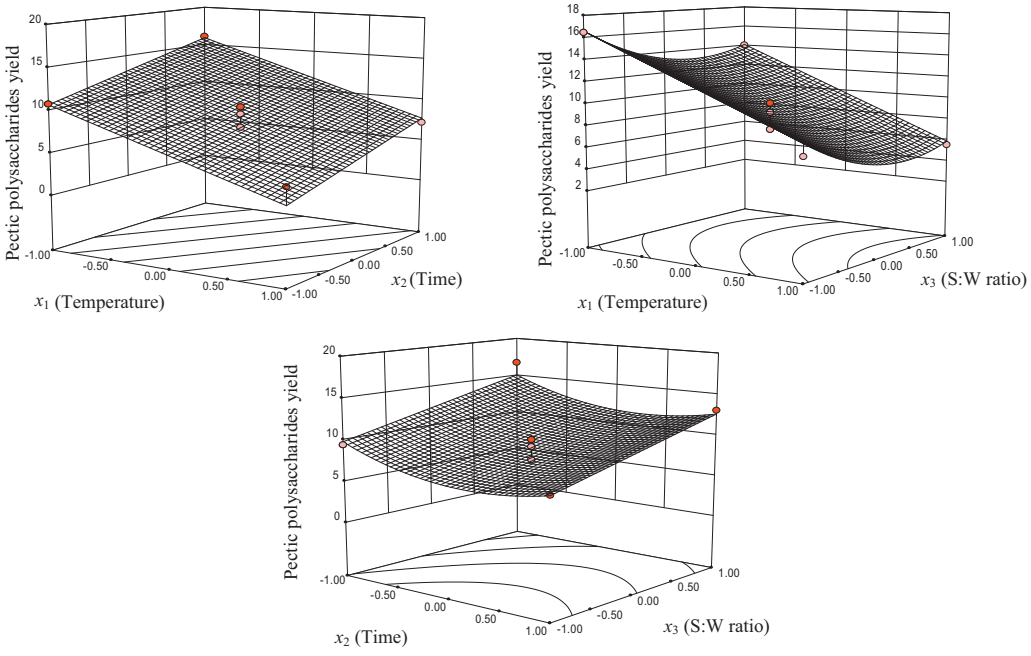

Fig. 1. Response surface plot of pectic polysaccharides yield as a function of time, temperature and S:W ratio.

Table 4
Results of ANOVA of polygalacturonic acid yield.

Source	Sum of squares	DF	Mean square	*F* value	*p*-value
Model	54.49	5	10.90	27.98	<0.0001
x_1-Temperature	36.63	1	36.63	94.07	<0.0001
x_2-Time	3.33	1	3.33	8.55	0.0169
x_3-S:W ratio	8.12	1	8.12	20.86	0.0014
$x_1 * x_3$	2.14	1	2.14	5.50	0.0436
x_2^2	4.26	1	4.26	10.94	0.0091
Residual	3.50	9	0.39		
Lack of fit	3.24	7	0.46	3.45	0.2428
Pure error	0.27	2	0.13		
R^2	0.9396				
Adj-R^2	0.9060				

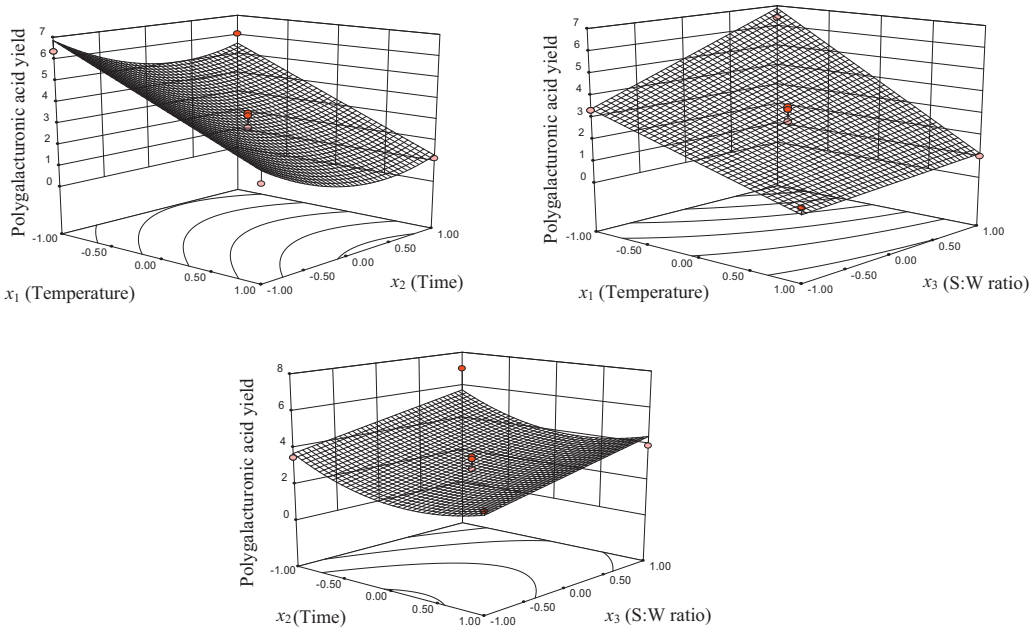

Fig. 2. Response surface plot of polygalacturonic acid yield as a function of time, temperature and S:W ratio.

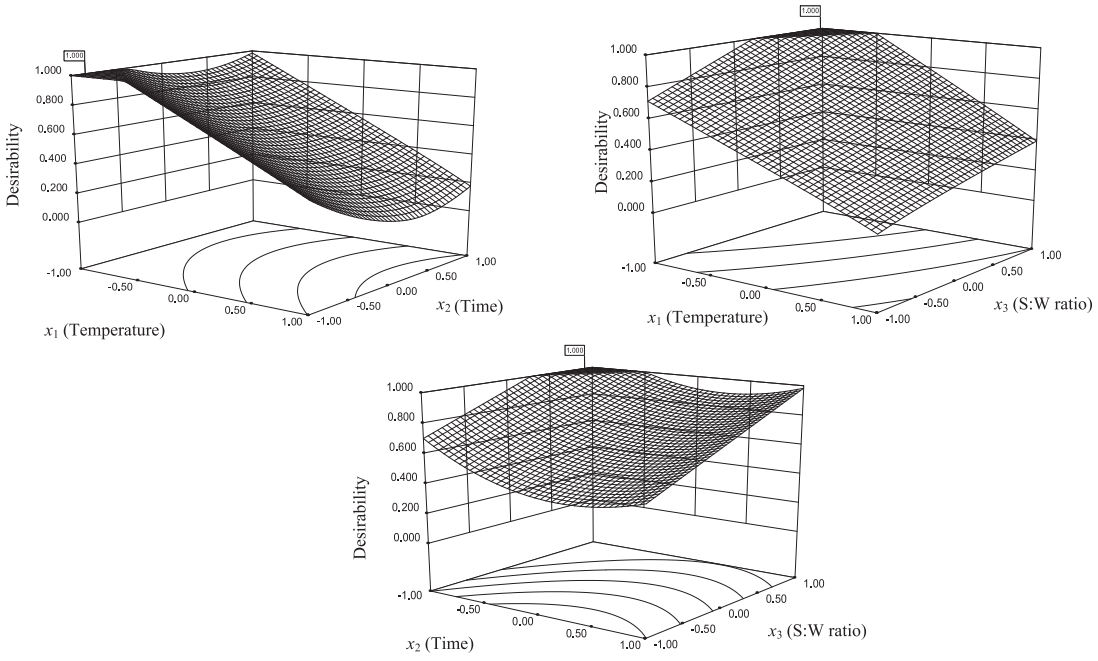

Fig. 3. Response surface plot of desirability as a function of time, temperature and S:W ratio.

Table 5
Chemical analysis of pectic polysaccharide extracted at optimal conditions and commercial pectin.

	Mw (kDa)	Galacturonic acid (wt% dry weight)	Degree of methylation (%)	Protein content (wt% dry weight)	Ash content (wt% dry weight)	Neutral sugar composition (wt% dry weight)						Unknown (wt% dry weight)
						Rha	Ara	Xyl	Man	Glc	Gal	
Extracted pectic polysaccahride	64.2	48.20 ± 1.26	60.23 ± 0.23	0.43 ± 0.02	2.79 ± 0.01	0.66 ± 0.04	2.07 ± 0.11	2.07 ± 0.09	0.82 ± 0.03	19.85 ± 0.94	5.44 ± 0.23	17.67 ± 1.37
Commerical pectin	539.1	66.01 ± 0.54	85.54 ± 0.12	1.36 ± 0.03	1.11 ± 0.01	0.68 ± 0.02	1.02 ± 0.10	1.01 ± 0.04	0.03 ± 0.01	5.46 ± 0.12	3.30 ± 0.11	20.02 ± 1.10

firstly hydrolyzed into water and degraded then, which has been proved in other biomass hydrolysis in hot-compressed water (Lu et al., 2009). Larger amount of glucose indicate that part of the glucose-enriched hemicelluloses or amorphous cellulose in apple pomace (Popper & Fry, 2008; Voragen, Sehols, & Pilnik, 1986; Watt, Brasch, Larsen, & Melton, 1999) was possibly simultaneously separated and linked together with pectic polysaccharides.

3.3.2. Thermal and rheological properties

It is shown in Fig. 4 that the endothermic peak of the extracted pectic polysaccharides is at 116.09 °C and the heat of fusion is 89.60 J/g for the extracted pectic polysaccharides while it is 124.38 °C and 92.3 J/g for commercial pectin. Generally endothermic peak and heat of fusion reflect the ability to retain water, which is related to these hydrophilic groups of the sample. Thus the decrease of endothermic transition temperature and heat of fusion possibly because lower *Mw*, degree of methylation and galacturonic acid (Iijima, Nakamura, Hatakeyama, & Hatakeyama, 2000). On the other hand, the exothermic peak reflects the sample degradation properties generally related to its chemical constituents profile. Thus fairly close exothermic peaks of the extracted pectic polysaccharides (245.50 °C) and commercial pectin (246.25 °C) indicated that they had same chemical constituents profile. Moreover, there are two small endothermic peaks of commercial pectin which possibly caused by the higher content of protein or other unknown substance.

It is shown in Fig. 5 that both extracted pectic polysaccharides and commercial pectin show pseudoplastic fluid characteristics similar as other polysaccharides rheological properties caused by their random coil formation (Zhang et al., 2013). The viscosity of the extracted pectic polysaccharides solution is slightly lower than commercial pectin at lower shear rate region. However, when the shear rate increase to 1 S^{-1}, there is a sharp viscosity decrease of the extracted pectic polysaccharides while it is not found for commercial pectin, which is possibly due to loose pectic polysacchrides entanglement caused by the lower *Mw*, galacturonic acid and degree of methylation of the extracted pectic polysaccharides (Liu et al., 2010; Zhang et al., 2013). The viscosity characteristic of the extracted pectic polysaccharides indicates that it has better shear-thinning characteristics as food thickener. If the extracted pectic polysaccharides are added into the pasty or pulp food products, these food products might be more easily flow when they are shaken or other external force is applied.

Furthermore, it is shown in Fig. 6A that the curves of the extracted pectic polysaccharides and commercial pectin do not have any rapid decline in the values of storage modulus (G') and loss modulus (G''), therefore 1% strain was chosen to determine the frequency dependence of G' and G''.

As shown in Fig. 6B, both G' and G'' moduli of the extracted pectic polysaccharides are lower than the commercial pectin's, which indicate that less polymer chain interaction of the extracted pectic polysaccharides lead to decrease in its viscoelastic properties. The G'' moduli are larger than G' moduli at low frequencies while reverse can be seen at higher frequencies, which indicate that the flow behavior is more viscous than elastic at low frequencies and reverse can be observed at higher frequencies. Moreover, the crossover point of G' and G'', which is used to define the beginning of the elastic behavior or approaching gel state (Sengkhamparn et al., 2010), are 0.631 Hz and 3.16 Hz for the extracted pectic polysaccharides and commercial pectin respectively. Thus it is indicated that the extracted pectic polysaccharides show predominantly elastic responses ($G' > G''$) when the frequency increase to 0.613 Hz, which also suggest that the solution of the extracted pectic polysaccharides is changed to more elastic performance at lower frequency compared with commercial pectin.

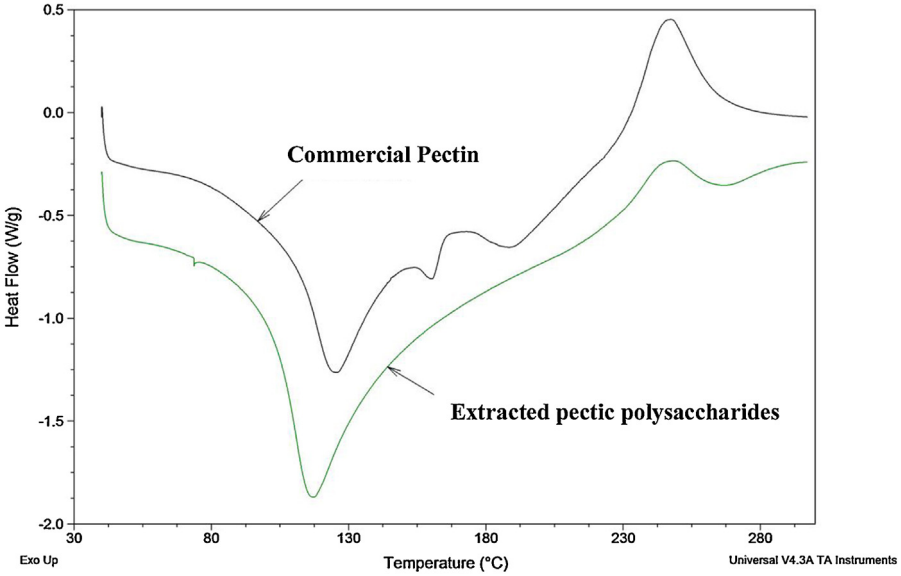

Fig. 4. DSC thermograms of extracted pectic polysaccharide and commercial pectin.

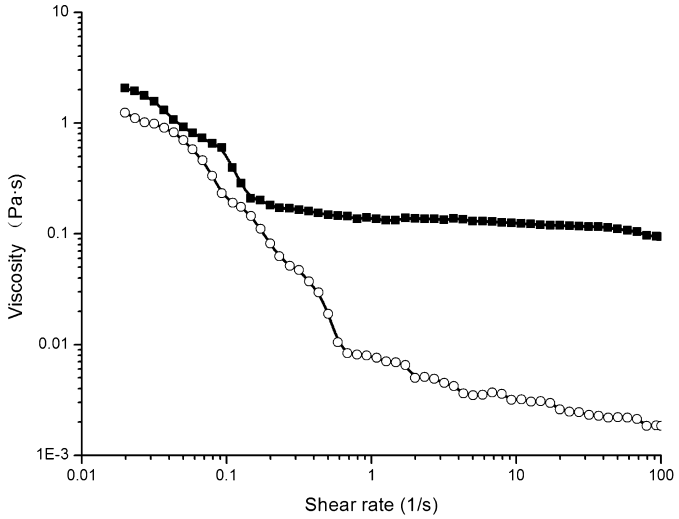

Fig. 5. The flow behavior of extracted pectic polysaccharide and commercial pectin. (■) Commercial pectin and (○) extracted pectic polysaccharide.

3.3.3. *Textural properties of extracted pectic polysaccharides gel*

It can be seen in Table 6 that firmness, cohesiveness, consistency and viscosity index of the extracted pectic polysaccharides gel are lower than commercial pectin gel. Generally the *Mw*, degree of methylation and galacturonic acid content of pectin, which affect the intra- and inter- action of pectin in gel formation, positively correlate with these gel indexes. The *Mw*, degree of methylation and galacturonic acid content of the extracted pectic polysaccharides are lower than commercial pectin because the extracted pectic polysaccharides would be hydrolyzed to shorter chain during extraction by hot-compressed water similar as other polysaccharides hydrolysis in hot-compressed water (Lu & Saka, 2010; Lu et al., 2009), which lead to weaker gel formation.

3.3.4. *In vitro antioxidant activity of pectic polysaccharides extracted at optimal conditions*

Pectic polysaccharides and other polysaccharides with polyhydroxyl groups were looked as promising antioxidants (Kang et al., 2006; Mateos-Aparicio, Mateos-Peinado, Jimenez-Escrig, & Ruperez, 2010). Two widely used antioxidant activity assay methods (DPPH assay and ABTS$^{\bullet+}$ assay) were used to evaluate the radical scavenging activities of the extracted pectic polysaccharides

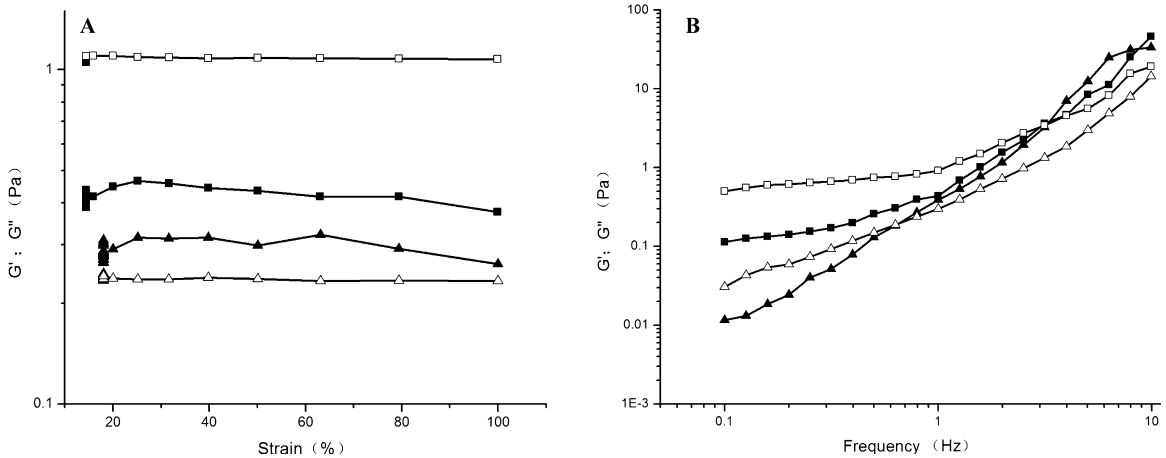

Fig. 6. G' and G'' of pectin samples (A, strain sweeps at 0.1 S^{-1} frequency of modulus. B, frequency sweeps at 1% strain.) (■) Commercial pectin (G'); (□) commercial pectin (G''); (▲) extracted pectic polysaccharide (G'); (△) extracted pectic polysaccharide (G'').

Table 6
Textural properties of extracted pectic polysaccharide gel and commercial pectin gel.

	Firmness (g)	Cohesiveness (g)	Consistency (g s)	Viscosity index (g s)
Extracted pectic polysaccahride	4.87	−1.125	227.691	−2.176
Commercial pectin	18.957	−8.703	289.029	−88.654

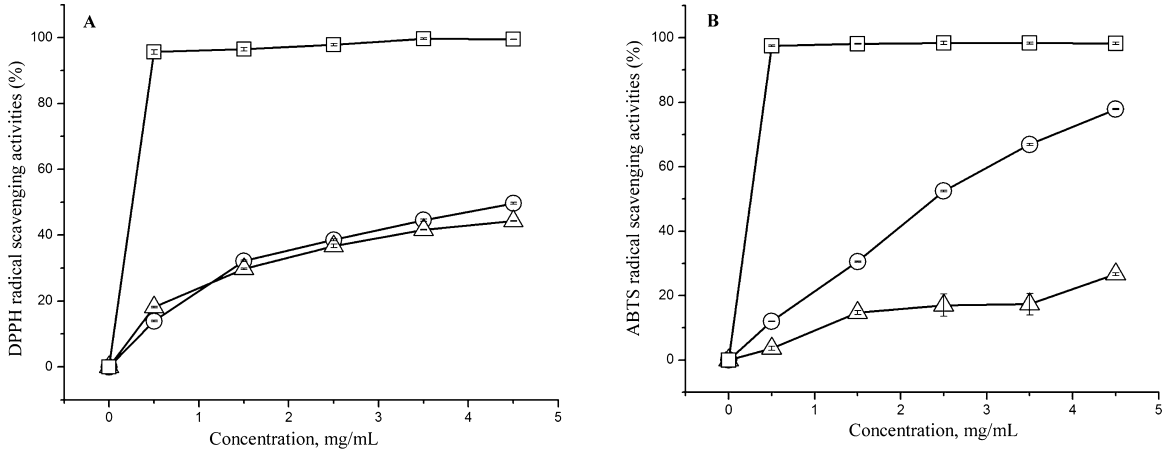

Fig. 7. In vitro antioxidant capability of extracted polysccharides (A: DPPH^{-} radical scavenging effects B: ABTS$^{\bullet+}$ radical scavenging effects) (□, quercetin; ○, extracted pectic polysaccharide; △, commercial pectin).

and commercial pectin (Fig. 7A and B). It has been found that results obtained by the two methods have strong positive correlation (Floegel, Kim, Chung, Koo, & Chun, 2011; Thaipong, Boonprakob, Crosby, Cisneros-Zevallos, & Byrne, 2006), which was also found in our study. Pearson correlation coefficient between two methods' results was 0.969 at 0.01 significant level for the extracted pectic polysaccharides while it was 0.941 at 0.01 significant level for commercial pectin. Actually both DPPH assay and ABTS$^{\bullet+}$ assay are commonly used to evaluate the in vitro antioxidant capabilities, generally they are assayed simultaneously for corroborating each other. Besides, it is found that the antioxidant activities of the extracted pectic polysaccharides assayed by ABTS$^{\bullet+}$ method are higher than corresponding ones assayed by DPPH method possibly because ABTS$^{\bullet+}$ assay is more suitable for the hydrophilic antioxidant evaluation while DPPH assay is more applicable for hydrophobic antioxidant evaluation (Floegel et al., 2011). The differences of antioxidant activity assayed by two methods are possibly caused by difference of the solvent in the two methods, in which ethanol was used for DPPH assay while aqueous ethanol solution was used in ABTS$^{\bullet+}$ assay. Since pectin is easily soluble in water while slightly and/or hardly soluble in ethanol, thus assayed antioxidant capabilities by ABTS$^{\bullet+}$ assay are generally higher than DPPH assay. The radical scavenging activities of commercial pectin assayed by the two methods are similar which possibly because its higher *Mw* make its dissolution performance similar in the two assay methods even in the aqueous-ethanol solvent system of ABTS$^{\bullet+}$ assay.

It can be seen from Fig. 7A and B that both DPPH radical scavenging activities and ABTS$^{\bullet+}$ radical scavenging activities of the extracted pectic polysaccharides and commercial pectin are dose-dependent, in which free radical scavenging activities of the extracted pectic polysaccharides are significantly higher than commercial one's ($p < 0.05$). The EC50 of the extracted pectic polysaccharides and commercial pectin are 4.69 mg/mL and 6.50 mg/mL according to DPPH assay, which indicate that the scavenging activity of the extracted pectic polysacchride is approximate 1.4-fold higher than commercial pectin. The EC50 of the extracted pectic polysaccharides and commercial pectin are 2.38 mg/mL and 16.83 mg/mL according to ABTS$^{\bullet+}$ assay, which indicate that the scavenging activity of extracted pectic polysaccaharide is approximate 7.1-fold higher than commercial pectin. Accordingly, the radical scavenging activity of the extracted pectic polysaccharides is higher than that of commercial one. The higher in vitro antioxidant capabilities of the extracted pectic polysaccharides are possibly because its lower *Mw*, degree of methylation, galacturonic acid content and linked hemicellulose's constituents. Lower *Mw* indicates shorter chain length of pectic polysaccharides which possibly make it easier package free radical and increase their interaction. Lower DM and galacturonic acid content mean that relative more hydroxyl groups can be provided by the extracted pectic polysaccharides which will be beneficial for scavenging free radical.

3.3.5. *Inhibitory effect on HT-29 colon adenocarcinoma cells of the extracted pectic polysaccharides*

It is shown in Fig. 8 that the extracted pectic polysaccharides has significantly higher inhibitory activity against the HT-29 adenocarcinoma colon cells than commercial pectin in a dose-dependent manner when sample concentration is higher than 1 mg/mL ($p < 0.05$). Furthermore, the dose required for 50% growth inhibition (IC50 values) of the extracted pectic polysaccharides is 7.705 mg/mL, while it is 15.965 mg/mL for commercial pectin. Previous reports demonstrated that pectin inhibited cell proliferation and induced apoptosis in several cancer cell lines (Westereng, Michaelsen, Samuelsen, & Knutsen, 2008), in which pectin with Ara-Gal rich parts showed the highest potent activity (Cheng et al., 2011; Westereng, Yousif, Michaelsen, Knutsen, & Samuelsen, 2006). Compared with commercial pectin, the possible functional side chain constituents including arabinose, xylose, galactose and mannose of the extracted pectic polysaccharides are higher, which might be the reason why the extracted pectic polysaccharides has higher inhibitory capability. However, the mechanism of antiproliferation activity of the extracted pectic polysaccharides should be further investigated, in which polysaccharides' spatial structure and tumor signal pathway as well as their relationship should be considered.

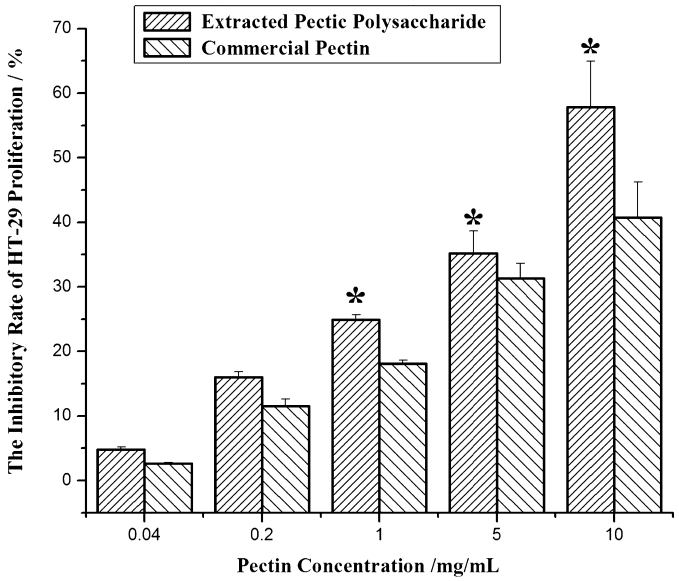

Fig. 8. Effects of pectin on HT-29 cell proliferation assayed by MTT (*$p < 0.05$ vs. commercial pectin).

4. Conclusions

It was confirmed that hot-compressed water can also be used for pectic polysaccharides extraction from apple pomace and optimum conditions were obtained by RSM. The *Mw*, galacturonic acid content, degree of methylation and protein of the extracted pectic polysaccharides were lower than commercial pectin, while ash content and neutral sugars were higher. It was found in dynamic rheological analysis that the viscosity of the pectic polysaccharides solution was slightly lower than commercial pectin at lower shear rate region while it decreased sharply when the shear rate increased, which indicated weaker inter- and intra-molecular action of the extracted pectic polysaccharides. Besides, the lower G' and G'' moduli of the pectic polysaccharides further verified the weaker chain interaction, which was also corroborated in gel textural properties as lower firmness, cohesiveness, consistency and viscosity index of the gel prepared by extracted pectic polysaccharides. Moreover, the extracted pectic polysaccharides showed higher in vitro antioxidant capability and inhibitory effect on HT-29 colon adenocarcinoma cells than commercial pectin, which were promising functional properties for food or pharmaceutical application. In conclusion, hot-compressed water is effective for pectic polysaccharides extraction, in which physicochemical and functional properties of the extracted pectic polysaccharides changed possibly because of extraction process. Interestingly some promising characteristics emerged such as higher in vitro antioxidant activity and inhibitory effect on colon cancer cell.

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