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Pulsed electric field-assisted modification of pectin from sugar beet pulp

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This current work is concerned with the modification of sugar beet pulp (SBP) pectin assisted by pulsed electric filed (PEF) without solvent. Pectin-arachates with degree of esterification (DE) ranging from 49 to 84 were prepared in one-step modification. The results showed that the DE of pectin derivatives increased significantly with the PEF intensity from 18 to 30 kV cm$^{(1}$ and total specific energy input from 124 to 345 J mL$^{(1}$. Evidence of modification of pectin was provided by FT-IR, X-ray diffraction patterns and NMR spectra. Thermogravimetric investigation of modified pectin indicated a higher thermal stability than the untreated one. Results revealed that PEF technology is a promising method for industrial manufacture of pectin derivatives.

1. Introduction

Pectins are natural polysaccharides and widely available in the cell walls of most plants. The main sources of commercial pectin are apple pomace and citrus peel, another source also attempted was sugar beet (*Betula vulgaris* L.) pulp, byproduct of sugar-refining industry, owing to its high pectin content (15–30% of pectin compared to apple pomace 10–18%, dry weight basis), and its ready availability in large quantities (Contreras Esquivel, Hours, Aguilar, Reyes Vega, & Romero, 1997; Norsker, Jensen, & Adler-Nissen, 2000). Pectin consists primarily of 1,4-a-D-galacturonic acid and its esters, interrupted in places by 1,2-a-L-rhamnose residues with side-chains of neutral sugars (Drusch, 2007; Gromer, Kirby, Gunning, & Morris, 2009).

Low water resistance and bad mechanical properties, however, are limiting factors for their applications in pharmaceutical and medical industries. For these reasons, chemically modification of pectin to modulate their properties is necessary. In the processes, one of the major problems is the need of volatile organic solvents widely employed in chemical reactions, which are contradictory with the view of "green chemistry". However, solvent free approach would be more suitable for the reaction with great advantages of lower cost, reduced energy, simplified reaction and improved yield (Bamoharram, Heravi, Roshani, & Charkhi, 2011; Einhorn-Stoll & Kunzek, 2009; You, Wang, Bo, & Chen, 2011). Therefore, the synthesis of pectin derivatives in the absence of solvent is good for the environmental impact.

Recently, pulsed electric field (PEF) technology receives considerable attention from food industry to inactive enzymes and pathogenic microorganisms, for improving the quality and shelf-life stability of milk, fruit juice, or wine (Jaeger, Meneses, & Knorr, 2009; Lyng, Riener, Noci, Cronin, & Morgan, 2009; Puertolas, Lopez, Condon, Alvarez, & Raso, 2010). However, there are few reports of PEF technology on structural and functional modification of macromolecular substances. The recent studies showed that electric field intensity, pulse width, and number of pulses are the main factors determining the physicochemical properties of chitosan and corn-starch (Han, Zeng, Zhang, & Yu, 2009; Luo, Han, Zeng, Yu, & Kennedy, 2010). This raises an interesting question of whether or not the SBP pectin could be modified with PEF. Therefore, this study aims at PEF-assisted modification of pectin with arachidic anhydride, especially the significant effects of PEF on the modification of pectin were investigated in detail. The extent of modification was measured by the degree of esterification (DE), and the modified samples were then characterized by FT-IR, X-ray diffraction, NMR, and TG-DTA.

2. Materials and methods

2.1. Materials

SBP was offered by Luyuan Sugar Industry Co., Ltd (Xinjiang, China). The arachidic anhydride and all solvents were purchased from Aladdin reagents Co., Ltd (Shanghai, China).

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2.2. *Preparation of SBP pectin*

SBP pectin was extracted with citric acid at 80 °C, pH 2.0 for 2 h. The resulting slurries were centrifuged and filtrated to get the supernatants. After dispersed into 4 volumes of 95% ethanol at 4 °C for 3 h, the pectic precipitate was washed twice with 70% ethanol followed by 95% ethanol, oven-dried and finely ground for pectin powder preparation.

2.3. *PEF treatment*

A bench-scale, continuous PEF system (SCUT-PEF Team, South China University of Technology, China) was used in this experiment. The treatment chamber is composed of two parallel stainless-steel plate electrodes and a tubular Teflon. The electrode diameter is 0.30 cm and electrode gap is 0.30 cm. By placing a perforated Teflon parallel to the planar electrode in the volume between the electrodes, the electric field could be enhanced in the openings of the Teflon. The waveforms of input voltage and current were measured with a two-channel digital oscilloscope (Tektronix TDS220, Beaverton, OR).

Pectin solution (25 g L$^{(1}$) was pumped by a peristaltic pump (Watson Marlow 323E/D Pump, USA), and its flow rate was controlled by a rotameter (Model FM-01, Ningbo Jiutian Meter Company, China). The electric conductivity of the solution was 475 μs cm$^{(1}$. The sample temperature was measured with two K-type thermocouples inserted in the inlet and outlet of chambers. A cooling bath, keeping at 0 °C, was used to cool the PEF-treated samples immediately. The pulse intensities applied were 18, 24 and 30 kV cm$^{(1}$, corresponding to the total specific energy input of 124 J mL$^{(1}$, 221 J mL$^{(1}$ and 345 J mL$^{(1}$, respectively. The pulse duration, frequency, and flow speed was 40 (s, 1 kHz and 1 mL s$^{(1}$, respectively. After PEF treatments, the samples were oven-dried and finely ground for the following reactions. All experiments were carried out in triplicate. Before and after each treatment, the whole system was cleaned and disinfected with 75% (v/v) ethanol solution and rinsed with distilled water.

The number of pulses (n = 20.16) and the total treatment time (T = 806 (s) were obtained according to the following functions:

$$n = \frac{Vf}{u} \tag{1}$$

$$T = n\tau \tag{2}$$

where V represents the volume of the treatment chamber (mL), f is the pulse frequency (Hz), u is the flow of the pectin solution (mL s$^{(1}$) and (is the pulse duration ((s).

The total specific energy input was calculated with the following formula:

$$Q_P = nE^2\tau\sigma \tag{3}$$

where E is the pulse intensity in kV cm$^{(1}$ and (the medium conductivity in S cm$^{(1}$ (Abram, Smelt, Bos, & Wouters, 2003; Unal, Yousef, & Dunne, 2002).

2.4. *Synthesis of modified pectin samples*

The pectin derivatives were prepared from PEF-treated pectin with arachidic anhydride in the absence of any solvent. The mixture of pectin and arachidic anhydride (1:2–2:1, w/w) was heated in an oil bath at 160 °C for 20–40 min, in the presence of K_2CO_3 (0.1 equiv). After cooling down room temperature, the final crude product was washed with dichloromethane. The obtained solid was dissolved in water, and titrated to a neutral pH with 0.5 N HCl. This solution was then dialyzed (membrane cut off 7000) for 1 day in distilled water and finally vacuum freeze dried to get the desired product.

2.5. *Characterization of modifying pectin*

2.5.1. *Determination of DE*

The DE of samples was determined by titrimetric method (Yapo, 2009). Briefly, 20 mL of 1% (w/v) pectin aqueous solution was titrated with 0.2 N NaOH using phenolphthalein indicator. Then, 20 mL of 0.5 N NaOH was added with stirring for 30 min to de-esterify pectin, after which 20 mL of 0.5 N HCl was added to exactly neutralize the NaOH. This mixture was titrated with 0.2 N NaOH using phenolphthalein indicator.

2.5.2. *Fourier transform infrared (FT-IR) spectrum*

FT-IR spectra were obtained on a Vector 33 FT-IR spectrometer (Bruker Company, Germany), using a KBr disc containing 1% finely ground samples. Thirty-two scans were taken of each sample recorded from 4000 to 400 cm$^{(1}$ at a resolution of 2 cm$^{(1}$ in the transmission mode.

2.5.3. *X-ray diffraction (XRD) patterns*

The XRD patterns of the pectin samples were performed with a D8 Bruker-advance X-ray powder diffractometer equipped with a Cu K(radiation ((= 1.5418 Å). An acceleration voltage of 40 kV and a current of 40 mA were used. The 2(range used was from 4° to 50° in steps of 0.04° with a count time of 2 s.

2.5.4. *NMR spectra*

The NMR spectra were recorded on a Bruker DRX-400 spectrometer at the frequency of 400 MHz with 5 mm MAS BBO probe. NMR samples consisted of pectin or pectin-derivatives dissolved in 99.8% D_2O and the measurements were carried out at 25 °C. 1D proton spectra were recorded with 256 scans and a relaxation delay of 2 s.

2.5.5. *Thermal analysis*

Thermal stability of pectin derivatives was performed using TG-DTA on a simultaneous thermal analyzer (SDT Q600, TA Instruments). All the thermogravimetric experiments were repeated at least thrice to confirm reproducibility of the data. By using alumina as the reference, about 10 mg of each sample was taken in a platinum crucible (50 (L). The scans were run from 25 °C to 500 °C at a rate of 10 °C min$^{(1}$ under nitrogen flow.

2.5.6. *Statistical analysis*

Statistical analyses of results were performed through ANOVA (level of significance, $p < 0.05$) followed by Duncan's significant difference test using SPSS software (version 13.0, SPSS Inc., Chicago, USA).

3. Results and discussion

3.1. *Effect of experimental conditions on the DE of SBP pectin*

To our knowledge, no studies have reported on the effect of PEF treatment in SBP pectin. The main focus of the present study was to investigate the assisted effect of PEF on the modification of SBP pectin. Reaction parameters, such as PEF intensity, mass ratio of arachidic anhydride/pectin, and reaction time, in the synthesis of pectin-arachates, were shown in Table 1 and presented in Scheme 1.

As seen from Table 1, the application of PEF has a significant effect ($p < 0.05$) on the modification of SBP pectin. The PEF intensity at 18 kV cm$^{(1}$, 24 kV cm$^{(1}$ and 30 kV cm$^{(1}$, led to a significant increment ($p < 0.05$) of DE from 61 to 69, and to 76 for pectin derivatives, respectively. It can also be calculated that the DE dramatically

Table 1
DE of pectin-arachate after PEF treatment.

Reaction conditions			Pectin-arachates		
PEF intensity (kV cm$^{(1}$)	SEI (J cm$^{(1}$)	Mass ratio[a]	Reaction time (min)	No.	DE
0	0	1:2	30	1	49 ± 1a
18	138	1:2	30	2	61 ± 2b
24	246	1:2	30	3	69 ± 1c
30	384	1:2	30	4	76 ± 1d
30	384	1:1	30	5	79 ± 2de
30	384	2:1	30	6	80 ± 3e
30	384	1:1	20	7	70 ± 2c
30	384	1:1	40	8	82 ± 1ef
30	384	1:1	50	9	84 ± 2f

SEI: total specific energy input; values are the means ± standard deviation ($n = 3$); mean values with different letters are significantly different ($p < 0.05$).

[a] Mass ratio of arachic acid/pectin.

increased by 24%, 40% and 55%, respectively, compared with modification without PEF treatment. In such cases, significant DE increase was achieved by applying just lower electric field intensity and less specific energy inputs.

For excellent energy efficiency, PEF treatments carried out at higher field intensities should reduce energy consumption without compromising the effectiveness of the PEF process. On the other hand, the continuous flow systems should be chosen to improve efficiency (Malicki, Oziemblowski, Molenda, Trziszka, & Bruzewicz, 2004). The results clearly highlight that the desired DE of pectin derivatives could be achieved with more intense intensity, thus resulting in both higher energy efficiency and lower operational costs. Pataro, Senatore, Donsi, and Ferrari (2011) observed similar behavior when inactivating *Saccharomyces cerevisiae* and *Escherichia coli* cells using PEF treatment. The higher the field strength applied, the lower the energy input required, which depended on the intrinsic characteristics of the microbial species involved.

The reason for this enhancement was possibly attributed to the favorable effect of PEF on pectin modification. Pectins were involved in the redox electrochemical reactions at the electrode–electrolyte interface around the anode and cathode, which led to the formation of reactive oxygen species (ROS) such as (OH and H_2O_2 (Sato & Ohshima, 2006; Wang, Mi, Yao, & Li, 2008). The electrochemical reactions included oxidation of the water molecules in the anode, producing highly reactive species ((OH and H_2O_2), and oxidation of the solid metals in the anode, and then transforming into soluble metallic ions, which could catalyze the formation of (OH form H_2O_2 via the Fenton reaction (Sun, Bai, Zhang, Liao, & Hu, 2011). The formed ROS would play an important role in the degradation of pectin. The structure of unstable neutral sugar side-chains, glycosidic bond and benzene ring of pectin would be attacked by the electrochemical reactions during PEF treatment, and thus forming degradation of pectin and inducing amount of active groups, which was benefit for esterification reaction between modified pectin and arachidic anhydride. The effects of PEF on the structure of compounds have also been reported in the literature, showing that reactive oxygen species such as (OH and H_2O_2, generated in the PEF, play an important role in the degradation of anthocyanins and cleave benzene ring in phenolic compounds (De, Chaudhuri, & Bhattacharjee, 1999; Ruenroengklin, Yang, Lin, Chen, & Jiang, 2009).

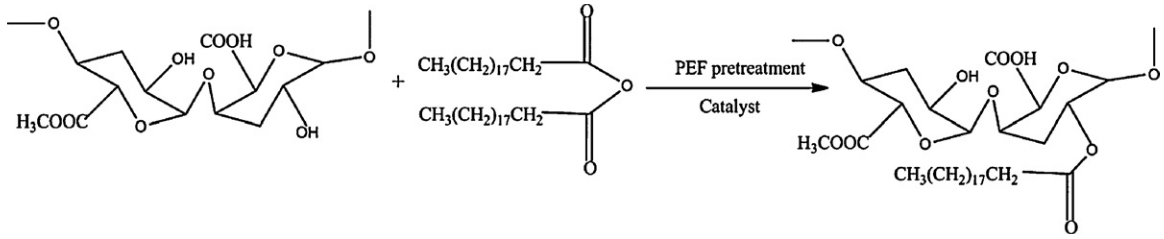
However, the mechanism of pectin induced by PEF treatment was different with that induced by ecological enzymatic process. The enzymatic transformation of polysaccharides could be mainly divided into two processes (Greenwood & Milne, 1968). One is a single-chain process, where enzyme acts linearly on one chain to another, and thus converting all substrate sites of chains. The other one is a multiple chain process, where enzyme converts a single residue. It releases from the enzyme–substrate complex, and enters into the other polysaccharide chain, and finally converts another single residue. The methyl esters, acetyl groups, and neutral sugar side-chains of pectin were influenced by the enzyme types (pectin methyl esterase, pectin acetyl esterase, rhamnogalacturonase, etc.), purity, and treatment conditions (pH, salt, concentration, etc.) (Sampedro, Rodrigo, & Martinez, 2011).

Raising the mass ratio of arachidic anhydride/pectin (MRO) from 1:2 to 1:1 or from 1:1 to 2:1 resulted in a slight but no significant increase of DE ($p < 0.05$). In contrast, the DE of pectin derivative obtained from MRO of 1:2 was significantly lower ($p < 0.05$) than that obtained from MRO of 2:1. The results indicated that the greater availability of arachidic anhydride had accessed to reactive sites at higher concentration of the esterifying agents.

The DE increased significantly ($p < 0.05$) from 70 to 79 as the reaction time variety from 20 to 30 min. However, the reaction time exceed 30 min had no significant effect ($p < 0.05$) on the DE, with the exception of reaction time between 30 min and 50 min. This was a direct consequence of increased reaction time on the diffusion of arachidic anhydride and pectin.

3.2. FT-IR spectra

FT-IR spectra of pure pectin as well as pectin-arachates are shown in Fig. 1. It reveals main changes in those regions particularly relating to the structure and composition of the inserted modifications. Bands in the region between 3402 and 1017 cm$^{(1}$



Scheme 1. Pulsed electric field-assisted synthetic of pectin-arachate without solvent.

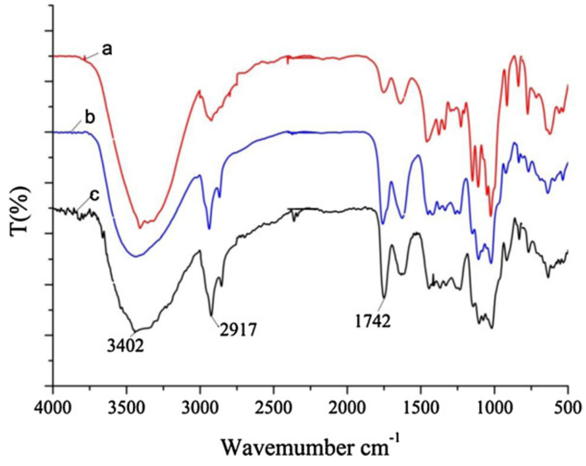


Fig. 1. FT-IR spectra of pectin (a), pectin-arachate sample 4 with DE = 76 (b), and the sample 5 with DE = 79 (c).

are typically associated with native pectin, which is consistent with previous literature (Kacurakova, Capek, Sasinkova, Wellner, & Ebringerova, 2000). In major details, the decrement of O—H band (3402 cm$^{(1}$) accounts for the decrease of the hydroxyl pectin groups upon reaction with the arachidic anhydride. On the other hand, the increment of C—H stretching region (2917 cm$^{(1}$) and the appearance of a new band (2855 cm$^{(1}$ for pectin-arachate) were also observed. Indeed, with increment of DE, the absorption intensity of —OH bond (3402 cm$^{(1}$) was weakened while and C—H stretching region (2917 cm$^{(1}$) was enhanced, which indicated that the intramolecular hydrogen bonds of pectin were involved in the reaction with arachidic anhydride, resulting in decrease of —OH bond and increase of the backbone of C—H, respectively. An increase in the C═O (1742 cm$^{(1}$) was also observed, which indicated that the DE of pectin-arachates increased.

3.3. XRD patterns

XRD patterns of pure pectin and pectin-arachate samples were shown in Fig. 2. It was observed from the XRD diffractogram of pure pectin that sharp peaks at 2(equals to 8.9°, 12.8°, 18.9°, 28.4° and 40.1° (, corresponds to those observed in the literature (Mishra, Datt, & Banthia, 2008). The pectin-arachate samples, however, show different peaks attributable to PEF treatment and crystalline arachidic anhydride. The PEF treatment would destroy the crystalline region of the pectin, and thus eliminate its main sharp peaks. A similar result was also observed by Luo et al. (2010). They found that the crystalline of chitosan sample was significantly damaged when the electric field strength was applied at 25 kV cm$^{(1}$. In addition to PEF effect, the broad peaks between 4.0° and 9.0° (were shown. Particularly, the intensity of the peaks at 5.6° and 7.0° (increased with increasing value of DE.

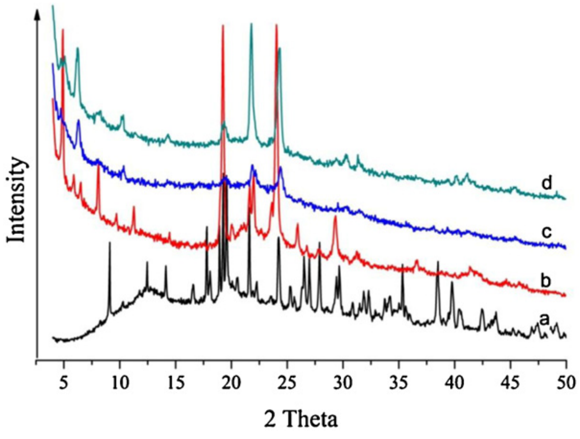


Fig. 2. XRD pattern of pectin (a) and pectin-arachate samples at different DE (b for 49, c for 61, d for 70).

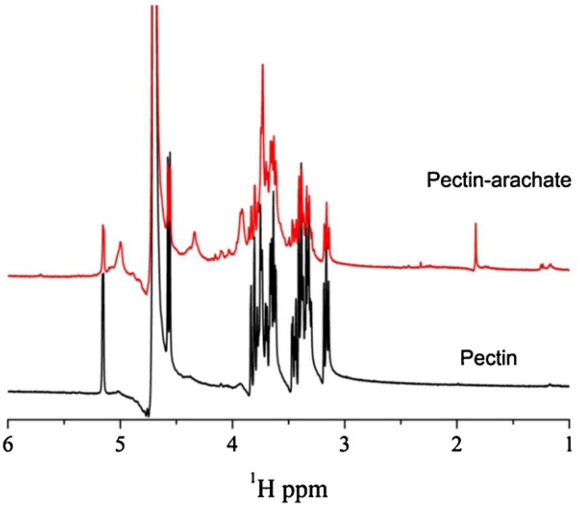


Fig. 3. 1D proton NMR spectra of pectin and its derivatives.

3.4. NMR spectra

NMR spectroscopy is capable of providing detailed information directly on the characterization of pectin and its derivatives. It could be seen from Fig. 3 that a rather good quality of NMR spectra is obtained under these experimental conditions. Bands in the region between 3.3 and 5.1 ppm are typically associated with native pectin, which is consistent with previous literature (Rosenbohm, Lundt, Christensen, & Young, 2003). Comparison of NMR spectra of pectin and its analog clearly demonstrates chemical modification of pectin. In fact, NMR spectra of modified pectins are characterized by broad signals belonging to the arachidic acid chain in the region between 1 and 2.5 ppm. Evidently, the presence of broad signals at 1.27 (CH_2), 1.63 (CH_2CH_2COO), 2.16 (CH_2COO), provided evidence of arachic acid chain, which indicated that the reaction shown in Scheme 1 did occur.

3.5. Thermal analysis

Loss of weight of sample was performed to investigate possible changes of thermal degradation of pectin derivatives. Fig. 4 showed the loss of weight of the pure and modified pectins as a function of temperature. All of the samples present a characteristic two-step thermal degradation at the given conditions. The first weight loss (8% of mass loss for b, 9% of mass loss for c) of modified pectins, occurs at 80 °C, corresponds to the adsorbed water, is slightly lower compared with that shown by the pure pectin (10% of mass loss), which indicated that PEF induced pectin-arachate reduced the adsorbed water. Moreover, the chemical modification produces an increase in the evaporation temperature, going from 80 °C for pure pectin to 86 °C for b and 88 °C for c, respectively. The second degradation stage (between 200 and 400 °C) due to pyrolitic decomposition shows a slightly lower mass loss and a lower midpoint temperature for the modified samples, implying that pectin-arachates with a higher DE increased the thermal stability.

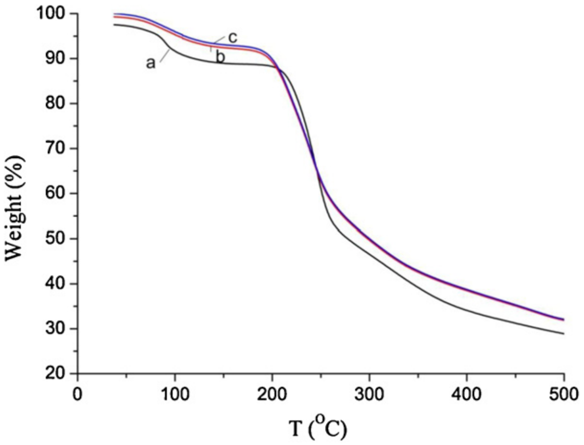


Fig. 4. Thermal analysis of pectin (a) and pectin-arachate samples at different DE (b for 69, c for 61, d for 76).

4. Conclusions

The initial aim of the presented work was a systematic investigation of the influence of PEF on modification of SBP pectin with arachidic anhydride, under solvent free conditions. The parameters included PEF intensity (18, 24 and 30 kV cm$^{(1}$), total specific energy input (124, 221 and 345 J mL$^{(1}$), MRO (1:2, 1:1 and 2:1), and reaction time (20, 30, 40 and 50 min). It was showed that the DE of pectin derivatives increased significantly with the increment of PEF intensity variety from 18 to 30 kV cm$^{(1}$ and total specific energy input in a range from 124 to 345 J mL$^{(1}$. The results were confirmed by FT-IR, XRD patterns and NMR spectra. The thermal stability of modified pectin was found to be a slightly higher than that of pure pectin.

The present research focused on the PEF-assisted modification of pectin. The reason for obtaining favorable effect was that the structure and group activity of pectin were modified when more intense electric field intensity or higher total specific energy input was applied. It would be a valuable contribution to extend the existing application of PEF beyond milk, fruit juice, or wine.

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