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Preparation and characterization of a quaternary ammonium derivative of pectin

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ABSTRACT

Cationic derivatives of pectin were prepared by reacting pectin with 3-chloro-2-hydroxypropyltrimethylammonium chloride (CHPTAC) in presence of sodium hydroxide (NaOH). The chemical structures of derivative were characterized by using elemental analysis, FT-IR, and ^{13}C NMR spectroscopy. The results revealed that the degree of substitution (DS) of quaternized pectin (QP) could be controlled by adjusting the molar ratio of CHPTAC to pectin, the molar ratio of NaOH to CHPTAC and reaction temperature occurred during quaternization. The moisture absorption (R_a) and moisture retention (R_h) abilities of QP are closely related to the DS value. With the DS value increasing, R_a and R_h increased. In vitro antimicrobial activity assessment exhibited QP showed pronounced inhibitory effect against the three bacteria (*Staphylococcus aureus*, *Escherichia coli*, and *Bacillus subtilis*). The improved functionalities of the derivative might be explained by its polycationic characteristics.

1. Introduction

In the last few years, considerable attention has been devoted to the applications of natural plant polysaccharides because of their unique properties (Crini, 2005; Edgar et al., 2001; Suh & Matthew, 2000). Recently, a large number of studies showed that natural plant polysaccharides and their derivatives were widely used as a type of desired biomaterials in many fields, such as drug delivery, immunization, controlled release, as well as in food application (Fox, Li, Xu, & Edgar, 2011; Liu, Fishman, & Hicks, 2006; Ramberg, Nelson, & Sinnott, 2010; Souto-Maior, Reis, Pedreiro, & Cavalcanti, 2010; Velisek & Cejpek, 2005). Pectin is a high-molecular weight, biocompatible, non-toxic, and anionic natural polysaccharide extracted from cell walls of higher plants (Zouambia, Moulai-Mostefa, & Krea, 2009). Chemically it consists of α-(1–4)-bond D-galacturonic acid units, occasionally interrupted by α-(1–2)-bond L-rhamnose units (smooth regions) and branched areas (hairy regions) represented by rhamnogalacturonan I and rhamnogalacturonan II (RGI and RGII) with lateral chains of galactan, arabinan, arabinogalactan, and other more complicated fragments (Günter & Ovodov, 2011; Ralet, Lerouge, & Quéméner, 2009). The degree of methoxylation (DM) is used to classify the pectins as high methoxyl pectins (DM > 50) and low methoxyl pectins (DM < 50) (Tripathi, Mehrotra, & Dutta, 2010). Pectin is known for a functional ingredient, gelling/thickening agent, and a stabilizer ingredient in the food industry due to its ability to form aqueous gels and has been used in jams and jellies, fruit preparations, fruit drink concentrates, fruit juice, desserts and fermented dairy products (Canteri-Schemin, Fertonani, & Waszczynskyj, 2005; Françoise, Kablan, & Kamenan, 2009). Moreover, characters of excellent gelling properties and good biocompatibility, as well as biodegradability entitle pectin to be a novel polymer material, which is employed in pharmaceutical industry, health promotion and treatment. It has been used potentially as a carrier for drug delivery to the gastrointestinal tract, such as matrix tablets, gel beads, film-coated dose form (Liu et al., 2006; Tripathi et al., 2010).

Physical or chemical modification of pectin can lead to new products with significantly functional properties. Previously, several pectin derivatives were prepared by crosslinking (Souto-Maior et al., 2010), amidation (Mishra, Datt, Pal, & Banthia, 2008), thiolation (Perera, Hombach, & Bernkop-Schnurch, 2010), sulfation (Cipriani et al., 2009) and then their properties and application were assessed. The applications of pectin have been also extended greatly from food and food additives to various fields, such as drug delivery (Mishra et al., 2008; Souto-Maior et al., 2010), antithrombotic agent (Cipriani et al., 2009), and mucoadhesive (Sharma & Ahuja, 2011).

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It is reported that quaternization is an efficient means of imparting new functional properties to polysaccharides (Geresh, Dawadi, & Arad, 2000). So far, quaternization has been widely applied to a variety of polysaccharides, such as chitosan (Yevlampieva et al., 2011), konjac glucomannan (Tian, Wu, Liu, & Xie, 2010), starch (Xu, Cao, Wu, & Wang, 2006), alginate (Kim, Kim, & Rhee, 2010), and cellulose (Niidei et al., 2010). However, quaternization of pectin is little reported. Therefore, in this study, a quaternary ammonium derivative of pectin was prepared by reacting with CHPTAC.

In the previous reports (Badawy, 2010; Kim et al., 2010; Sajomsang, Gonil, & Tantayanon, 2009), the antimicrobial ability of natural polysaccharides is improved by quaternization. As a polymer having quaternary ammonium salt group, QP possess potential antimicrobial properties. Hyaluronic acid (HA), an important functional ingredient in cosmetics, is unique for its excellent moisture-absorption and moisture-retention abilities, but the total amount is limited, and the price is high. The results showed QP could replace HA for its better moisture-absorption and moisture-retention abilities.

The purpose of this work was to prepare a pectin derivative by quaternization. The results reported in this article may contribute to finding the application of QP in pharmaceutical, packaging, preservatives, and cosmetic fields.

2. Experimental

2.1. Materials

CHPTAC was purchased from Guofeng fine chemical Co. Ltd., Shandong, China and it was used as etherifying reagents without further purification. Pectin (DM > 50) was purchased from Huilong pectin Corp., Quzhou, China. The content of galacturonic is 90.5%. And the molecular weight calculated from the static light scattering method (Fan et al., 2011) was about 12.8×10^4. All other reagents were of analytical grade and were used without further purification.

2.2. Synthesis of the derivative

In a typical reaction procedure, pectin (5 g) was dissolved in deionized water (250 mL), and then various molar ratio of NaOH/CHPTAC aqueous solution was dropped into the pectin solution through pressure-equalizing dropping funnel. After that, magnetic stirring was continuous for 17 h at certain temperature. The solution was neutralized with aqueous hydrochloric acid and then purified by dialysis through a 10 000–8000 molecular weight cut-off dialysis tubing for three days. The dialyzed product was finally freeze-dried with lyophilizer to obtain the purified pectin derivative. The dried samples were stored in vacuum desiccators over P_2O_5 for further analysis. According to Table 1, Experiments were conducted under different reaction conditions, such as the molar ratio of CHPTAC/pectin, the molar ratio of NaOH/CHPTAC, and temperature to obtain nine quaternized pectin derivatives (coded as QPs).

Table 1
Conditions and results of quaternary ammonium derivatives of pectin.

Sample ID	Molar ratio[a]	Molar ratio[b]	Temp (°C)	Nitrogen (%)	DS
QP-1	4	1.2	50	2.54	0.44
QP-2	5	1.0	50	2.90	0.53
QP-3	5	1.2	40	3.08	0.58
QP-4	5	1.2	50	3.74	0.79
QP-5	5	1.2	60	4.56	1.13
QP-6	6	1.2	50	4.64	1.17
QP-7	5	1.1	50	6.19	2.36
QP-8	7	1.2	50	6.25	2.43
QP-9	5	1.2	70	6.36	2.56

[a] The molar ratio of CHPTAC/pectin.
[b] The molar ratio of NaOH/CHPTAC.

2.3. Characterization of the products

Nitrogen contents (N %) of QPs were measured with an elemental analyzer (CHN-O-Rapid, Foss Hera us GmbH, Hanau, Germany). The DS value of QP was determined by nitrogen contents and calculated according to the following equation:

$$DS = \frac{176 \times N\%}{(14 - 151.5 \times N\%)}$$

FT-IR spectra of QP samples and pectin were performed with a Nicolet 170SX (USA) Fourier transform infrared spectrometer. The test specimens were prepared by the KBr-disk method.

The ^{13}C NMR spectrum of the QP sample was recorded on Bruker AMX-500 NMR spectrometer at an ambient temperature. The sample was dissolved in D_2O. Tetramethylsilane (TMS) was used as internal standard. Chemical shifts were expressed in δ (ppm) relative to the resonance.

2.4. Moisture absorption and retention test (Chen, Du, & Zeng, 2002)

Prior to the moisture-absorption testing, the samples were dried over P_2O_5 in vacuo for 24 h. The water-absorption ability was evaluated by the percentage of weight increase of dry sample (R_a):

$$R_a\,(\%) = 100 \times \frac{(W_n - W_0)}{W_0}$$

W_0 and W_n are the weights of sample before and after putting it into a saturated $(NH_4)_2SO_4$ desiccator (81% relative humidity) and in a saturated Na_2CO_3 desiccator (43% relative humidity) at 20 °C after 48 h of the test.

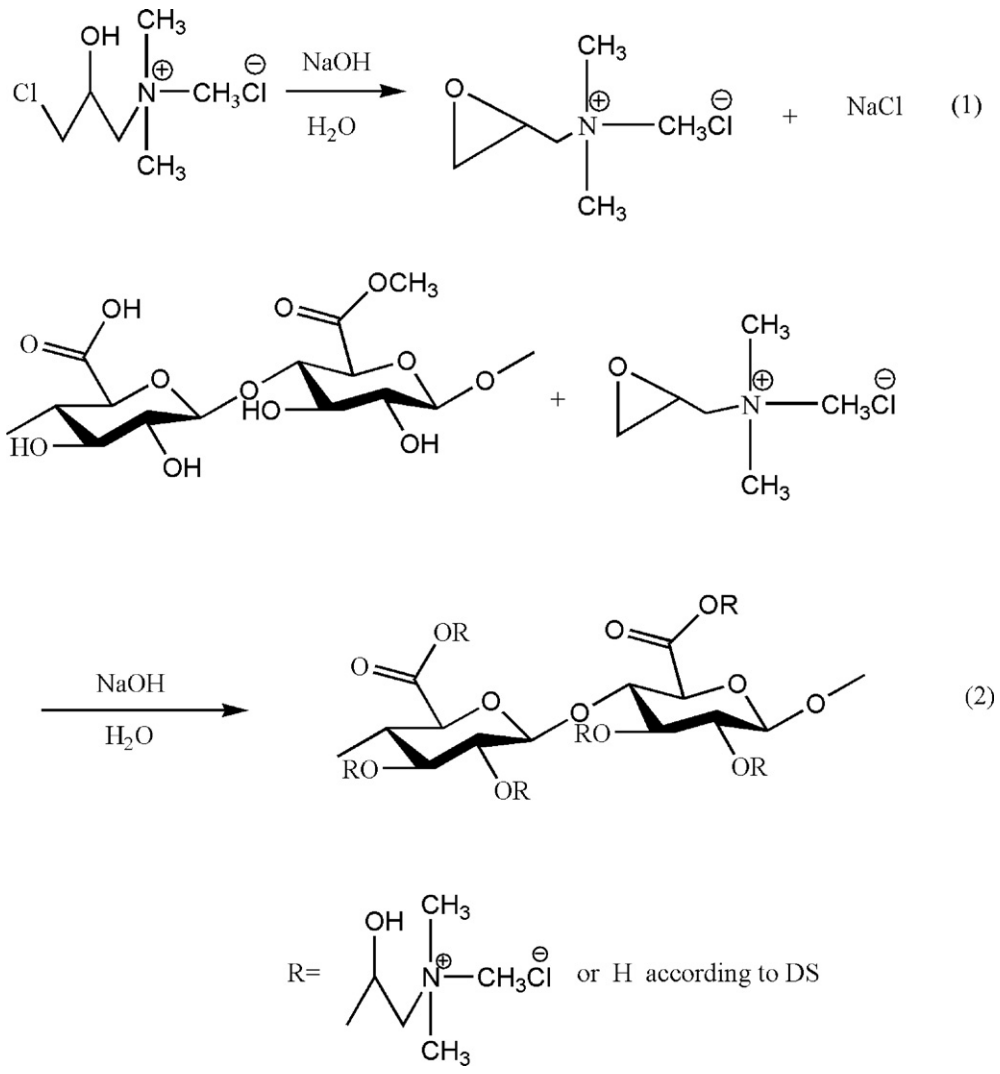
In the moisture-retention test, wet samples were prepared by adding 10% water to each sample. The moisture-retention ability was evaluated by the percentage of residual water of wet sample (R_h):

$$R_h\,(\%) = 100 \times \frac{H_n}{H_0}$$

H_0 and H_n are the weights of water in sample before and after putting in the silica gel desiccator at 20 °C 48 h of the test.

2.5. Antimicrobial activity assessments

In vitro antimicrobial activity of pectin and QP was assessed against three strains of bacteria (*Staphylococcus aureus*, *Escherichia coli*, and *Bacillus subtilis*) and three fungal strains (*Aspergillus niger*, *Mucor* sp., and *Rhizopus* sp.) by zone of inhibition. The strains were kept at 4 °C for the antimicrobial tests. Petri dishes were inoculated with 0.1 ml test bacteria solutions and fungal solutions, and activated respectively at 37 °C and at 28 °C for 24 h. The filter paper discs disinfected of 6 mm in diameter was impregnated with sterile distilled water and saturated with pectin and QP, followed by lying on the agar plates. The agar plates of bacteria were incubated at 37 °C for 24 h. At the same time, the agar plates of fungus were incubated at 28 °C for 24 h. Blank sterile filter paper discs and sterile water were used as control in this study.



Scheme 1. Reaction scheme for the quaternization of pectin with CHPTAC.

3. Results and discussion

3.1. Synthesis of the derivative

Scheme 1 illustrates the quaternization of pectin by using CHPTAC as etherifying agent. Under the alkaline conditions, epoxide is produced in situ from CHPTAC. QP is then formed through reaction between the pectin and the epoxide. The reaction conditions for quaternization of pectin are summarized in Table 1. The content of nitrogen and the degree of quaternization (expressed as DS value) under various reaction conditions are shown in Table 1. As can be seen, the N content and DS value of the quaternized derivatives increased with increasing molar ratio of CHPTAC to pectin, as well as the reaction temperature. Furthermore, the optimal molar ratio of NaOH/CHPTAC is 1.1, which is the same as quaternization of konjac glucomannan (Yu, Huang, Ying, & Xiao, 2007). The reasons might be as follows: first, the lower molar ratio of NaOH/CHPTAC could make the first step reaction incomplete and the epoxide is produced insufficiently. Second, the higher concentration of sodium hydroxide solution can hydrolyze CHPTAC and produce the diol. Obviously, quaternized pectin derivatives with the DS values of 0.44–2.56 could be obtained by adjusting the molar ratio of CHPTAC/pectin from 4 to 7, the molar ratio of NaOH/CHPTAC from 1.0 to 1.2 and the reaction temperature from 40 °C to 70 °C.

3.2. Characterization of the derivative

Fig. 1 shows the FT-IR spectra of the native citrus pectin and the quaternized pectin. The native pectin showed a broad band at about 3419 cm^{-1}, assigned to stretching vibration modes of O–H groups. The bands at 2937 and 1051 cm^{-1} were assigned to stretching vibration of $-CH_2-$ groups. Three peaks at 1747, 1636, and 1443 cm^{-1} in the carboxylic groups region were assigned to the C=O stretching of methyl-esterified carboxyl groups, to antisymmetric and symmetric stretching modes of COO^-, respectively (Kurita, Fujiwara, & Yamazaki, 2008; Monfregola, Leone, Vittoria, Amodeo, & De Luca, 2011; Pappas et al., 2004). In the case of QP, the broad band at 3399 cm^{-1} was assigned for O–H stretching vibration. The peak of 2938 and 1057 cm^{-1} referenced as stretching vibration peaks of $-CH_2-$ group. The peak of 1747 cm^{-1} of pectin disappeared due to removal of the ester group from the backbones of pectin during reaction. Meanwhile, the peak of 1611 cm^{-1} of QP was assigned to stretching vibrations of the carboxyl group. The most striking

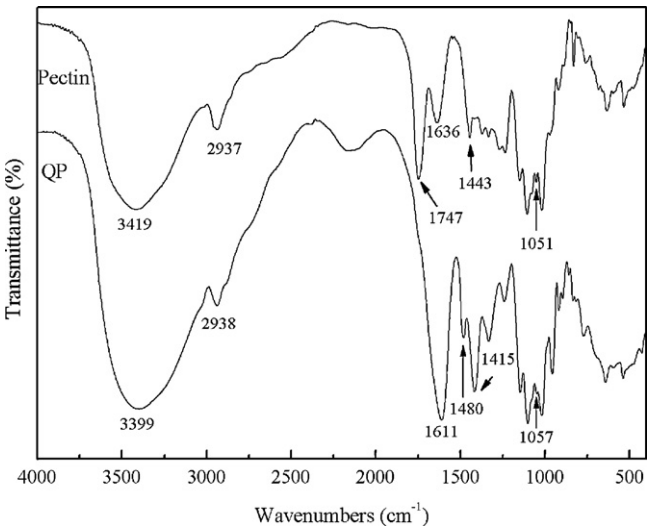


Fig. 1. FT-IR spectra of the native citrus pectin and the quaternized pectin (QP).

difference between native pectin and QPs positioned at 1480 cm^{-1}, which corresponded to the methyl groups of ammonium. Moreover, the peak of QCs positioned at 1415 cm^{-1} was referenced as the C–N stretching vibration (Pal, Mal, & Singh, 2005). FT-IR spectra have given an evidence of the introduction of the quaternary ammonium salt group on the pectin backbone.

The ^{13}C NMR spectrum of QP in D_2O at 25 °C is shown in Fig. 2. The peaks were assigned according to the native citrus pectin (Synytsya et al., 2004; Zhao et al., 2006) and the quaternary ammonium derivatives of konjac glucomannan (Yu et al., 2007), cellulose (Song, Sun, & Zhang, 2008), and starch (Pi-xin et al., 2009). The peak at lower field (175.5 ppm) was assigned to C6 of COOH group. The chemical shift at 99.0 ppm attributed to C1. Unsubstituted and substituted C2 and C3 appeared between 68.2 and 68.8 ppm. The peak at 77.9 ppm corresponded to C4, while C5 appeared at 71.4 ppm which was overlapped by the chemical shift of C7. The signals at 63.7 and 66.5 ppm were believed to belong to the carbons of C8 and C9, respectively. A relatively sharp high intensity signal at 54.2 ppm was attributed to a quaternary ammonium *N*-methyl carbon nucleus (C10). Therefore, the results of ^{13}C NMR spectrum further proved the successful synthesis of quaternized pectin.

3.3. Moisture absorption and retention properties

The moisture absorption and retention properties of QPs which were examined and compared with hyaluronic acid (HA) are shown

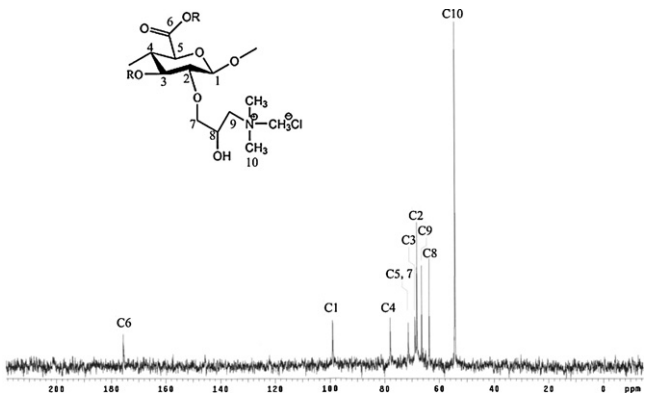


Fig. 2. ^{13}C NMR spectrum of quaternized pectin.

Table 2
Moisture-absorption and retention property of QP with different DS values.

Sample ID	DS	R_a (%) (dry sample)		R_h (%) (wet sample)
		RH 81%	RH 43%	Silica gel
Pectin	/	23.45	11.74	45.48
QP-1	0.44	40.81	36.45	48.31
QP-2	0.53	51.99	41.30	50.24
QP-3	0.58	54.51	42.84	51.49
QP-4	0.79	62.30	50.26	55.37
QP-5	1.13	81.02	55.61	58.42
QP-6	1.17	82.21	57.44	59.16
QP-7	2.36	87.37	59.00	64.34
QP-8	2.43	91.45	60.88	67.68
QP-9	2.56	100.35	66.38	70.78
HA	/	56.2	24.8	72.8

QP, quaternized pectin; HA, hyaluronic acid; RH, relative humidity.

in Table 2. Obviously, the moisture absorption and retention abilities of QP depend on the DS value. With an increase in DS, the moisture absorption and retention abilities of QP increased accordingly. And all QPs showed better R_a and R_h than pectin, indicating that the introduction of $-N^+(CH_3)_3$ group is a convenient and effective method to enhance both moisture absorption and retention abilities for pectin. The group has obvious charges, which are similar to those of HA, and the increased moisture absorption and retention abilities may be largely due to the charged group (Yang et al., 2010). QPs with the DS value from 0.79 to 2.56 showed better R_a than that of HA. And when the DS value is more than 2.0, QP showed good R_h. Therefore, QP has potential to be used as a moisture absorption and retention ingredient.

3.4. Antimicrobial activity

The capabilities of pectin, QP-3, and QP-7 in inhibiting the growth of the tested microbes are listed in Table 3. Obviously, pectin and QPs are both devoid of antifungal activity, whereas they showed inhibitory effect against the three bacteria investigated. As shown in Fig. 3, the higher zone of inhibition was recorded at QP-7 than QP-3 for *S. aureus* and *E. coli*. However, it is opposite for *B. subtilis*. This result implies that high DS value causes gain of antimicrobial activity for *S. aureus* and *E. coli*. While, with an increase in DS value, the antimicrobial activity for *B. subtilis* is decreased.

Although pectin showed inhibitory effect against the three bacteria, the water-solubility and pH value that is far from the human physiological pH value limited its application. The antimicrobial mechanism of the QP on test bacteria and fungus is probably complex and still not resolved. In the case of antibacterial mechanism against *S. aureus* and *E. coli*, it is generally believed that the positive charge of the quaternized group resulted in a polycationic structure absorbed onto the negatively charged cell surface of bacteria led to great alteration of the structure of outer membrane

Table 3
Antibacterial and antifungal activity of natural pectin and quaternized pectin.

Microorganisms	Antibacterial and antifungal activity			
	QP-3	QP-7	Pectin	Control
Bacteria				
S. aureus	+	+	+	–
E. coli	+	+	+	–
B. subtilis	+	+	+	–
Fungi				
A. niger	–	–	–	–
Mucor sp.	–	–	–	–
Rhizopus sp.	–	–	–	–

'–': no inhibition; '+': inhibition.

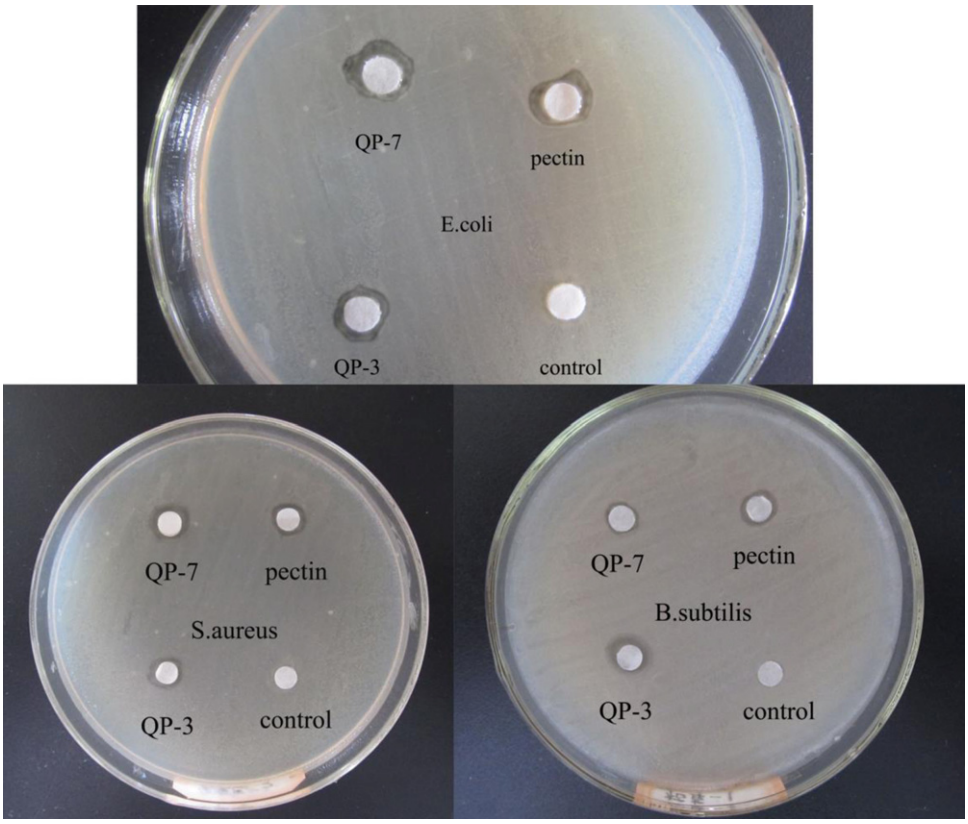


Fig. 3. Effect of natural and quaternized pectin on growth of three bacteria strains.

which caused release of major proportion of proteinaceous material from the cell. Cytoplasmic membrane is disrupted followed by the binding of quaternized pectin, and then releasing cytoplasmic constituents, such as K^+ ions, DNA and RNA, ultimately leading to bacterial death (Jiang, Wang, Yu, & Chen, 2005; Sajomsang, Tantayanon, Tangpasuthadol, & Daly (2009b); Sun, Du, Fan, Chen, & Yang, 2006). Thus, high DS value causes gain of antimicrobial activity for *S. aureus* and *E. coli*. Although *B. subtilis* and *S. aureus* are both Gram-positive bacteria, the effect on the antimicrobial activity of the QPs against them is different. The facts indicated that the different bacterium which is used has a great effect on the results of antimicrobial activity of the QPs. This is highly related to the structure and physiological activity of various bacteria. In addition, there maybe not only the effect of quaternized group of QPs but also another antimicrobial mechanism against *B. subtilis*, which is not totally the same as *S. aureus* and *E. coli*. However, most works (Dizman, Elasri, & Mathias, 2006; Lei, Yang, Jia, & Zhang, 2010) of antimicrobial activity were aimed at *S. aureus* and *E. coli*. The study of antimicrobial mechanism against *B. subtilis* was less reported. Thus, further research is still needed. As regards antifungal mechanism, it might be due to direct interference of nutrilite synthesis or blocking of fissiparism. Furthermore, major component of cell walls of bacteria is peptidoglycan and teichoicacid or lipoprotein and lipopolysaccharide, whereas major constituent of cell walls of fungi is chitin and glucan. The difference of inhibiting effect of QP on growth of bacteria and fungi tested may be due to the difference of structure and component of cell (Yu et al., 2007). More work is needed to confirm this hypothesis.

4. Conclusions

Cationic derivatives of pectin were successfully synthesized by reacting pectin with CHPTAC in NaOH aqueous solutions. Water-soluble QPs with DS value of 0.44–2.56 could be obtained by adjusting the molar ratio of CHPTAC to pectin from 4 to 7, the molar ratio of NaOH to CHPTAC from 1.0 to 1.2, and the reaction temperature from 40 to 70 °C. The QP was evaluated in terms of moisture absorption, moisture retention, and antibacterial activity test. The results indicated that R_a and R_h of QP are closely related to the DS value. With the DS value increasing, the R_a and R_h increased. And all QPs showed better R_a and R_h than pectin. Furthermore, QPs with the DS value from 0.79 to 2.56 showed better R_a than that of HA. When the DS value is more than 2.0, QP showed good R_h. In vitro antimicrobial activity assessments exhibited QP showed pronounced inhibitory effect against the three bacteria (*S. aureus*, *E. coli*, and *B. subtilis*). Therefore, the QP is promising derivative of pectin which applied in pharmaceutical, packaging, preservatives, and cosmetic fields.

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