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Abstract

Cross-linked porous starch has become a subject of research interest recently. The degree of cross-linking and the freeze-thaw stability are critical for cross-linked porous starch with respect to its practical applications. Herein, cross-linked porous corn starch (c-PCS) was prepared by the pre-alkalization of porous corn starch (PCS) using NaCl/NaOH which was then cross-linked using epichlorohydrin (ECH). The effects of key experimental parameters involved in both the pre-alkalization process (i.e., the mass ratio of NaOH to PCS ($m_{NaOH}/m_{PCS}$), the mass ratio of NaCl to PCS ($m_{NaCl}/m_{PCS}$), the temperature ($T_a$), and the reaction time ($t_a$)) and the cross-linking reaction (the ratio of ECH volume to the mass of PCS ($V_{ECH}/m_{PCS}$), the ratio of solution volume to the mass of PCS ($V_{s}/m_{PCS}$), pH value, the temperature ($T_c$), and the reaction time ($t_c$)) on the cross-linking degree and freeze-thaw stability of c-PCS were investigated in details. Results show that optimum conditions for the pre-alkalization and cross-linking reaction to obtain c-PCS are as follows: $m_{NaCl}/m_{PCS}$ is 0.06, $m_{NaOH}/m_{PCS}$ is 0.02, $T_a$ is 40 °C and $t_a$ is 1.0 h, $V_{ECH}/m_{PCS}$ is 0.003 (mL/g), $V_{s}/m_{PCS}$ is 7 (mL/g), pH is 10, $T_c$ is 20 °C, and $t_c$ is 3.0 h. The morphology, crystalline structure and thermal stability of the as-obtained c-PCS were also investigated, suggesting that the surface became rougher, the crystallinity was decreased, and the thermal stability was enhanced resulting from the cross-linking reaction.

Keywords: Cross-linked starch; Porous; Freeze-thaw stability; Epichlorohydrin.

1. Introduction

Porous starch refers to starch with a porous honeycomb structure.[1,2] The micro-pores of porous starch extend from the surface of starch granules to the center, rendering it an ideal carrier to encapsulate functional materials, such as medicament, perfumes, pigment, health care substance.[3,4] Besides, the porous starch shows good adsorption performance for metal ions, vegetable oils, etc., due to its high surface area and porous structure.[5] Therefore, porous starch has been explored as micro-capsules or adsorbents for versatile applications, e.g. in the fields of food,[6] medicine,[7] environmental remediation,[8,9] cosmetics,[10] and so forth.

Porous starch can be prepared via physical, chemical, and enzymatic methods.[11] For example, under the action of acids or the enzyme, the strength of the particle structure in the starch was weakened, which made the water molecules easier to diffuse and therefore resulted in a change of physicochemical characteristics of the native starch.[12] However, part of the starch granule structure was also damaged after the modification. Physical properties including freeze-thaw stability, shear resistance, and viscosity stability were reported to be inferior to those of the pristine starch; the adsorption capacity of the starch was also reduced, which restricted its industrial applications.[13]

To improve the mechanical strength of porous starch, cross-linking has been proved to be an effective approach. Bryant first reported cross-linked porous starch using sodium hypochlorite in 1933.[14] Since then, the cross-linking of native starch employing various reagents such as phosphoryl chloride,[15] chlorine,[16] hypochlorous acid,[17] and dichlorobutylene[18] has been studied. In recent years,
increasing research on the characteristics of cross-linked porous starch has been conducted. Gao et al.\textsuperscript{[23]} reported that granular cold-water soluble waxy corn starch (N-GCWS) and granular cold-water soluble porous waxy corn starch (P-GCWS) were prepared by waxy corn starch (WS) treated with alcohol-alkali. The results showed that the turbidity, freeze-thaw stability, viscosity, and oil absorption capacity of the surface N-GCWS and P-GCWS starch were higher than that of WS. Yussof et al.\textsuperscript{[24]} found the cross-linking had a considerable impact on the hydrolysis of corn and tapioca starch by reducing the swelling power and solubility of the starch granules. Moreover, the thermal stability of the starch granules was also enhanced since the expansion of cross-linked starch granules was inhibited, and the starch was therefore more stable and exhibited a higher gelatinization temperature in comparison with the native starch.\textsuperscript{[21]}

Freeze-thaw stability is a key parameter with respect to the practical application of cross-linked porous starch. Unfortunately, the effect of experimental parameters on the degree of cross-linking and the freeze-thaw stability of the product has not been reported yet to the best of our knowledge. In this context, we prepared cross-linked porous corn starch (c-PCS) from porous corn starch (PCS) which was first pre-alkalized using NaCl/NaOH and then cross-linked using epichlorohydrin (ECH). The effects of key experimental parameters (i.e., the mass ratio of NaOH to PCS ($\text{m_{NaOH}}$/$\text{m_{pcs}}$), the mass ratio of NaCl to PCS ($\text{m_{NaCl}}$/$\text{m_{pcs}}$), and the temperature ($T_c$) and reaction time ($t_c$) during the pre-alkalization process, the ratio of ECH volume to the mass of PCS ($V_{ECH}$/$\text{m_{pcs}}$), the ratio of solution volume to the mass of PCS ($V_s$/$\text{m_{pcs}}$), pH value, the temperature ($T_e$), and the reaction time ($t_e$) in the cross-linking reaction), on the degree of cross-linking and the freeze-thaw stability of c-PCS were investigated in details. X-ray diffraction (XRD) and differential scanning calorimetry (DSC) were further employed to investigate the crystallinity and thermal stability of c-PCS.

2. Materials and methods
2.1 Materials
Cross-linked microporous starch was prepared using the method in our previous study.\textsuperscript{[22]} NaCl and citric acid were provided by Tianjin Kwangfu Fine Chemical Industry Research Institute (Tianjin, China). ECH and sodium dihydrogen phosphate were purchased from Tianjin Chemical Reagent Co., Ltd. (Tianjin, China), hydrochloric acid (chemical pure) was supplied by Baiyin Liangyou Chemical Reagent Co. (Gansu Province, China). Corn starch, food grade, was purchased from Xuejing Biochemical Co., Ltd. (Shandong Province, China). Both $\alpha$-Amylase (biochemical grade) with an activity of 3.7 U/mg and glucoamylase (biochemical grade) with an activity of 100 U/mg were provided by Aoboxing Biotechnology Co., Ltd. (Beijing, China). NaOH was purchased from Shuangshuang Chemical Industry Co., Ltd. (Shandong Province, China). All the other chemicals were analytical reagent grade and were used as-received unless otherwise noted.

2.2 Methods
2.2.1 Cross-linking density measurement
Sedimentation method is an indirect approach to calculate the cross-linking density.\textsuperscript{[23]} First, 2.0 wt.% starch gel was prepared by adding 0.500 g dry c-PCS into 25 mL distilled water. The beaker was put into water bath maintaining at 85 °C for 2 min under slight stirring, and then taken out and cooled down to room temperature. Then 10 mL gel was transferred to a centrifuge tube and was centrifuged for 8 min at 15000 RPM. Finally, the tube was taken out from the centrifuge and the supernatant ($V$, in mL) was separated from the precipitate. Therefore, the sedimentation ($S$, in mL) could be obtained following Equation 1:

$$ S = 10 - V $$  \hspace{1cm} (1)

2.2.2 Freeze-thaw stability measurement
Freeze-thaw stability is a key indicator of structure stability for c-PCS, which can be calculated from the percentage of water separation from the gel after frozen and thawed.\textsuperscript{[24]} First, 6.0 wt% starch suspension was prepared by adding 1.500 g dry microporous starch into 25 mL distilled water. The gel was obtained by heating the suspension in the water bath at 85 °C for 5 min, and then taken out and cooled down to room temperature. The gel was transferred to a frozen centrifuge tube (the weight of the tube was denoted as $M_0$) and the total weight was recorded ($M_0-M_1$), from which the weight of starch gel could be calculated ($M_{0-M_1}$). Then the tube was put into the freezer chamber for 24 h, and then was taken out for thawing. The tube was centrifuged for 8 min at 15000 RPM and the total weight after removing the supernatant was recorded ($M_2$). The water separation from the gel before and after thawing was ($M_0 - M_1$). The syneresis was calculated using the following Equation 2:

$$ W = (M_0 - M_1) / (M_0 - M_2) \times 100\% $$ \hspace{1cm} (2)

2.2.3 Morphology and structure
Scanning electron microscopy (SEM) (JEOL JSM-6701F) analysis shows the microstructure after the cross-linking of the corn starch, and the functional groups of the cross-linking of the corn starch were examined by Fourier Transform Infrared Spectrometer (FTIR) on American Nicolet Corp.,
Model 170-SX with a scanning range of 4000-400 cm\(^{-1}\). The crystalline structures of the cross-linked porous corn starch were examined by XRD operating with a Cu-K\(\alpha\) radiation source filtered with a graphite monochromator at a voltage of 40 kV and current of 20 mA.

### 2.2.4 Gelatinization temperature \((T_g)\) measurement

The gelatinization temperature of c-PCS was determined by a Sapphire DSC (Perkin–Elmer, America).\(^{[25]}\) Briefly, the starch sample and distilled water at a mass ratio of 1:10 was prepared. This mixture was stirred on a magnetic stirrer for 30 min. Then about 20 mg of the starch paste was added to an aluminum pan and tightly sealed. The samples were scanned from 45 to 95 °C at a heating rate of 5 °C/min under nitrogen atmosphere. The gelatinization temperatures of peak temperature \((T_g)\) were recorded.

### 3. Results and Discussions

The cross-linking treatment not only enhanced the adsorption performance, but also increased the stability of porous starch, and therefore expands its practical applications. Investigating the effect of key cross-linking reaction parameters on both the sedimentation and syneresis helps to establish the relationship between processing parameters and the degree of cross-linking and structure stability, separately. Various reaction parameters were studied, including \(m_{\text{NaCl}}/m_{\text{pcs}}\), \(m_{\text{NaOH}}/m_{\text{pcs}}\), \(T_s\) and time \(t_s\) during the pre-alakalization reaction, \(V_{\text{ECH}}/m_{\text{pcs}}\), pH value, \(T_c\) and \(t_c\) during the cross-linking reaction.

#### 3.1 Effect of alkalinization reaction parameters on cross-linking degree and structure stability

##### 3.1.1 The mass ratio of NaCl to PCS \((m_{\text{NaCl}}/m_{\text{pcs}})\)

Fig. 1a shows the influence of the mass ratio of \(m_{\text{NaCl}}/m_{\text{pcs}}\) on the sedimentation and syneresis (other reaction parameters: \(m_{\text{NaOH}}/m_{\text{pcs}} = 0.02\), \(T_s = 30 \degree C\), \(t_s = 2.0\) h, \(V_{\text{ECH}}/m_{\text{pcs}} = 0.001\) (mL/g), \(V_s/m_{\text{pcs}} = 6\) (mL/g), pH = 9, \(T_c = 30 \degree C\), \(t_c = 2.0\) h). It is observed that the sedimentation initially decreases with increasing \(m_{\text{NaCl}}/m_{\text{pcs}}\) (lower than 0.06) and then begins to level off. The phenomenon is explained by the functionality of NaCl during the alkalinization process. By introducing NaCl, the ionic interaction between sodium ions and starch polymers reduced the plasticizing effect of water, and therefore inhibited the gelatinization or increased the gelatinization temperature of PCS.\(^{[26]}\) When insufficient NaCl was added, i.e., at low values of \(m_{\text{NaCl}}/m_{\text{pcs}}\) in Fig. 1a, partial gelation of PCS occurred under the influence of hydroxide ions during alkalinization reaction, which was unfavorable for the following cross-linking reaction, giving rise to low cross-linking degree. The chemical bonds formed in the cross-linking process were reported to cause a reinforcement of granule interactions that restrained the solubility and swelling of starch. Cross-linked starch exhibited negative line correlation between sedimentation and degree of cross-linking, that is, the sedimentation decreases linearly with increasing the cross-linking density.\(^{[27]}\) Thus, the sedimentation was high when the cross-linking degree was low. With increasing the amount of NaCl, the gelatinization was effectively inhibited, facilitating the cross-linking reaction.\(^{[28]}\) Therefore, higher degree of cross-linking was obtained, resulting in declined sedimentation. When the amount of NaCl reached saturated, it did not contribute to the alkalinization anymore. Correspondingly, the cross-linking degree did not change, and the curve began to level off.

During the freeze-thaw cycling process, starch molecules prone to re-associate into crystalline structure and new intermolecular hydrogen bonds were formed. As a result, free water in the preparation of starch gel was separated from starch, a process known as syneresis.\(^{[27]}\) The starch is required to retain its physicochemical properties as much as possible for applications, e.g., frozen food storage, for which the freeze-thaw stability provides necessary information. Syneresis in the cross-linked starches was reported to a certain extent to decrease with increasing the level of cross-linking.\(^{[29]}\) The reason is that cross-linking strengthened the intermolecular hydrogen bonds, thereby not only interrupting the linearity of the chain of amylose molecules, but also resulting in much more disorder distribution of partial amylopectin branch, and thus hindered the formation of

![Fig. 1](image-url) The effect of (a) \(m_{\text{NaCl}}/m_{\text{pcs}}\) and (b) \(m_{\text{NaOH}}/m_{\text{pcs}}\) on the sedimentation and syneresis.
intermolecular hydrogen bonds which made it difficult to rearrange and associate for amylase molecules. Therefore, the syneresis demonstrated similar trend as the sedimentation in Fig. 1a, which could also be explained by the effect of \( m_{\text{NaCl}}/m_{\text{pcs}} \) on the cross-linking degree. The optimum \( m_{\text{NaCl}}/m_{\text{pcs}} \) as suggested by Fig. 1a is 0.06.

### 3.1.2 The mass ratio of NaOH to PCS (\( m_{\text{NaOH}}/m_{\text{pcs}} \))

The effect of \( m_{\text{NaOH}}/m_{\text{pcs}} \) on the sedimentation and syneresis was displayed in Fig. 1b (other parameters: \( m_{\text{NaCl}}/m_{\text{pcs}} = 0.06, T_s = 30 \, ^\circ\text{C}, t_s = 2.0 \) h, \( V_{\text{ECH}}/m_{\text{pcs}} = 0.001 \, (\text{mL/g}), V_{s}/m_{\text{pcs}} = 6 \, (\text{mL/g}), \text{pH} = 9, T_c = 30 \, ^\circ\text{C}, t_c = 2.0 \) h). NaOH was used to enhance the reactivity of PCS with ECH during alkalization. The hydroxyl groups of starch molecules reacted with NaOH to yield sodium salt.\(^{[30]}\) When inadequate NaOH was added, the hydroxyl groups of starch molecules were not fully activated, leading to incomplete cross-linking, so that the cross-linking degree initially increased with increasing \( m_{\text{NaOH}}/m_{\text{pcs}} \) in Fig. 1b. Since the structure stability depends on the cross-linking degree, syneresis follows the same trend. Nevertheless, gelatinization began to occur with further increasing the amount of NaOH (\( m_{\text{NaOH}}/m_{\text{pcs}} \) was higher than 0.02) considering the limited inhibitory effect of NaCl which was kept at a constant concentration (\( m_{\text{NaCl}}/m_{\text{pcs}} = 0.06 \)). Complete gelatinization was observed when excessive NaOH were used, rendering the following cross-linking reaction completely impossible. Therefore, the sedimentation and syneresis increase with further increasing \( m_{\text{NaOH}}/m_{\text{pcs}} \) and an optimum \( m_{\text{NaOH}}/m_{\text{pcs}} \) of 0.02 could be established.

### 3.1.3 Alkalization temperature (\( T_s \))

Fig. 2a depicts the effect of \( T_s \) on the sedimentation and syneresis (the other parameters: \( m_{\text{NaOH}}/m_{\text{pcs}} = 0.02, m_{\text{NaCl}}/m_{\text{pcs}} = 0.06, t_s = 2.0 \) h, \( V_{\text{ECH}}/m_{\text{pcs}} = 0.001 \, (\text{mL/g}), V_{s}/m_{\text{pcs}} = 6 \, (\text{mL/g}), \text{pH} = 9, T_c = 30 \, ^\circ\text{C}, t_c = 2.0 \) h). It is observed that the sedimentation and syneresis both initially decrease rapidly and then start to increase slowly, which could be explained by two competing effects by increasing the temperature. First, at low temperatures (lower than 40 \(^\circ\text{C} \)) when gelatinization was still under control with the aid of NaCl, the positive effect of increasing the temperature dominated, \( i.e., \) the alkalization reaction rate increased with increasing the temperature, giving rise to more complete cross-linking reaction. At temperatures higher than 40 \(^\circ\text{C} \), gelatinization of porous starch occurred. With further increasing the temperature, gelatinization of porous starch became much more severe, making it difficult for the following cross-linking reaction. An optimum alkalization temperature of 40 \(^\circ\text{C} \) is therefore determined from Fig. 2a.

### 3.1.4 Alkalization time (\( t_a \))

The influence of \( t_a \) on the sedimentation and syneresis was investigated in Fig. 2b (other parameters constant: \( m_{\text{NaOH}}/m_{\text{pcs}} = 0.02, m_{\text{NaCl}}/m_{\text{pcs}} = 0.06, T_s = 40 \, ^\circ\text{C}, V_{\text{ECH}}/m_{\text{pcs}} = 0.001 \, (\text{mL/g}), V_{s}/m_{\text{pcs}} = 6 \, (\text{mL/g}), \text{pH} = 9, T_c = 30 \, ^\circ\text{C}, t_c = 2.0 \) h). As shown in Fig. 2b, but then increase slowly. It is reasonable that it took time for the full activation of hydroxyl groups of starch molecules, which was favorable for the next cross-linking reaction. Therefore, it is reasonable that the sedimentation and syneresis first decrease with increasing the reaction time in Fig. 2b. Nevertheless, the porous starch would partially gelatinize with prolonged reaction time after the alkalization was complete.\(^{[31]}\) Accordingly, the sedimentation and syneresis increased slowly. An optimum alkalization time of 1.0 h could be confirmed by Fig. 2b.

### 3.2 Effect of cross-linking reaction parameters on the sedimentation and syneresis

#### 3.2.1 The ratio of ECH volume to the mass of PCS (\( V_{\text{ECH}}/m_{\text{pcs}} \))

ECH was reported to be commonly used for the cross-linking of polysaccharides.\(^{[32]}\) After the alkalization of PCS (Reaction 3), the sodium salt of starch reacted with ECH to yield corn starch-epoxy (Reaction 4). Diether bridges were formed when corn starch-epoxy further reacted with the hydroxyl groups of another PCS molecule (Reaction 5). The cross-linking was
conducted under heterogeneous conditions where the solid PCS granules reacted with the liquid phase containing NaOH/NaCl and ECH.\[^{33}\]\n
\[
\text{PCS-OH} + \text{NaOH} \rightleftharpoons \text{PCS-O'Na}^+ + \text{H}_2\text{O} \tag{3}
\]

\[
\text{PCS-O'Na}^+ + \text{ClCH}_2\text{CH}_2\text{CH}_2\text{OH} + \text{NaCl} \rightleftharpoons \text{PCH}_2\text{S-O-CH}_2\text{CH}_2\text{O-PC} + \text{NaCl} \tag{4}
\]

\[
\text{PCH}_2\text{S-O-CH}_2\text{CH}_2\text{O-PC} + \text{PCS-OH} \rightleftharpoons \text{PCH}_2\text{S-O-CH}_2\text{CH}_2\text{O-PCS} \tag{5}
\]

The plot of $V_{\text{ECH}}/m_{\text{pcs}}$ against the sedimentation and syneresis is presented in Fig. 3a (the other experimental parameters: $m_{\text{NaOH}}/m_{\text{pcs}} = 0.02$, $m_{\text{NaCl}}/m_{\text{pcs}} = 0.06$, $T_a = 40^\circ \text{C}$, $t_a = 1.0 \text{ h}$, $V_s/m_{\text{pcs}} = 6$ (mL/g), pH = 9, $T_c = 30^\circ \text{C}$, $t_c = 2.0 \text{ h}$). The sedimentation is observed to increase with increasing the volume of epoxy first and then becomes relatively constant. The phenomenon is explained that the cross-linking degree of PSC increased by increasing the volume of ECH before PSC became fully cross-linked. Nevertheless, it was no use by further increasing the amount of ECH when almost all hydroxyl groups were cross-linked.

As aforementioned, the syneresis decreased with the increasing degree of cross-linking. Therefore, the trend of the syneresis curve is consistent with that of the sedimentation in the beginning. However, when the cross-linking degree of starch granules reached a considerable level, it became difficult for the water to penetrate into the starch molecular to form a homogenous gel, which was a prerequisite for the syneresis measurement. As a result, the values of syneresis measured were usually very high. Therefore, the syneresis was observed to increase with increasing $V_{ECH}$. The optimum $V_{ECH}/m_{pcs}$ is determined to be 0.003 mL/g.

### 3.2.2 The ratio of solution volume to the mass of PCS ($V_s/m_{pcs}$)

While keeping other experimental parameters fixed ($m_{\text{NaOH}}/m_{\text{pcs}} = 0.02$, $m_{\text{NaCl}}/m_{\text{pcs}} = 0.06$, $T_a = 40^\circ \text{C}$, $t_a = 1.0 \text{ h}$, $V_{\text{ECH}}/m_{\text{pcs}} = 0.003$ (mL/g), pH = 9, $T_c = 30^\circ \text{C}$, $t_c = 2.0 \text{ h}$), the dependence of the sedimentation and syneresis on $V_s/m_{\text{pcs}}$ were studied and plotted in Fig. 3b. Upward parabolas were obtained, indicating that solvent volume has influence on both the alkalization and cross-linking process. When a little amount of solvent was added, the concentration of NaOH was high, resulting in partial gelatinization of PCS, which is undesirable for cross-linking process. Meanwhile, less solution volume led to poor dispersion of starch granules,\[^{34}\] causing inefficient cross-linking reaction. However, too much solvent brought in lower concentration of NaOH and ECH, which were not favorable either for the alkalization or the cross-linking reaction. Therefore, an appropriate $V_s/m_{\text{pcs}}$ is required to obtain an optimum degree of cross-linking. From Fig. 3b, the optimum $V_s/m_{\text{pcs}}$ of 7 (mL/g) is confirmed.

![Fig. 3](image_url) The effect of (a) ECH volume, (b) solution volume and (c) pH value on the sedimentation and syneresis.
3.2.3 pH value
The plots of pH value against the sedimentation and syneresis are shown in Fig. 3c (other experimental parameters: \(m_{\text{NaOH}}/m_{\text{pcs}} = 0.02, m_{\text{NaCl}}/m_{\text{pcs}} = 0.06, T_a = 40 \, ^\circ \text{C}, \; t_a = 1.0 \, \text{h}, V_{\text{ECH}}/m_{\text{pcs}} = 0.003 \, (\text{mL/g}), V_s/m_{\text{pcs}} = 7 \, (\text{mL/g}), \; T_c = 30 \, ^\circ \text{C}, \; t_c = 2.0 \, \text{h})\). Due to the fact that ECH only has a higher reactivity in the alkaline environment for cross-linking with starch, both the sedimentation and syneresis were relatively high when pH was around 7.[35] With increasing pH, the sedimentation and syneresis were decreased. At pH values higher than 10, side reactions such as ring-open reaction of ECH and the reaction between ECH and the solvent occurred,[36] which was not conductive for the cross-linking of PCS. Correspondingly, both the sedimentation and syneresis increased. Furthermore, the structure of starch molecules would also be destroyed due to the gelatinization caused by high pH values. Therefore, the optimum pH value is determined to be 10.

3.2.4 Cross-linking temperature (\(T_c\))
Fig. 4a displays the effect of \(T_c\) on the sedimentation and syneresis (other experimental parameters: \(m_{\text{NaOH}}/m_{\text{pcs}} = 0.02, m_{\text{NaCl}}/m_{\text{pcs}} = 0.06, T_a = 40 \, ^\circ \text{C}, \; t_a = 1.0 \, \text{h}, V_{\text{ECH}}/m_{\text{pcs}} = 0.003 \, (\text{mL/g}), V_s/m_{\text{pcs}} = 7 \, (\text{mL/g}), \; \text{pH} = 10, \; t_c = 2.0 \, \text{h})\). By increasing \(T_c\), both the sedimentation and syneresis increased and reached a remarkably high value to the end. This is because even though high temperature was good for the reaction to proceed, it also gave rise to side reactions aforementioned.[38] In addition, gelatinization of the starch molecules occurred at high temperature.[37] Therefore, it can be concluded that low \(T_c\), i.e., 20 \, ^\circ \text{C}, is favorable for the cross-linking reaction.

3.2.5 Cross-linking time (\(t_c\))
The plot of cross-linking time \(t_c\) against the sedimentation and syneresis is shown in Fig. 4b (other experimental parameters: \(m_{\text{NaOH}}/m_{\text{pcs}} = 0.020, m_{\text{NaCl}}/m_{\text{pcs}} = 0.06, T_a = 40 \, ^\circ \text{C}, \; t_a = 1.0 \, \text{h}, V_{\text{ECH}}/m_{\text{pcs}} = 0.003 \, (\text{mL/g}), V_s/m_{\text{pcs}} = 7 \, (\text{mL/g}), \; \text{pH} = 10, \; T_c = 20 \, ^\circ \text{C})\). Sufficient reaction time was required for the cross-linking of PCS since ECH was a slow-acting cross-linking agent,[38] which explains the decrease of the sedimentation and syneresis with increasing \(t_c\) from 0.5 to 3.0 h in the beginning of Fig. 4b. A short plateau occurs in \(t_c\) values ranging from 3.0 to 5.0 h, suggesting that the cross-linking reaction was almost complete and the reaction time had negligible effect on the sedimentation and syneresis. By further prolonging \(t_c\), i.e., from 5.0 to 16.0 h, hydrolysis of PCS took place and the granular structure was damaged. Moreover, the integrity of PCS was impaired when subjected to overdue shear force by continuous stirring.[34] As a result, the values of the sedimentation and syneresis in slowly increased with increasing \(t_c\). Therefore an optimum \(t_c\) of 3.0 h is determined.

3.3 Physicochemical properties of c-PCS
3.3.1 SEM images
The microstructure after the cross-linking of the corn starch was observed by SEM images. It is obvious that the cross-linked porous corn starch (Figs. 5c-d) became much rougher compared to both the pristine corn starch (Fig. 5a) and porous corn starch (Fig. 5b), which was presumed to be caused by an increased amorphous region in the starch molecules.[22] The linear amylase was interrupted and/or the branched amylpectin became messier by the cross-linking, transforming part of the crystalline region into amorphous region, which was also reported by other researchers.[39]

3.3.2 FTIR
Fig. 6 shows the FTIR spectra of (a) c-PCS, (b) PCS and (c) pristine corn starch. There are no remarkable changes observed in the characteristic peaks since the newly formed ether bonds during the cross-linking reaction are similar to those of native corn starch and PCS. The peaks at 3392, 2927, and 1646 cm\(^{-1}\) correspond to the stretching vibration of –OH, the stretching vibration of –CH, and the bending vibration of –OH…O– formed between PCS molecules, respectively.[40] The adsorption bands in the range of 1415 to 1367 cm\(^{-1}\) are assigned to the bending vibration of –CH,[41] and the typical starch spectra from 1160 to 1013 cm\(^{-1}\) remain relatively unchanged.

![Fig. 4 The effect of (a) cross-linking temperature and (b) cross-linking time on the sedimentation and syneresis.](image-url)
3.3.3 XRD images

To confirm the crystalline structure of c-CPS granules, X-ray diffraction patterns of obtained samples were recorded and displayed in Fig. 7. Both the native starch (Fig. 7a) and cross-linked porous starch (Fig. 7b) were A-type amylopectins,[42] suggesting that the crystalline type had not changed after the modification. It is worthy noting that the peak intensity of c-CPS decreased, indicating that the cross-linking reaction gave rise to a decreased crystallinity, which is in good agreement with the SEM images. The decreased crystallinity is attributed to the cross-link reaction. With the cross-linking reaction proceeding, the regularity of the amyllose was interrupted, and the crystallized region was transformed into amorphous region.[43]

Fig. 5 SEM images of (a) pristine corn starch, (b) PCS and (c-d) c-PCS.

Fig. 6 The FTIR spectra of (a) the pristine corn starch, (b) PCS and (c) c-PCS.

3.3.4 Gelation Temperature ($T_g$) measurement via DSC

Thermal properties of native corn starch and c-CPS were obtained by the DSC. The endothermic peaks observed represents the gelatinization properties and the corresponding temperature is identified as $T_g$.[34] Fig. 8 displays DSC plots of native corn starch (a) and c-CPS (b). It can be seen that the gelatinization temperature of c-CPS was 69.3 °C, which is higher than 63.7 °C of the native starch. This is because cross-linking bonds were brought in between the starch molecules, which increased the intermolecular force and thus enhanced the gelatinization temperature.[18]

Fig. 7 XRD patterns of (a) the pristine corn starch and (b) c-PCS.

Fig. 8 DSC thermograms of (a) the pristine corn starch and (b) c-PCS.

4. Conclusions

Cross-linked porous corn starch has been successfully prepared using epichlorohydrin as the cross-linking agent after the alkalization of porous starch corn. The effect of key experimental parameters involved in the alkalization and cross-linking reaction process, that is, the mass ratio of NaCl to PCS ($m_{NaCl}/m_{pcs}$), the mass ratio of NaOH to PCS
(m\textsubscript{NaOH}/m\textsubscript{pcs}), the alkalization temperature (T\textsubscript{a}), the alkalization time (t\textsubscript{a}), the ratio of ECH volume to the mass of PCS (V\textsubscript{ECH}/m\textsubscript{pcs}), the ratio of solution volume to the mass of PCS (V\textsubscript{s}/m\textsubscript{pcs}), pH value, the cross-linking temperature (T\textsubscript{c}), and the cross-linking time (t\textsubscript{c}), on the degree of the cross-linking and the freeze-thaw stability of the porous granules structure has been investigated in details. The optimum experimental conditions are determined: m\textsubscript{NaOH}/m\textsubscript{pcs} = 0.06, m\textsubscript{NaOH}/m\textsubscript{pcs} = 0.02, T\textsubscript{a} = 40°C and t\textsubscript{a} = 1.0 h, V\textsubscript{ECH}/m\textsubscript{pcs} = 0.003 (mL/g), V\textsubscript{s}/m\textsubscript{pcs} = 7 (mL/g), pH = 10, T\textsubscript{c} = 20°C, and t\textsubscript{c} = 3.0 h. The cross-linked porous corn starch exhibits rougher morphology, decreased crystallinity, and a higher gelatinization temperature. This study paves the way for the preparation of cross-linked corn starch using epichlorohydrin.

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Conflict of Interest
There is no conflict of interest.

Supporting Information
Not Applicable.

References

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