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Effect of crosslinked condition on characteristics of chitosan/tripolyphosphate/genipin beads and their application in the selective adsorption of phytic acid from soybean whey

Chin-Yo Yang[a], Chu-Hsi Hsu[a,b], Min-Lang Tsai[a,*]

[a] Department of Food Science, National Taiwan Ocean University, 2 Pei-Ning Road, Keelung 20224, Taiwan, ROC
[b] Department of Food and Beverage Management, Yuanpei University, 306 Yuanpei Street, Hsinchu 30015, Taiwan, ROC

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ABSTRACT

In this study, tripolyphosphate/genipin co-crosslinked chitosan beads were prepared in different pH solutions and applied for selective adsorption of phytic acid from soybean whey solution. The co-crosslinking degrees (81.20–59.22%) of beads decreased with increased pH value of solution. The major chemical linkage between chitosan and genipin and a little ionic interaction between chitosan and tripolyphosphate gave CB7 (co-crosslinked in pH 7 solution) the best mechanical strength among these beads. The best adsorption ratio of phytic acid of the co-crosslinked beads occurred in pH 1 solution. The adsorption ratios of CB7 for phytic acid, trypsin inhibitor and lectin from pH 2 soybean whey solution were 30.23%, 3.76% and 1.53%, respectively. The highest desorption ratio of phytic acid from the CB7 desorbed in pH 9 solution was 93.98%; selective isolation for phytic acid can definitely be processed by CB7.

1. Introduction

Chitosan is a high molecular weight polysaccharide and is composed by glucosamine and *N*-acetyl-glucosamine. It is a widely distributed biopolymer since it is readily available via cationic polyelectrolyte in acid solution and because it is non-toxic, biocompatible and biodegradable. Chitosan is considered to be a versatile and environmentally friendly raw material. It can be applied in food, agriculture, biochemistry, wastewater treatment, paper, textiles, cosmetics, nanoparticles, hydrogel, liquid crystals, membranes, microcapsules, etc. (Harish Prashanth & Tharanathan, 2007; Rinaudo, 2006).

Chitosan is often considered to be an adsorbent due to its amino and hydroxyl groups as well as adsorption of protein, dye, metal, etc. (Babel & Kurniawan, 2003; Casal, Montilla, Moreno, Olano, & Corzo, 2006; Dotto & Pinto, 2011; Muzzarelli, 2011; Wu, Tseng, & Juang, 2000; Zhang, Yang, & Guo, 2011). Advantages of chitosan-based material used as sorbent include the fact that it is an extremely cost-effective environmentally friendly natural polymer with outstanding metal and dye-binding capacities, high efficiency and selectivity in detoxifying both very dilute or concentrated solutions, exhibiting excellent diffusion properties and resulting in high-quality treated effluent; it is a versatile sorbent and easily regenerated if required (Crini, 2005).

The chitosan bead crosslinks with crosslinking agent could improve these drawbacks and increase the use of recycling. Tripolyphosphate (TPP) is a non-toxic polyanion which can interact with chitosan via electrostatic forces to form ionic crosslinked networks (Mi, Sung, Shyu, Su, & Peng, 2003). Genipin is a natural crosslinking agent. It is an aglucone of geniposide extracted from *Gardenia jasminoides* and obtained from geniposide via enzymatic hydrolysis. Genipin is considered an ideal biomedical material due to its being 10,000 times less toxic than glutaraldehyde (Muzzarelli, 2009; Sung, Huang, Huang, & Tsai, 1999). Mi et al. (2003) have explored the mechanism of the co-crosslinked reaction of chitosan and TPP/genipin.

Soybean whey is a by-product from the preparation of soybean products such as tofu, soy protein isolate, etc. It was deemed a waste product, and disposal constitutes an environmental and industrial problem (Peñas, Préstamo, Polo, & Gomez, 2006). Soybean whey is composed of trypsin inhibitor, lectin, lipoxygenase, urease, β-amylase, phytic acid, etc. (Sorgentini & Wanger, 1999). The value of soybean will increase if the above compounds are recovered and used.

Phytic acid (myo-inositol hexakisphosphate, IP6) is found in abundance in cereals, beans, nuts, oilseeds, tubers, pollen, spores, and organic soil (Febles, Arias, Hardisson, Rodríguez-Alvarez, & Sierra, 2002). Phytic acid is often considered an anti-nutrient due to its ionization behaviour and ability to chelate multivalent cations

* Corresponding author. Tel.: +886 2 2462 2192x5122; fax: +886 2 2463 4203.
E-mail address: tml@mail.ntou.edu.tw (M.-L. Tsai).

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such as Zn^{2+}, Ca^{2+}, and Fe^{3+}, thus preventing mineral absorption (Wodzinski & Ullah, 1996). Phytic acid has antioxidant and anticancer effects and can lower blood cholesterol and lipids. Both *in vivo* and *in vitro* experiments have demonstrated the inhibitive and therapeutic effects of the dephosphorylation of phytic acid on colorectal cancer, colon cancer and rectal carcinoma (Dost & Tokul, 2006).

The aims of this study were explored the mechanical properties, size and crosslinking degree of chitosan beads co-crosslinked with TPP/genipin in different pH solutions and selective isolation of phytic acid from soybean whey solution by co-crosslinked chitosan bead.

2. Experimental

2.1. Materials

Squid (*Illex argentinus*) pens and soybean (*Glycine max* Merrill) were donated by Shin Dar Bio-Tech. Co. Ltd. (Taoyuan, Taiwan) and Hua Shang Food Enterprise Co. Ltd. (Taoyuan,Taiwan), respectively. The chitosan (degree of deacetylation: 80%, molecular weight: 900 kDa) was prepared from squid pens as described by Tsai, Chang, Yu, Lin, and Tsai (2011). Potassium bromide, acetic acid, and iron (III) chloride hexa hydrate were purchased from Merck (Darmstadt, Germany). Sodium tripolyphosphate (TPP), sodium hydroxide, sodium phytate, sodium azide, Kunitz trypsin inhibitor, lectin, and sodium acetate anhydrous were purchased from Sigma–Aldrich (St. Louis, MO, USA). Ninhydrin was purchased from Mallinckrodt Baker (Phillipsburg, NJ, USA). Genipin was purchased from Challenge Bioproducts (Taichung, Taiwan).

2.2. Preparation of non-crosslinked chitosan beads

The chitosan solution (1%) was applied through a 25 G × 1 in. syringe needle mounted by the syringe pump at a controlled flow rate of 0.42 ml/min to 1 N NaOH (15% ethanol) to produce chitosan beads of 1.8 mm in diameter. The chitosan beads were washed until neutral and stored at 4 °C (Shu & Zhu, 2000).

2.3. Preparation of TPP/genipin co-crosslinked chitosan beads

The chitosan solution (1%) was dripped into 0.01 M TPP/0.01 M genipin solution with pH 2, 3, 5, 7 and 9, respectively and stored for 24 h to proceed co-crosslinking. After crosslinking, the solidified beads were stirred for two days in ultrapure water (Millipore) to remove residual TPP and genipin, and then stored at 4 °C (Mi et al., 2003).

2.4. Determination of crosslinking degree of chitosan bead

The crosslinking degree of chitosan beads was determined by ninhydrin assay, which can determine the percentage of free amino groups in crosslinked chitosan beads (Mi, Tan, Liang, Huang, & Sung, 2001). The ninhydrin solution was prepared as follows: Solution A: 1.05 g citric acid, 10 ml (1 M) NaOH, and 0.04 g $SnCl_2{\cdot}2H_2O$ were mixed, then de-ionized water was added until 25 ml; Solution B: 1 g ninhydrin was dissolved in 25 ml ethylene glycol monomethyl ether. Solutions A and B were mixed and stirred for 45 min and then stored in a dark bottle. The chitosan beads were lyophilized for 6 h and then weighed. Lyophilized beads (5.0 mg) were placed into a 1.5 ml Eppendorf tube and then 0.5 ml ninhydrin solution was added. The solution was heated for 20 min at 100 °C and then cooled to room temperature. The mixture was stirred and centrifuged at 4500 rpm for 5 min at 25 °C. Then, the absorbance at 570 nm was measured with an ELISA reader (Micro-plate Reader, μQuant-MQX 200, Kcjunior software, Bio-Tec Instruments, Winooski, VT, USA). The concentration of free amino groups was proportional to the absorbance. With D-glucosamine as a standard, a calibration curve was established and the concentration of the free amino groups of samples was calculated. Every sample was tested three times. The calculation equation for crosslinking degree was as follows:

$$\text{Crosslinking degree}\,(\%) = \frac{x-y}{x} \times 100$$

where x is the mole of free amino groups of non-crosslinked chitosan beads, and y is the mole of free amino groups of co-crosslinked chitosan beads.

2.5. Determination of mechanical strength of chitosan bead

The mechanical strength of the chitosan beads was determined with a texture analyzer (TA-XT2, Haslemere, England). The needle probe moved at 1.0 mm/s to perform penetration testing. Every sample was tested five times to obtain the average. The units of breaking force, deformation and gel strength, were expressed as g, mm and g mm, respectively.

2.6. Determination of phytic acid concentration

The concentration of phytic acid was measured by a modified method proposed by Gao et al. (2007). In brief, 50 μl of sample was mixed with 150 μl of Wade reagent (5 ml 0.015% $FeCl_3{\cdot}6H_2O$ + 5 ml 0.15% sulfosalicylic acid) and thoroughly mixed on a vortex for 10 min. Then, the absorbance at 500 nm was measured with an ELISA reader.

A series of calibration standard solutions containing 0, 15, 30, 60, 120, 150 and 300 μM phytic acid were prepared from sodium phytate. Each 50 μl of standard solutions was mixed with 150 μl of Wade reagent and mixed by a vortex; subsequently, the absorbance at 500 nm was measured with an ELISA reader. The standard curve was established via plotting of absorbance intensity versus concentration of phytic acid.

2.7. Adsorption ratio of phytic acid of chitosan bead

Chitosan beads were dipped in blank solutions at different pH values (1, 3, 5, 7, and 9) for 4 h. The beads were then taken out and drained. Chitosan beads (30 mg) were added into phytic acid solutions at pH 1, 3, 5, 7, and 9 (C_0 = 300 μM, V = 10 ml). After adsorption at 25 °C for 24 h, the concentration of phytic acid (C) was determined with an ELISA reader. The calculation equation for adsorption ratio was as follows:

$$\text{Adsorption ratio}\,(\%) = 1 - \frac{C}{C_0} \times 100$$

2.8. Preparation of soybean whey

The soybeans were soaked in pure water for 5 h, hulls cleaned, and dried at 45 °C. Then the soybeans were ground into flour. Subsequently the soybean flour was defatted with tenfold weight hexane for 8 h. The defatted soybean flour was parted and dried by fume cupboard. Then, the defatted soybean flour was extracted at room temperature for 2 h with ultrapure water (Millipore) adjusted to pH 8 with 2 N NaOH (water:flour = 10:1). It was then centrifuged at 10,400 × *g* for 15 min at 20 °C. The supernatant was adjusted to pH 4.5 with 1 N HCl and kept for 2 h at 4 °C, and subsequently centrifuged at 10,400 × *g* for 20 min at 4 °C. The supernatant (pH 4.5) was filtered through No. 1 filter paper (Toyo Roshi Kaisha, Ltd., Tokyo, Japan), and adjusted to pH 8 with 2 N NaOH, kept for 1 h at room temperature and subsequently centrifuged at 12,400 × *g* for

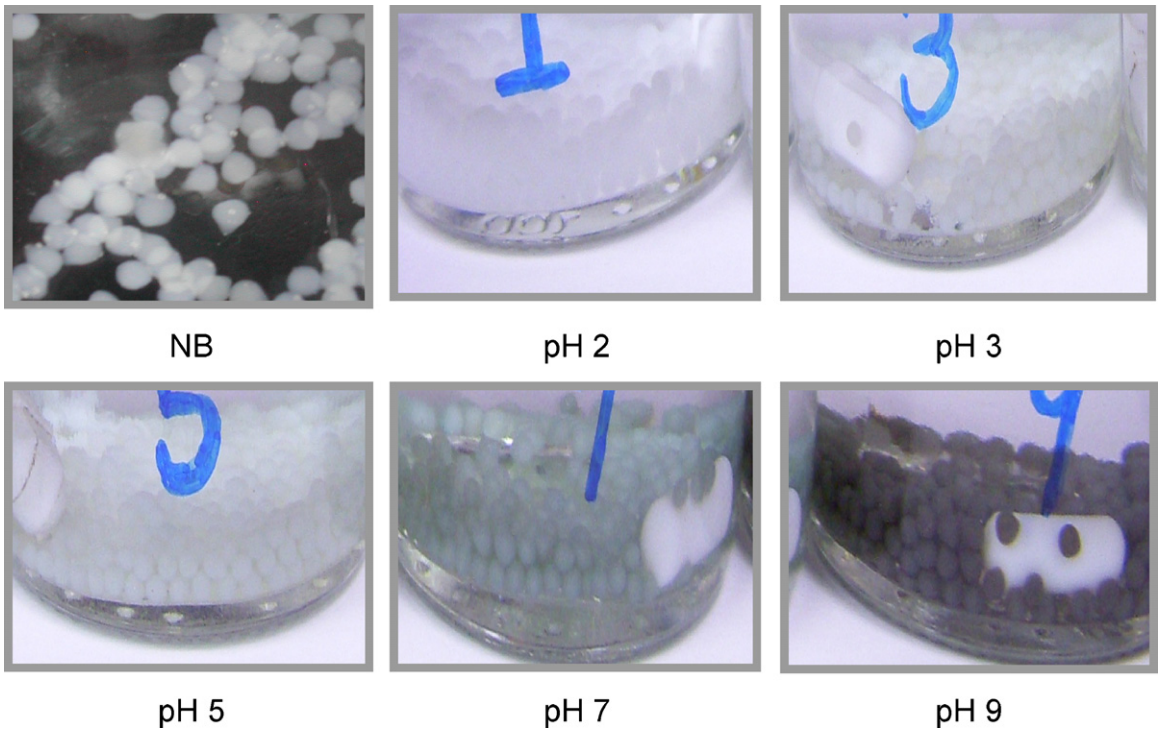

Fig. 1. Photographs of non-crosslinked chitosan beads (NB) and the chitosan beads crosslinked with tripolyphosphate/genipin at different pH conditions after 24 h.

15 min at 20 °C. The supernatant was precipitated with ammonium sulfate to 90% saturation, followed by centrifuging at 12,400 × *g* for 15 min at 20 °C. The precipitate (soybean whey) was washed with water and dialyzed for 24 h at 4 °C and finally lyophilized (Freeze dry system, Labconco, MO, USA) (Sorgentini & Wanger, 1999).

2.9. Determination of trypsin inhibitor and lectin

The concentration of trypsin inhibitor and lectin was measured with a modified method reported by Castro-Rubio, Marina, and García (2007). The HPLC system equipped with L-2130 Pump and L-7455 Detector (Hitachi, Tokyo, Japan). A POROS R2/H perfusion column (100 mm × 2.1 mm I.D., Perseptive Biosystems, Framingham, MA, USA) was used for the separation of proteins. Mobile phase A consisted of ultrapure water and 0.05% (v/v) TFA. Mobile phase B was ACN with 0.05% (v/v) TFA. These separations were performed at a flow-rate of 0.5 ml/min (mobile phase A + B) using a solvent gradient from 5% to 16% B in 8 min, 16% to 20% B from 8 to 12.5 min, 20% to 24% B from 12.5 to 13.5 min, 24% to 40% B form 13.5 to 17.5 min, 40% to 45% B form 17.5 to 22.5 min, 45% to 50% B from 22.5 to 26.5 min, and 50% to 95% B form 26.5 to 27 min. The injected volume was 20 μl, the operation temperature was 60 °C and UV detection was performed at 254 nm. The standard curves were established via plot of peak area versus concentration of trypsin inhibitor and lectin, respectively.

2.10. Adsorption and desorption of phytic acid

The CB7 beads were dipped in blank solutions at pH 2 for 4 h. The beads were then taken out and drained. The 0.1 g CB7 was added into 30 ml of pH 2 soybean whey solution. The solution was stirred at 100 rpm for 24 h at 25 °C. Then, the CB7 was taken out, the concentrations of phytic acid, trypsin inhibitor and lectin, of adsorbed soybean whey solution were determined with the Wade reagent method and HPLC, respectively.

At 25 °C, the desorptions of phytic acid from adsorbed phytic acid CB7 were carried out in solutions at pH 7, 8 and 9, stirred at 100 rpm for 24 h. Then, the concentration of phytic acid was determined and desorption ratio was calculated:

$$\text{Desorption ratio (\%)} = \frac{\text{desorbed amount of phytic acid in solution}}{\text{initial amount of phytic acid in CB7}} \times 100$$

2.11. Statistical analysis

Analysis of variance was used to determine any significant difference ($p < 0.05$). Duncan's new multiple range test was used to further test their difference by SAS system for windows Version 8.1 (SAS Institute Inc., Cary, CA, USA, 1988).

3. Results and discussion

3.1. Physicochemical properties of chitosan beads

Fig. 1 shows that photographs of a non-crosslinked chitosan beads (NB) and chitosan beads co-crosslinked with tripolyphosphate (TPP)/genipin at pH 2, 3, 5, 7, and 9 (CB2, CB3, CB5, CB7 and CB9) after 24 h, respectively. The results showed that the colour of the beads was influenced by the pH values. The NB, CB1 and CB3 were whiter. With the increase in pH values of solutions, the colour of the beads changed from light green (pH 5) to dark green (pH 7) to deep green (pH 9), indicating different crosslinking degrees for different pH values. According to this phenomenon, Mi et al. (2003) explained that TPP will ionize and form a pentavalent anion in water. In acidic solutions, the phosphate groups interacted with the protonated amino groups of chitosan to form ionic crosslinked networks. However, the carbonyl group of genipin will bind the amino groups of chitosan in neutral or alkaline solution; or the O in the chemical structure of genipin will be replaced by the N in the

Table 1
The size, crosslinking degree, breaking force, deformation, and gel strength of chitosan beads co-crosslinked with TPP/genipin in different pH solutions.

Type	Size (mm)	Crosslinking degree (%)	Breaking force (g)	Deformation (mm)	Gel strength (g mm)
CB2	2.38 ± 0.08d	80.14 ± 1.30 d	0.54 ± 0.05 a	0.06 ± 0.00 a	0.03 ± 0.00 a
CB3	2.22 ± 0.13 c	76.65 ± 2.90 c	0.60 ± 0.07 a	0.38 ± 0.20 b	0.23 ± 0.11 a
CB5	2.02 ± 0.04 b	74.64 ± 0.45 b	0.80 ± 0.16 a	0.60 ± 0.07 c	0.49 ± 0.13 a
CB7	2.00 ± 0.12 b	65.29 ± 1.28 b	5.38 ± 0.98 c	1.31 ± 0.19 d	7.08 ± 1.81 c
CB9	1.94 ± 0.11 b	59.22 ± 1.38 a	3.46 ± 0.42 b	1.27 ± 0.19 d	4.39 ± 0.79 b
NB	1.76 ± 0.09 a	0.00	0.56 ± 0.11 a	0.08 ± 0.04 a	0.04 ± 0.02 a

NB expressed non-crosslinked chitosan beads. CB2 expressed chitosan bead co-crosslinked TPP/genipin under pH 2 solution. The rest may be deduced by analogy. Values are mean ± S.D. ($n = 5$). Different letters (a–d) in the same column indicate significant differences ($p < 0.05$) between samples.

amino groups of chitosan, which is a covalent bond with greater strength.

Table 1 shows the size, crosslinking degree, breaking force, deformation, and gel strength of chitosan/TPP/genipin co-crosslinked beads prepared using different pH solutions. The bead sizes were between 1.94 and 2.38 mm; the size decreased as the pH values increased. The size of NB (prepared at pH 12) was 1.76 mm, the smallest size among these beads (Table 1). The results correspond with those of Kamiński, Zazakowny, Szczubialka, and Nowakowska (2008) where the genipin crosslinked chitosan microsphere was swollen when dipped into solutions at pH values below 6.5 and contracted when the solutions were at pH values higher than 6.5. The amino groups of chitosan chain were protonated in acidic solutions so the conformation was extended; thus, the size of beads was larger. In neutral and alkaline solutions, since the chitosan chain was electrically neutral, the conformation was contracted. The covalent bonds produced by the crosslinking of genipin also contributed to the contracted conformation; thus, the bead size was smaller.

Table 1 also shows that the crosslinking degrees of the co-crosslinked chitosan beads were 59.22–80.14%. Fig. 2a indicates that the degree of crosslinking decreased as the pH value of the solutions increased. The results were similar to those of Mi et al. (2003). In acidic solution, chitosan failed to react with genipin due to the cations on the chitosan chain and the fact that they only performed ionic crosslinking with TPP (physical interaction); lower pH solution causes the chitosan beads to experience a higher degree of crosslinking (74.64–80.14%). However, at pH values higher than 6.5, the crosslink reaction among chitosan and TPP was rare; the crosslink reaction among chitosan and genipin was predominant (Mi et al., 2003). The chemical reaction between chitosan and genipin was more difficult than was a physical reaction between chitosan and TPP, due to the requirement of more energy. This caused the crosslinking degree of chitosan beads down to 65.29% and 59.22% in pH 7 and 9 solutions, respectively. The crosslinking degrees of CB7 and CB9 were lower than the regression equation of pH versus crosslinking degree which also demonstrated this phenomenon (Fig. 2a). The chemical crosslinked degree between genipin and chitosan was not changed due to pH variation (theoretically when the solution pH is higher than 7); however, the crosslinking degree of CB7 being larger than CB9 (Table 1) may be caused by a little ionic crosslinking between chitosan and TPP.

Fig. 2b–d shows the relationships of the mechanical properties (breaking force, deformation and gel strength) versus the crosslinking degree of chitosan/TPP/genipin beads. Under acidic conditions (pH 2, 3 and 5), the mechanical strength of chitosan beads was weaker due to the physical interaction alone. There was a negative correlation between the relationships of the mechanical properties and the crosslinking degree of beads. Under neutral and alkaline conditions (CB7 and CB9), the mechanical strength of beads was stronger than CB2, CB3 and CB5 due to increased chemical crosslinking. The interaction of beads co-crosslinked in pH 9 almost

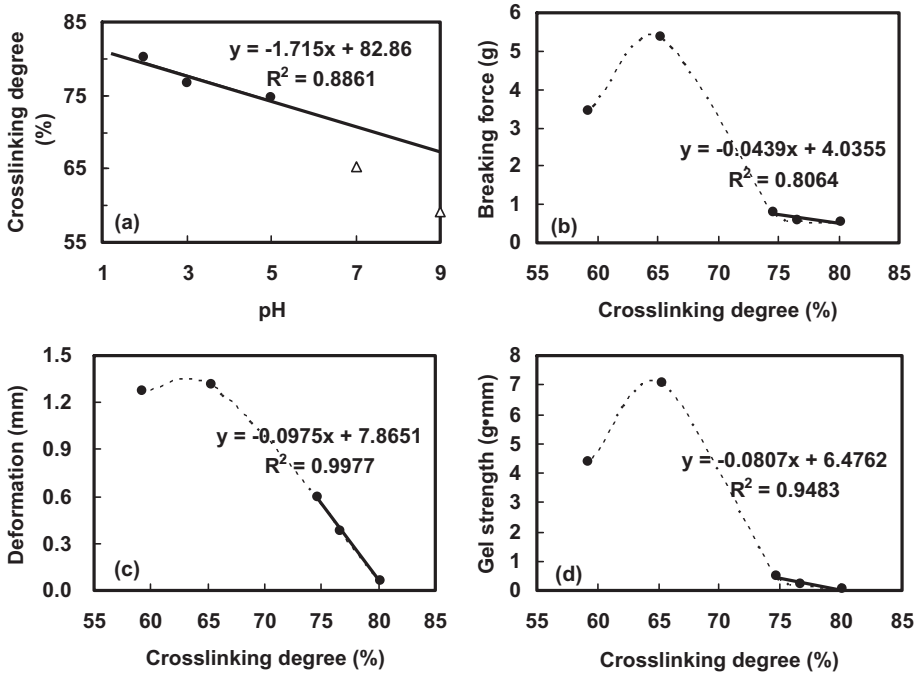

Fig. 2. (a) Effect of solution pH on the crosslinking degree of chitosan/TPP/genipin beads. Effects of crosslinking degree on the breaking force (b), deformation (c), and gel strength (d) of chitosan/TPP/genipin beads, respectively.

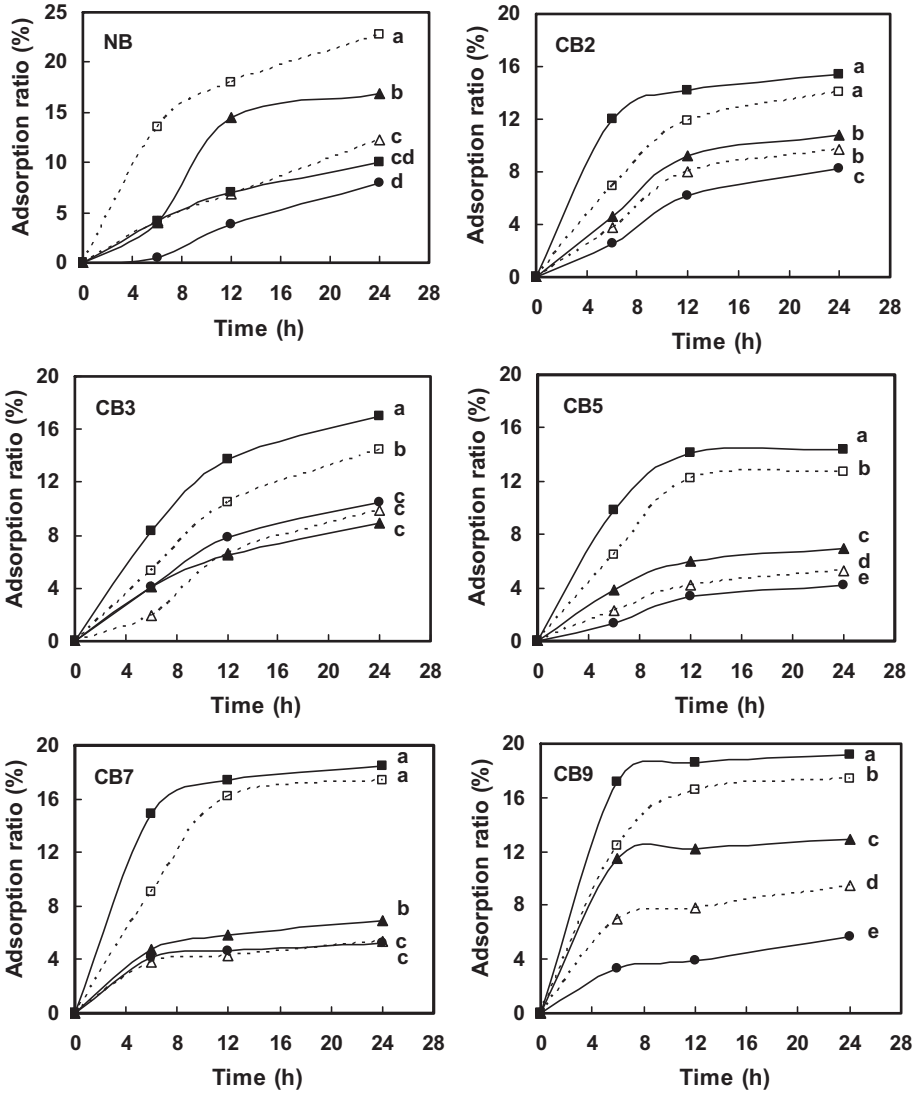

Fig. 3. The phytic acid adsorption ratio (%) of chitosan bead (30 mg) adsorbed in different pH of solution (■: pH 1; □: pH 3; ▲: pH 5; △: pH 7; ●: pH 9). NB expressed non-crosslinked chitosan beads. CB2 expressed chitosan bead co-crosslinked TPP/genipin under pH 2 solution. The rest may be deduced by analogy. Different letters (a–e) in the same figure indicate significant differences ($p < 0.05$) between samples.

exhibited chemical linkage. As a result, the texture of the beads was hard and brittle due to the deformation of CB9 being smaller than that of CB7 (Fig. 2c). A little ionic crosslinking between chitosan and TPP caused elasticity of CB7, causing CB7 to have better mechanical properties than CB9 has.

3.2. Phytic acid adsorption properties of chitosan beads

Fig. 3 indicates the phytic acid adsorption behaviour of chitosan beads investigated at different pH values. The initial concentration of phytic acid was 300 μM. The results show that the NB demonstrated favourable adsorption capabilities, adsorbing 22.74% and 16.91% of phytic acid under acidic conditions pH 3 and 5, respectively, for 24 h. However, the adsorption ratios in pH 1 were not satisfactory (<10.07%) due to the intolerance of NB to strong acid. The acid dissolution of the structure of the chitosan beads led to a decrease in the adsorption ratio. In neutral and alkaline solutions, the adsorption ratio of phytic acid was 12.26% and 7.93%, respectively.

Fig. 3 shows that all the TPP/genipin co-crosslinked chitosan beads (CB2, CB3, CB5, CB7, and CB9) had the highest adsorption ratios of phytic acid in pH 1 solution. The adsorption ratios were 15.36% for CB2, 16.97% for CB3, 14.40% for CB5, 18.41% for CB7, and 19.21% for CB9. Fig. 3 also shows that the adsorption ratios of TPP/genipin co-crosslinked chitosan beads for phytic acid decreased as the pH values increased. This trend may be related to the degree of protonation of chitosan molecules. When the degree of protonation of the chitosan chain increased (i.e., more cations), the adsorption capability of phytic acid (anions) was stronger. In addition, at pH 1 and 3 solutions, compared with other CBs, CB7 and CB9 had favourable adsorption ratios for phytic acid due to lower crosslinking degrees of chitosan beads (Table 1). At pH 1 and 3 solutions, the adsorption ratios of CB7 and CB9 were 18.41%, 17.41% and 19.21%, 17.41%, respectively, displayed greater adsorption ratios for phytic acid due to more protonated amino groups of chitosan chain in the lower crosslinking degree of beads. At pH 5, 7 and 9 solutions, the ionic interaction between chitosan and phytic acid was weaker due to a lower protonation degree of the chitosan chain, causing all of the CBs to have poorer adsorption ratios for phytic acid.

Fig. 3 shows that the adsorption ratio of CB2, CB3, and CB5 still increased after adsorption for 12 h; however, the adsorption ratios of CB7 and CB9 almost reached saturation after adsorption for 12 h. This may be correlated to the type of co-crosslinked reaction

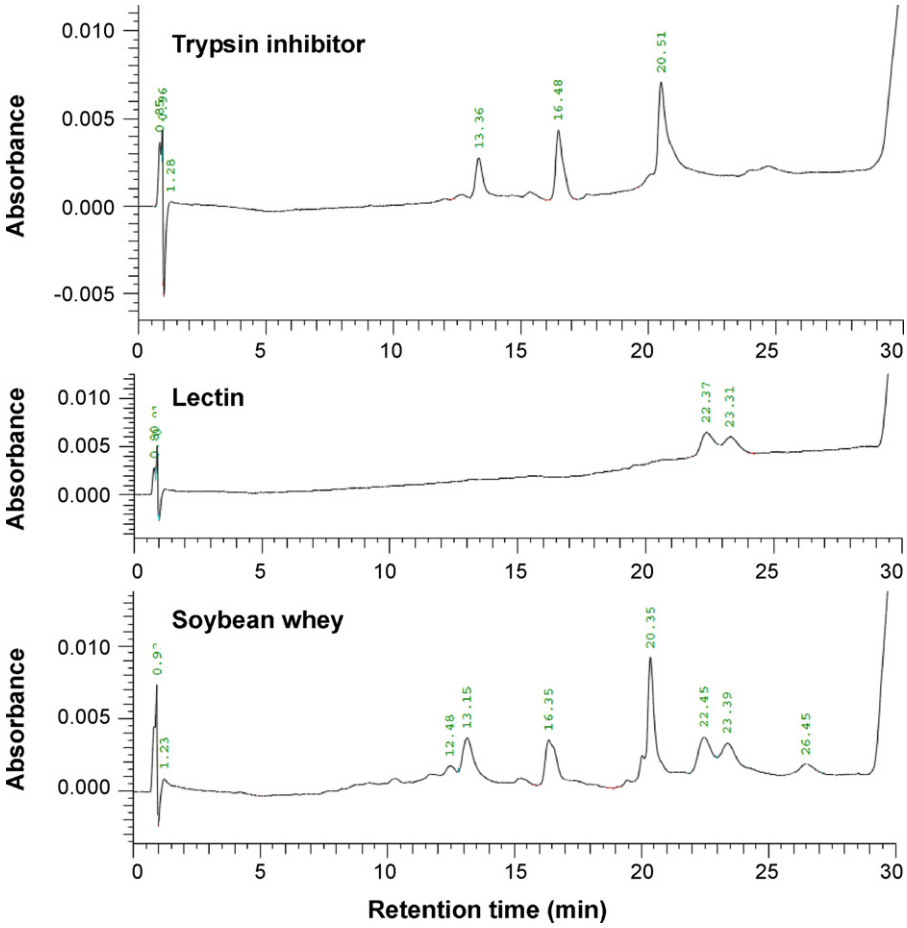

Fig. 4. The elution patterns of reverse-phase high performance liquid chromatography of trypsin inhibitor (KTI), lectin and soybean whey.

between chitosan and TPP/genipin. The main linkage between chitosan and crosslinking agent of CB2, CB3 and CB5 was ionic interaction. In acidic solution, phytic acid may be competing with TPP to interact with the amino group of chitosan. The phytic acid interacting with chitosan seemed predominant, and caused the adsorption ratio to increase. We assumed that the adsorption capability of every kind of CB was same under same solution condition. The calculated adsorption ratios of CB2, CB3 and CB5 at pH 1 solution were 9.36%, 11.00% and 11.95%, respectively, roughly calculated from adsorption ratio and amount of free amino group (non-reacted with TPP or genipin) of CB9. However, the experimental adsorption ratios of these beads: 15.36%, 16.97%, and 14.40%, respectively, were higher than calculated values. It is thus clear that the reaction of phytic acid and chitosan was predominant. However, the main linkage between chitosan and crosslinking agent of CB7 and CB9 was chemical bonding. The phytic acid competing with TPP to interact with the amino group of chitosan should not happen in the adsorption process. So, the adsorption ratios of CB7 and CB9 almost reached saturation after adsorption for 12 h.

3.3. Selective adsorption of chitosan bead for soybean whey

Fig. 4 shows the elution patterns of reverse-phase high performance liquid chromatography of trypsin inhibitor (KTI), lectin and soybean whey. The results indicate that the retention times of peaks of soybean whey at 13.15, 16.35 and 20.35 min were nearly the same as the peaks of trypsin inhibitor; the peaks at 22.45 and 23.39 min were almost same as the peaks of lectin, since the major proteins of soybean whey were trypsin inhibitor and lectin. The result was similar to that of Sorgentini and Wanger (1999).

In this study, the CB7 was chosen to carry out the adsorption process of phytic acid from soybean whey solution due to the mechanical properties and the adsorption ratio of CB7 were better than those of other CBs.

At 25 °C, the adsorption of phytic acid, trypsin inhibitor and lectin from soybean whey solution (pH 2) by CB7 bead was carried out for 24 h. The results show that the initial concentration and adsorbed concentration of phytic acid were 270.4 and 188.7 μM, respectively. This indicates that the CB7 could effectively adsorb phytic acid of 81.7 μM in 24 h; the adsorption ratio was 30.23%. However, the initial concentration and adsorbed concentration of trypsin inhibitor changed insignificantly; they were 1835 and 1766 μg/ml, respectively. The result of lectin was similar to the result of trypsin inhibitor. The concentration of lectin changed insignificantly, from 196 to 193 μg/ml. The adsorption ratios of trypsin inhibitor and lectin were very low: the values were 3.76% and 1.53%, respectively. The results indicate that CB7 had an acceptable adsorption ratio of phytic acid in pH 2 soybean whey solution at 25 °C. But trypsin inhibitor and lectin were almost not adsorbed by CB7 in pH 2 soybean whey solution at 25 °C. In short, the selective adsorption of phytic acid can be executed by CB7 in pH 2 soybean whey solution at 25 °C.

At 25 °C, the desorption of phytic acid from adsorbed phytic acid CB7 bead was carried out in pH 7, 8 and 9 solutions for 24 h. The desorption ratio of phytic acid from the CB7 desorbed in pH 7, 8 and 9 solution were 74.07%, 80.25% and 93.98%, respectively. This indicates that the desorption ratio of phytic acid increased with the increased solution pH value. In higher pH solution, the electrostatic

force between the amino group of chitosan and phytic acid was weaker due to lower protonation of chitosan. This led to phytic acid being more easily desorbed from chitosan, and with a higher desorption ratio.

4. Conclusions

In the TPP/genipin co-crosslinked process, the co-crosslinking degree of chitosan beads was decreased with increased solution pH value. In acidic solution, the mechanical properties of co-crosslinked beads and crosslinking degree exhibit negative correlation. The major chemical linkage between chitosan and genipin and a little ionic interaction between chitosan and TPP caused CB7 (co-crosslinked in pH 7 solution) to have the best mechanical strength among these beads. The best adsorption ratios (15.36–19.21%) of phytic acid of all co-crosslinked beads were in pH 1 solution. CB7 could adsorb 30.23% phytic acid from pH 2 soybean whey solution, but the adsorption ratios of trypsin inhibitor and lectin were very low, with a high desorption ratio (93.98%) of phytic acid from the CB7 desorbed in pH 9 solution. The selective isolation for phytic acid from soybean whey solution could be processed by CB7 due to good mechanical properties, accepted adsorption ratio and high desorption ratio.

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