JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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A Study of Controlled Uptake and Release of Anthocyanins by Oxidized Starch Microgels

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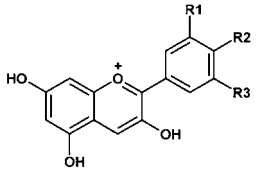
ABSTRACT: Anthocyanins are well-known antioxidants, but they are sensitive to environmental conditions. Herein we used oxidized starch microgel to prevent their early degradation and deliver them to the target place. The aim of this study was to investigate the uptake and the release ability of anthocyanins by the oxidized starch microgels and measure their in vitro gastrointestinal release. The gels were made of oxidized potato starch polymers, which were chemically cross-linked by sodium trimetaphosphate (STMP). In this study, the uptake and release behaviors of anthocyanins by starch microgel were investigated under various pH and salt concentrations. The microgel of high degree of oxidation and high cross-link density had a high uptake capacity for anthocyanins at low pH and salt concentration; 62 mg anthocyanins had been absorbed per gram of dry DO100% (degree of oxidation 100%) microgel at pH 3 with ionic strength 0.05M. The in vitro study of the release was investigated under stimulated gastrointestinal fluid. The anthocyanins were identified and quantified by UV/vis detection. The results indicated that the oxidized starch microgels had a potential for being a carrier system for protecting anthocyanins from degradation in the upper gastric tract and for delivering them to the intestine. This paper provides a good reference for an intestinal-targeted delivery system of vulnerable functional ingredients by oxidized starch microgel.

KEYWORDS: *encapsulation, microgel, oxidized potato starch polymer, anthocyanins*

INTRODUCTION

Anthocyanins are glycosides and acylglycosides of anthocyanidins. Anthocyanidins vary with different hydroxyl or methoxyl substitutions in their basic structure flavylium (2-phenylbenzopyrilium).[1] Currently, there are reports of more than 500 different anthocyanins and 23 anthocyanidins.[2] Anthocyanins are O-glycosylated, with different sugar substituents and acylated groups, of which only six are the most common in vascular plants (Figure 1).[3] Anthocyanins are flavonoids commonly found in plants which produce blue, red, or purple colors. They are the most important water-soluble pigments in plants.[4]

The stability of anthocyanins is easily influenced by environmental conditions such as temperature, pH, solvent, light exposure, the structure of the pigment itself, and the presence of other molecules.[5] In aqueous solution, several components can be involved in the complex equilibria. Flavylium cation (AH^+) is the predominant component at low pH, and it will be deprotonated to form the quinoidal base or the colorless pseudobase carbinol at high pH, accompanied by a reduction of the bioavailability of anthocyanins.[6,7] Therefore, a novel technique is needed to protect them from degradation and enhance their bioavailability.



Anthocyanidin	R1	R2	R3	MW
Pelargonidin (Pg)	H	OH	H	271
Cyanidin (Cy)	OH	OH	H	287
Delphinidin (Dp)	OH	OH	OH	303
Peonidin (Pn)	OMe	OH	H	301
Petunidin (Pt)	OMe	OH	OH	317
Malvidin (Mv)	OMe	OH	OMe	331

Figure 1. Chemical structures of six common anthocyanidins.

In recent decades, there has been intense interest in anthocyanins, prompted by the increasing evidence that anthocyanins have beneficial health effects.[8] In vitro and in vivo studies have shown that anthocyanins possess antioxidant, anti-inflammatory, and chemopreventive activities.[9,10] The reported chemopreventive activity took effect in the gastrointestinal tract.[11] For example, anthocyanin-rich extract from black raspberry suppresses proliferation of human oral squamous cells and carcinoma cells. It reduces the number of chemically induced tumors in the esophagus and especially in the colon.[9,11,12] For these reasons, typical U.S. diets contain a range of 12.5−215 mg of anthocyanins/day, which is higher than the amount of many flavonoids found in food and beverage.[13−15] Blueberry (*Vaccinium myrtillus* L.) is one of the richest sources, which contains 20−27 kinds of anthocyanins from different cultivars.[16−18] In this paper, anthocyanins are extracted from blueberry pomace. This makes a good use of the waste of blueberry.

Received: January 23, 2013
Revised: May 7, 2013
Accepted: May 26, 2013
Published: May 27, 2013

dx.doi.org/10.1021/jf400275m | *J. Agric. Food Chem.* 2013, 61, 5880−5887

Anthocyanins must be sufficiently rich in diets in order to provide noticeable pharmacological effects. Numerous studies in animals and in humans have demonstrated that ingested anthocyanins are poorly absorbed and largely disappear in the gastrointestinal tract (GIT) within several hours after consuming a meal.[19−21] Anthocyanins may convert to phenolic acids and aldehydes by intestinal microbiota and lose its activity.[22] Therefore, encapsulation of anthocyanins might provide an effective protection of the integrity of anthocyanins and subsequently increase the concentrations of bioactive anthocyanins in the small intestine and boost their beneficial effects such as the inhibition of the growth of tumor cells.[23]

The common method for encapsulation of extracted plant anthocyanins is spray drying. The most common matrix materials used are polysaccharides such as maltodextrin, inulin,[24] gum Arabic, tapioca starch,[25] citrus fiber,[26] and materials such as glucose syrup[27] and soy protein isolate.[28] The encapsulated anthocyanins are stabilized against degradation due to oxygen and light exposure. An alternative encapsulation system such as whey protein, which can maintain their structure and greatly enhance their bioavailability, is expressly needed.[29] However, protein is not an ideal material for encapsulation because it is easy to denature under processing. Polysaccharides such as starch are stable to heat, acid, base, and other conditions. Our previous research showed that negatively charged oxidized starch microgel could absorb oppositely charged ingredients such as protein. It could take up and release protein in a controlled manner because the microgel is responsive to the solvent conditions.[30,31] The oxidized starch can be a novel delivery system for positively charged anthocyanins (Figure 2). The electrostatic interaction

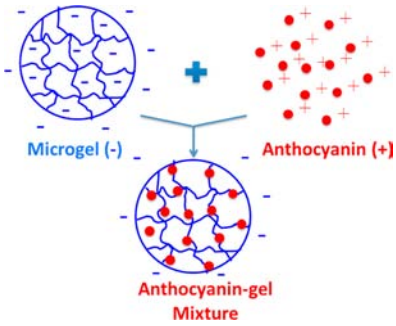


Figure 2. Encapsulation of positively charged anthocyanins by the negatively charged starch microgel.

between oxidized starch microgel and anthocyanins can be tuned by pH and salt concentration. This tunable interaction can be used for a controlled uptake and release of anthocyanins from starch gel.

The objective of this study is to investigate the controlled uptake and release ability of anthocyanins by the oxidized starch microgels. First, we determined the optimal uptake conditions by measuring the anthocyanins uptake capacity as a function of pH and salt concentration. Second, we selected the microgel of optimal degree of oxidation (DO) and cross-link density ($R_{\text{cross-linker/polymer}}$), which give optimal uptake capacity. Next, we studied the release of anthocyanins from microgels under different pH and salt concentrations. Lastly, the in vitro release of anthocyanins from starch microgel under simulated gastric and intestinal fluids was investigated.

MATERIALS AND METHODS

Materials. Native potato starch was kindly provided by AVEBE, The Netherlands. The oxidation catalyst 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) was purchased from Merck, Germany. The cross-linker sodium trimetaphosphate (STMP) was supplied by Sigma-Aldrich. Blueberry pomace was provided by Jilin Agricultural University, Jilin, China. Pancreatin was purchased from Beijing Hongrunbaoshun Technology Co., Ltd., Beijing, China. All other chemicals used were of analytical grade. Purified Milli-Q water was used throughout.

Extraction of Anthocyanins. Anthocyanins were extracted from blueberry pomace by using acidified methanol, followed by filtration, evaporation, and lyophilization. It was stored under dry condition at −24 °C. The extract contained small amount of polyphenols, tannins, carbohydrates, and fibers.

Microgel Synthesis. Starch polymer was selectively oxidized at the 6-position to obtain a polyglucuronate with >95% selectivity at complete conversion of the primary alcohol groups by TEMPO-mediated oxidation. In this way, starch polymers of 30, 50, 70, and 100% degree of oxidation (DO) were prepared.[32] Microgels were prepared by cross-linking the oxidized starch polymer with STMP at pH 10. First, 20 g of oxidized starch polymer was dissolved in 95 mL of distilled water at room temperature, which took around 30 min. Then, the cross-linker STMP and sodium hydroxide were added to the polymer solution, and the mixture was heated to 40 °C and kept at that temperature for 10 min without stirring, which led to a gel formation. After the gel was formed, the gel was put into an oven at 40 °C for 1 h to allow the cross-linking reaction to take place. Then the gel was kept overnight in a cold room at 0 °C. The whole piece of gel was grinded through a sieve (1 mm) covered with a nylon cloth of 200 mesh (mesh size 0.074 mm) to obtain reasonably uniform microgel particles.[32,33] Citric acid−phosphate buffer (0.02 M, range from pH 1−7) was used for controlling the pH. Sodium chloride was added to obtain the appropriate ionic strength.

Saturation Anthocyanins Uptake Capacity Measurements. Absorbed amounts Γ [mg/g] of anthocyanins per weight of dry starch microgel were measured using a UV−vis spectrophotometer (Persee, Beijing, China). First, 1 g of extract anthocyanins were dissolved in 10 mL of purified Milli-Q water with constant magnetic stirring stirring at 300 rpm for 30 min (DF-1-1S, Gongyi, Henan, China). Then the solution was centrifuged (Sigma, Germany) at 10000 rpm (16000*g*) (g is gravitational force) for 6 min. The supernatant was stored at −4 °C without light for following experiments. Then 10 mg of dry gel particles were suspended into 9 mL of buffer at various pH values and salt concentrations. Anthocyanins solution (1 mL) was added into 9 mL of microgel solution and gently stirred for 3 h (enough time to reach saturation). Subsequently, the samples were centrifuged at 10000 rpm (16000*g*) for 5 min, and the concentration of anthocyanins in the supernatant (C_{ant}) was determined by UV spectrophotometry. The total anthocyanins absorption at saturation level Γ (mg ant/g dry gel) in the microgel particles was calculated from the mass balance.

The amount of anthocyanins left in solution, m_{left} [mg], could be calculated as

$$m_{\text{left}} = C_{\text{ant}} \times V_{\text{supernatant}} \quad (1)$$

in which $V_{\text{supernatant}}$ was the volume of the supernatant after centrifugation. The amount of anthocyanins absorbed in the microgel, m_{abs} [mg] could be calculated from the total anthocyanins added m_{add} [mg] and m_{left} [mg],

$$m_{\text{abs}} = m_{\text{add}} - m_{\text{left}} \quad (2)$$

The absorbed amount per gram dry gel mass, Γ [mg/g], was given by

$$\Gamma = m_{\text{abs}}/m_{\text{dry gel}} \quad (3)$$

Anthocyanins Release under Dilution. Samples of microgel−anthocyanins mixtures were prepared under the same conditions as described above. They were stirred for 3 h and centrifuged at 10000 rpm (16000 g) for 5 min. The sediments (anthocyanins−gel complexes) were diluted with 10 mL of fresh buffer and gently stirred

dx.doi.org/10.1021/jf400275m | J. Agric. Food Chem. 2013, 61, 5880−5887

for 4 h. After a second centrifugation, the absorbance of supernatant was measured. The percentage of anthocyanins released $A_{release}$ was calculated from the measured anthocyanins concentration (C_{ant}) as

$$A_{release} = (C_{ant} \times 10mL)/(\Gamma \times m_{dry\,gel}) \times 100\% \quad (4)$$

In Vitro Release of Anthocyanins from Microgels. Based on the Chinese Pharmacopoeia,[34] the simulated stomach milieu without enzyme at pH 1.2 was performed by using 1 L of aqueous solution comprising 2 g of sodium chloride and 7 mL of concentrated hydrochloric acid; likewise, the simulated intestine milieu at pH 6.8 was performed by disolving 6.8 g of potassium dihydrogen phosphate in 500 mL of water . The pH of the solution was adjusted to 6.8 with a 0.10 N aqueous solution of sodium hydroxide. Pancreatin (10 g) was added to the above solution, and the resulting solution was diluted to 1000 mL with water.

In vitro release kinetics of anthocyanins from microgels was monitored during incubation with continuous agitation at 50 rpm about their horizontal axes, in an end-over-end shaker equipped with a temperature control system (DSHZ-300A, Taicang, China). Capsules and release media were warmed prior to the start of the experiment to 37 ± 1 °C, and they were maintained at this temperature throughout each incubation. Samples of both sediments (anthocyanins−gel complexes) and the same amount of anthocyanins absorbed in the microgel were added to the incubation media. This concentration was set as 100%, and concentrations in all samples were related to this value. Following standard pharmacopoeia methods,[34] the compounds were incubated for 120 min in simulated gastric fluid. After incubation in stomach-mimicking medium, the microgels were separated and changed into simulated intestinal fluid for 3 h.

At preset time points (0, 15, 30, 45, 60, 75, 90, and 120 min for incubations in simulated gastric fluid; 0, 15, 30, 45, 60, 120, and 180 min for those in simulated intestinal fluid), 2 mL of solution samples were taken. Each sample was centrifuged at 12000 rpm (24000*g*) for 3 min and the supernatant per 1 mL was mixed with 9 mL of pH buffers of 1.0 and 4.5 to stop the release and stabilize the anthocyanins. The resulting supernatants were stored at −4 °C in the dark until analysis (see below).

Total Anthocyanins Content Measurement. The total anthocyanins content was measured by the modified pH differential method.[35] The anthocyanins solution was dissolved in a 0.025 M potassium chloride buffer, pH 1.0, and 0.4 M sodium acetate buffer, pH 4.5, with a dilution factor. Sample spectral absorbance measurements were read at 520 and 700 nm, respectively. The absorbance (A) of the diluted sample was then calculated as follows:

$$A = (A_{520} - A_{700})_{pH1.0} - (A_{520} - A_{700})_{pH4.5} \quad (5)$$

The monomeric anthocyanins content in the original sample was expressed as cyanidin-3-glucoside equivalents according to the following formula:

$$\text{Anthocyanins content (mg/L)} = (A \times MW \times DF \times 1000)/(\varepsilon \times L) \quad (6)$$

where MW (449.2) of cyanidin 3-glucoside was used because the anthocyanin content was calculated in cyanidin-3-glucoside equivalents, the molar absorptivity, ε, is 26900, DF is the dilution factor, 1000 is the factor to convert g to mg, and A is absorbance.

Statistical Analysis. Triplicates have been done for all the experiments to ensure the accuracy, and error bars represent the standard error for three measurements of each data

RESULTS AND DISCUSSION

Anthocyanins Uptake by Oxidized Starch Microgels. By TEMPO oxidation, the primary alcohol groups on potato starch are selectively oxidized to carboxyl groups. The microgel carrier consists of cross-linked negatively charged starch polymer. It can absorb and bind positively charged compounds, e.g., lysozyme, through electrostatic attraction.[31−33] In this paper, positively charged anthocyanins were absorbed by oxidized starch microgel by electrostatic attraction. Besides the hydrophobic interaction, hydrogen bonding may also play a role in the interaction between anthocyanins and oxidized starch gel.

Figure 3A shows a picture of dispersion of DO100% microgel particles encapsulating anthocyanins, which was made after mixing anthocyanins and DO100% microgel at pH 3 and ionic strength of 0.2 M for overnight equilibrium. It is clearly visible that anthocyanins and DO100% microgel formed a complex which was the sediment on the bottom. The supernatant contains unabsorbed anthocyanins. Comparing with the blank in Figure 3B, when no gel particles were added, the anthocyanins solution showed a homogeneous dark-red color. As we found in our previous investigation, the microgels can be dispersed in water because there are negative charges on their surface and the density is similar to water.[31] But when anthocyanins were absorbed, the charges on the gel were neutralized. The particles aggregated and settled down.

Figure 3. Photograph of anthocyanins−DO100% microgel mixtures (A) and the anthocyanins without gel (B) at pH 3 and ionic strength 0.20 M. The concentration of anthocyanins solution for both cases is around 0.07 mg/mL.

Figure 4 presents optical images of microgel particles before and after the absorption of oppositely charged anthocyanins. The empty microgel particles have irregular shapes and transparent. Sizes are distributed between 10 and 50 μm. After anthocyanins had been absorbed, microgels had a homogeneous red color, indicating that the anthocyanins molecules were distributed homogeneously all over the microgel. Our previous research found that most pores of microgel range from 4 to 25 nm,[33] and the molecular weight of anthocyanins was 449 g/mol. Anthocyanins molecules were easy to diffuse practically into all parts of the microgel particles.

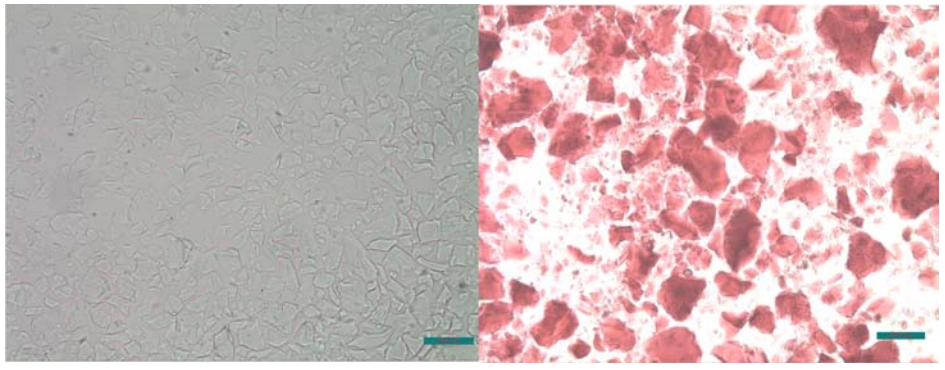


Figure 4. Optical microscopic image of microgel particles shows the absorbed and unabsorbed anthocyanins dispersed in water. The scale bar indicated is 100 μm. DO100% microgel particle in the equilibrium state at pH 3.0 before the addition of anthocyanins (left). The same particle in the equilibrium state after adding anthocyanins (right).

The above results of the preliminary experiment showed that our negatively charged starch microgel was capable of absorbing positively charged anthocyanins. The optimal absorption condition was studied by investigating the uptake capacity as a function of pH and salt concentration. The influence of the physical chemical properties of microgel on the uptake capacity was studied as a function of various degrees of oxidation and cross-link density.

pH Dependence on Anthocyanins Uptake. To find out the optimal conditions for anthocyanins uptake by oxidized starch microgels, the microgel of degree oxidation 70% (DO70%) with intermediate cross-link density ($R_{\text{cross-linker/polymer}}$ of 0.20) was chosen to study the anthocyanins uptake capacity as a function of pH.

The absorption amount of anthocyanins by microgel particles at saturation was interpreted as the uptake capacity (Γ expressed in mg anthocyanins/g dry gel). As shown in Figure 5, the anthocyanins uptake capacity of microgel was the

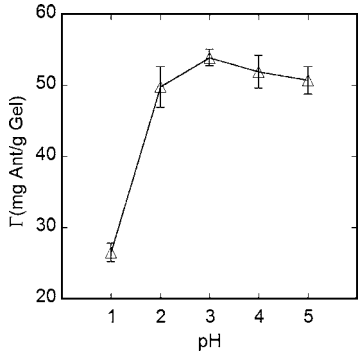


Figure 5. Anthocyanins uptake capacity Γ (mg ant/g gel)for the DO70% microgels as a function of pH. $R_{\text{cross-linker/polymer}}$ is 0.20 (4 g STMP per 20 g of oxidized starch polymer).

minimum at pH 1. The anthocyanins uptake capacity Γ (mg of ant/g of gel) increased with increasing pH and reached the maximum at pH 3. It has been reported that the amount of flavylium cation of anthocyanins was much higher at low pH than at high pH.[36] The positive charges of anthocyanins solution decreased with increasing pH and reached the minimum at pH 4.[37,38] The charge densities of microgels increased with the increasing pH and reached a plateau value at pH around 5.[32] Our previous paper also indicated uptake capacity was mainly determined by electrostatic interactions between two oppositely charged microgels and protein.[31] The highest uptake capacity at pH 3 indicated that the electrostatic interaction was the strongest at pH 3. It may be due to the compromise of both charges of microgel and anthocyanins, which resulted in a maximum uptake capacity. On the basis of our aforementioned finding, we employed pH 3 as the optimal pH condition for anthocyanins uptake experiments

Salt Dependence on Anthocyanins Uptake. To determine the salt effect on anthocyanins uptake capacity by starch microgel, we measured the anthocyanins uptake capacity as a function of NaCl concentrations (M) for DO100% microgel with cross-link density of $R_{\text{cross-linker/polymer}}$ 0.40 at pH 3.0, ionic strength of 0.05. Figure 6 shows the anthocyanins uptake

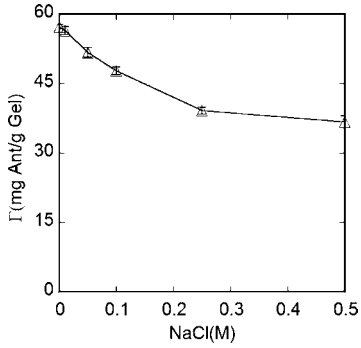


Figure 6. Anthocyanins uptake capacity Γ (mg ant/g gel) of microgel particles as a function of NaCl concentration (M) for DO100%, $R_{\text{cross-linker/polymer}}$ is 0.40 (8 g STMP per 20 g of oxidized starch polymer) at pH 3.0 citric acid−phosphate buffer, ionic strength 0.05 M.

capacity decreased with increasing salt concentration. The decreasing uptake capacity occurred because salt screened both charges on microgel and anthocyanins, leading to decreased electrostatic interaction.[39] When the salt concentration was higher than 0.25 M, the screening effect hardly affected the anthocyanins uptake capacity. Anthocyanins still could be absorbed by microgels at 0.5 M salt concentration. Our previous study also showed that the saturation protein uptake at low pH was almost independent of ionic strength. It might be caused by nonelectrostatic interaction such as hydrogen bonding or hydrophobic interaction between the protein and gel.[31] This is good for food applications. Because the binding was quite strong, the encapsulated anthocyanins concentration was well retained inside the microgel.

Dependence of Degree of Oxidation (DO%) on Anthocyanins Uptake. As shown in Figure 7, the uptake capacity Γ

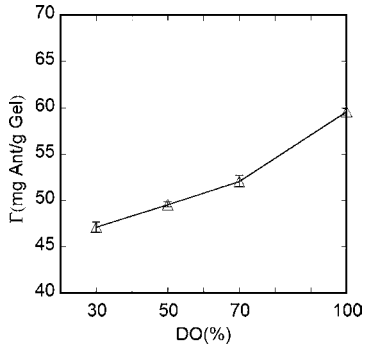


Figure 7. Anthocyanins uptake capacity Γ (mg of ant/g of gel) as a function of microgels of varying degree of oxidation: DO30%, DO50%, DO70%, DO100%, and $R_{\text{cross-linker/polymer}}$ of 0.20 (4 g STMP/20 g polymer) at pH 3.0 citric acid−phosphate buffer, ionic strength 0.05 M.

(mg of anthocyanins/g of gel) increased with increasing DO. About 60 mg of anthocyanins were absorbed per gram of dry DO100% microgel at pH 3 with ionic strength 0.05 M. It was known that the maximum charge density of oxidized polymers practically increased linearly with increasing DO.[32] High DO microgel had higher negative charge density than the low DO ones, resulting in a stronger attraction to anthocyanins. Consequently, high DO microgel absorbed more anthocyanins molecules.

Dependence of Cross-Link Density on Anthocyanins Uptake. As shown in Figure 8, the anthocyanins uptake

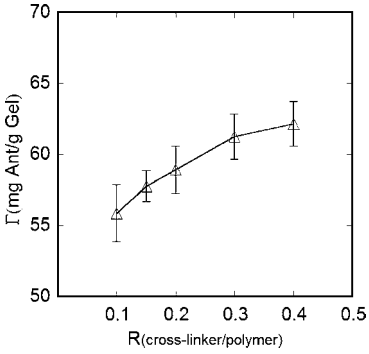


Figure 8. Anthocyanins uptake capacity Γ (mg of ant/g of gel) as a function of DO100% microgels of varying $R_{\text{cross-linker/polymer}}$: 0.10, 0.15, 0.20, 0.30, 0.40 at pH 3.0 citric acid−phosphate buffer, ionic strength 0.05 M.

capacity of the microgel increased with increasing cross-link density ($R_{\text{cross-linker/polymer}}$). The previous study showed that the charge densities (C/g of gel) of the microgel were hardly influenced by the cross-link density.[32] Our previous studies showed that the lysozyme uptake capacity decreased with increasing cross-link density due to the decreased pore size.[31] Anthocyanins molecules (MW 449 g) are much smaller than lysozyme (average MW: 14 KDa). The small pore size could not affect the absorption of anthocyanins. On the contrary, the small pore size of microgels increased the stability of anthocyanins molecules in the polysaccharide network and prevented the molecules escaping from the microgels.

Anthocyanins Release from Oxidized Starch Microgels. *pH Dependence on Anthocyanins Release from Microgels.* The above results showed that the optimal anthocyanins uptake conditions were at pH 3 with low salt concentration. The starch gel of high degree of oxidation and high cross-link density are suitable for delivering anthocyanins. By applying the optimal conditions for anthocyanins uptake on a DO100% microgel with a high cross-link density, the release properties had been investigated as a function of pH and salt concentration.

Figure 9 shows the percentage of anthocyanins released from DO100% microgel as a function of pH after 4 h equilibrium in

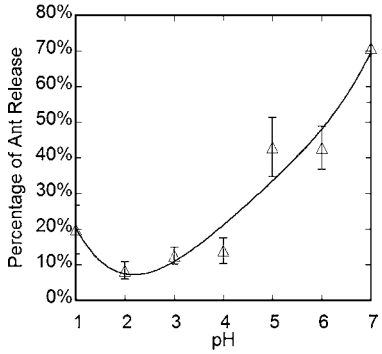


Figure 9. Percentage of anthocyanins released from DO100% microgel as a function of pH, $R_{\text{cross-linker/polymer}}$ is 0.40 (8 g STMP per 20 g of oxidized starch polymer) at citric acid−phosphate buffer, ionic strengths of 0.05 M.

buffer solution. The gel particles were saturated with anthocyanins before the releasing experiment. The percentage of anthocyanins release increased from 10% to 70% from pH 2 to pH 7, respectively. Our previous study indicated that the affinity was mainly determined by the weakly charged species.[31] In this case, the affinity was determined by the charge of anthocyanins. The positive charge of anthocyanins molecules decreased with increasing pH. The binding affinity decreased with increasing pH, which led to the increasing anthocyanins released from microgel. Otherwise, the pore size of microgel increased with increasing pH,[31] and the anthocyanin molecules were much easier to escape from the microgel.

The percentage of anthocyanins released from microgel was about 20% at pH 1, which was higher than 10% at pH 2. At pH 1, microgel practically had no negative charges.[31,32] 80% of anthocyanins remained inside the noncharged microgel after dilution with the release buffer. It indicated that hydrophobic interaction and hydrogen bonding might also be an important driving force for anthocyanins absorption by starch microgel at low pH.

Salt Dependence on Anthocyanins Release from Microgels. Figure 10 shows the percentage of anthocyanins released from microgel as a function of NaCl concentration. The percentage of anthocyanins release increased with increasing salt concentration and reached a plateau value at 0.2 M of salt concentration. This was due to the screening of electric charges of microgel by the presence of salt. Our previous study showed that nearly all charges were screened at 0.2 M of NaCl concentration.[32] Because the experiment has been performed at pH 3, where the binding affinity was very high and the release time may too short for the complete release of anthocyanins which tightly binds to the microgel. This explained why 80% of

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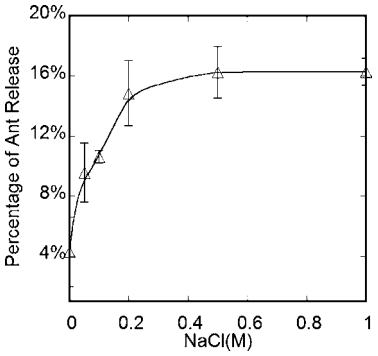

Figure 10. Percentage of anthocyanins released from DO100% microgel as a function of NaCl concentration (M), $R_{cross\text{-}linker/polymer}$ is 0.40 (8 g STMP per 20 g of oxidized starch polymer) at pH 3.0 citric acid−phosphate buffer, ionic strengths of 0.05 M.

anthocyanins still remained inside the microgel at 1.0 M NaCl concentration. As shown in Figure 6, that microgel still could absorb large amount of anthocyanins at 0.5 M NaCl. This was due to the nonelectrostatic interaction such as hydrophobic interaction and hydrogen binding which contributed to the binding of anthocyanins to microgel.

Anthocyanins Release under Stimulated Gastric and Intestinal Conditions. The above results showed that anthocyanins were strongly bound to microgel at acidic conditions and weakly bound to microgel at neutral pH. It indicated that anthocyanins absorbed by microgels could be protected and retained in the stomach where the pH was low and released in the intestine where the pH was neutral. To evaluate the effect of microgel as the intestine-target delivery system, the release experiment was performed in vitro in physiological simulated gastric and intestinal fluids for DO70% and DO100% microgels.

Figure 11 shows the percentage of anthocyanins released from microgels in simulated gastric fluid during incubation. The

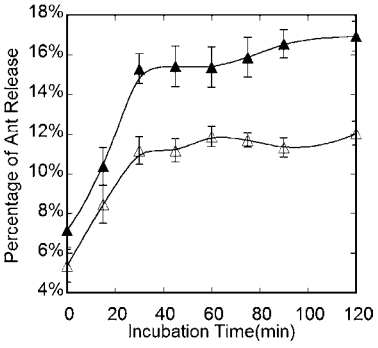

Figure 11. Percentage of anthocyanins released from microgels during incubation in simulated gastric fluid. Chosen microgels: DO100%, $R_{cross\text{-}linker/polymer}$ is 0.40 microgel (Δ); DO70%, $R_{cross\text{-}linker/polymer}$ is 0.20 microgel (▲). The pH of simulated gastric is about 1.2 without enzyme.

percentage of release reached a plateau after 30 min and remains stable afterward. The percentage of release was 11% for DO100% and 16% for DO70% microgel. This result was similar to the results presented in Figure 9. At pH about 1.2 in simulated gastric fluid, microgel practically had no negative charges and the pore size of the microgel became very small. Anthocyanins could not escape from microgels. The result also showed DO70% microgel released more anthocyanins than DO100% microgel. Because DO70% microgel had larger pore size than DO100% microgel at the same cross-link density ($R_{cross\text{-}linker/polymer}$), the anthocyanins molecules were escaped much more easily. On the other hand, the DO100% had more negative charges than DO70%, indicating that the binding affinity was higher for DO100% than DO70%.

After incubation in stomach-mimic medium, the microgels were separated and changed to simulated intestinal fluid. The difference between the stomach and the small intestine is that the pH and salt concentration are substantially higher. As presented in Figure 12, most anthocyanins were released in the

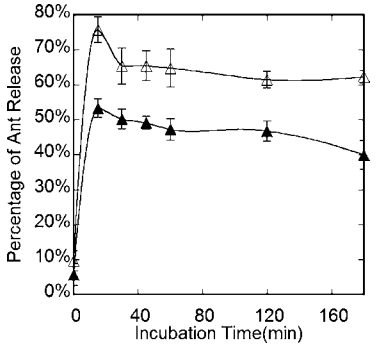

Figure 12. Percentage of anthocyanins released from microgels during incubation in simulated intestinal fluid. Chosen microgels: DO100%, $R_{cross\text{-}linker/polymer}$ is 0.40 microgel (Δ); DO70%, $R_{cross\text{-}linker/polymer}$ is 0.20 microgel (▲). The pH of simulated gastric is about 6.8 with pancreatic enzyme.

first 15 min. The percentage of anthocyanins release was 75.82% for DO100% and 55.31% for DO70%, respectively. In Figure 12, the anthocyanins released remained nearly constant and reached a steady state from 20 to 180 min. The slight decreasing is an artifact due to the error of experimental determination. When the microgels were transferred from stomach to intestinal fluid, the pH of environment was increased from 1.2 to 6.8, the positive charges of anthocyanins rapidly reduced, and the pore size of microgels became larger, which led to release of anthocyanins quickly. The salt concentration increased from stomach-mimic media to intestine-mimic media. Therefore the increasing of salt concentration could also promote the release of anthocyanins. According to literature,[9,10,36] the total anthocyanins concentration decreased over time because of degradation at neutral pH conditions. The anthocyanins solution without microgel in intestine-mimic conditions was also analyzed as a blank to assess the percentage of anthocyanins degradation in solution over time. The release kinetics was adjusted by the factor of degradation in solution. Therefore the degradation effect should not have been affected by our releasing results.

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Funding

We acknowledge the financial support of the National Natural Science Foundation of China (NSFC21176018,

NSFC31201334), the Special Fund for Agro-Scientific Research in the Public Interest (201103037), the Program for New Century Excellent Talents (NCET-05-0117), the Funding Project of Competence Development Program for Beijing VET Teachers and PHR201107151 (IHLB) for financial support.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We would very much like to thank Dr. Renko de Vries of Wageningen University of The Netherlands for the helpful discussion about the results. We also thank Tang Pingwah for helping with English.

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