Releasing characteristics of anthocyanins extract in pectin–whey protein complex microcapsules coated with zein

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Abstract This study investigated pectin-based capsules as delivery systems for purple rice bran anthocyanin extract (AE) during exposure to simulated gastrointestinal conditions. Four different capsules loaded with AE were prepared by ionotropic gelation/extrusion, including (1) pectin capsules (PE), (2) pectin capsules coated with zein (PE/ZE), (3) pectin–whey protein isolate complex capsules (PE + WP), and (4) pectin–whey protein isolate complex capsules coated with zein (PE + WP/ZE). CaCl₂ in an ethanol solution with or without zein was used as a crosslinking solution. Swelling and release characteristics of all capsules under simulated gastric fluid at pH 1.2 (SGF) and simulated intestinal fluid at pH 6.8 (SIF) for 120 and 180 min, respectively, were examined. PE + WP, PE + WP/ZE, and PE/ZE capsules had higher encapsulation efficiency than PE capsules. After incubation, PE + WP/ZE and PE capsules had the lowest swelling ratio in SGF and SIF, respectively. PE + WP/ZE capsules had the lowest AE release in SGF, while PE capsules had the highest. Both PE + WP and PE + WP/ZE capsules

had significantly lower AE release in SIF than PE and PE/ZE capsules. The study demonstrated that PE + WP and PE + WP/ZE capsules have potential to function as a slow release delivery system for AE.

Keywords Pectin · Anthocyanin · Encapsulation · Releasing of anthocyanin · Swelling ratio of capsules

Introduction

Anthocyanins are water soluble pigments responsible for the blue, purple, and red colors of flowers, fruits, and vegetables. Due to their high antioxidant capability, anthocyanins have gained interest in health promoting functions. They exhibit anti-inflammatory activity, inhibit carcinogens and tumors, and reduce serum cholesterol (He and Giusti 2010). However, they have poor bioavailability after oral consumption due to instability of the compound and pH-dependent degradation. The maximum concentrations of anthocyanins in human blood and urine are reported only in the nM to low μ M range (Pojer et al. 2013). To overcome the problem, encapsulation techniques have been applied in order to maintain their levels and protect them from unfavorable conditions, resulting in improvement of anthocyanin concentrations in the small intestine, bioavailability, and health promoting effects (Munin and Edwards-Lévy 2011).

Pectin is a natural polymer used to encapsulate several bioactive compounds. It can form three dimensional rigid and water insoluble hydrogels by calcium-induced ionotropic gelation. Pectin hydrogels have been used as a delivery system for colon-specific drugs as they can stay intact in gastric fluids, but disintegrate to release the encapsulated compound in intestinal fluids (Oidtmann et al.

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2012). However, due to the high porosity of pectin and expansion of its pores in physiological conditions, the use of pectin hydrogels are limited to low water soluble compounds (Liu et al. 2006). To overcome this limitation, pectin combined with whey protein isolate to form pectin–whey protein complex and subsequently coating with zein could be an alternative. The complex of pectin and whey protein isolate is formed by electrostatic interactions between positive charges of protein and pectin's negative charges (Gentès et al. 2010). The pectin–whey protein complex was found to remain intact from pH 3 to pH 7 (Jones et al. 2009). Certain studies showed that the complex could function as a delivery vehicle for vitamin D, fatty acids, and vitamin B (Zimet and Livney 2009). However, releasing characteristics of the encapsulants from the complex were not determined. Additionally, zein, an alcohol-soluble protein derived from corn. It has been shown to successfully bind to pectin hydrogels, helping reduce hydrogel swelling and subsequently delay releasing of indomethacin and bovine serum albumin loaded in pectin hydrogels under mimicked conditions from mouth to colon (Liu et al. 2006). Although there are many studies using pectin hydrogels as a vehicle for drug delivery, the use of a combination between pectin–whey protein complex and zein to encapsulate high water soluble compounds and studies of the encapsulant releasing have not been investigated. Therefore, this study is aimed to evaluate releasing characteristics of anthocyanin, a high water soluble compound, loaded in pectin–whey protein isolate complex capsules coated with zein, compared to pectin–whey protein isolate complex capsules, pectin capsules coated with zein, and pectin capsules. The release of anthocyanin and stability of the hydrogel capsules were assayed in simulated gastric and intestinal fluids.

Materials and methods

Anthocyanin extraction

The anthocyanin used in this research was extracted from purple rice (*Blanca Isabel*) bran, kindly provided by Rush Rice Products, L.L.C (Baton Rouge, LA). Prior to anthocyanin extraction, the rice bran was defatted according to the method described by Alfaro et al. (2015). Thirty grams of defatted purple rice bran was then mixed with 300 mL of 50 mL/100 mL ethanol at 50 °C for 2 h with 200 rpm agitation (Shin et al. 1992). After centrifugation at 6500 rpm for 15 min, the extract (supernatant) was placed in a vacuum rotary evaporator (Rotavapor RE121, BUCHI Labortechnik AG, Flawil, Switzerland) to remove ethanol at 50 °C. The extract was subsequently freeze dried (Heto PowerDry LL3000, Thermo Scientific, Laurel, MD) and

the resulting purple rice bran anthocyanin extract (AE) powder was stored at −20 °C.

Preparation of pectin gel solution and pectin–whey protein isolate complex solution

Pectin gel solution (5 g/100 mL) was prepared by adding low-methoxyl pectin (TIC PRETESTED[®] pectin LM 32 powder), kindly provided by TIC Gums Inc (Belcamp, MD), to distilled water with vigorous stirring and kept overnight in order to ensure hydration. Pectin–whey protein isolate complexes were prepared by mixing stock solutions of 10 g/100 mL pectin and 4 g/100 mL whey protein isolate (Davisco Business Unit, Eden Prairie, MN) adjusted pH to 7.0 at the ratio of 1:1. The mixture was then adjusted to pH 4.75 and heated at 83 °C for 15 min in order to form pectin-WP complexes (Jones et al. 2009).

Encapsulation of anthocyanin extract in pectin-based capsules

AE was encapsulated into four types of capsules, including pectin capsules (PE), PE capsules coated with zein (PE/ZE), pectin–whey protein isolate complex capsules (PE + WP), and PE + WP capsules coated with zein (PE + WP/ZE). PE and PE + WP capsules were prepared by following the method described by Lee et al. (2009) and Liu et al. (2006) with some modifications. AE (1 g) was separately mixed with the pectin gel solution (20 g) or pectin–whey protein isolate complex solution (20 g) for 5 min. The mixtures were then extruded through a 23G needle by a syringe pump (Pump 11, Harvard Apparatus, Holliston, MA) at a 1.2 mL/min flow rate to drop into 80 mL of ethanol solution (75 mL/100 mL) containing 4 g/100 mL of calcium chloride (Fisher Scientific Inc., Pittsburgh, PA), called as a crosslink solution. After dropping, the capsules were immediately formed and were continuously stirred for additional 30 min. Then the capsules were collected and dried at 37 °C for 4 h. PE/ZE and PE + WP/ZE capsules were prepared as same as PE and PE + WP capsules but the crosslink solution added with 1 g/100 mL of zein was used. All dried capsules were kept at −20 °C for further analysis.

Antioxidant activity and total phenolic contents of anthocyanin extract

Antioxidant activity and total phenolic content of AE were determined by the method described by Jun et al. (2012). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (Sigma Aldrich, St. Louis, MO) was used to construct a standard curve (0–0.5 mM). Antioxidant activity was expressed as % Antioxidant

activity = [(absorbance at 515 nm of control – absorbance at 515 nm of sample)/absorbance at 515 nm of control] × 100. The results were reported as Trolox equivalents. For total phenolic contents, Gallic acid (Sigma Aldrich, St. Louis, MO) at concentrations of 0–600 µg/mL was used to construct a standard curve. The results were expressed as gallic acid equivalents.

Determination of anthocyanin contents

Total monomeric anthocyanin content of AE was measured using a spectrophotometric pH differential method according to AOAC official method 2005.02 (Lee et al. 2005). Anthocyanin content was expressed as cyanidin 3-*O*-glucoside equivalents (µg/mg of AE).

Encapsulation efficiency

Encapsulation efficiency (EE) of PE, PE/ZE, PE + WP, and PE + WP/ZE capsules was examined by following the method described by Santos et al. (2013). To avoid anthocyanin degradation in phosphate buffer pH 7.4, an alternative method based on the concentration of unencapsulated AE was performed. Anthocyanin contents in crosslinking solutions were measured by method mentioned in “[Determination of anthocyanin contents](#)” section. For the crosslinking solution containing zein, the samples were centrifuged at 10,000×*g* for 5 min to remove zein precipitates prior to analysis. A calibration curve was constructed to correlate the measured absorbance with the known concentration of unencapsulated AE in the crosslinking solutions. EE was determined according to Eq. 1.

$$EE(\%) = \frac{\text{mg anthocyanin extract added/L} - \text{mg unencapsulated anthocyanin/L}}{\text{mg anthocyanin extract added/L}} \times 100 \quad (1)$$

Swelling and release media

In order to examine the potential of the pectin-based capsules as a colon targeted delivery system, swelling and release characteristics of pectin-based capsules loaded with AE were investigated under simulated gastric and intestinal fluids. In accordance with the United States Pharmacopeia, simulated gastric fluid (SGF at pH 1.2) consisted of 2 g of sodium chloride (Fisher Scientific Inc., Pittsburgh, PA), 7 mL of hydrochloric acid (Fisher Scientific Inc., Pittsburgh, PA), and distilled water used to adjust the total volume to 1 L. For simulated intestinal fluid (SIF at pH

6.8), monobasic potassium phosphate (Sigma Aldrich, St. Louis, MO) (6.805 g) and sodium hydroxide (Fisher Scientific Inc., Pittsburgh, PA) (0.896 g) were dissolved in distilled water and the total volume was adjusted to 1 L.

Swelling study

Swelling is one of the important properties of capsules made by hydrogels. It affects release of the compound in hydrogel capsules (Liu et al. 2004). In this study, swelling characteristics of pectin based capsules were determined by following the method described by Sriamornsak et al. (2010) with some modification. 100 mg of pectin-based capsules was placed in small vials containing 10 mL of SGF or SIF. The samples were shaken at 100 rpm in an orbital shaker incubator at 37 °C for 120 and 180 min in SGF and SIF, respectively. The capsules were removed from the media, surface dried with a filter paper, and reweighed on an analytical balance (Pioneer™, Ohaus, Parsippany, NJ) at different time intervals. The same capsules were used during the whole experiment. The swelling ratio of the gel beads was calculated by Eq. 2.

$$\text{Swelling ratio} = \frac{\text{Wet weight of the capsules at time } t}{\text{Initial dry weight of the capsule}} \quad (2)$$

Release study

Release of AE from pectin-based capsules was investigated in SGF and SIF for 120 and 180 min, respectively as described by Yu et al. (2009) and Kim et al. (2010) with some modifications. 30 mg of capsules were placed into

small vials containing 3 mL of SGF or SIF. The vials were then incubated with 100 rpm agitation at 37 °C in an orbital shaker incubator. The simulated fluids were sampled to determine anthocyanin contents during incubation. Three individual vials were prepared for each sampling time. The content of AE release was determined by the method mentioned in “[Determination of anthocyanin contents](#)” section. The percentage of released extract was calculated by Eq. 3. The initial anthocyanin loaded in the capsules was determined by macerating the capsules (30 mg) in 3 mL of HCl/NaCl buffer pH 1.2 after soaking for 2 h. The solid residue was washed with the known

volume of buffer three times and then centrifuged at $10,000\times g$ for 5 min. The liquid was pooled and measured for anthocyanin contents.

Released extract(%)

$$= \frac{\text{Amount of anthocyanin released from capsules at each time } t}{\text{Amount of anthocyanin initially loaded in the capsules}} \times 100 \quad (3)$$

Scanning electron micrographs of pectin-based capsules

Pectin-based capsules were mounted on aluminum SEM stubs and then coated with gold: palladium (60:40) in an Edwards S150 sputter coater. The morphology of FLP-PRF was observed under a scanning electron microscope (JSM-6610LV, JEOL Ltd. Japan).

Statistical analysis

All values were means and standard deviations of three determinations. SAS (Statistical Analysis System) software (version 9.4) (SAS Institute Inc., Cary, NC, USA) was used to compare sample mean values. Analysis of Variance (ANOVA) and Tukey's studentized range test was performed at a significance level of $\alpha = 0.05$.

Results and discussion

Anthocyanin extract derived from purple rice bran

Anthocyanin extract (AE) that was derived from purple rice bran contained $14.22 \pm 0.33 \mu\text{g}$ anthocyanins/mg extract. Anthocyanin from rice bran has been of interest to many researchers. A short synopsis of relevant research follows: anthocyanin contents are varied, depending on rice variety, bran layer fractions, growing environments, and extraction solvents (Laokuldilok et al. 2011). According to Jang and Xu (2009), purple rice bran hydrophobic extract consists of more than 90% cyanidin-3-glucoside followed by cyanidin-3-galactoside and peonidin-3-glucoside. Cyanidin-3-glucoside was reported to have the capability of anti-diabetic and anti-inflammatory effects (Min et al. 2010). Jang and Xu (2009) mentioned that high anthocyanin contents in purple rice bran extract contribute to high levels of its total phenolic contents and antioxidant activity. In the present study, total phenolic contents and antioxidant activity were $327.5 \pm 25.7 \mu\text{g}$ of gallic acid equivalents/mg extract and $215.4 \pm 4.6 \mu\text{mol}$ of Trolox equivalents/mg extract, respectively. Thus, purple rice bran could become a cheap source of anthocyanin and AE could be used as a functional ingredient.

Encapsulation efficiency

As shown in Fig. 1, mixing pectin with whey protein isolate (WP), coating with zein, and also mixing with WP and coating with zein increased the encapsulation efficiency (EE) compared to encapsulation with pectin alone. The EEs for PE + WP, PE/ZE, PE + WP/ZE and PE were 63.10 ± 6.82 , 65.47 ± 8.12 , 68.77 ± 3.46 and 45.65 ± 5.72 , respectively. This indicated high diffusional loss of AE from the capsules to CaCl_2 crosslinking solution during PE capsule formation. The loss was mainly due to the concentration gradient of the compound between the capsules and CaCl_2 solution (López Córdoba et al. 2013). Lee et al. (2009) found that EE of pectin beads loaded with catechin was in the range of 21–49%, depending on pectin concentrations and catechin loading contents. It was possible that WP could fill the void of PE capsules and block diffusion of AE to the surrounding medium, while zein provided a physical barrier for the capsules, delaying penetration of the surrounding solution through the capsules and limiting AE migration. In regards to zein coating, Liu et al. (2006) found that zein increased EE of bovine serum albumin from 60% to over 80%. The research evidenced that zein could bind to the pectin network, migrate into the capsule, and attach to the packed fibers.

Swelling of pectin-based capsules loaded with purple rice bran anthocyanin extract

Hydration and swelling are related to pre-disintegration of hydrogel capsules in physiological conditions, resulting in spread out encapsulated compounds (Sriamornsak et al. 2010). The core of pectin based capsules is Ca-pectin

Fig. 1 Encapsulation efficiency of pectin-based capsules loaded with purple rice bran anthocyanin extract. PE pectin capsules, PE/ZE pectin capsules coated with zein (1 g/100 mL), PE + WP pectin–whey protein isolate complex capsules, PE + WP/ZE pectin–whey protein isolate complex capsules coated with zein (1 g/100 mL). ^{a, b} Means $\pm$ SD with different letters indicate significant difference ($P \leq 0.05$)

hydrogels. When they are exposed to gastric and intestinal juices which contain electrolyte solutions such as sodium chloride and sodium citrate, ionotropy will occur between Ca^{2+} and Na^{+} resulting in the conversion to liquid which tends to expand the hydrogel's volume and cause 'burst release' of the entrapped compound (Liu et al. 2004). In this study, the swelling ratio of pectin-based capsules loaded with AE in simulated gastric fluid (SGF, pH 1.2) at different times is illustrated in Fig. 2 and in simulated intestinal fluids (SIF, pH 6.8) in Fig. 3. The capsules were incubated for 120 and 180 min, respectively, as it is reported that the transit time of foods is 0.5–2 h in the stomach and 3 h in the small intestine (Daniels and Allum 2005). However, the transit times are dependent on time

since previous consumption of food and age of the individual.

As shown in Fig. 2, the capsules tended to absorb water and become swollen until equilibrium was obtained. According to Sriamornsak et al. (2010), swelling behavior is mainly based on relaxation of the polymer network in the presence of osmotic pressure. When the osmotic pressure equals the forces of the cross-linking bonds that stabilize the network structure, water uptake ceases in the capsules. All capsule treatments rapidly swelled within 15 min after rehydration in SGF at pH 1.2. PE capsules showed the highest swelling ratio which was 5.66 ± 0.02 , followed by PE/ZE (4.49 ± 0.11). PE + WP had a significantly lower swelling ratio (4.25 ± 0.20) than PE and PE/ZE, but was not significantly different than PE + WP/ZE (4.00 ± 0.18). After 15 min, the capsules swelled slowly until they reached the equilibrium state. As explained by Sriamornsak et al. (2010), water filled the void regions of the polymer network formed during dehydration. After rehydration for 120 min, the capsules still remained intact. The swelling ratios of PE, PE/ZE, PE + WP, and PE + WP/ZE capsules were 6.34 ± 0.09 , 4.69 ± 0.12 , 4.37 ± 0.15 , and 4.02 ± 0.17 , respectively. As same as the swelling ratio at 15 min, PE + WP showed no significant difference when compared to PE/ZE and PE + WP/ZE. The results indicated that WP and zein reduced water absorption of the capsules in the acidic condition. Complexes formed from pectin and WP may reduce the void regions in the capsules. De Jong et al. (2009) postulated that the lower swelling of PE + WP was possibly because aggregation of the protein and interaction between WP and pectin could improve the gel strength, however they stated that the mechanism needs additional study. According to Paliwal and Palakurthi (2014), zein is an amphiphilic protein, having a hydrophobic inner core. Its structure can serve as a potent water barrier.

Moreover, the pectin-based capsules swelled slower under SIF pH 6.8 than at SGF pH 1.2, requiring a longer time to reach equilibrium. However, the swelling ratios of all capsules except PE were considerably higher than at pH 1.2 (Fig. 3). After 180 min rehydration, the highest swelling ratio was found in PE/ZE (16.31 ± 0.28) followed by PE + WP/ZE (12.01 ± 0.87), PE + WP (6.66 ± 0.18), and PE (4.34 ± 0.15). Sriamornsak et al. (2007) explained that at pH 1.2 the carboxyl groups of pectin are protonated, causing interconversion of carboxylate anions to free carboxyl groups (pKa of pectin was about 3–4), resulting in decrease of the charge density in the pectin chains and leading to reduction of gel electrostatic repulsion. The effect could tighten the inner core, resulting in less water uptake by the gel at the acidic condition. In addition, zein has an isoelectric point at around pH 6.2. Based on the dual effect of salt on the electrostatic and hydrophobic

Fig. 2 The swelling ratio of pectin-based capsules loaded with purple rice bran anthocyanin extract in simulated gastric fluid pH 1.2. PE pectin capsules, PE/ZE pectin capsules coated with zein (1 g/100 mL), PE + WP pectin–whey protein isolate complex capsules, and PE + WP/ZE pectin–whey protein isolate complex capsules coated with zein (1 g/100 mL)

Fig. 3 The swelling ratio of pectin-based capsules loaded with purple rice bran anthocyanin extract in simulated intestinal fluid pH 6.8. PE pectin capsules, PE/ZE pectin capsules coated with zein (1 g/100 mL), PE + WP pectin–whey protein isolate complex capsules, and PE + WP/ZE pectin–whey protein isolate complex capsules coated with zein (1 g/100 mL)

interactions of proteins, at pH above its isoelectric point the net negative charges of protein are increased. This caused zein to lose its physical stability and elevate electrostatic repulsion. As explained by Chou and Morr (1979), the effect allowed additional water imbibition within the protein network, resulting in greatly increased volume. This possibly explained the higher swelling ratio of PE/ZE and PE + WP/ZE capsules when compared to the uncoated samples in SIF at pH 6.8. It was surprising that PE capsules had a lower swelling ratio at pH 6.8 than at pH 1.2. This might be related to lack of gel elasticity of PE capsules. After drying, PE capsules were very brittle, unlike the other capsules. Loss of elasticity of PE capsules could be because the capsules were prepared in an ethanol solution. Moe et al. (1993) mentioned that ethanol could precipitate alginate, resulting in gel collapse and consequently decreased volume of the gel.

Release of anthocyanin extract loaded in pectin-based capsules

Release of a compound from hydrogel capsules is divided into two phases, fast release and decline release periods (Sriamornsak et al. 2010). Figure 4 showed release of AE from pectin-based capsules when they were under SGF at pH 1.2. Initially, AE was released from the capsules rapidly. Swelling and diffusion are two major mechanisms governing the release in this state (Sriamornsak et al. 2010). The release rate started declining after 30 min rehydration. After 120 min, PE + WP/ZE capsules showed significantly lower AE release, which was $68.6 \pm 6.8\%$, than PE + WP ($78.9 \pm 6.5\%$), PE/ZE ($91.2 \pm 5.1\%$), and PE ($95.3 \pm 2.4\%$). When the pectin-based capsules were exposed to SIF at pH 6.8, release of

Fig. 4 Release of purple rice bran anthocyanin extract loaded in pectin-based capsules after exposure to simulated gastric fluid pH 1.2. PE pectin capsules, PE/ZE pectin capsules coated with zein (1 g/100 mL), PE + WP pectin–whey protein isolate complex capsules, and PE + WP/ZE pectin–whey protein isolate complex capsules coated with zein (1 g/100 mL)

Fig. 5 Release of purple rice bran extract loaded in pectin-based capsules after exposure to simulated intestinal fluid pH 6.8. PE pectin capsules, PE/ZE pectin capsules coated with zein (1 g/100 mL), PE + WP pectin–whey protein isolate complex capsules, and PE + WP/ZE pectin–whey protein isolate complex capsules coated with zein (1 g/100 mL)

AE was slower than in SGF, as shown in Fig. 5. Similarly to the acidic condition, after 180 min rehydration PE + WP and PE + WP/ZE released AE more slowly than the capsules without WP, releasing 55.8 ± 1.8 and $60.0 \pm 5.7\%$, respectively, however they were not significantly different from each other. High AE release was measured from PE capsules ($81.0 \pm 5.2\%$), which was not significantly different than that for PE/ZE capsules ($86.0 \pm 3\%$). Higher release of AE in the pectin-based capsules in SIF compared to SGF could be because at a condition less than a pH of 3, carboxyl groups of pectin are protonated, and anthocyanins exhibit positive charges (Castañeda-Ovando et al. 2009). This would reduce the surface charges of the polymer, inducing release of AE. Lu et al. (2015) reported that the percentage of anthocyanin release was higher when pH of the medium was below the isoelectric point or pKa of a matrix polymer. For example, anthocyanins in oxidized glucomannan microspheres, an anionic polymer, had greater release when they were incubated at pH 2 than at pH 6. Our results indicated that PE + WP capsules with and without zein reduced release of AE in both SGF and SIF. These results are in accord with results reported by other researchers. Coughlan et al. (2004) reported that pectin–WP complexes have a greater number of intermolecular crosslinkages between the matrix than individual pectin or WP, serving as a better water barrier with greater tensile properties. Early release of anthocyanin was found in amidated pectin (1 g/100 mL) capsules and WP capsules formed by the emulsification/heat gelation method. Complete release was detected within the first 20 min in SGF (Oidtmann et al. 2012). Nithitanakool et al. (2013) have reported that mango kernel extract is totally released from calcium pectinate capsules after incubation in SGF and SIF for 2 h and 4 h.

Fig. 6 Scanning electron micrographs of PE (a, b), PE/ZE (c, d), PE + WP (e, f), and PE + WP/ZE (g, h) capsules. PE pectin capsules, PE/ZE pectin capsules coated with zein (1 g/100 mL),

PE + WP pectin–whey protein isolate complex capsules, and PE + WP/ZE pectin–whey protein isolate complex capsules coated with zein (1 g/100 mL). Arrows indicate zein particles

The high release of AE from PE and PE/ZE capsules we obtained could also be due to wide cracks observed on the capsules' surface (Fig. 6a–d). According to Wang et al. (2013), anthocyanin molecules are very small. The molecular weight is 449 Da, facilitating easy diffusion through the capsules. In our work, the PE + WP capsules' surfaces had smaller cracks than the PE capsules, their surface was more homogenous and slightly rougher (Fig. 6e, f). Zein randomly covered PE/ZE capsules, however there were not many zein molecules observed on PE + WP/ZE capsules (Fig. 6g, h). Using 1 g/100 mL of zein may not have been sufficient to layer the whole capsules. This could explain why no significant differences were observed in the quantity of AE released from the capsules coated with zein when compared with uncoated capsules. Optimizations of zein contents on pectin-based capsules are needed.

Conclusion

Purple rice bran anthocyanin extract (AE) could be entrapped into pectin-based capsules. Encapsulation efficiency (EE) of AE in pectin capsules was improved by either complex formation between pectin and whey protein isolate (WP) or coating the pectin capsules with zein. The complex capsules formed by pectin and WP with or without zein coating, PE + WP and PE + WP/ZE capsules, reduced swelling of pectin capsules in SGF at pH 1.2, potentially resulting in decrease of early AE release in the stomach. Swelling of pectin-based capsules was pH-dependent. Compared to swelling in SGF (pH 1.2), higher swelling was observed in SIF (pH 6.8). The complex capsules helped sustain AE when the capsules were

exposed to SGF and SIF. However, an effect of zein coating on lowering AE release was not found. This could be due to insufficient zein content. This study showed that PE + WP capsules could be used as a delivery system for AE. Future research on optimization of zein contents on PE-WP capsules will be needed.

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