In Vitro Evaluation of Pectin-Coated Starch Granules for Colonic Delivery

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ABSTRACT

Glutinous rice starch was prepared in a coated granule formulation by wet granulation for use as a colon-specific starch delivery system. The starch granules were coated with pectin types LC 710 and AMD 382 by ionotropic gelation technique at various ratios at a concentration of 10% weight by weight and dropped in $CaCl_2$ solution. The pectin coated starch granules with LC 710 alone (1:0) showed the highest effective protection against starch hydrolysis from simulated gastric fluid (0.1 N HCl pH 1.2, 2 h) and small intestine fluid (phosphate buffer solution pH 6.8, 2 h) and then the complete release of the starch granule in simulated colonic fluid (phosphate buffer solution pH 7.4, 4 h). In contrast, granules coated with AMD 382 alone (0:1) did not tolerate the simulated gastric fluid. When granules were coated with a combination of the two pectin types, the effectiveness of starch protection depended on the ratio, with higher ratios of LC 710 over AMD 382 offering more protection.

Glutinous rice starch, prepared in granule form and coated with pectin LC 710, offers potential for development as a nutraceutical product given its ability to tolerate simulated stomach acids and small intestine enzymes and then release the starch when exposed to simulated colonic fluid, where it would be available as a probiotic substrate.

Keywords: Pectin, Starch granules, Colonic delivery, Ionotropic gelation technique

INTRODUCTION

Interest in the development of colon-specific drug delivery systems has increased with the search for better treatments of specific local pathologies as well as for systemic therapy of both conventional and labile molecules. Several methods of potential colonic drug delivery systems, including bead matrices, micro-particles and nano-particles have been studied (Maestrelli et al., 2008). To successfully deliver drugs orally to treat diseases of the colon requires protecting

the drugs from absorption and the environment of the upper gastrointestinal tract while allowing them to be subsequently released upon entering the proximal colon, the optimal site for colon-targeted delivery of drugs (Chourasia and Jain, 2003).

Pectin are non-starch linear polysaccharides that consist of α-1,4-galacturonic acid and 1,2 D-rhamnose with D-galactose and D-arabinose side chains (Desai, 2005). Pectins divide into two groups according to their gelation properties (Novosel et al., 2000). Low-methoxyl pectin with a degree of methylesterification (DE) less than 50% can form strong gels when the galacturonic acid chains are cross linked with calcium ions or multi-valent cations to produce Ca-pectinate networks called "egg box" structures. These display a variety of properties, including reducing the water-solubility of pectin and tolerating acidic media (Sriamornsak et al., 2007). High-methoxyl pectin has more than 50% DE, with a pH-value between 2.8-3.6, and hydrogen bonds and a hydrophobic interaction that bind the individual pectin chains together. The gelling-mechanism is called a low-water-activity gel or sugar-acid-pectin gel (Novosel et al., 2000). This form of gel has served as a carrier to protect and control the release of drugs (Sriamornsak and Nunthanid, 1998; 1999).

This study used glutinous rice starch as the test starch because bacteria, in particular *Lactobacillus amylovorus* TISTR 1110, can utilize it as a carbon source, adhering and hydrolyzing the glutinous rice starch granules. In addition, humans prefer to consume glutinous rice starch, especially in northern and northeastern Thailand. The starch was coated in granular form for *in vitro* study as a colon-specific starch delivery system.

The purpose of the present study was to develop a colonic drug delivery system for glutinous rice starch for use as a prebiotic using polysaccharides (pectin) to protect the granular starch from leaking in a media-simulated stomach and small intestine while allowing all of the granular starch to leak in media-simulated colonic fluid for use as a probiotic.

MATERIALS AND METHODS

Materials

Cho-Heng Company, Thailand supplied the glutinous rice starch. Pectin LC 710 (low-methoxyl pectin with a DE less than 50%) and pectin AMD 382 (high-methoxyl pectin with a DE more than 50%) were purchased from Danisco, Denmark. All other chemicals used in this experiment were of reagent grade.

Preparation of starch

The starch granule formulation was prepared by wet granulation, using polyvinyl pyrorlidone (PVP-K90) as the binder. Binder solution (10% PVP) was mixed with starch until the appropriate wet mass was obtained, and then passed through a 16-mesh sieve. The wet granules were dried in an oven for 24 h at 40°C and subsequently sieved through a 20-mesh by an oscillating granulator. After sieve analysis, the fraction of granule left on the 20-mesh was selected for coating. Before coating, starch granules were assessed for hardness and friabi-

lity. The general appearance of 20-mesh glutinous rice starch granules in shape, size, and weight was assessed and captured by digital camera. In addition, the morphological characteristics of the external surface of the uncoated glutinous rice starch granules were examined with a scanning electron microscope.

Coating of starch granules with ionotropic gelation technique

Two types of pectin, LC-710 (LM) and AMD-382 (HM) were combined at various proportions to obtain the desired combination ratio (w/w) of 1:0, 0:1, 1:1, 1:2, 2:1, 1:3, 3:1, 1:4 and 4:1. The pectin coated starch granules were prepared by the method of Das and Ng (2010), with modification. Using a homogenizer, the pectin was homogeneously dispersed in deionized water at a concentration of 10% (w/w). Starch granules were added in viscous solution and then pressure-dropped through a plastic syringe into 10% (w/v) calcium chloride solution under gentle stirring at room temperature. The pectin-coated starch granules that formed were allowed to stand in the solution 20 min for cross-linking, then filtered and washed with distilled water. Subsequently, they were dried at 37°C for 12 h.

Morphology of coated granules

A digital camera captured the shape, size, and weight of the coated granules before and after drying. Weight loss from drying (from a random selection, n=50) was calculated using the following equation:

$$\% \text{ weight loss} = \left(\frac{\text{weight}_{wet} - \text{weight}_{dry}}{\text{weight}_{wet}} \right) \times 100\ \%$$

The experiment was performed in triplicate and data were expressed as the mean ± standard deviation (SD).

Morphological examination of the surface appearance and a cross section of the inner structure of the coated granules were investigated using a scanning electron microscope, both before and after the experiments. To verify the internal structure of the coated granules, they were cut in half with a steel blade and then examined by scanning electron microscope, using low-vacuum mode for the freshly prepared pectin-coated starch granules and high-vacuum mode for the dried pectin-coated starch granules.

Test of tolerance of pectin coated starch granule to simulated gastrointestinal fluid

The pectin-coated starch granules were evaluated for their ability to protect the starch along the gastrointestinal tract by mimicking its pH conditions (Mura et al., 2003). Starch leakage from the coated granules was tested using the method, with modification, of Das and Ng (2010).

The dry granules (n=100) were placed into a bottle with screw cap and suspended in 30 ml of each test solution in sequence: 0.1 N HCl (pH 1.2) to simulate gastric fluid for 2 h; phosphate buffer solution (pH 6.8) to mimic small intestine fluid for 2 h; and phosphate buffer solution (pH 7.4) to simulate colonic fluid for 4 h. The bottles were continuously shaken in a water bath at 37°C at 100 rpm. After testing with the simulated gastric and small intestine fluid, the coating of the granules was examined. Granules with intact coating were retained for subsequent testing with the simulated colonic fluid solution. Granules with incomplete coating that showed signs of starch leakage were removed. The results report the number of intact pectin-coated starch granules under various conditions from triplicate experiments.

Statistical Analysis

Results were expressed as the average of three independent experiments, i.e., mean ± standard deviation, using SPSS version 12.0 for Windows the difference of the means between the test groups was compared by one-way analysis of variance (ANOVA), followed by post-hoc analysis. P values less than 0.05 were considered significant.

RESULTS

Shape, size, and weight of starch granules before coating

Scanning electron micrographs (Figure 1) show the granular structure of glutinous rice starch before and after binding with PVP-K 90. Figure 2 shows photographs of glutinous rice starch after granulation. The prepared starch after granulation displayed various shapes under light microscope (Figure 3). The hardiness value of the 20-mesh glutinous rice starch granules was 289.45±21.24 g (n = 50 from triplicate experiments). Frangibility was achieved at 0.37% (< 0.8%) according to BP 1993.

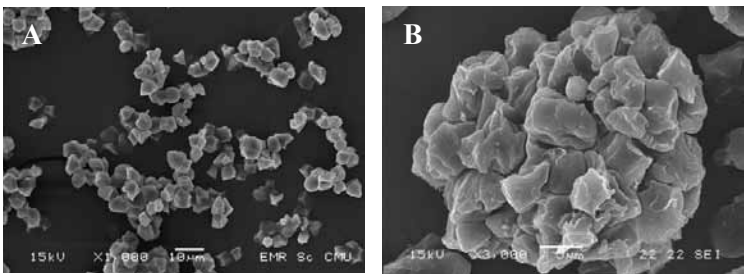

Figure 1. Scanning electron micrographs of native glutinous rice starch before (A) (1000x magnification) and after granulation with PVP-K 90 (B) (3000x magnification).

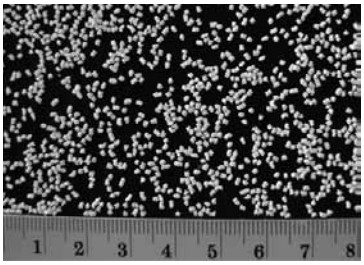

Figure 2. Photographs of 20-mesh glutinous rice starch granules before coating.

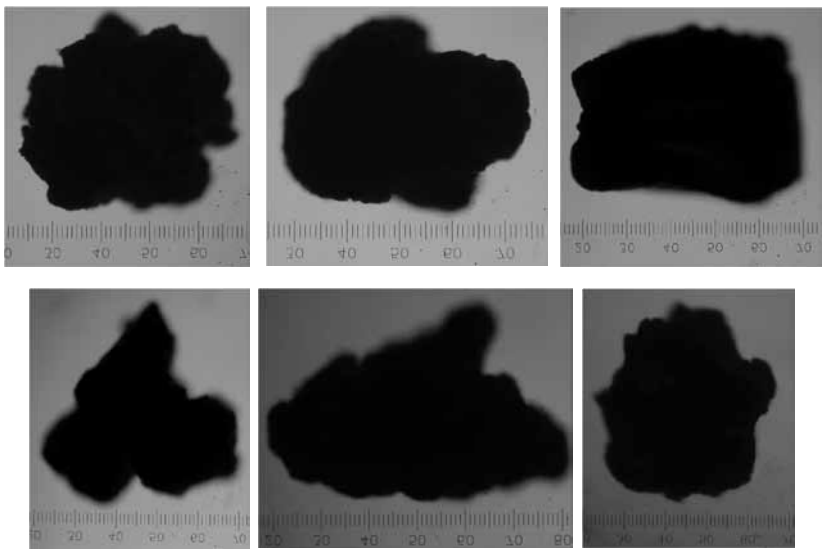

Figure 3. The various shapes of uncoated starch granules (10x magnification).

Shape, size, and weight of pectin-coated starch granules

The starch granules were coated with pectin LC 710 and AMD 382 at various ratios of 1:0, 0:1, 1:1, 1:2, 2:1, 1:3, 3:1, 1:4 and 4: 1 by ionotropic gelation technique. Figure 4 shows freshly prepared and coated granules after drying at 37°C for 12 h. The preparations formed drop-like-shape granules, 4-5 mm across. However, they shrank after drying to 2-3 mm. The shape depended on the ratio of pectin coating. The pectin-coated starch granules with LC 710 were off-white in color and with AMD were light colored.

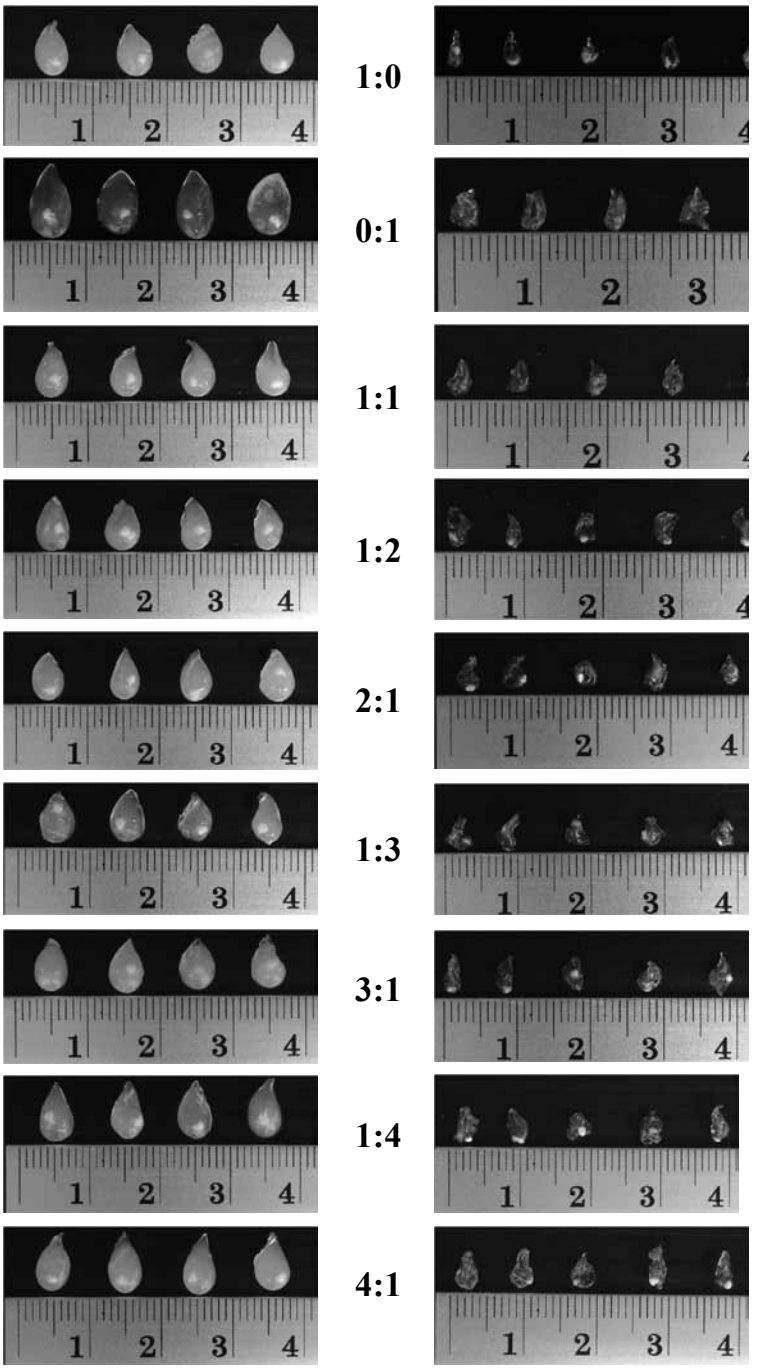

Figure 4. Photographs of pectin-coated starch granules with a combination of pectin LC 710 and AMD 382 coatings in various ratios.

Note: The left column shows freshly prepared pectin-coated starch granules and the right column after drying at 37°C for 12 h.

Table 1 shows the average wet weight, dry weight, and percentage weight loss during drying. The mean wet weight of pectin-coated starch granules varied from 3.89±0.15 to 5.32±0.22 g, depending on the pectin ratio. After drying at 37°C for 12 h, the mean dry weight of pectin-coated starch granules decreased in a range from 0.54±0.02 to 0.87±0.04 g. The mean percentage weight loss of pectin-coated starch granules after drying ranged from 83.08±0.53 to 85.99±0.20%.

Table 1. Wet weight, dry weight, and % weight loss of pectin-coated starch granules.

Pectin **LC 710:AMD 382**	Wet weight (g)	Dry weight (g)	% Weight loss
1 : 0	4.36±0.02	0.61±0.04	85.74±0.79
0 : 1	5.32±0.22	0.87±0.04	84.07±1.09
1 : 1	4.01±0.08	0.61±0.02	84.59±0.21
1 : 2	3.89±0.15	0.54±0.02	85.99±0.20
2 : 1	4.20±0.10	0.65±0.04	84.21±1.14
1 : 3	4.60±0.11	0.71±0.01	84.37±0.64
3 : 1	4.96±0.14	0.84±0.06	83.08±0.53
1 : 4	5.31±0.03	0.82±0.08	84.48±1.54
4 : 1	4.71±0.21	0.76±0.00	83.61±0.52

Note: Values are expressed as mean ± SD (n=50) from triplicate experiments.

Morphology of pectin-coated starch granules

The external surface and internal structure (cross-sectional) morphology of pectin-coated starch granules at various ratios was examined using a scanning electron microscope (Figure 5). The surface of pectin LC 710 alone (1:0) showed a smooth surface. The cross-section of pectin-coated starch granules also showed clusters of starch granules that were coated with pectin completely. In contrast, pectin AMD 382 alone (0:1) exhibited mixed surface types including smooth, rugged, and fissured. The internal structure showed fissures on the surface of this pectin type and some of the starch granules leaked out from the cluster. The coating surface appeared cracked in all ratios of pectin combination. The cross-section showed the same pattern as pectin AMD 382.

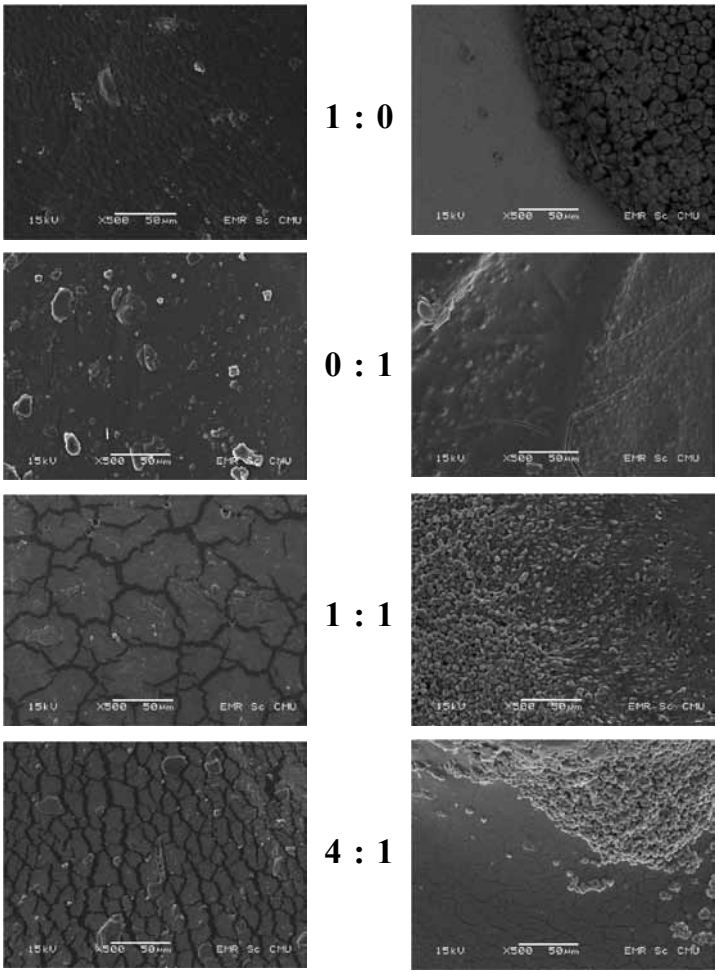

Figure 5. Scanning electron micrographs of pectin-coated starch granules at various ratios.

Note: The left column shows external surface and the right column shows internal structure (cross-section). Magnifications are shown in the individual micrographs.

Test of tolerance of coated starch granule to simulated gastrointestinal fluid

The pectin-coated starch granules were evaluated for their ability to protect the starch along the gastrointestinal tract using simulated media to mimic the pH conditions.

The 1:0 pectin-coated starch granules remained intact, releasing no starch in the 0.1 N HCl (pH 1.2) solution after 2 h. In contrast, the 0:1 pectin-coated starch granules released 100% of the starch after 2 h in the simulated gastric fluid. The intact granules from different combinations of two grades of pectin formulation in simulated gastric fluid followed the order of 4:1 > 3:1 > 2:1 > 1:1 > 1:2 > 1:3 > 1:4, as shown in Table 2.

Within 2 h of immersion in phosphate buffer solution (pH 6.8), simulating small intestine fluid, both 1:3 and 1:4 pectin-coated starch granules leaked all of the starch. In contrast, 75.7±16.0% of the 1:0 pectin-coated starch granules remained intact, protecting the starch. The percentage of 4:1, 3:1, 2:1, 1:2 and 1:1 pectin-coated starch granules that retained their coating was 51.0±13.7, 45.3±11.7, 40.7±10.3, 8.7±7.6 and 5.0±8.7, respectively.

The starch granules that retained their coatings after the simulated gastric and small intestine fluids were then immersed for 4 h in simulated colonic fluid conditions using a phosphate buffer solution (pH 7.4). After 4 h, the starch completely leaked out of all coating formulations (Figure 6).

Morphology of pectin coated-starch granule (1:0) after *in vitro* starch leaking test

The surface morphology and cross-sectional surface structure of 1:0 pectin-coated starch granules after the leaking test was evaluated by scanning electron microscope in a low vacuum evaporator mode (Figure 7).

Coating the starch granules with 1:0 pectin prevented starch granules from leaking in the 0.1 N HCl (pH 1.2) after 2 h. All the pectin-coated starch granules displayed the same regular shape, smooth surface and packing of starch granules inside as at the beginning of the experiment. After 2 h exposure to the phosphate buffer solution (pH 6.8), simulating the small intestine fluid, the appearance of the pectin-coated starch granules changed. The surface was irregular and eroded. Viewed in cross-section, most of the starch granules shifted to a star or coral-like shape and seemed to be separated from the pectin before completely dissolving in the phosphate buffer solution (pH 7.4), simulating the colonic media, for 4 h.

Table 2. The percentage of granules of various coating formulations remaining intact after sequential exposure to the simulated gastrointestinal tract fluids.

Test medium (hours of contact)	Pectin (LC 710 : AMD 382)								
	1 : 0	0 : 1	1 : 1	1 : 2	2 : 1	1 : 3	3 : 1	1 : 4	4 : 1
0.1 N HCl pH1.2 (2h)	100.00±0.00	0.00±0.00	52.33±8.50	30.00±15.62	70.66±9.45	34.33±13.65	71.33±12.05	24.66±5.03	76.00±10.44
PBS pH 6.8 (2h)	75.66±16.01	n/a	8.66±7.57	5.00±8.66	40.66±10.26	0.00±0.00	45.33±11.71	0.00±0.00	51.00±13.74
PBS pH7.4 (4h)	0.00±0.00	n/a	0.00±0.00	0.00±0.00	0.00±0.00	n/a	0.00±0.00	n/a	0.00±0.00

Note: Values (n=100) are expressed as mean±SD (triplicate experiments). PBS: phosphate buffer solution. n/a: not applicable as no coated granules remained after the previous stage.

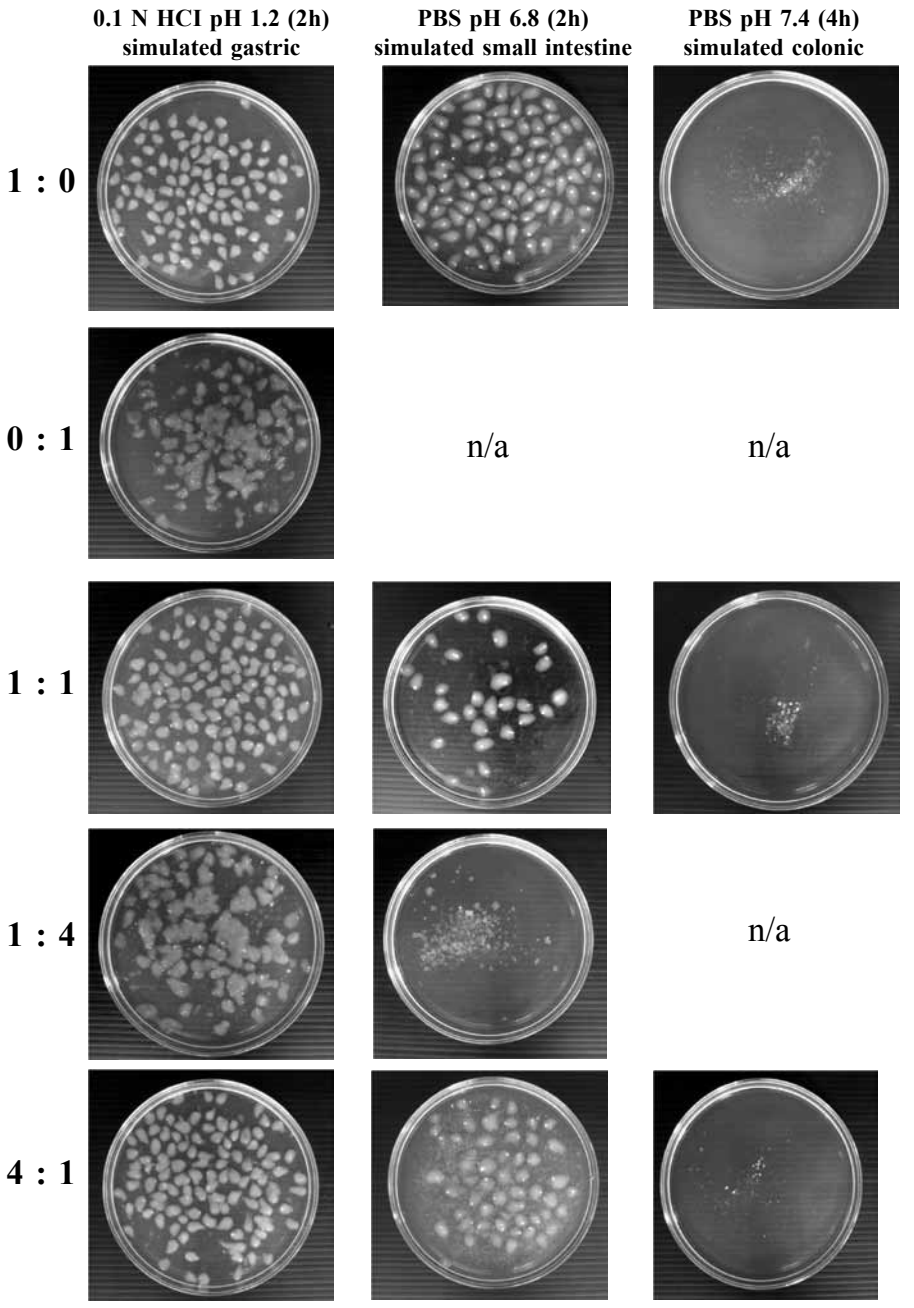

Figure 6. Leaking test of pectin-coated starch granules at various coating ratios.
Note: n/a - not applicable as no coated granules remained after the previous stage.

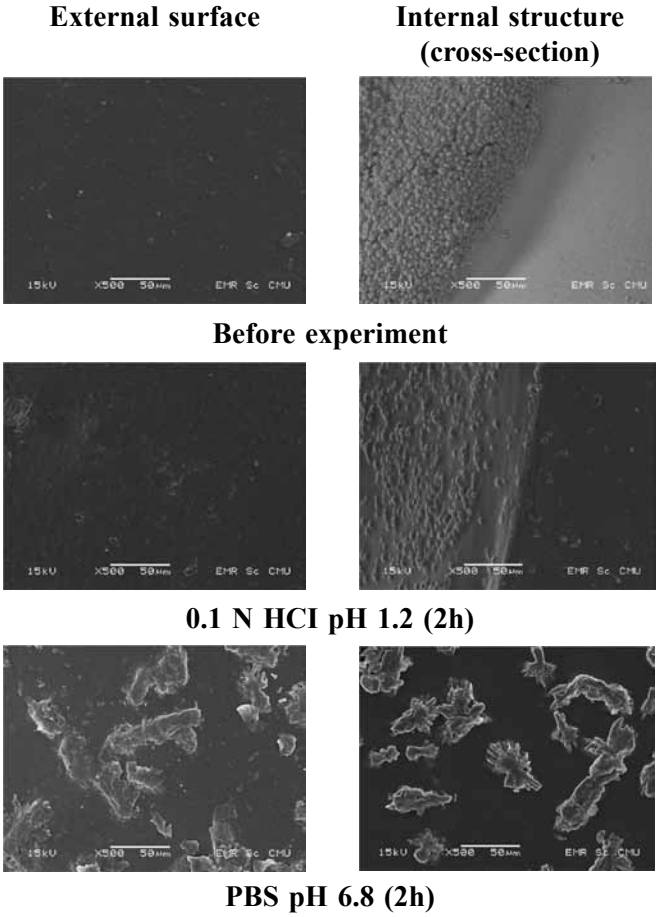

Figure 7. Scanning electron micrographs (500x magnification; low vacuum mode) of pectin-coated starch granules before and after experiment.

DISCUSSION

The shape of pectin-coated starch granules coated with pectin LC 710 and AMD 382 at various ratios appeared drop-like. The mean wet weight of pectin-coated starch granules was 6-7 times greater than the dry weight because of water loss during drying at 37°C for 12 h. The mean percentage weight loss of pectin-coated starch granules was in the range of 83.1±0.5 to 86.0±0.2.

Using a scanning electron microscope, the external surface and internal structure of pectin-coated starch granules displayed various textures, depending on the coating formulation. When coated with pectin LC 710 alone, the surface was smooth. When coated with AMD 382, either alone or in combination with LC 710, the surfaces were mixed, including smooth, rough, fissured, and cracked. The different surface morphology might have resulted from the specific degree of esterification of pectin.

During the first 2 h in acid conditions, mimicking gastric fluid, the starch granules coated with LC 710 (10% w/w) alone remained 100% intact. LC 710 protected the starch in the granules from leaking in the simulated gastric fluid. The scanning electron microscope revealed intact internal and external structures, confirming this finding. In contrast, for the granules coated with AMD 382 alone, a large amount of starch was detected in the acid medium of simulated gastric fluid. This type of pectin coating did not form a strong gel against the acid environment. The starch may have leaked through fissures in the coating as seen by scanning electron microscope. The starch granules coated with pectin in ratios of LC 710 : AMD 382 showed the most to least starch leakage in the following order: 1:4 > 1:3 > 1:2 > 2:1 3:1 > 4:1. The leaking presumably occurred through penetration of acid through cracks, fissures and small pores in the outer surface. The leaking decreased with increasing ratios of LC 710 in the coating. LC 710 forms a stronger gel than AMD 382.

Following 2 h in the phosphate buffer solution (pH 6.8), simulating small intestine fluid, the starch leaked completely from the 1:3 and 1:4 coating combinations. In the other combinations, the starch granules leaked least to most as the ratio of LC 710 in the pectin coating reduced from 1:0 > 4:1 > 3:1 > 2:1 > 1:2 > 1:1, respectively. The 1:0 ratio, or all LC 710 coating, retained about 75% of the granular starches inside the pectin-coated starch granules. Again, LC 710 was the most suitable for protecting the starch granules in an acid medium.

Similarly, simulating colonic fluid conditions with a phosphate buffer solution (pH 7.4) for 4 h, the remained-intact starch granules of all formulations from previous immersion in 0.1 N HCl and phosphate buffer solution (pH 6.8) completely leaked within 4 h. This showed that 10% (w/w) pectin-coated starch granules protected starch granules at the simulated upper gastrointestinal tract and let the starch leak from the system in the simulated lower gastrointestinal tract within the appropriate time.

The scanning electron microscope analysis (low vacuum evaporator) for pectin-coated starch granules after the *in vitro* starch leaking study in simulated gastric fluid after 2 h subjected to 0.1 N HCl pH 1.2 showed a swelling of pectin-coated starch granules with a smooth homogeneous surface and their integrity maintained. Scanning electron microscope images of the cross-section of granules also revealed a compact Ca-pectinate network wrapping all of the starch granules. LC 710 in 10% (w/w) plays a crucial role in protecting and retarding the starch granules from leaking from the system prematurely. Following 2 h of immersion in phosphate buffer solution pH 6.8 mimicking intestinal fluid, erosion and rough surfaces were found. Evidence from the internal structure showed loose Ca-pectinate covering the starch granules with the granules modified to star or coral-like shapes. This phenomenon might be due to surface erosion, then dissolving of some of the Ca-pectinate network creating morphological changes in the starch granule by gelatinization with calcium chloride. This investigation was in agreement with the result of Koch and Jane (2000), who reported that $CaCl_2$ 4M concentration attacked native starch granules such as potato, maize, and wheat on the surface and gelatinized the starch on the inside. The gelatinization tempera-

ture of the starch and varies by species and salt concentration. For formulations with intact starch granules in the phosphate buffer solution (pH 6.8), almost all of them let the starch leak in the simulated colonic fluid at pH 7.4. Pectin has higher solubility at this pH because of the ionization of its carboxylic groups at higher pH values (Liu et al., 2003).

This experiment suggested that using pectin LC 710 alone exhibited the appropriate properties to form a strong gel to protect starch granules during the *in vitro* leaking study for colonic delivery when compared with AMD 382 and their combinations. Moreover, scanning electron micrographs, both external surface and internal structure, indicated that pectin-coated starch granules with LC 710 alone protected intact granular starch better than both AMD 382 alone and LC 710 combined with AMD 382 at various ratios.

Glutinous rice starch, prepared in granule form and coated with pectin LC 710, offers potential for development as a nutraceutical product given its ability to tolerate simulated stomach acids and small intestine enzymes and then release the starch when exposed to simulated colonic fluid, where it would be available as a probiotic substrate.

ACKNOWLEDGEMENTS

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