A Thesis for the Degree of Master of Science

Physicochemical and digestion properties of psyllium husk powder-starch-alginates based mixtures

차전자피가루, 옥수수전분, 알진산 혼합물의 이화학 및 소화 특성

August, 2016

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Seoul National University

농학석사학위논문

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by

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Submitted in Partial Fulfillment of the Requirement
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ABSTRACT

Native corn starch is utilized not only in many processed food products, but also in nonfood industrial applications. However, they have limited industrial uses due to their low process tolerance within a commercial manufacturing process. Hence, formulating starch with soluble dietary fiber is viewed as an efficient way of improving its poor processability. Psyllium husk powder can be a useful source for formulating with starches due to its highly viscous form when in contact with water and its unique therapeutic effect. This study aimed to elucidate the influences of psyllium husk powder concentration on physicochemical and digestion properties of starch and to further–develop psyllium-starch-alginate beads for applications of natural polymer in food. Formulating starches with increasing psyllium husk powder concentration delayed digesting time up to 4 hrs compared to the control. The addition of psyllium husk powder brings a slow release of glucose in simulated system of small intestine. The extent of glucose binding to fiber increased from 20.54 to 79.95 as the psyllium husk powder concentration increased from 1% (w/v) to 2% (w/v). The apparent viscosity continuously increased, considerably contributing to overall rheological flow behavior.

Psyllium husk powder played an important role in reducing digestibility of starch and glucose permeability. The physical properties of psyllium husk powder including water holding capacity, viscosity, and swelling capacity mainly contributed to a promising controlled glucose release behavior of psyllium husk powder-starch-alginate beads. Binary mixtures of psyllium husk powder, starch, and sodium alginate can provide potential controlled polymer-based matrix systems.

Keywords: normal corn starch, psyllium husk powder, alginate, encapsulation, starch digestion, glucose release

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INTRODUCTION

Starch is the most common source of carbohydrate in human diet. Starch is mainly composed of amylose and amylopectin. Amylose is a linear molecule of α-1, 4 linked anhydroglucose residues with a few branches whereas amylopectin is a branched molecule comprising chains of α-1, 4 linked anhydroglucose residues linked by α-1, 6 glycosidic bonds (Gidley *et al*., 2010). The release of glucose from starch digestion plays an important role in energy metabolism. However, health related issues such as obesity, irritable bowel syndrome, diabetes, or cardiovascular disease have been associated with consumption of native corn starches (Zhang *et al*., 2016). Native corn starch is biodegradable and low-cost polysaccharide. Uses of starch permeate the entire economy including bakeries, or restaurants. However, raw starches still have limited industrial usages due to their poor processability, water resistance, and loss in viscosity during a commercial manufacturing process. These drawbacks have been improved by blending the starch with non-starch polysaccharides. Hence, starch-based polymers have been suggested as an attractive material for many processing applications. Numerous attempts to improve mechanical properties of starch-

based polymers have been conducted (Mariotti *et al*., 2009; Rassis *et al*., 2002; Schoebitz *et al*., 2012). Soluble dietary fiber has been widely implemented to stabilize starch-based materials.

Psyllium husk powder, the seed of the *Plantago*, has been classified as an indigestible source of soluble dietary fiber. This soluble dietary fiber has been issued for its therapeutic effect on hypoglycemia, constipation, irritable bowel syndrome, and inflammatory bowel disease such as ulcerative colitis, diabetes, and cholesterol lowering (Pastors *et al*., 1991). U.S Food and Drug Administration has authorized the use of psyllium in food products and recommended 10 g/ day intake for its effectual use against hypercholesterolemia. This psyllium husk powder has the ability to swell 10 to 14 times of its original volume when they are in contact with water. It was revealed that every 100 g of psyllium proved 71 g of soluble dietary fiber while a similar amount of oat bran contains only 5 g of soluble dietary fiber (Singh *et* al., 2007). The major components of psyllium husk powder are 15% of non-polysaccharide material and 85% of a single polysaccharide. The single polysaccharide mainly comprises of arabinoxylan (Singh., 2007). Hence, psyllium husk powder can be a useful source for formulating with starch. However, psyllium-containing products are viewed as having poor

palatability. When the psyllium-containing products are in contact with water, the products are considered to be objectionable because of its ragged texture and high viscosity. Therefore, Psylllium husk powder formulated with starch can be encapsulated with alginate. Alginate beads containing starch achieve a controlled and sustained release of glucose. Encapsulating starch-psyllium husk powder based mixtures with sodium alginate would help obstruct a substantial swelling and rapid enzymatic degradation of native starch in biological systems.

The objectives of this study were to elucidate the effects of varying psyllium husk powder concentrations on physicochemical and digestion properties of starches, and to show promising controlled glucose release behavior of psyllium husk powder-starch-alginate based-beads. This investigation of psyllium husk powder-starch-alginates based beads can help to provide food materials for applications of natural polymers in food.

MATERIALS AND METHODS

1. Materials

Normal corn starch was provided by Ingredion (Westchester, IL, USA). Pancreatin from porcine pancrease was purchased from Sigma Aldrich (St. Louis, MO, USA). α-Amylase activity was 230 units/mg solid. Amyloglucosidase (AMG 300L, activity 300 AGU/mL) was obtained from from Novozymes (Bagsvaerd, Denmark). One amyloglucosidase unit (AGU) is defined as the amount of enzyme that cleaves 1 μmol of maltose per minute under standard assay conditions. Glucose oxidase-peroxidase (GOD-POD) assay kit was purchased from Embiel Co. (Gunpo, Korea). Psyllium husk powder (PSY) was acquired from NOW FOODS (Bloomingdale, IL, USA).

2. Methods

2-1. *In vitro* digestibility of normal corn starch within soluble dietary fiber mixed system

The *in vitro* digestibility of normal corn starch (NCS) and that of NCS within PSY mixed systems were examined based on the method of Englyst *et al*., (1992) with a slight modification. Pancreatin (3.0 g) was added to 36 mL of distilled water and stirred for 10 min. The dispersion of pancreatin was centrifuged at 1,500 g for 10 min at 4°C. The supernatant (30 mL) and amyloglucosidase (0.6 mL) were mixed with 5.4 mL of distilled water. The enzyme solution was stored in a water bath at 37°C for 10 min prior to the usage.

NCS (0.09 g) was placed into each of conical tubes with three glass beads and 2.25 mL of 0.1M sodium acetate buffers was added. PSY-starch-alginate beads samples (0.9 g) were placed in each of 50 mL conical tubes with three glass beads. Samples were placed in a shaking incubator (37°C, 240 rpm) for 10 min.

To determine the starch digestibility, 2.25mL of enzyme solution was added to each, and then incubated in a shaking incubator for 30, 60, 120, 180, 240, 300, and 360 min. The reaction was terminated by boiling samples for

10 min. To determine the glucose released from starch hydrolysis, the conical tube was centrifuged at 10,000 g for 10 min, and glucose concentration in the supernatant was measured using a GOD-POD kit.

2-2. Determination of glucose permeability within dialysis tubing systems

The effect of PSY concentration on glucose diffusion was investigated by measuring the permeability of glucose within dialysis tubing systems (Ou *at el.,* 2001). Glucose solution (2% w/v, 10 mL) was mixed with: 0.1 g or 0.2g of PSY (1% w/v or 2% w/v PSY, respectively). Each of PSY mixed glucose solutions was transferred into dialysis tube (flat width 25 mm, molecular weight cut-off 14,000, Sigma-Aldrich). For each analysis, 10 mL of sample solutions (glucose only, 1% w/v PSY, 2% w/v PSY) were dialyzed against 100 mL of distilled water in a shaking incubator (37°C, 240 rpm). The diffused glucose content in 1.0 mL of dialysate was determined after 30, 60, 120, 180, 240, 300, and 360 min using a GOD-POD kit.

2-3. Determination of glucose-adsorption capacity

The glucose-adsorption capacity was determined based on the method of Ou *at el.* (2001) with a slight modification. Sample solutions were prepared using 2% (w/v) PSY- dissolved solution at different glucose concentrations (1%, 2%, 3%, and 5% w/v). Each of 10 mL sample solutions was transferred into a dialysis tube (flat width 25 mm, molecular weight cut-off 14,000, Sigma-Aldrich) and dialyzed against 100 mL of distilled water in a shaking incubator (37 C, 240 rpm) for 6 hr. The diffused glucose content in 1.0 mL of dialysate was measured using a GOD-POD kit.

2-4. Determination of glucose dialysis retardation index (GDRI)

A 10 mL aliquot of 2% (w/v) glucose solution mixed with 0.1 g or 0.2 g of PSY was dialyzed against 100 mL of distilled water in a shaking incubator (37 C, 240 rpm). The glucose content in the dialysate was determined after 30, 60, 120, and 180 min using a GOD-POD kit. A control test was performed without soluble dietary fiber.

The GDRI and glucose bound was calculated according to the methods of Ou *et al*. (2001), and Ahmed *et al*. (2011) using the following equation:

$$\text{GDRI} = 100 - [\left(\frac{\text{Glucose content with the addition of soluble dietary fiber}}{\text{Glucose content of the control}}\right)] \times 100$$

$$\text{Glucose bound} = \frac{(\text{G1} \times \text{V1}) - [\text{G2} \times (\text{V1} + \text{V2})]}{\text{weight of the sample}} \times 100$$

G1: glucose concentration in retentate before start of diffusion

G2: glucose concentration in dialysate after 6 hours

V1: volume of retentate

V2: volume of dialysate

2-5. Rheological properties

The rheological properties were determined using an oscillatory rheological measurement (Rheostress 1, Thermo HAKKE, Karlsruhe, Germany) with a cone-plate system (35 mm diameter, 0.052 mm gap) to understand the changes in viscosity. Mixtures of NCS and PSY were prepared by mixing 0.09 g of NCS 0.09 g, 0.18 g, and 0.27 g of PSY (to reach the ratio of NCS: PSY to 1:1, 1:2, and 1:3, respectively with 2.25 mL of distilled water.

The oscillatory rheometer time sweep experiment was performed to measure viscosity for NCS and PSY mixture samples based on the method of Sigh *et al* (2010). The sample was loaded on a cone and plate system and preheated from 4°C to 37°C in 10 min at 0.6 mm/min in order to equilibrate the sample temperature same as digestion reaction temperature. The edge of the sample was covered with a thin layer of silicon oil to prevent water evaporation during measurements. The test was conducted at 37°C with a strain and frequency of 1% and 1 Hz, respectively.

2-6. Preparation of psyllium husk powder-starch-alginate beads

Psyllium husk powder-starch-alginate beads were prepared following the modified method of Rose *et al*. (2009). The measured amount of normal corn starch (2% w/v) was added to the 2% (w/v) sodium alginate solution. To prepare the PSY-starch-alginate-based beads, the mixtures containing PSY (1%, 1.5%, and 2% w/v) and NCS (2% w/v) were added to the sodium alginate solution. After mixing uniformly with a stirrer at 400 rpm for 10 min, the mixture was pumped through a tube (i.d. x 4mm, F1825103, Gilson, Middleton, WI, USA) using a peristaltic pump (Miniplus 3, Gilson,Villiers le Bel, France), dropping into a 2% (w/v) calcium chloride solution under

continuous stirring to form beads. The beads were kept in the 2% (w/v) calcium chloride solution for 10 min and then washed with distilled water three times.

2-7. *In vitro* digestibility of starch-entrapped beads

The *in vitro* digestibility of starch-entrapped alginate based beads was determined based on a slightly modified method of Englyst *et al* (2001). Pancreatin (3.0 g) was added to 36 mL of distilled water and stirred at 450 rpm for 10 min. The dispersion of pancreatin was centrifuged at 1,500 g for 10 min at 4°C. The supernatant (30 mL) and amyloglucosidase (0.6 mL) were mixed with 5.4 mL of distilled water in a beaker. Starch-entrapped beads samples (0.9 g) were placed in each of 50 mL conical tubes with three glass beads. A 2.25 mL aliquot of 0.1M sodium acetate buffer was added, and vortexed. Samples were placed in a shaking incubator (240 rpm, 37°C) for 10 min prior to a digestion experiment. At the same time, enzyme solution was also equilibrated in a water bath at 37°C for 10 min.

To determine the bead digestibility, 2.25 mL of enzyme solution was added to each of conical tubes, and the samples were incubated in a shaking incubator for 30, 60, 120, 180, 240, 300, and 360 min. The reaction was then

terminated by boiling samples for 10 min. To determine the glucose released from starch, the conical tube was centrifuged at 10,000 g for 10 min and glucose concentration of hydrolyzates was measured using a GOD-POD kit.

2-8. Stereomicroscopy

A stereomicroscope (stemi-DV4, Car Zeiss, Oberkochen, Germany) was used to examine the size and images of psyllium husk powder-starch-alginate beads.

2-9. Surface morphology of starch entrapped beads: scanning electron microscope (SEM) analysis

The starch-entrapped bead samples reinforced with PSY were examined using a scanning electron microscope (FESEM SUPRA 55VP, Carl Zeiss, Oberkochen, Germany). All of the bead samples were prepared as described in section 2-6. Prior to the SEM analysis, all of the bead samples were freeze-dried and placed on stub, then coated with copper palladium.

2-10. Statistical Analysis

All experimental data were analyzed using analysis of variance (ANOVA) and expressed as mean ± standard deviation of replicate measurement. Significant differences among mean values were compared using the Duncan's multiple range test ($p < 0.05$). Statistical analysis was conducted by IBM SPSS statistics version 21.0 (IBM, Armonk, NY, USA)

RESULTS AND DISCUSSION

1. *In vitro* digestibility of normal corn starches within soluble dietary fiber mixed system

Inhibitory factor affecting starch digestion may be attributed to either insoluble or soluble dietary fiber concentration (Braaten *et al*., 1994; Chau *et al*., 2003; Zhang *et al*., 2016). However, it has not been clearly revealed what extent varying concentrations of psyllium husk powder, a type of soluble dietary fiber, influence the starch digestion. Hence, the *in vitro* digestibility of NCS was examined at 2 different PSY concentrations as a function of incubation period, and the data are given in Table 1.

Figure 1 shows hydrolysis of starch curves for PSY- NCS mixtures that were compared with the NCS. The control had the highest starch digestion (87.96 %) at 360 min. However, the degree of starch digestion of samples mixed with PSY always remained lower among samples than the control. The increase in PSY concentration from 1 % to 2% predominantly lowered the *in vitro* digestibility of starch. The higher the concentration of PSY, the higher the ability to retard the starch digestion occurs. The PSY did affect lowering starch digestion due to its several characteristics; fibrous,

hydrophilic, highly viscous, and mucilaginous properties when in contact with water (Kaialy *et al*., 2013). Especially, PSY is extremely hygroscopic, having the ability to swell 10 to 14 folds of its original volume. This hygroscopic characteristic of PSY will possibly impart viscous solution entrapping the starch molecules (Cybulska and Doe, 2002). Viscous soluble dietary fiber helps to reduce the *in vitro* digestion of starch, reducing the interaction with amylase. However, still an exact mechanism of how the PSY contribute to the overall digestibility of starch remains uncertain despite the several research reports (Isaksson *et al*., 1982; Dunaiff and Schneeman, 1981).

Hence, Table 1 explains functional role of PSY on starch digestion with specific numerical values. The NCS reached a plateau at 120 min of digestion (73.07 %). The digestibility of control (30.81%) at 30 min was more than 1.6 folds the level for PSY 2% (w/v) (19.27%), which presumably reflects the time available for a rapid breakdown of starch into glucose and absorption occurring in the small intestine (Englyst *et al*., 1996). As shown in Table 1, the level of starch digestion (38.21%) of PSY 1% mixture for 180 min was almost identical to that of PSY 2% containing the starch digested (38.51%) for 360 min. Increasing PSY concentration can delay starch

digesting time up to 4 hrs when compared to NCS only sample. No significant ($p > 0.05$) differences were observed between the degree of starch digestion of PSY 1%, and PSY 2%. Thus, manipulating and a further test on the PSY concentrations with a range between 1% and 2% might be required to develop an advantageous food targeting materials for controlling the starch digesting time.

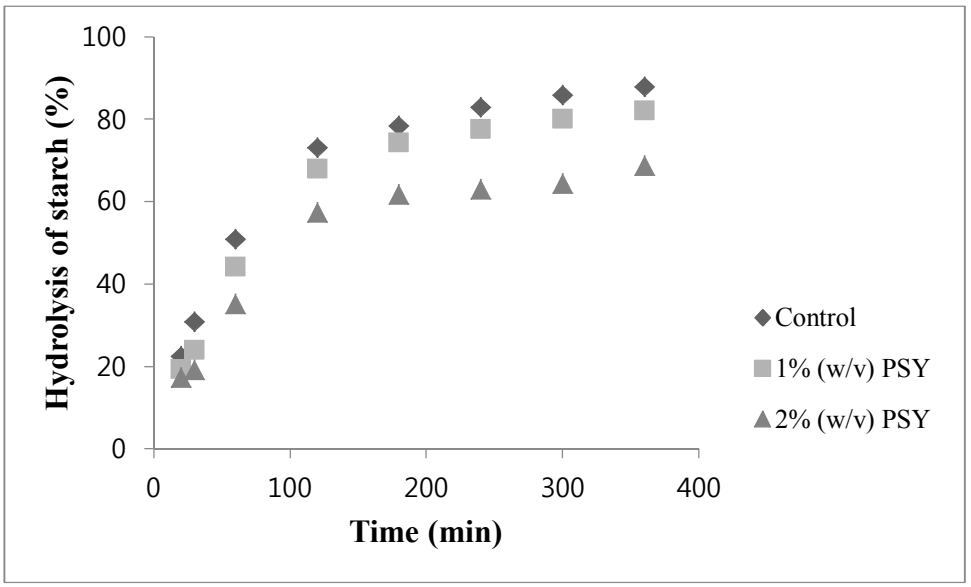


Figure 1. Digestion pattern of NCS, 1% (w/v) PSY, and 2% (w/v) PSY (0-360 min). Control = normal corn starch (NCS); PSY 1% = NCS within 1 % (w/v) PSY concentration system; PSY 2% = NCS within 2 % (w/v) PSY concentration system

Table 1. Extent of starch digestion at different levels of psyllium husk powder

	Extent of starch digestion								
Sample[1)]	10 min	20 min	30 min	60 min	120 min	180 min	240 min	300 min	360 min
Control	17.46[a] ±0.30	22.52[a] ±0.43	30.81[a] ±0.65	50.85[a] ±0.16	73.07[a] ±0.65	78.46[a] ±0.57	82.93[a] ±0.67	85.90[a] ±0.26	87.96[a] ±0.65
1% (w/v) PSY	16.23[b] ±0.40	19.38[b] ±0.23	24.10[b] ±0.36	44.25[b] ±0.50	68.06[b] ±0.84	74.33[b] ±0.51	77.68[b] ±0.41	80.08[b] ±0.19	82.07[b] ±0.78
2% (w/v) PSY	16.51[b] ±0.59	17.34[c] ±0.12	19.27[c] ±0.49	35.18[c] ±0.78	57.41[c] ±0.18	61.77[c] ±0.48	63.06[c] ±0.76	64.48[c] ±0.77	68.76[c] ±0.19

[1)] Control = normal corn starch (NCS); PSY 1% = NCS within 1 % (w/v) PSY concentration system; PSY 2% = NCS within 2 % (w/v) PSY concentration system.

[2)] The mean values with different superscripts in columns differ significantly from each other ($p<0.05$)

2. Glucose permeability within dialysis tubing systems

2.1. Determination of glucose-absorption capacity

Figure 2 and Table 2 demonstrate the static model of glucose absorption by facilitated diffusion (Thorens, 1993). Hydrolysis of α-1, 4 and α-1, 6 glycosidic bonds occur in the small intestine, resulting in the breakdown of starch into glucose molecules (Englyst *et al*., 1992). In the small intestine, not only the digestion occurs, but also the absorption takes place (Thorens, 1993). A testing hypothesis was that viscosity of samples is the primary rate-determining parameter for dynamic absorption of glucose into human blood cell after starch digested. Hence, the dialysis tubing system further investigates if the variations in PSY concentration as a method of modifying the viscosity would delay glucose absorption.

The permeability of glucose increased over time in all of the samples (Figure 2, Table 2). The viscosity of glucose solution transferred to a dialysis tube depends on one of two important factors: concentration of the soluble dietary fiber or molecular weight (MW) of soluble dietary fiber (Zhang *et al*, 2016). According to Zhang *et al*. (2016), the effect of different oat β-glucan concentrations (5 mg/mL to 10 mg/mL) on diffusion rate is greater than the

effect of different OBG MW (OBG 500, 40, 30, 18, and 7 g mol^{-1}). The allowed level of PSY concentration treatments was set from 0% (w/v) to 2% (w/v) mainly considering its highly viscous gel-forming structure with a minute dose.

Variation in PSY concentration was used to compare the glucose diffusivity in samples since it has been suggested that viscous form of dietary fiber improves blood glucose lowering effect (Jenkin *et al*., 1978; Vuksan *et al*., 2000; Torsdottir *et al*., 1991). As shown in Table 2, glucose concentration in the permeate solution at 2% (w/v) PSY system was dropped to 50% of control at the first 30 min. The glucose concentration in the permeate solution from 2% (w/v) PSY system (29.86 mg/ml) was lower than that from 1% (w/v) PSY system (40.32 mg/ml) at 180 min. The reduction in concentration of glucose in the permeate solution revealed that the PSY concentration does significantly affect retardation of glucose absorption (P<0.05). The hampering effect of PSY on diffusion of glucose through dialysis membrane could be mainly due to an increase in viscosity (Ahmed *et al*., 2011; Zhang *et al*., 201). Viscosity means resistance to flow; the migration of glucose can be diminished by PSY due to its viscosity. Viscous solutions reduce the mixing effects of gastro-intestinal contractions (Jarjis *et*

al., 1984). The addition of 2% PSY allowed 82.2 % (38.51 mg/mL) drop of migrated glucose concentration from a control sample (46.85 mg/mL) at 360 min. The permeate glucose concentration from 1% PSY containing system at 120 min was 83.54% of control, while that of 2$ PSY system was 60.54%, compared with control. Variation in PSY concentration was used to compare the glucose diffusivity in samples since it has been suggested that viscous form of dietary fiber improves blood glucose lowering effect (Vuksan *et al*., 2000; Torsdottir *et al*., 1991). Overall, Table 1 showed a continuous decline in the glucose concentrations from 1% (w/v) and 2% (w/v) PSY system compared to the control. This continuous reduction at each of time interval is expected because the increase of PSY concentration as the viscosity of soluble dietary fiber mainly contributes to the retardation of glucose uptake. Once the viscosity increases, the glucose molecules are trapped within the highly viscous portion of products, delaying their access to digestive enzymes (Asp, 1996). However, not all soluble dietary fibers show the same physiological effect. For example, it was observed that guar gum does reduce postprandial glycaemia, but does not highly influence stool bulking. PSY, though, has shown both of glucose lowering effect and of laxative agent effect (Vinik and Jenkins, 1988).

Table 2. Glucose concentration in the permeate solution at different levels of psyllium husk powder system

	Glucose concentration (mg/mL)						
Sample[1)]	30 min	60 min	120 min	180 min	240 min	300 min	360 min
Control	22.43±0.41[a]	33.41±0.16[a]	42.89±0.88[a]	45.62±0.24[a]	46.05±0.44[a]	46.46±0.55[a]	47.74±1.31[a]
1% (w/v) PSY	16.82±0.27[b]	26.61±0.18[b]	34.77±0.87[b]	40.32±0.56[b]	40.88±0.45[b]	42.66±0.68[b]	43.88±0.60[b]
2% (w/v) PSY	11.40±0.37[c]	18.52±0.91[c]	25.20±0.99[c]	29.86±0.68[c]	35.09±0.55[c]	36.75±0.53[c]	38.51±0.21[c]

[1)] Control = 2% (w/v) glucose solution; 1% (w/v) PSY = 1 % (w/v) PSY concentration system based on 2% (w/v) glucose solution; 2% (w/v) PSY = 2 % (w/v) PSY concentration system based on 2% (w/v) glucose solution

[2)] The mean values with different superscripts in a column differ significantly from each other ($p<0.05$)

3. Determination of glucose dialysis retardation index (GDRI)

The glucose dialysis retardation index (GDRI) values at two PSY concentration systems (1% w/v and 2% w/v) are a useful tool to discern that the glucose absorption in the small intestine was not only contributed by viscosity of fibers, but also by the PSY adsorption capacities (Ou et *al*., 2001; Ahmed *et al*., 2011). In order to determine the functionality of PSY and its effect on the glucose absorption with respect to time, glucose adsorption capacity was determined; the GDRI values of 1% PSY and of 2 % PSY samples are compared in Figure 2.

The GDRI of 2% PSY sample was approximately two-fold greater than that of 1% PSY sample after 30 min incubation period. The GDRI values of 2 % PSY system were generally higher than the values for 1% PSY system in overall reaction (0-180 min). Hence, samples within 2% PSY system exhibited greater effect on the delay in glucose absorption than the 1% PSY system. Figure 2 shows that 2 % PSY system significantly contributes to higher glucose adsorption capacity over time ($p<0.05$). The glucose adsorption capacity of 2 % PSY system was approximately 3- fold higher than that of 1 % PSY system after 180 min. Since the PSY forms highly viscous gel as it comes into contact with water, viscosity induced from PSY

may hinder the glucose molecules to penetrate through dialysis membrane tube. The glucose molecules were entrapped within highly viscous form of fiber matrix, which may lead to the retarding glucose absorption in small intestine (Jenkins *et al*., 1978).

Table 3 shows that the glucose adsorption capacity increased with an increase in glucose concentration. Since the 2 % PSY system has shown greater effect on glucose adsorption capacity than the 1 % PSY system, different glucose concentrations (1%, 2%, 3%, and 5%) were examined based on the 2 % PSY system. The 2% PSY system could bind glucose both at a lower concentration of glucose (1% w/v) and at a higher concentration of glucose (5% w/v). The values of glucose molecules bound to fiber ranged from 20.54 to 79.95.

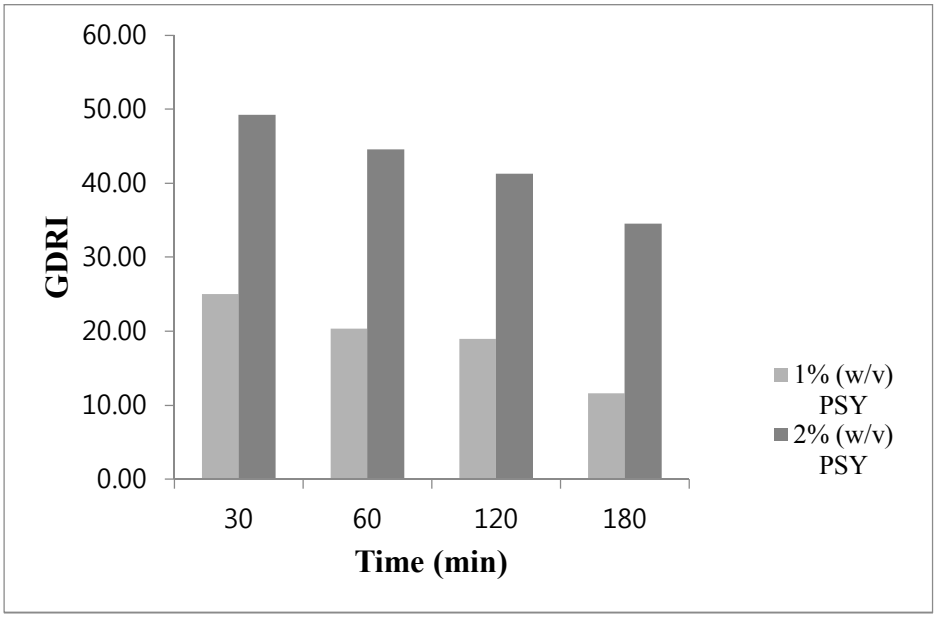


Fig 2. The effects of psyllium husk powder concentration on glucose dialysis retardation index (GDRI) as a function of reaction time

Table 3. Glucose adsorption capacity at 2% PSY concentration system in different glucose concentration

	Glucose bound			
Glucose solution concentration (w/v)	1%	2%	3%	5%
2% (w/v) PSY system	20.54± 0.74[d]	38.50± 0.21[c]	56.59± 0.72[b]	79.95± 0.54[a]

[1)] The mean values with different superscripts differ significantly from each other (p<0.05).

4. Rheological properties-time sweep analysis

Table 4 and Table 5 show the rheological characteristics including apparent viscosity (Pa·s), the loss modulus (Pa), and storage modulus (Pa) of samples. The results found that time sweep experiment for starch and soluble dietary fiber mixture samples do not translate to the similar trends in apparent viscosity, storage, and loss moduli values (Dartois *et al*., 2010). Dartois *et al* (2010) have previously suggested that the pattern of storage modulus, and loss modulus values for starch-guar gum samples were stable as 1.41 Pa and 1.12 Pa, respectively similar to the guar gum only sample (21.20 Pa and 15.75 Pa, respectively) during the 50 min of the experiment. At 1800 s, the apparent viscosity of NCS-PSY mixtures increased from 61.90 Pa·s to 319.35 Pa·s as the PSY concentration changed from 1% (w/v) to 2% (w/v). The apparent viscosity of PSY (1:2) mixture sample increased from 168.19 Pa·s to 184.51 Pa·s, and that of PSY (1:3) mixture sample increased from 259.26 Pa·s to 319.35 Pa·s over the range of 300s -1800s. As the PSY content level increased, the apparent viscosity continuously increased as well during 1800 s, significantly contributing to overall rheological flow behavior. PSY plays an important role in reducing digestibility of starch and glucose permeability because of its large contribution to overall viscosity.

Table 5 shows *G'* and *G"* values obtained by a time sweep analysis (0-1800 s) for PSY and NCS-PSY mixture samples. Both the values of *G'* and *G"* were reduced approximately 90 % by 1800 s. The relationship between *G'* and *G"* helps to understand the strength of mixed NCS-PSY systems at 37°C. The *G"* values of PSY sample were higher than the *G"* during 1800 s measurements. This implies that the PSY suspension itself exhibited solid-like behavior (Farahnaky *et al*., 2010). A previous study revealed that psyllium gel is considered to be a weak gel because *G'* is larger than *G"* for all mucilage concentrations (Farahnaky *et al*., 2010). The significant increase in both *G'* and *G"* at each time intervals (30, 600, 1200, and 1800) was observed when the PSY content level within the sample mixture became triple. It implied that rheological behavior of NCS could be changed by PSY addition, and the amount of PSY affected the level of change. At 1800 s, both *G"* values of PSY and NCS:PSY (1:1) samples decreased over time while both *G'* and *G"* values of PSY (1:3) increased. Considering that both *G'* and *G"* were generally increasing in accordance with apparent viscosity over time, an observed increase illustrates that NCS-PSY mixture behavior can be changed by PSY concentration.

Table 4. Changes in apparent viscosity of psyllium husk powder (PSY); normal corn starches and psyllium husk powder mixtures at varying PSY contents

Time (s)	Apparent viscosity (Pa·s)			
	PSY	PSY (1:1)	PSY (1:2)	PSY (1:3)
300	55.79±6.60[a]	64.43±3.25[ab]	168.19±5.18[b]	259.26±10.57[d]
600	58.56±0.53[a]	69.36±2.65[a]	177.48±5.53[ab]	276.84±10.53[c]
1200	56.01±1.00[a]	65.89±2.86[ab]	176.55±5.00[ab]	301.57±8.84[b]
1800	57.24±2.71[a]	61.90±3.03[a]	184.51±4.05[a]	319.35±5.54[a]

[1)] PSY (control) = 0.09 g of psyllium husk powder; PSY (1:1) = 4% (w/v) PSY concentration; PSY (1:2) = 8% (w/v) PSY concentration; PSY (1:3) =12% (w/v) PSY concentration.

[2)] The mean values with different superscripts in a column differ significantly from each other (p<0.05)

Table 5. Changes in rheological properties of psyllium husk powder (PSY); normal corn starches in the varying psyllium husk powder (PSY) contents

	G' (Pa)				*G"* (Pa)			
Sample/ Time (s)	PSY	PSY (1:1)	PSY (1:2)	PSY (1:3)	PSY	PSY (1:1)	PSY (1:2)	PSY (1:3)
300	294.80±11.09[a]	304.49±4.05[a]	779.49±2.10[b]	1163.14±52.51[c]	231.70±0.62[a]	206.67±7.50[c]	535.48±22.70[c]	865.92±42.12[b]
600	279.35±11.26[zb]	289.96±3.99[b]	931.50±19.91[a]	1213.77±54.03[c]	218.96±0.34[a]	265.27±11.51[b]	617.16±7.25[b]	889.02±49.86[b]
1200	273.05±8.25[b]	267.80±7.60[c]	956.17±21.74[a]	1396.22±54.53[b]	212.12±3.02[a]	269.85±6.74[ab]	671.95±20.62[a]	936.04±43.65[b]
1800	274.20±3.00[b]	252.28±6.67[d]	978.74±40.68[a]	1719.13±53.14[a]	205.43±3.89[a]	281.90±5.03[a]	675.07±19.77[a]	1090.91±64.02[a]

[1)] PSY (control) = 0.09 g of psyllium husk powder; PSY (1:1) = 4% (w/v) PSY concentration; PSY (1:2) = 8% (w/v) PSY concentration; PSY (1:3) =12% (w/v) PSY concentration. [Storage modulus, loss modulus; *G'* (Pa), *G"*(Pa)]

[2)] The mean values with different superscripts in a column differ significantly from each other ($p<0.05$)

5. *In vitro* digestibility of starch-entrapped alginate based beads

The loading of normal corn starch onto psyllium based polymeric matrix for controlled glucose release was performed in this section. The influence of increased psyllium husk powder concentrations on the *in vitro* digestibility of PSY-starch-alginate beads is numerically described in Table 6. PSY contains highly viscous soluble dietary fiber and it has been proposed as a possible treatment for high blood glucose levels (Chau *et al*., 2003; Gohil *et al*., 2014; Liu *et al*., 2010; Singh *et al*., 2010). Compared with the control bead, the higher PSY concentration showed significantly higher glucose lowering effects (P<0.05). The incorporation of 2 % PSY from 0 % PSY led to 73.44% glucose reduction. The released glucose level elevated while it decreased 44.00 % and 55.60 % for 1% PSY and 1.5 % PSY-containing beads, respectively after 30 min. The total released glucose concentration in the control at the end of *in vitro* digestion period was 20.32 mg/mL, while it reached 16.93 mg/mL for the 2 % PSY samples. In previous studies, psyllium husk powder as a source of soluble dietary fiber decreased postprandial glucose concentrations with a minute single dose of psyllium husk powder (Jenkins *et al*., 2000; Wolever *et al*., 1991; Watters and Blaisdell, 1989; Uribe *et al*., 1985; Ziai *et al*., 2005).

As shown in Table 6, the addition of PSY to the starch-entrapped alginate based beads clearly affected the final values of hydrolysis to a major extent. The most reasonable cause for this was that the starch granules were tightly packed inside the highly viscous form of soluble dietary fiber from psyllium husk powder. In the case of 2% (w/v) PSY bead samples, the highly viscous form of PSY would have restricted the surface of starch granules from access of enzymes. PSY shows the most significant effect with the least adverse effects among viscous soluble dietary fibers (Pastors *et al*., 1991). It has also been suggested that the reduction in released glucose concentration from bead samples is primarily due to the swelling extent (Jenkins *et al*., 2000; Wolever *et al*., 1991). One of the most important factors that affect the swelling of polymeric networks was the components of copolymers. The arabinoxylan, the main constituent of psyllium husk powder, has the swelling characteristics, providing greater viscosity in the intestine than other types of soluble dietary fiber (Pastors *et al*., 1991). Consequently, the physical properties of PSY including water holding capacity, viscosity, or swelling capacity not only determine the rate of glucose release during a food process, but also affect the quality of the final products.

Table 6. Concentration of glucose released from psyllium husk powder-starch-alginates based beads

	Glucose concentration (mg/mL)						
Time (min) Sample/	30	60	120	180	240	300	360
Control	2.41±0.20^{f}	7.40±0.20^{e}	13.26±0.11^{d}	16.47±0.49^{c}	18.74±0.20^{b}	19.50±0.66^{ab}	20.32±0.50^{a}
1% PSY	1.35±0.17^{e}	5.61±0.47^{d}	12.27±0.48^{c}	15.86±2.62^{b}	17.46±1.66^{ab}	17.53±0.96^{ab}	19.03±1.30^{a}
1.5% PSY	1.07±0.34^{e}	4.76±0.46^{d}	11.31±0.80^{c}	14.03±0.23^{b}	15.67±0.60^{a}	16.09±0.45^{a}	16.87±0.47^{a}
2% PSY	0.64±0.04^{f}	4.25±0.32^{e}	10.57±0.29^{d}	14.33±0.63^{c}	14.57±0.46^{bc}	15.63±0.36^{b}	16.93±0.50^{a}

[1)] Control = normal corn starch entrapped alginates based beads; 1% (w/v) PSY=1% (w/v) PSY concentration; 1.5% (w/v) PSY=1.5 % (w/v) PSY concentration; 2% (w/v) PSY=2% (w/v) PSY concentration starch-alginate based beads.

[2)] The mean values with different superscripts in a column differ significantly from each other (p<0.05).

6. Shape and size of psyllium husk powder-starch-alginate based-beads

Photographs of freshly prepared psyllium husk powder-starch-alginate beads are presented in Figure 3. Selecting the proper concentration of sodium alginate (2% w/v) and starch (2% w/v) provided uniform bead formation throughout the experiment. The control, 1% (w/v) PSY beads, and 2% (w/v) PSY beads showed spherical shape with a mean diameter of 4.00 mm. After oven drying (45 °C, 12 hr), sodium alginates bead, starch-alginate beads, and PSY-starch-alginate beads showed a decreased average diameter, 2.00 mm. Drying involves physical stress, causing a water outflow from structure of beads. The appropriate size of beads is an important determining factor for stability and reactivity of food in solutions (Gbassi *et al.*, 2013). Since the Food and Drug Administration of the USA (FDA) published the appropriate maximal bead size as 2.8 mm (10 % variation of the target, 2.5 mm) based on chewing and swallowing particle size, the resulted dried-beads sample (2.00 mm) was well suited to the ranges of defined bead size (FDA, 2012). Unlike the fresh bead samples, oven-dried bead samples showed irregular shape. Figure 3c displays a more oblate or prolate spheroid shape of beads. The inclusion of normal corn starch gave the most regular shape where horizontal section reaches a line of circle (Figure 3b) while lopsided shape of

beads (Figure 3a) were formed for the alginate-based beads without starches or soluble dietary fiber sources (Dehaven *et al*., 2014).

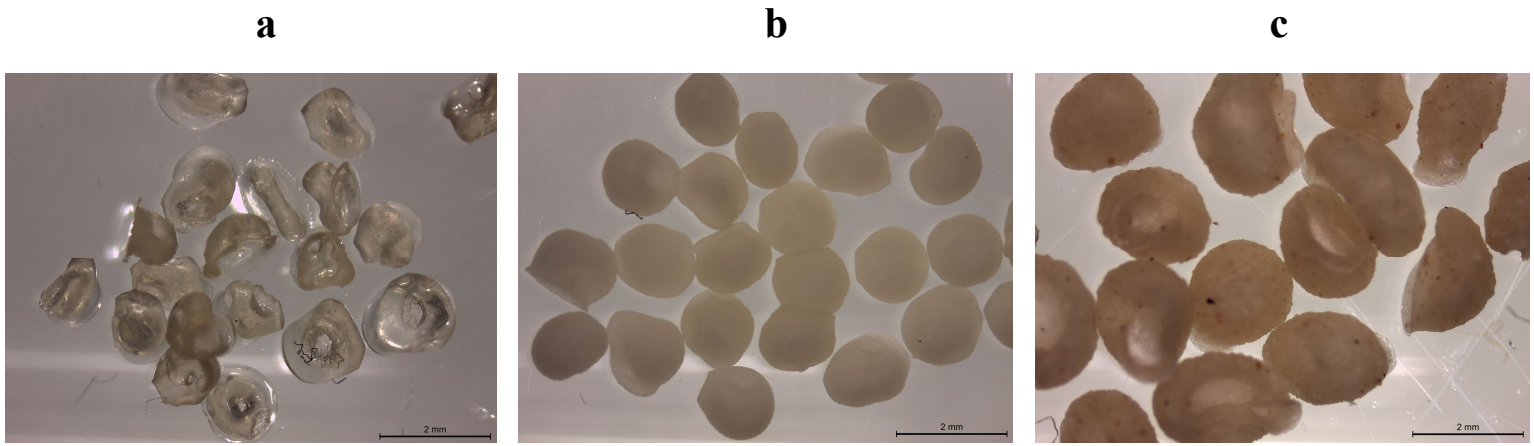


Figure 3. Photographs of oven-dried beads of polysaccharides at the magnification of 8× a) sodium alginate beads b) starch entrapped alginate beads c) psyllium husk powder-starch-alginates based beads

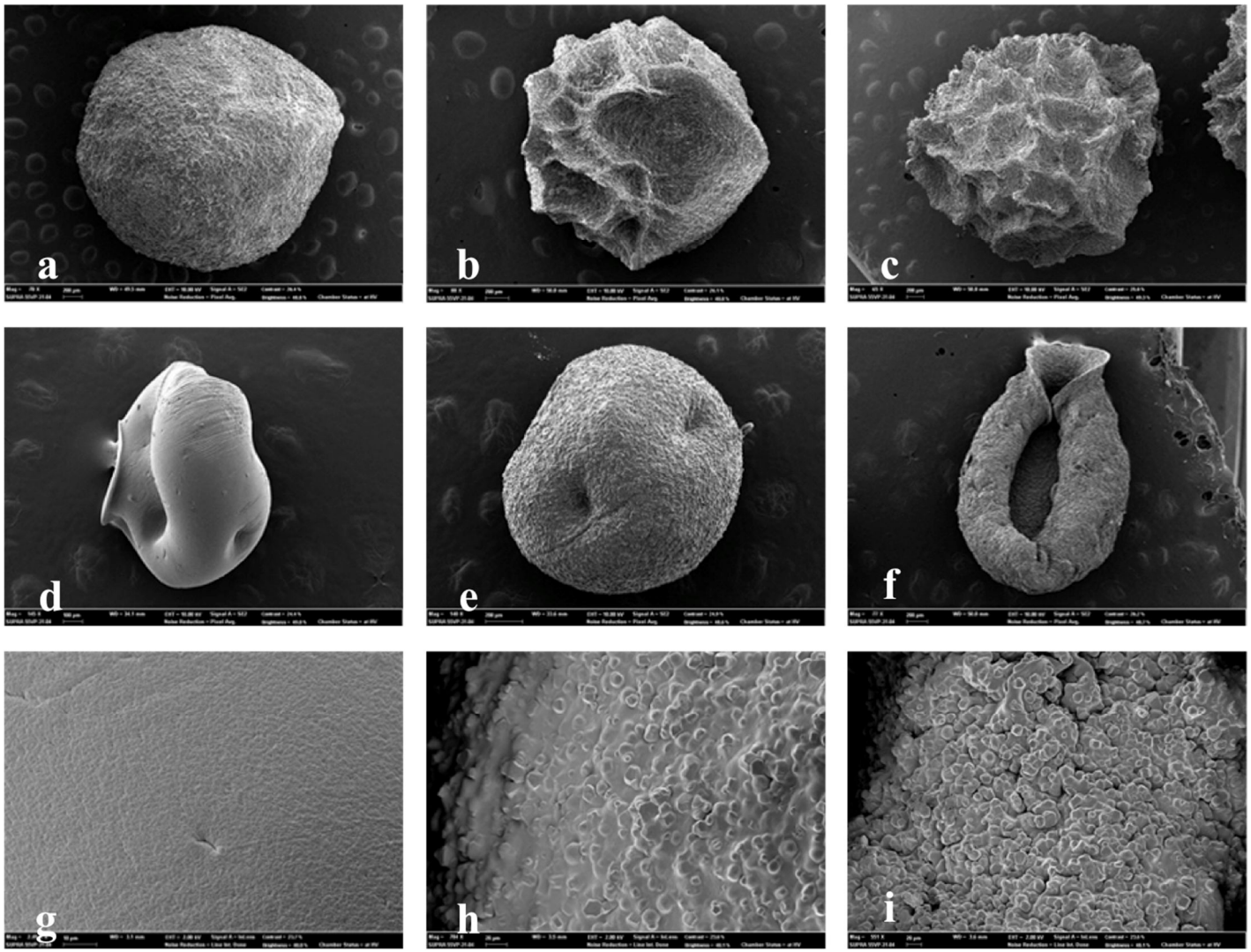
7. Surface morphological properties of starch-entrapped alginate based beads

The surface morphologies of PSY-starch-alginate beads observed by SEM at different magnifications are presented in Figure 8. Freeze-dried bead samples were predominantly different from those dried in an oven. After freeze-drying, the most spherical shape was observed (Figure. 4a). However, the beads formulated with PSY generally showed uneven and rugged features (Figure. 4b and Figure. 4c). The 2% (w/v) PSY beads sample (Figure. 4c) showed the coarsest surface. The main contribution to the irregular shape of beads after freeze-drying is the bead's water content before freeze-drying (Nussinovitch *et al*., 2004). As the PSY concentration increased, the beads' water content increased due to the hygroscopic characteristic of PSY. The water evaporation occurs quickly with a freeze-drying method, whereas it slowly progressed under oven-drying conditions. This slow water movement caused the colloidal particles to settle slowly, producing a more spherical and smooth shaped beads. Figure 4c revealed a surface structure, imperfect honeycomb- like structure observed from lyophilized arabinoxylan hydrogels containing caffeine (Iravani *et al,* 2011). Another reason for rough shape with honeycomb-like structure was the influence of high molecular weight of soluble dietary fiber. The inclusion of

smaller-sized fillers results a smoother surface whereas that of larger-sized fillers result a rougher surface (Nussinovitch *et al*., 2003). Since PSY includes an enormously high molecular weight component, arabinoxylan compared to other types of soluble dietary fiber, rougher features on the surface of beads are presumably expected. The projections appeared on the surface, but collapse was not shown due to the inclusion of other polysaccharides, starch and alginates (Figure 4c) (Rassis *et al*., 2002; Radley, 2012). Therefore, PSY seemed to be a potential inert filler to interact with different components of polysaccharides that can be used to tune the surface structure.

Figure. 4d represents oven-dried alginates based beads without the inclusion of fillers such as starch or PSY, showing a shrinked surface with a collapse. Figure. 4e represents a more round shape with a less shrinkage. In this case, starch acted as an inert filler even after oven-drying process. However, Figure. 4f displayed an irreversible structure with a dramatic change in shape of beads after oven drying. This folded structure is possibly attributed to the heterogeneous gelation mechanism. During the gelation process, alginate diffused from the core toward the interface between droplets and gelling solution in order to form shell structure with calcium ions. Hence, a heterogeneous structure of beads possibly resulted in a loose

core, leading to a collapse of beads during the drying process (Santagapita *et al*., 2011).


a
b
c
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e
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g
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i

Figure 4. Surface morphology of freeze-dried beads; a) normal corn starch-entrapped alginate based beads (magnification=70×), b) 1% (w/v) PSY starch-alginates based beads (magnification=88×), c) 2% (w/v) PSY starch-alginates based beads (magnification=65×); surface morphology of oven-dried beads; d) sodium alginates beads (magnification=167×), e) normal corn starch-entrapped alginates based beads (magnification=140×), f) 2% (w/v) PSY starch-alginates based beads (magnification=77×), g) sodium alginates beads (magnification=2.49 K×), h) normal corn starch-entrapped alginates based beads (magnification=784 ×), i) 2% (w/v) PSY starch-alginates based beads (magnification=551×).

CONCLUSION

The influence of psyllium husk powder on the physicochemical and digestion properties of normal corn starches was examined. Formulating starches with an increasing level of psyllium husk powder in the simple suspension significantly delayed the starch digesting time and showed a more pronounced glucose permeability lowering effects in glucose dialysis membrane. Since arabinoxylan is the main constituent of psyllium husk powder, which imparts high water holding capacity and swelling capacity, viscosity in the small intestines was the primary reason for the slow release of glucose over digesting periods. As a result, binary mixtures of starch and psyllium husk powder with sodium alginate by ionotropic gelation methods can be utilized as a material for controlling a substantial swelling or rapid enzymatic degradation of native starch in biological systems, masking the poor palatability of psyllium husk powder.

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국문초록

천연 옥수수 전분은 많은 가공 식품의 제조 원료로서 사용되며, 타 산업 부문에서도 다양한 용도로 쓰이고 있다. 그러나 생전분은 산업적 제조 과정에서 공정에 대한 저항성이 낮아 효율적으로 활용되고 있지 않다. 생전분과 가용성 식이섬유의 혼합물은 전분의 낮은 가공 적성을 향상시키는 효과적인 방법으로 간주된다. 따라서 이 연구에서는 수용성 식이 섬유의 함량이 많은 차전자피가루를 옥수수 전분과 혼합하였을 때 나타나는 소화 및 이화학 특성을 밝히며, 아울러 이를 바탕으로 하여 차전자피가루-전분-알진산 혼합물 소재를 개발하고자 하였다. 혼합물의 소화특성을 분석한 결과, 차전자피가루를 2% 농도로 첨가한 경우 전분의 소화 시간을 최대 4 시간까지 연장시키는 효과를 나타내었으며, 글루코스 흡착능 또한 20.54 에서 79.95 로 크게 증가하였다. 차전자피가루는 물과 접촉하여 높은 점성을 보였으며, 혼합물의 유변학적인 거동에 크게 영향을 미쳤다. 이로 인해 효소의 접근성을 저해함으로써 소화성이 감소되었다. 차전자피가루와 옥수수 전분 혼합물을 알진산으로 결합하여

비드를 형성하였을 때에도, 대조구에 비해 전분의 소화에 따른 포도당의 방출이 유의적으로 감소하였다 (p<0.05). 결론적으로, 차전자피가루-전분-알긴산 혼합물 비드는 글루코스의 방출 정도를 조절할 수 있는 새로운 소재로서 식품산업에서 다양한 용도로 활용할 수 있을 것이다.

주요어: 옥수수전분, 차전자피가루, 알진산, 전분 소화성, 점도

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