RESEARCH ARTICLE

Encapsulated amylosucrase-treated starch with enhanced thermal stability: Preparation and susceptibility to digestion

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Understanding in vitro digestibility and release experiments with starch-entrapped calcium alginate microspheres provide the possibility of controlling starch digestibility in the human gastrointestinal tract. Native and amylosucrase (AS)-treated waxy corn starches (5000 U, 20 000 U, and control) were encapsulated with three different ratios of sodium alginate: 0.5, 0.7, and 1.0%. The slowly digestible and RS fractions of the encapsulated starch were 57.5 and 97.7%, respectively. After cooking, the proportions of these fractions still ranged from 55.7 to 96.1%, depending on the type of starch and concentration of sodium alginate, whereas the un-encapsulated starch contained between 2.9 and 48.3% slowly digestible and RS after cooking. In vitro release was studied in both uncooked and cooked encapsulated samples. When incubated in simulated gastric fluid, the beads swelled and started to float but did not erode, even after thermal treatment. Incubation in simulated intestinal and colonic fluids caused the beads to release starch in both uncooked and cooked starch-encapsulated samples. Encapsulated AS control and native waxy corn starch samples released much more starch than AS-modified starches. The total amount of starch released did not exceed 20% for uncooked samples and 25% for cooked samples.

Keywords:
Alginate beads / Amylosucrase / Encapsulated starch / Low glycemic properties / Thermal stability

1 Introduction

Starch is a primary carbohydrate, an important source of human nutrition that is digested by pancreatic α-amylase in the gastrointestinal (GI) tract. A popular method to analyze the kinetics of starch digestion has been reported by Englyst et al. [1]. Starch is categorized according to the rate of glucose release and absorption into rapidly digestible starch (RDS, the amount of glucose released within 20 min of hydrolysis), slowly digestible starch (SDS, the amount of glucose released between 20 and 120 min of hydrolysis), and RS (the starch portion that is not digested in the small intestine). SDS leads to a slower entry of glucose into the bloodstream. Therefore, SDS can stabilize and sustain blood glucose concentration and provide physiological advantages in diabetes management, mental performance, and satiety. As described above, RS is the starch fraction that is not digested in the small intestine but is fermented in the large intestine by colonic bacteria, with beneficial health functions including hypoglycemic and hypocholesterolemic effects, inhibition of fat accumulation, and absorption of minerals [2, 3]. Both SDS and RS have a low glycemic index, which has a moderate but advantageous impact on postprandial blood glucose and on sustained blood glucose levels, and they reduce the risk of developing diabetes and cardiovascular diseases [2, 3]. In contrast, RDS is rapidly digested and absorbed in the small intestine, leading to a rapid increase followed by an equally rapid drop in blood glucose levels.

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Abbreviations: GI, gastrointestinal; **RDS,** rapidly digestible starch; **SCF,** simulated colonic fluid; **SDS,** slowly digestible starch; **SGF,** simulated gastric fluid; **SIF,** simulated intestinal fluid.

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Colour online: See the article online to view Fig. 1 in colour.

The preparation of SDS and RS mainly by heat, chemical, and enzymatic treatments has been reported for starches from various sources [4–6]. As enzymatic modifications, Shin et al. [4] reported that waxy and normal starches treated with amylosucrase (AS) from *Neisseria polysaccharea* showed an increase in the SDS fraction by up to 25%. A particular action of AS is the elongation of amylose and branched chains of amylopectin by 13–19 glucose units. Because of its action, the proportion of the branched chains with short and/or medium length decreases, and the proportion of long branched chains increases. Previous studies showed that the structural changes by AS could correlate positively and/or negatively with resistance of starch molecules to hydrolytic enzymes [4, 7]. Especially, an extended branched chain in amylopectin molecules can interrupt the formation of crystallites, resulting in a less perfect crystalline structure that favors the formation of the SDS fraction [4, 5, 7].

Despite research on SDS preparations, no commercial SDS products are available on the market due to their thermal instability. Since the SDS fraction consists of less perfect crystallites with a lower density and amorphous components, it can be transformed into RDS after thermal processing [7]. Hence, improving the thermal stability of SDS is required to retain its beneficial physiological functionalities as a food ingredient.

Alginate is a natural biopolymer extracted from brown algae. In the presence of divalent cations like Ca^{2+}, alginate gels easily due to the cross-linking of guluronic acid with divalent cations after the formation of an egg-box junction [8]. This alginate system has been studied for food industry applications to encapsulate, protect, and deliver bioactive food components such as antimicrobials, antioxidants, flavors, lipids, and raw starch [9, 10]. Studies on the behavior of alginate beads in the GI tract showed that they resist the low pH of the stomach but swell at the neutral pH of the small intestine [11]. Recently, Venkatachalam et al. [12] employed a calcium alginate system to coat native starch, to examine how it can control glucose release in the human GI tract, and reported that alginate starch beads efficiently controlled the glucose release and remarkably increased the SDS and RS fractions, which would attenuate glycemic and insulinemic responses in vivo.

Therefore, encapsulated microspheres with improved thermal stability that contain high amounts of SDS and RS might be useful to slow glucose release and aid metabolic regulation of both hyperglycemia and hypoglycemia.

In the present study, we modified waxy corn starch using AS to increase the portions of SDS and RS, and then encapsulated it with calcium alginate to protect the starch and control access to starch-digesting enzymes. We also examined the susceptibility to digestion of encapsulated native and AS-treated starches in simulated GI fluids to assess the behavior of calcium alginate-coated starch beads in the human GI tract.

2 Materials and methods

2.1 Materials

Waxy corn starch was purchased from Samyang Genex Corp. (Incheon, Korea). Calcium chloride dehydrate was obtained from Showa Chemical Co., Ltd. (Tokyo, Japan) and sodium alginate from Osaka Chemical Co. (Osaka, Japan). Sucrose was obtained from Junsei Chemical (Tokyo, Japan). Pancreatin (P7545, amylase activity 265 enzyme units/mg) and amyloglucosidase (AMG 300 L, activity 300 AGU/mL) were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and Novozymes (Bagsvaerd, Denmark), respectively. AS from *N. polysaccharea* was provided by the Food Microbiology and Bioengineering Laboratory of Kyunghee University. All chemicals were of analytical grade.

2.2 AS activity assay

The AS gene from *N. polysaccharea* was cloned and expressed in *Escherichia coli*. The AS was purified by affinity chromatography using Ni-NTA (nickel-nitrilotriacetic acid) resin (Qiagen, Hombrechtikon, Switzerland), and AS activity was determined as previously described [4].

2.3 Preparation of AS-treated starch

Starch suspension (5%, w/w) was prepared by mixing starch with 50 mM Tris–HCl buffer (pH 7.5) to a final volume of 150 mL. The suspension was boiled for 30 min. After cooling to 30°C, AS (5000 U or 20 000 U/30 mL) was added to the suspension. The gelatinized starch paste with AS was incubated in a water bath at 30°C for 20 h, followed by reaction termination with the addition of three volumes of ethanol. Precipitated AS-treated starch was recovered by centrifugation at $10\,000 \times g$ for 10 min and subsequent supernatant removal. The AS-treated starch was then washed three times with distilled water and centrifuged at $10\,000 \times g$ for another 10 min. The pellet was freeze-dried, ground, and passed through a 100-mesh sieve. The control was prepared using the same procedure as AS-treated starch but without enzymatic modification.

Amylopectin branch chain-length distribution was determined using high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD), as previously described [4].

2.4 Preparation of starch-entrapped calcium alginate microspheres

Starch-entrapped microspheres were prepared as in Rose et al. [13] with slight modification. The mixtures of sodium alginate, waxy corn starch or AS-treated waxy corn starch

(5000 U, 20 000 U, and AS-control) and water were prepared at three different ratios: 1:9:90, 0.7:9:90.3, and 0.5:9:90.5. After sodium alginate was completely dissolved in water, the starch was suspended. The suspension was pumped through a 23-gauge hypodermic needle using a peristaltic pump (Cole-Parmer, model 7518-00, Vernon Hills, IL, USA) into a calcium chloride (2% w/v) bath under continuous stirring. The microspheres were kept in the calcium chloride bath for 3 h and then harvested and filtered by Whatman Grade 42 filter paper. After washing with distilled water extensively, the microspheres were dried in an oven at 40°C for 24 h.

The mean diameter of each calcium alginate bead preparation was determined by measuring 20 beads under an optical microscope (DE/Axiovert 40, Carl Zeiss, Göttingen, Germany). Total starch content of the microspheres was measured using the Megazyme Total Starch Determination Kit (K-TSTA, Megazyme International Ireland, Ltd., Bray, Ireland) after grinding in a ball mill (Pulverisette, FRITSCH, Idar-Oberstein, Germany) to destroy the starch-alginate microsphere.

2.5 Determination of digestibility of native and AS-treated starches and starch-entrapped microspheres

The digestibility of uncooked and cooked native, AS-treated, and AS-control waxy corn starches was measured according to a slightly modified version of the method in Brumovsky and Thompson [14]. Starch-entrapped microspheres equivalent to 90 mg of starch were used as specified in Table 1. Pancreatin (3 g) was added to 36 mL of distilled water and stirred well for 10 min. This dispersion was precipitated by centrifugation at $1500 \times g$ for 10 min. The supernatant (30 mL) and amyloglucosidase (0.6 mL) were mixed with 5.4 mL of distilled water in a beaker. To determine starch digestibility, the microspheres equivalent to 90 mg of starch were dispersed into a 15 mL conical tube with 2.25 mL 0.1 M sodium acetate buffer and three glass beads at a pH of 5.2. The starch sample in a conical tube was heated in a water bath at 95°C for 30 min, and is called the cooked sample from here on. The enzyme solution and conical tube were equilibrated in a shaking incubator (240 rpm at 37°C) for 10 min. Then, 2.25 mL of enzyme solution was added to each conical tube and the starch sample was incubated in a shaking incubator for 10 or 240 min. The reaction was then terminated by boiling for 10 min. To determine the glucose produced by starch hydrolysis, the conical tube was centrifuged at $10\,000 \times g$ for 10 min. The glucose concentration of the hydrolyzates remaining after starch digestion was measured using a glucose oxidase-peroxidase (GOD-POD) kit. The RDS fraction was defined as the amount of glucose released after 10 min of digestion. The glucose released between 10 and 240 min of digestion was designated as the SDS fraction, and the RS fraction was determined as the unhydrolyzed starch remaining after 240 min of digestion.

2.6 Release studies

The in vitro starch release profiles of starch-entrapped beads were studied by incubating 100 mg beads in 10 mL simulated gastric fluid (SGF, pH 1.4) in four conical flasks kept in a shaking water bath at 37°C and 70 rpm. After 4 h of incubation, the beads in each of the conical flasks were filtered using Whatman Grade 42 filter paper, transferred to 10 mL simulated intestinal fluid (SIF, pH 6.8), and kept for 3 h. The medium was then transferred to simulated colonic fluid (SCF, pH 7.4). The release media, SGF (pH1.0), SIF (pH 6.8), and SCF (pH 7.4), were prepared as previously reported [15]. At different time intervals, 1 mL of the solution was withdrawn and replaced by fresh medium. The total starch released from the beads was determined using a phenol-sulfuric acid assay [16].

2.7 Statistical analysis

All experiments were performed in triplicate and results are reported as the mean ± SD. Differences between the means

Table 1. Contents of RDS, SDS, and RS in uncooked and cooked AS-treated starches (unit: %)

	Uncooked AS-treated starches				Cooked AS-treated starches			
Sample	RDS	SDS	RS	SDS + RS	RDS	SDS	RS	SDS + RS
Native waxy corn	85.3 ± 1.6a	5.3 ± 2.9c	9.4 ± 2.6b	14.7 c	97.1 ± 1.4a	0.1 ± 1.0c	2.8 ± 2.4c	2.9c
5000 U-AS	**60.0** ± 1.0b	**33.3** ± 0.4b	**6.7** ± 1.2b	40.0b	**95.5** ± 2.9a	**0.3** ± 2.0c	**4.2** ± 1.3c	4.5c
20 000 U-AS	**24.6** ± 1.1c	**42.7** ± 0.7a	**32.7** ± 1.3c	75.4a	**51.7** ± 1.5c	**14.9** ± 3.3a	**33.4** ± 3.3a	48.3a
AS-Con	**58.4** ± 1.3b	**5.9** ± 1.4c	**35.7** ± 1.5a	41.6b	**79.0** ± 2.5b	**7.4** ± 0.5b	**14.5** ± 2.8b	21.9b

RDS, SDS, RS, and AS denote rapidly digestible starch, slowly digestible starch, resistant starch, and amylosucrase, respectively.
The values with different letters in the same column are significantly different ($p < 0.05$) by Duncan's multiple range test.
5000 U, 20 000 U: amount of AS used.
AS-Con, control for AS-treated starch.

of samples were analyzed by Duncan's multiple range tests at a significance level of 0.05 using the Statistical Analysis System software (version 9.1, SAS Institute Inc., Cary, NC, USA).

3 Results and discussion

3.1 In vitro digestibility of native and AS-treated starches

After treatment of waxy corn starch with 5000 or 20 000 U AS, the SDS fraction increased by up to 42.7%, while RDS was reduced to 24.6% (Table 1). AS catalyzes the elongation of external chains of acceptors at their non-reducing ends, displaying specificity for α-glucans with (1 → 4)-α-linkages or (1 → 4)-α-linked glucosyl chains branched with (1 → 6)-α-linkages [17]. Therefore, as shown in Table 2, AS treatment decreased the proportion of short chains (DP 6–12) and increased the proportion of long chains (DP 25–36) in amylopectin molecules. This branch chain elongation permits the formation of ordered structural crystallites which may be hindered by α-(1,6)-linked branch points in amylose-free waxy type starches [4, 18]. However, the branch chain length distribution of this amylose-like polymer is different from real amylose because the DP of amylose ranges from 300 to 3000, depending on its origin [19, 20], while that of AS-treated starches is much shorter (Table 2). Further, recrystallization is affected by certain length of branch chain, which is DP 19–26, corresponding with the most abundant branch chain after AS-treatment [4, 7]. The formation of crystallites in starch molecules decreases the rate of enzymatic digestion, a possible reason for the increased SDS and decreased RDS fractions of the AS-treated starches [5].

However, after the 5000 and 20 000 U AS-treated waxy corn starches were cooked, the SDS fractions decreased to 0.3 and 14.9%, respectively (Table 1). Thus, the structure of SDS was disrupted during cooking because the SDS fraction consists of less perfect crystallites and amorphous components, which can be thermally unstable [5, 19].

The control starch, prepared like the AS-treated starch but without enzymatic modification, contained 58.4% RDS and 35.7% RS. The preparation of the control starch included boiling to increase enzyme accessibility, which gelatinized the starch. It was then retrograded through the rearrangement and recrystallization of amylose and amylopectin branch chains during incubation at 30°C for 20 h [20]. Retrograded starch is more resistant to hydrolysis by digestive enzymes [21]. This may be a reason that the control starch had more RS than the native starch and less SDS than the AS-treated starches.

Our study confirmed that SDS is thermally unstable in both native and AS-treated starches, and that SDS is converted into RDS in the process (Table 1). Therefore, in the current study, native and AS-treated starches were encapsulated in calcium alginate to improve thermal stability and to create a physical barrier to control glucose release in the GI tract.

3.2 Composition, morphology, and size distribution of starch-encapsulated alginate beads

The concentration of sodium alginate influenced the encapsulation of starch in calcium alginate beads. With increasing sodium alginate concentration, the percent starch in the dried microspheres decreased and calcium alginate in the microspheres increased (Table 3).

Starch-entrapped microspheres showed similar morphology and size at the same concentration of sodium alginate (Fig. 1). As the sodium alginate concentration increased, the microspheres became slightly smaller, with well-formed and rounded shapes. Oval-shaped microspheres were produced at lower concentrations of sodium alginate (0.5 and 0.7%). The shorter radius of these oval microspheres was similar to the average size of the spherical-shaped microspheres (~1.40 mm), and the difference between the long and short radii was >0.3 mm. These results are in agreement with a previous report that starch beads become more spherical as the concentration of sodium alginate increases [22]. Furthermore, we observed different surface morphology between waxy and AS-treated waxy corn starch, with the former

Table 2. The branch chain length distributions of AS-treated starches

Sample		Percent distribution (%)			
		DP 6–12	DP 13–24	DP 25–36	≥DP 37
Waxy corn	Native	24.6 ± 0.7	50.3 ± 0.3	17.9 ± 0.7	7.2 ± 0.2
	5000 U	9.5 ± 0.8	51.7 ± 0.5	30.2 ± 0.7	8.2 ± 1.2
	20 000 U	4.1 ± 0.6	41.8 ± 0.8	41.0 ± 1.1	12.9 ± 0.7

AS and DP denote amylosucrase and degree of polymerization, respectively.
5000 U, 20 000 U: amount of AS used.
Data were expressed as mean value ± SD.

Table 3. Formulation of alginate suspensions prior to starch entrapment and composition of final starch-entrapped microspheres after drying ($n = 3$)

Suspension			Final composition			
Sodium alginate	Starch	Water	Sample	Calcium alginate (%)[a)]	Starch (%)[b)]	Moisture (%)
1	9	90	WC + EN	17.4	76.6 ± 1.0	6.0
			WC + 5000 EN	16.1	77.2 ± 1.0	6.7
			WC + 20 000 EN	15.7	77.7 ± 0.5	6.6
			WC + ConEN	16.6	76.9 ± 0.5	6.5
0.7	9	90.3	WC + EN	14.2	79.8 ± 0.6	6.1
			WC + 5000 EN	14.3	79.3 ± 0.7	6.4
			WC + 20 000 EN	15.8	77.8 ± 0.9	6.4
			WC + ConEN	15.6	78.2 ± 0.9	6.2
0.5	9	90.5	WC + EN	11.3	82.7 ± 0.1	6.0
			WC + 5000 EN	11.4	82.6 ± 0.5	6.1
			WC + 20 000 EN	12.8	81.0 ± 1.0	6.2
			WC + ConEN	11.6	82.2 ± 0.8	6.2

WC + EN: encapsulation with native waxy corn starch.
WC + 5000 EN: encapsulation with 5000 U amylosucrase-treated waxy corn starch.
WC + 20 000 EN: encapsulation with 20 000 U amylosucrase-treated waxy corn starch.
WC + ASConEN: encapsulation with control for amylosucrase-treated waxy corn starch.
a) Calculated by difference, i.e. 100% − (% starch + % moisture).
b) Expressed as mean value ± SD, on a wet weight basis.

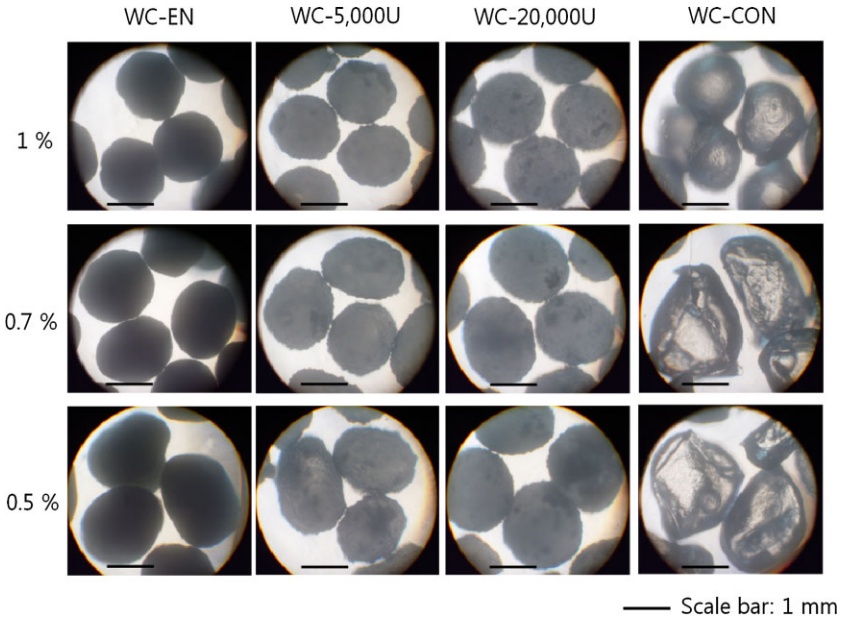

Figure 1. Morphology and size distribution of starch-encapsulated microspheres. Microspheres of waxy corn starch and AS-treated waxy corn starch (5000 U, 20 000 U, and AS-control) were prepared at three concentrations of sodium alginate (0.5, 0.7, and 1 %); and their morphology and size distribution were observed by optical microscopy.

beads having a smooth surface and the latter, a rough surface.

3.3 In vitro starch digestion assay

In uncooked samples, the digestibility of all starches varied with the amount of AS used for the reaction and sodium alginate used for encapsulation (Table 4A). After encapsulation, the fractions of SDS and RS increased by 32.3–35.1% for native waxy corn starch, and by 15.9–54.3% for AS-treated starches.

After cooking, the SDS + RS fraction in the encapsulation with native waxy corn starch (WC + EN) increased from 57.5 to 87.8% with an increase in sodium alginate concentration (Table 4B). In the AS-treated samples, the SDS and RS fractions remained between 71.2 and 96.2%, depending on the amount of AS and alginate used. More calcium alginate in the spheres provided a denser matrix around the starch, and

Table 4. Fractions of RDS, SDS, and RS in both uncooked and cooked native, AS-treated, and AS-control entrapped waxy corn starches (unit:%)

(A) Uncooked samples

	1% Sodium alginate				0.7% Sodium alginate				0.5% Sodium alginate			
Sample	RDS	SDS	RS	SDS + RS	RDS	SDS	RS	SDS + RS	RDS	SDS	RS	SDS + RS
WC + EN	4.4 ± 0.6bc	46.9 ± 0.7b	48.7 ± 0.7d	95.6ab	7.1 ± 0.5 b	56.8 ± 4.2a	36.2 ± 3.7c	93.0b	7.2 ± 1.4b	69.4 ± 1.5a	23.3 ± 2.8c	92.8b
WC + 5000 EN	5.7 ± 1.6b	19.4 ± 0.7c	74.9 ± 0.8b	94.3b	7.4 ± 0.2b	20.9 ± 2.2b	71.8 ± 2.3b	92.6b	7.7 ± 0.1b	16.4 ± 1.5c	76.0 ± 1.4b	92.4b
WC + 20 000 EN	2.3 ± 1.7c	4.2 ± 0.8d	93.5 ± 1.1a	97.7a	2.5 ± 0.1c	11.7 ± 0.5c	85.8 ± 0.4a	97.5a	3.0 ± 1.2c	14.0 ± 1.6c	83.0 ± 0.4a	97.0a
WC + ASConEN	32.9 ± 1.3a	61.0 ± 3.0a	6.1 ± 2.6d	67.1c	34.2 ± 2.4a	59.8 ± 0.9a	6.0 ± 1.6d	65.8c	42.5 ± 2.0a	51.6 ± 2.1b	5.9 ± 3.0d	57.5c

(B) Cooked samples

	1% Sodium alginate				0.7% Sodium alginate				0.5% Sodium alginate			
Sample	RDS	SDS	RS	SDS + RS	RDS	SDS	RS	SDS + RS	RDS	SDS	RS	SDS + RS
WC + En	12.2 ± 1.9c	75.0 ± 0.3a	12.8 ± 2.0c	87.8b	21.4 ± 2.4b	63.19 ± 4.0a	15.6 ± 1.6c	78.6b	42.5 ± 2.9a	53.5 ± 1.8a	4.0 ± 2.7c	57.5c
WC + 5000 EN	21.9 ± 1.4b	49.4 ± 1.8c	28.7 ± 1.4b	78.1c	24.4 ± 1.1b	44.9 ± 3.8c	30.7 ± 2.7b	75.6b	31.8 ± 1.6b	36.8 ± 2.3b	34.3 ± 0.8b	71.2b
WC + 20 000 EN	3.8 ± 1.3d	6.4 ± 0.8d	89.8 ± 1.0a	96.2a	7.0 ± 0.5c	16.1 ± 0.3d	76.9 ± 0.7a	93.0a	7.5 ± 0.8c	23.1 ± 2.3c	69.4 ± 2.8a	92.5a
WC + ASConEN	31.6 ± 2.8a	56.5 ± 2.1b	11.9 ± 2.2d	68.4d	41.4 ± 2.3a	53.7 ± 3.7b	4.9 ± 3.1d	58.6c	44.3 ± 3.8a	48.3 ± 4.3a	7.4 ± 2.6c	55.7c

RDS, SDS, RS, and AS denote rapidly digestible starch, slowly digestible starch, resistant starch, and amylosucrase, respectively.
The values with different letters in the same column are significantly different ($p < 0.05$) by Duncan's multiple range test.
WC + EN: encapsulation with native waxy corn starch.
WC + 5000 EN: encapsulation with 5000 U AS-treated waxy corn starch.
WC + 20 000 EN: encapsulation with 20 000 U AS-treated waxy corn starch.
WC + ASConEN: encapsulation with control for AS-treated waxy corn starch.

provided a thicker barrier to the diffusion of starch through the pores, the degradation of the calcium alginate matrix, and the penetration of digestive enzymes into the microspheres.

In cooked samples, the 20 000 U AS-treated (WC + 20 000) starches still maintained 92.5 to 96.2% of their SDS + RS fraction, and the 5000 U AS-treated (WC + 5000) starches maintained 71.2 to 78.1% of their SDS + RS fraction. Therefore, the amount of AS used strongly affected the SDS + RS fraction more than the amount of sodium alginate. However, in uncooked samples, RS contributed more to the SDS + RS fraction of the WC + 5000 and WC + 20 000 samples, whereas SDS contributed more to the SDS + RS fraction of the WC + ASCon samples at all three alginate concentrations. In cooked samples, SDS made a greater contribution to the SDS + RS fraction of WC + 5000 and WC + ASCon, while RS was the main contributor to the SDS + RS fraction in WC + 20 000.

These differences may be due to the differences in molecular structure caused by different amounts of AS used for each starch modification. Compared with native starch, the AS-treated starches showed three distinct structural characteristics concerning digestibility. First, as the amount of enzyme increased, the proportion of long chains increased and short chains decreased in the amylopectin. These long chains can form relatively perfect crystallites, resistant to reactions with starch-digestive enzymes [4, 18].

Second, the bigger molecular size of the AS-treated starches retarded their diffusion through the pores of the polymer network when compared with that of the native and control starches. Shin et al. [4] reported that the average molar mass (M_w) of AS-modified starches was approximately twice as large as those of native starches, and that the radius of gyration (R_z) also increased from 182 to 260 nm in waxy rice starch. An increase in starch molecular size can lead to reduced penetration and diffusion into the alginate matrices. The pore size of calcium alginate microbeads has been reported to range from 5 to 200 nm in diameter [23].

Lastly, the enzyme reaction conditions (30°C for 20 h) may have induced the rearrangement of chains in the gelatinized starch, resulting in the formation of retrograded starch. The long branched chains of AS-treated starches can produce a high degree of retrogradation. The SDS + RS fraction of ASCon was ~25 to 30% lower than that of AS-treated starch. This was because in ASCon, which did not undergo AS modification, no chain length elongation occurred. However, retrogradation proceeded through the same procedures as in the enzymatic reactions. From this result, it follows that the crystallites of AS-modified starches were the most stable of all samples and these starches had increased resistance to hydrolysis by digestive enzymes [4, 18]. A similar phenomenon was also observed in the encapsulated starch, even after cooking. The decreased amounts of SDS and RS in cooked

samples are likely to be due to the decreased rigidity of calcium alginate matrices. The weakened calcium alginate matrices were more penetrable by digestive enzymes and allowed starch to diffuse out of the matrices more easily.

Overall, different ranges of SDS and RS fractions were obtained using different alginate concentrations and varying amounts of AS. These treatments led to different molecular characteristics: the AS-control starch was retrograded, and AS-modified starches were retrograded with elongated chains. Therefore, AS-modified starches might be a useful resource to provide a desired proportion of SDS and RS to RDS. In addition, the in vitro digestion assay suggested that starch-encapsulated microspheres might release less glucose in the small intestine and bring more starch into the large intestine. The release profiles under simulated GI fluids measure the amount of glucose released in an alternative way and will be comparable to the in vitro digestion assay.

3.4 Starch release under GI tract conditions

3.4.1 Uncooked samples

When uncooked samples were placed in SGF for 4 h, <0.5% of the starch was released from the beads (Fig. 2A). The dry beads were swollen as the result of the hydration of the hydrophilic COO^- groups of alginate [24]. Swollen beads still

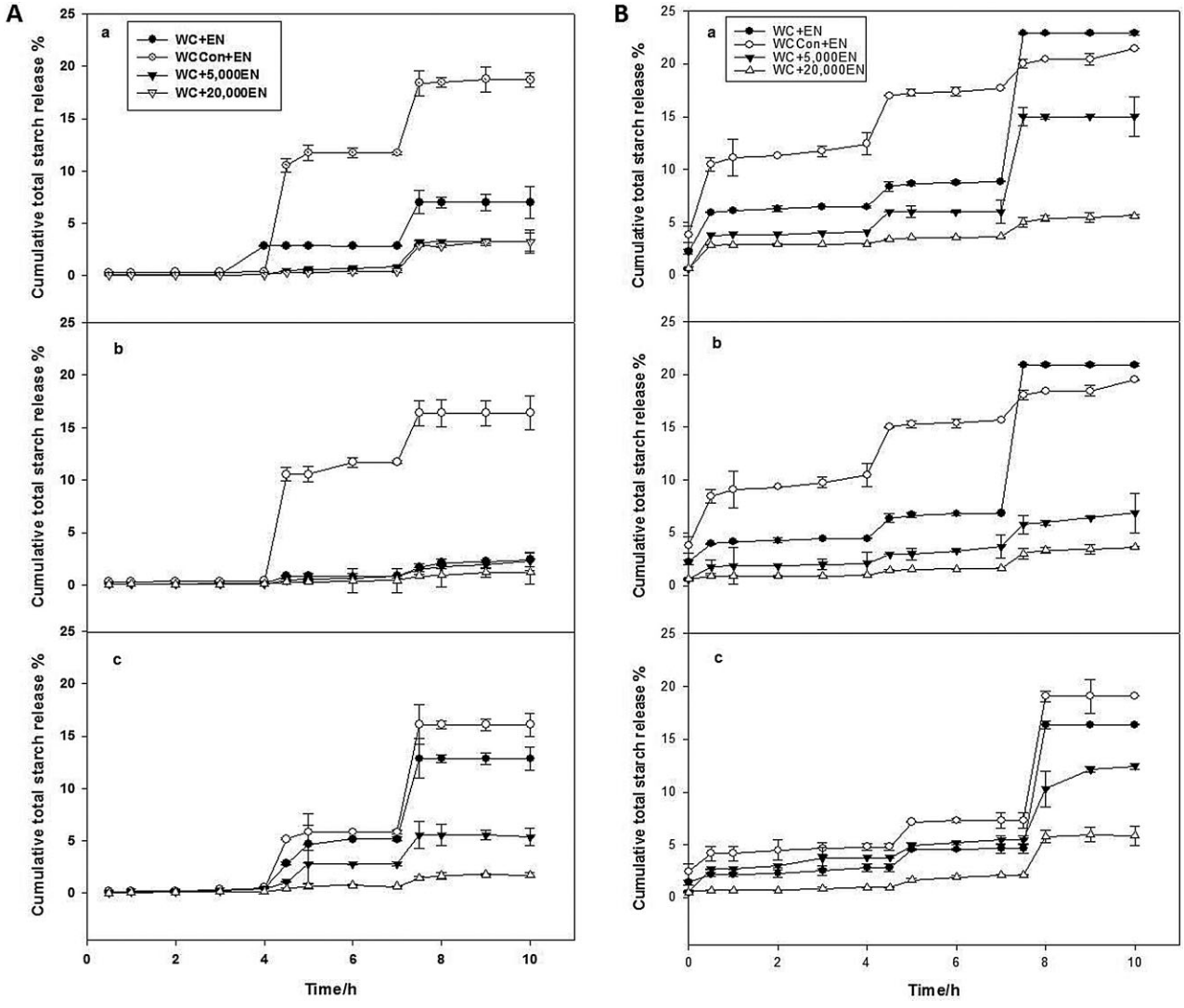

Figure 2. (A) The amount of total starch released from uncooked calcium alginate encapsulated beads of different formulations under different pH conditions immersed sequentially in SGF, SIF, and SCF for 4, 3, and 3 h, respectively. a: 0.5% sodium alginate, b: 0.7% sodium alginate, and c: 1.0% sodium alginate. (B) The amount of total starch released from cooked calcium alginate encapsulated beads of different formulations under different pH conditions immersed sequentially in SGF, SIF, and SCF for 4, 3, and 3 h, respectively. a: 0.5% sodium alginate, b: 0.7% sodium alginate, and c: 1.0% sodium alginate.

effectively incorporated and held starch. After transfer to SIF (pH 6.8), the beads started to erode and disintegrate. The percentage of starch released significantly increased within the first hour for WCCon + EN and WC + EN samples. The cumulative starch released from AS-treated samples was much lower than that of the others. The 20 000 U AS-treated sample released the least starch: <1% at three different alginate concentrations. When transferred to SCF (pH 7.4), the beads continued to erode and disintegrate. The percentage of starch released increased dramatically in both the WCCon + EN and WC + EN samples in the first hour of digestion. The total starch released was highest in the WCCon + EN sample, ranging from 15.2 to 18.8%, while the WC + EN sample released 2.4 to 12.7% of its starch. The AS-treated sample released less starch than non-AS-treated samples in SCF, similar to its SIF release patterns. Less starch was released from the WC + 20 000 EN samples, suggesting that branch chain elongation led to the formation of denser starch matrices and prevented starch release from the beads.

3.4.2 Cooked samples

The encapsulated starch beads maintained their spherical shape and had no observable sign of erosion or disintegration after cooking in deionized water for 20 min, as in an agreement of previous report [12]. The total starches released during cooking were 2.2 ± 0.2, 3.8 ± 0.5, 0.6 ± 0.3, and 0.6 ± 0.2% for WC + EN, WCCon + EN, WC + 5000 EN, and WC + 20 000 EN, respectively (data not shown). These values were similar at all three different alginate concentrations. The calcium alginate beads maintained stable gels, although gel rigidity decreased after heat processing [13, 25]. In a cooked sample kept in SGF for 4 h, <2% of the starch was released from the beads. After transfer to SIF (pH 6.8) the beads started to erode and disintegrate. The starch release patterns both in SGF and SIF were similar to those of uncooked samples. The WCCon + EN sample released dramatically more starch than any other sample.

The cumulative starch released from the AS-treated samples was much less than that from other samples. The 5000 U AS-treated samples released 2–4% of their starch, probably due to decreased rigidity of the gels, which increased the penetration of SIF solution into the beads and the diffusion of starch out of the beads. The 20 000 U AS-treated samples still incorporated starch inside the beads and released <1% in SIF. After transfer to SCF (pH 7.4), the beads eroded and disintegrated, and the release of starch markedly increased in the WCCon + EN, WC + EN, and WC + 5000 EN samples during the first hour.

The amount of starch released from cooked samples increased more than that from uncooked samples. However, no sample showed an increase over 25%, and the release pattern was similar to uncooked samples. The total starch released from WC + EN beads was much higher in cooked samples at all three different alginate concentrations. The amount released increased substantially in the SCF solution. In addition, the AS-treated samples released more starch than the other samples at all three different alginate concentrations, and the WC + 5000 EN released more starch than the WC + 20 000 EN. This agreed with the in vitro digestibility experiment, where a smaller SDS + RS fraction was obtained from the WC + 5000 EN sample than from the WC + 20 000 EN sample after cooking. In the current study, we assessed starch release in the GI tract with an in vitro method using simulated GI fluids. Further study, however, is needed to evaluate in vitro–in vivo correlation of these encapsulated starches as suggested by Hur et al. [10].

4 Conclusions

This in vitro digestibility and release study clearly showed that starch-encapsulated calcium alginate beads had low glycemic properties under various sample conditions. The AS-treated samples contained more SDS and RS, and these samples were the most resistant to starch release under conditions that mimic the human GI tract, even after cooking. The starch-encapsulated beads can also be customized for varying amounts of RDS, SDS, and RS by altering the encapsulated starch, to use native or retrograded starches, or starches modified by treatments such as AS. These encapsulated microspheres with improved thermal stability that contain high amounts of SDS and RS may prove useful in slowing glucose release and controlling the metabolic regulation of hyperglycemia and hypoglycemia.

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5 References

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