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In vitro and *in vivo* digestion of octenyl succinic starch

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ABSTRACT

This study aimed to understand effects of octenyl succinic anhydride (OSA) modification of normal corn (NCS) and high-amylose corn (HA7) starch on their enzymatic hydrolysis rates. After modification with 3% and 10% OSA, resistant starch (RS) contents of the cooked OS-NCS increased from 0.8% of the control starch to 6.8% and 13.2% (Englyst Method), respectively, whereas that of the cooked OS-HA7 decreased from 24.1% to 23.7% and 20.9%, respectively. When the cooked NCS, HA7 and OS (10%)-HA7 were used to prepare diets for rats at 55% (w/w) starch, RS contents of the diets were 1.1%, 13.2% and 14.6%, respectively. After feeding to the rats, 20.2–31.1% of the starch in the OS (10%)-HA7-diet was not utilized *in vivo* and was found in rat feces, which was substantially larger than that of the HA7-diet (≤4.9%) and NCS-diet (≤0.2%). The body weights of the rats, however, remained similar between different groups.

1. Introduction

Starch, one of the most abundant biomass on earth, is a major energy source for humans and animals. Starch is composed of two types of glucans: amylose and amylopectin. Amylose is an essentially linear molecule with α-1, 4 linked D-glucose units and a few branches of α-1, 6 linkages, whereas amylopectin is a highly-branched molecule with about 5% α-1, 6 branch linkages (Hizukuri, Takeda, Yasuda, & Suzuki, 1981). Native starch is commonly modified using chemical, physical, and enzymatic methods to improve its functional properties for different applications. One of the common modified starches used in the food industry is octenyl succinic (OS) starch, which is prepared using a chemical reaction of starch with octenyl succinic anhydride (OSA) (Bai & Shi, 2011; Caldwell & Wurzburg, 1953). The starch derivatized with the OS groups has amphiphilic properties and, thus, is suitable for various applications, including emulsification (Chiu, 1990; Nilsson & Bergenstahl, 2007; Woo, Maningat, & Bassi, 2004), encapsulation (Drusch & Schwarz, 2006; Morehouse, 1994; Murua-Pagola, Beristain-Guevara, & Martinez-Bustos, 2009), and as a fat replacement (Chung, Lee, Han, & Lim, 2010; Sarneel, Peremans, & Jonckers, 2008).

OSA modification of starch has been used to produce resistant starch (RS) (Han & BeMiller, 2007; He, Liu, & Zhang, 2008). RS refers to a portion of starch that is resistant to the enzymatic hydrolysis in the small intestine and passed into the colon to be fermented by the gut microflora (Englyst & Macfarlane, 1986; Englyst, Wiggins, & Cummings, 1982). RS has been classified into five different types: physically inaccessible starch (RS1), native granular starch with the B- or C-type crystalline structure (RS2), retrograded starch (RS3), chemically-modified starch (RS4) and amylose–lipid complex (RS5) (Englyst, Kingman, & Cummings, 1992; Hasjim et al., 2010; Muir, Young, & Odea, 1994). The RS derived from OSA modification is categorized as the RS4. Waxy corn starch modified with OSA (3%, dry starch basis, dsb) followed by a heat-moisture treatment resulted in a substantially less postprandial plasma-glucose response than the unmodified starch after being ingested by human subjects (He et al., 2008).

A recent human-feeding study showed that ingestion of bread made with RS5, a free-fatty-acid complexed high-amylose corn starch (HA7), resulted in significantly less postprandial plasma-glucose and insulin responses than ingestion of bread made with normal wheat flour, suggesting that the RS5 was more resistant to *in vivo* digestion than starch in the wheat flour (Hasjim et al., 2010). In addition, the cooked RS5 diet effectively inhibited azoxymethane (AOM)-induced preneoplastic lesions (colon cancer precursors) in rat colon, suggesting that the RS5 suppressed colon carcinogenesis (Zhao et al., 2011). The inhibitory effects of the RS5 on the development of preneoplastic lesions could be attributed to both the enzymatic resistance and hydrophobic property of the RS5. The RS5 with hydrophobic property could physically interact with other hydrophobic chemicals, including toxic compounds, and carry them out of the digestive tract of rats.

To test this hypothesis, we selected OS starch for a rat feeding study because of its enzymatic resistance and hydrophobic property. The objective of the present study was to understand effects of OSA modification of normal corn (NCS) and high-amylose corn

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(HA7) starch on their enzymatic hydrolysis rates. The *in vivo* hydrolysis rate of the OS-HA7 was compared with that of the NCS and HA7 in a rat feeding study. Thermal and pasting properties of the modified starches were also characterized, and their relationships with the enzymatic hydrolysis of the starches were elucidated.

2. Materials and methods

2.1. Materials

Normal corn starch (NCS, Cargill Gel™, 34.3% amylose) and high-amylose corn starch (HA7, AmyloGel™, 68.4% amylose) were purchased from Cargill Inc. (Minneapolis, MN). 2-Octen-1-ylsuccinic anhydride (OSA), porcine pancreatin and amyloglucosidase from *Aspergillus Niger* were purchased from Sigma–Aldrich Co. (St. Louis, MO). Casein, glucose, mineral mix (AIN-93), choline, methionine, vitamin mix (AIN-93) and corn oil used to prepare diets for rats were purchased from Harlan Laboratories (Madison, WI). Five-week-old male Fisher-344 rats were purchased from Charles River Laboratories (Wilmington, MA). D-Glucose Assay Kit and Total Starch Assay Kit were purchased from Megazyme International Ireland Ltd. (Wicklow, Ireland).

2.2. Preparation of OS-starch

NCS and HA7 were modified with 3% and 10% (w/w, dry starch basis, dsb) OSA following the method of Zhang et al. (2011). The starch was suspended in distilled water at 35% (w/w, dsb). The pH of the starch suspension was adjusted to 8.0 using a sodium hydroxide aqueous solution (3%, w/w), and the temperature was maintained at 35 °C. OSA was added dropwise to the starch suspension while maintaining the pH at 8.0 °C and 35 °C. After the reaction was completed, the pH of the starch suspension became stable and was then adjusted to 6.5 using hydrochloric acid (1.0 M). The starch was recovered using centrifugation, washed twice with distilled water and twice with 100% ethanol, dried at 37 °C and then ground. Each of the starches subjected to the same processing without addition of OSA was used as the control.

2.3. Degree of substitution and reaction efficiency

The degree of substitution (DS) of the OS-starch was determined using a titration method (Bai & Shi, 2011). Reaction efficiency (RE) of the starch modification was calculated as: % RE = 100% × (mass percentage of OS group in the OS-starch/mass percentage of OSA added to the starch for modification). The analysis was done in duplicate. The mass of the OS group was excluded from the mass of the OS-starch when the OS-starch was used on the dry starch basis for other analyses in this study.

2.4. Thermal properties of starch

Thermal properties of the OS-starch and the control starch were analyzed using a differential scanning calorimeter (Diamond DSC, Perkin-Elmer, Norwalk, CT) (Hasjim et al., 2010). The starch (~10 mg, dsb) was mixed with distilled-deionized water (3×, w/w, dsb) and heated from 10 °C to 150 °C at a rate of 10 °C/min. After the first thermal scan, the sample was cooled down at 40 °C/min and immediately rescanned to confirm the dissociation peak of amylose–lipid complex (ALC). The enthalpy change (ΔH) of the thermal transition was calculated on the dry starch basis. The analysis was done in duplicate.

Table 1
Compositions of rat diets.

Ingredient	Weight percentage (%)		
	NCS-diet	HA7-diet	OS (10%)-HA7-diet
NCS[a,b]	55.0	–	–
HA7	–	55.0	–
OS (10%)-HA7	–	–	55.0
Casein	20.0	20.0	20.0
Glucose	15.0	15.0	15.0
Mineral mix (AIN-93)	3.5	3.5	3.5
Choline	0.2	0.2	0.2
Methionine	0.3	0.3	0.3
Vitamin mix (AIN-93)	1.0	1.0	1.0
Corn oil	5.0	5.0	5.0
Total	100.0	100.0	100.0
Starch content of dry diet[c]	54.2	53.6	51.2

[a] The ingredients were all weighed on as-is weight basis.
[b] NCS: normal corn starch, HA7: high-amylose corn starch, OS (10%)-HA7: HA7 modified with 10% (w/w, dsb) OSA.
[c] Dry starch basis, excluding the mass of chemical derivatives, OS group.

2.5. Pasting properties of starch

Pasting properties of the OS-starch and the control starch were analyzed using a Rapid Visco-Analyzer (RVA, Newport Scientific, Sydney, Australia). Each starch sample (2.24 g, dsb; equivalent to dry weight of 2.24 g, 2.29 g, 2.37 g, 2.30 g and 2.41 g for the control starch, OS (3%)-NCS, OS (10%)-NCS, OS (3%)-HA7 and OS (10%)-HA7, respectively) was suspended in distilled-deionized water or 0.5% (w/w) sodium chloride solution to reach a total weight of 28.0 g and analyzed following the program reported by Ai, Medic, Jiang, Wang, and Jane (2011). The analysis was done in duplicate.

2.6. RS content of starch

RS contents of the raw and cooked OS-starch and the control starch were analyzed using the Englyst Method (Englyst et al., 1992) with modifications (Li, Jiang, Campbell, Blanco, & Jane, 2008). The starch sample (1.0 g, dsb; equivalent to dry weight of 1.0 g, 1.02 g, 1.06 g, 1.03 g and 1.07 g for the control starch, OS (3%)-NCS, OS (10%)-NCS, OS (3%)-HA7 and OS (10%)-HA7, respectively), with or without cooking, was hydrolyzed *in vitro* using porcine pancreatin extract and amyloglucosidase in a shaker water-bath (37 °C and 80 rpm). The concentrations of glucose released from the starch at time intervals of 20 min and 120 min were quantified using a D-Glucose Assay Kit containing glucose oxidase and peroxidase (GOPOD). Rapidly digestible starch (RDS), slowly digestible starch (SDS) and RS contents of the starch samples were calculated on the dry starch basis (Englyst et al., 1992). The analysis was done in duplicate.

2.7. Diet preparation for rats

The NCS, HA7 and OS (10%)-HA7 were used to prepare rat diets with 55% (w/w, as-is weight basis) starch, 20% casein, 5% corn oil, and other ingredients as shown in Table 1, following the method of Zhao et al. (2011). Each starch sample was boiled with distilled water (3×, w/w, dsb) under constant manual stirring. After cooling to room temperature, the cooked starch paste was thoroughly mixed with other ingredients to prepare rat diet (Table 1). Starch contents of the NCS, HA7 and OS (10%)-HA7-diet on the dry basis (db) were 54.2%, 53.6% and 51.2%, respectively (Table 1). The mass of the OS group was excluded from the mass of the OS (10%)-HA7 for the dry starch content calculation. RDS, SDS and RS contents of each diet were analyzed as described earlier. The analysis was done in triplicate.

2.8. Rat feeding and feces collection

Thirty male Fisher-344 rats (five-week-old) were received and housed as described by Zhao et al. (2011). All the rats were fed with the NCS-diet for the first two weeks after arriving at the facility (Week 0), and then fed with respective diets (10 rats/diet) for additional nine weeks. The first week when the rats were fed with selected diets was designated as "Week 1." The diets were fed to the rats with high moisture contents, which ranged from 49.3% to 59.7% of the freshly prepared diets, and from 35.4% to 52.3% of the diets after 2 days of feeding on the cage top rack. The daily feed-disappearance weights were calculated on the dry diet basis: daily feed-disappearance weight (db) = (weight of diet given to the rat, db − weight of diet recovered after 2 days, db)/2. On the last day of each week, the body weight of each rat was recorded, and the feces discharged by each rat was collected from the bottom of the cage, weighed, and stored at −20 °C before analysis. The animal study was performed in compliance with the guidelines of the Institutional Animal Care and Use Committee.

2.9. Starch content of feces

The feces collected in the same week from the rats fed with the same diet was combined, dried at 45 °C, and ground before analysis. The starch content of the feces was determined using a Total Starch Assay Kit following the AACC Method 76-13 (2000) with modifications. Because the OS derivatives of the modified starch interfered with the enzymatic hydrolysis of starch, it produced less glucose and resulted in a less starch content using the standard method. Thus, the feces (~100 mg) from the rats fed with the OS (10%)-HA7-diet was dispersed in 2.0 mL sodium hydroxide aqueous solution (0.5 M) for 0.5 h to completely remove the OS derivatives of the starch before the analysis of starch content. The alkali-treated feces dispersion was neutralized using hydrochloric acid (0.5 M), and the starch was hydrolyzed to glucose using thermostable α-amylase and amyloglucosidase and then quantified using the GOPOD method. To prevent any discrepancy caused by different methods used for the analysis, starch contents of the feces from rats fed with the other two diets were also measured using the same method. The analysis was done in duplicate. The percentage of starch not being utilized by the rats and their gut microflora was calculated as: % starch not being utilized = 100% × (starch content of feces, db × average daily feces weight, db)/(starch content of diet, db × average daily feed-disappearance weight, db).

2.10. Lipid content of feces

Lipid was extracted from the feces and quantified following the AOAC Method 996.06 (2000). The analysis was done in duplicate.

2.11. Cecum tissue weight, cecal content weight and pH

The cecum tissue weight, cecal content weight and pH were measured for each rat after the rats were sacrificed at the end of the feeding study (Kishida, Nogami, Himeno, & Ebihara, 2001; Zhao et al., 2011).

2.12. Statistical analysis

Statistical significance was analyzed using one-way ANOVA and multiple comparison test with Tukey's adjustment at p value <0.05. The statistical analyses were conducted in SAS (Version 9.2, SAS Institute, Inc., Cary, NC).

Table 2
Degree of substitution (DS) and reaction efficiency (RE) of normal corn (NCS) and high-amylose corn (HA7) starch modified with 3% or 10% (w/w, dsb) octenyl succinic anhydride (OSA).

Starch	DS[a]	RE (%)[b]
OS (3%)-NCS	0.019 ± 0.000	78.9 ± 0.6
OS (10%)-NCS	0.045 ± 0.001	55.0 ± 1.6
OS (3%)-HA7	0.022 ± 0.000	92.2 ± 0.6
OS (10%)-HA7	0.058 ± 0.002	69.4 ± 1.9

[a] Determined using a titration method (Bai & Shi, 2011).
[b] % RE = 100% × (mass percentage of OS group in the OS-starch/mass percentage of OSA added to the starch for modification).

3. Results and discussion

The NCS and HA7 were modified with 3% and 10% (w/w, dsb) OSA to assess suitable OS-starch for the rat feeding study. The DS and RE of the NCS and HA7 modified with 3% and 10% (w/w, dsb) OSA are shown in Table 2. With 3% OSA, the DS of OS-NCS and OS-HA7 were 0.019 and 0.022, respectively, and the RE were 78.9% and 92.2%, respectively. With 10% OSA, the DS of OS-NCS and OS-HA7 increased to 0.045 and 0.058, respectively, but the RE decreased to 55.0% and 69.4%, respectively. The decreased RE results with increased OSA concentrations were consistent with previously reported data because there was more OSA hydrolyzed into OS acid at a higher reagent concentration (Bai & Shi, 2011; Song, He, Ruan, & Chen, 2006). With the same concentration of OSA, the DS and RE of the OS-HA7 were greater than that of the OS-NCS. The reaction with OSA primarily occurred in the amorphous structures of starch granules, and there was a larger proportion of amorphous amylose present in the HA7 than the NCS (He, Song, Ruan, & Chen, 2006; Kuakpetoon & Wang, 2006).

Thermal properties of the control and the OS-starches are shown in Table 3. After modification with 3% and 10% OSA, onset gelatinization temperatures (T_o) and ΔH of the modified NCS and HA7 substantially decreased, indicating that the OS derivatives destabilized the double helices of starch molecules (Bao, Xing, Phillips, & Corke, 2003; Han & BeMiller, 2007). In addition, the second peak temperature of HA7 (T_{p2}), which corresponded to the dissociation of amylose–lipid complex (ALC) (Ai, Hasjim, & Jane, 2013; Jiang, Lio, Blanco, Campbell, & Jane, 2010), decreased from 99.8 °C to 91.9 °C with 3% OSA modification and disappeared with 10% OSA modification. When the starch samples were rescanned using the DSC, the ΔH of the ALC dissociation peak of the OS-NCS decreased from 1.1 J/g of the control starch to 0.7 J/g and 0.6 J/g with 3% and 10% OSA modification, respectively, and that of the OS-HA7 decreased from 4.0 J/g to 3.4 J/g and 1.2 J/g, respectively. These results indicated that the OS derivatives interfered with the ALC formation, which were consistent with the results reported for acetyl and hydroxypropyl starches (Eliasson, 1994; Liu, Arntfield, Holley, & Aime, 1997).

Pasting properties of the control and the OS-starches suspended in distilled-deionized-water medium are shown in Fig. 1. After the OSA modification, the pasting temperatures of the modified NCS and HA7 decreased, but their peak and final viscosities increased. When a NaCl solution (0.5%) was used as the medium, the viscosities of the unmodified NCS and HA7 control samples remained the same, whereas that of the OS-NCS and OS-HA7 decreased. The pasting temperature and peak viscosity of the 3% OSA modified starch became similar to that of the respective control starch. The results indicated that the remarkably higher viscosities of the OS-starches were caused by the repelling between negative charges of the OS groups.

RDS, SDS and RS contents of the raw and cooked control and OS-starches analyzed using the Englyst Method are shown in Table 4. Compared with the respective control starch, the RDS contents of the raw OS-NCS, raw OS-HA7 and cooked OS-NCS decreased,

Table 3
Thermal properties of normal corn (NCS) and high-amylose corn (HA7) starch modified with 3% or 10% (w/w, dsb) octenyl succinic anhydride (OSA).[a]

Starch	Gelatinization of starch[b]					Dissociation of amylose–lipid complex in rescan[b]			
	T_o (°C)	T_{p1} (°C)	T_{p2} (°C)	T_c (°C)	ΔH (J/g)	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
NCS, control	65.5 ± 0.2	70.3 ± 0.1	–	74.8 ± 0.1	12.9 ± 0.1	88.9 ± 0.2	95.5 ± 0.0	101.3 ± 0.2	1.1 ± 0.2
OS (3%)-NCS	62.6 ± 0.2	68.9 ± 0.1	–	74.2 ± 0.6	12.4 ± 0.8	89.5 ± 0.0	95.3 ± 0.2	100.7 ± 0.1	0.7 ± 0.1
OS (10%)-NCS	60.7 ± 1.1	69.4 ± 0.1	–	77.2 ± 0.1	11.6 ± 0.5	88.1 ± 0.3	95.4 ± 0.1	100.5 ± 0.8	0.6 ± 0.2
HA7, control	70.1 ± 0.0	74.8 ± 0.4	99.8 ± 0.1	108.1 ± 0.0	11.8 ± 0.2	86.2 ± 0.1	95.2 ± 0.2	106.5 ± 0.7	4.0 ± 0.1
OS (3%)-HA7	67.1 ± 0.2	74.0 ± 0.1	91.9 ± 0.1	105.2 ± 0.5	11.5 ± 0.1	78.6 ± 0.6	91.6 ± 0.6	102.9 ± 0.8	3.4 ± 0.0
OS (10%)-HA7	61.7 ± 0.8	71.3 ± 0.8	–	95.3 ± 2.8	10.5 ± 0.5	73.0 ± 0.1	82.4 ± 0.0	96.6 ± 1.0	1.2 ± 0.1

[a] Measured using differential scanning calorimetry.
[b] T_o: onset temperature, T_p: peak temperature, T_c: conclusion temperature, ΔH: enthalpy change.

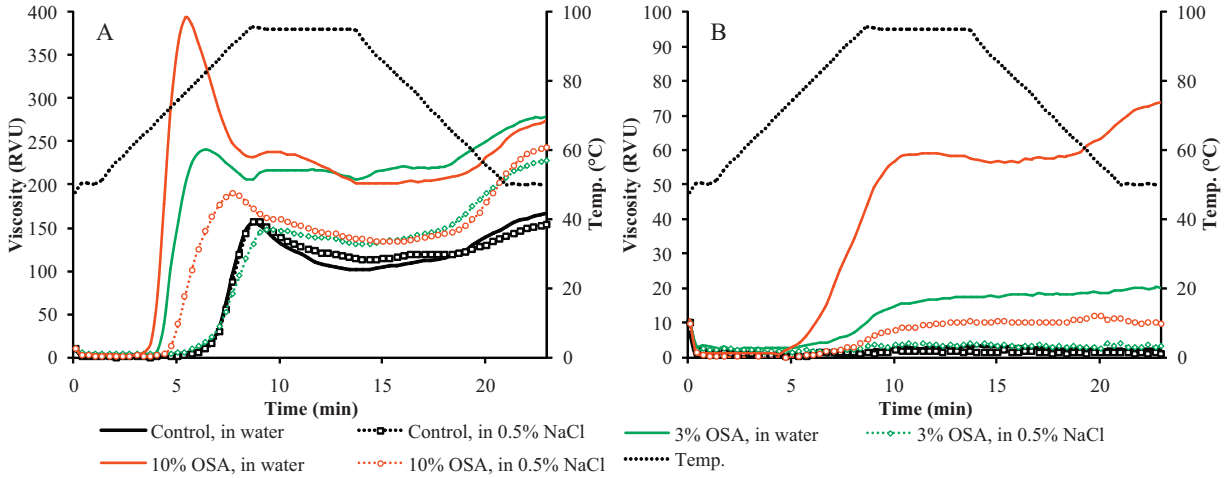

Fig. 1. Pasting properties of normal corn (A) and high-amylose corn starch (B) modified with 3% or 10% (w/w, dsb) octenyl succinic anhydride (OSA). Pasting profiles of the starches were analyzed using a Rapid Visco-Analyzer with 8% (w/w, dsb) starch suspension (28.0 g total weight) in distilled-deionized water or 0.5% (w/w) sodium chloride solution.

but their RS contents increased, suggesting that the OS derivatives interfered with enzymatic hydrolysis of starch (Han & BeMiller, 2007; He et al., 2008; Zhang et al., 2011). After cooking, the 3% and 10% OS-HA7, however, displayed increased RDS contents from 71.7% of the control starch to 75.7% and 78.0%, respectively, but decreased RS contents from 24.1% to 23.7% and 20.9%, respectively. The reduced RS contents of the OS-HA7 samples were attributed to that the modified HA7 had lower gelatinization temperatures (T_c decreased from 108.1 °C to 105.2 °C and 95.3 °C, respectively, Table 3) and less ALC formation (Table 3). The OS-HA7 samples were gelatinized to a greater extent and more swollen after cooking (Fig. 1). Consequently, the OS-HA7 became more susceptible to enzymatic hydrolysis than the HA7 control.

The RS contents of the diets containing boiled starch (51.2–54.2%, dsb) (Table 1), mixed with casein and corn oil, are shown in Table 4. Among the diets, the determined RS content of the OS (10%)-HA7-diet (14.6%) was remarkably larger than the RS content (10.7%) calculated on the basis of weight percentage of starch in the diet (Table 4). This substantial difference could be attributed to that the OS (10%)-HA7 carried hydrophobic octenyl groups, which physically interacted with the hydrophobic moiety of casein and corn oil present in the diet and became glomerate to further reduce the susceptibility of the starch to enzymatic hydrolysis.

Average daily feed-disappearance weights, body weights, daily feces weights, starch and lipid contents of the feces of rats fed with different diets are shown in Table 5. The daily feed-disappearance weights could be larger than the amount of feed ingested by the rats because some feed fell to the bottom of the cage and could not be recovered. In Week 0, all the rats were fed with the NCS-diet, and, thus, there were no significant differences in the feces weight

Table 4
Rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) contents of different starches and diets analyzed using the Englyst Method.

Sample[a]	Raw starch			Cooked starch			Diet[b]			
	RDS (%)	SDS (%)	RS (%)	RDS (%)	SDS (%)	RS (%)	RDS (%)	SDS (%)	RS (%)	Calculated RS (%)[c]
NCS, control	20.7 ± 0.3	42.3 ± 1.0	36.9 ± 0.8	97.2 ± 0.4	2.0 ± 0.2	0.8 ± 0.2	51.8 ± 0.5	1.3 ± 0.5	1.1 ± 0.5	0.4
OS (3%)-NCS	12.2 ± 0.4	37.3 ± 0.9	50.5 ± 1.3	90.1 ± 1.3	3.1 ± 1.1	6.8 ± 0.2	–	–	–	–
OS (10%)-NCS	9.0 ± 0.1	22.9 ± 0.2	68.1 ± 0.3	83.9 ± 1.0	3.0 ± 0.4	13.2 ± 1.4	–	–	–	–
HA7, control	14.9 ± 0.4	13.3 ± 0.6	71.7 ± 0.2	71.7 ± 1.4	4.2 ± 0.0	24.1 ± 1.4	35.1 ± 0.1	5.2 ± 0.3	13.2 ± 0.4	12.9
OS (3%)-HA7	13.5 ± 0.2	11.8 ± 0.6	74.6 ± 0.4	75.7 ± 0.3	0.6 ± 0.0	23.7 ± 0.3	–	–	–	–
OS (10%)-HA7	10.2 ± 0.2	9.9 ± 0.0	79.8 ± 0.2	78.0 ± 0.8	1.1 ± 0.1	20.9 ± 0.7	34.2 ± 0.4	2.4 ± 0.5	14.6 ± 0.6	10.7

[a] NCS: normal corn starch, HA7: high-amylose corn starch, OS: octenyl succinic.
[b] Diet was prepared following the formulae shown in Table 1. The NCS, HA7 and OS (10%)-HA7-diet contained 54.2%, 53.6% and 51.2% starch on the dry basis, respectively.
[c] Calculated RS content = RS content of cooked starch, dsb × starch content of diet, db.

Table 5
Average daily feed-disappearance weights, body weights, daily feces weights, starch and lipid contents of feces of rats fed with different diets.[a]

Group		Week 0[c]	Week 1	Week 3	Week 6	Week 9
Daily feed-disappearance weight (g/rat, db)	NCS[b]	5.58 ± 1.00 a	6.31 ± 1.09 a	7.60 ± 0.13 b	13.79 ± 0.90 a	15.00 ± 1.25 ab
	HA7	6.47 ± 1.28 a	7.21 ± 1.26 a	8.84 ± 0.23 a	13.52 ± 0.59 a	16.68 ± 1.56 a
	OS (10%)-HA7	6.92 ± 0.68 a	7.51 ± 0.67 a	8.56 ± 0.87 a	10.56 ± 1.66 b	14.51 ± 0.59 b
Body weight (g/rat)	NCS	107.7 ± 13.1 a	129.1 ± 11.2 a	183.6 ± 7.2 a	239.6 ± 7.4 a	276.8 ± 15.5 a
	HA7	108.3 ± 10.3 a	127.3 ± 10.2 a	178.6 ± 10.5 a	227.4 ± 12.8 b	263.8 ± 11.7 a
	OS (10%)-HA7	106.2 ± 12.6 a	128.2 ± 12.1 a	177.7 ± 12.4 a	228.8 ± 11.8 ab	265.7 ± 10.2 a
Daily feces weight (g/rat, db)	NCS	0.43 ± 0.22 a	0.52 ± 0.22 b	0.49 ± 0.12 b	0.55 ± 0.09 b	0.56 ± 0.11 b
	HA7	0.42 ± 0.30 a	0.43 ± 0.07 b	0.44 ± 0.10 b	0.68 ± 0.07 b	0.67 ± 0.12 b
	OS (10%)-HA7	0.55 ± 0.32 a	1.77 ± 0.26 a	2.46 ± 0.33 a	2.77 ± 0.51 a	2.75 ± 0.59 a
Starch content of feces (%, db)[d]	NCS	1.1 ± 0.2	0.6 ± 0.0	0.7 ± 0.1	0.7 ± 0.2	0.8 ± 0.1
	HA7	1.4 ± 0.7	44.1 ± 0.5	40.5 ± 0.5	32.5 ± 0.5	23.2 ± 0.5
	OS (10%)-HA7	0.3 ± 0.1	48.9 ± 1.3	55.4 ± 0.5	57.5 ± 0.1	54.6 ± 0.5
Starch not being utilized (%)[e]	NCS	0.2	0.1	0.1	0.1	0.1
	HA7	0.2	4.9	3.8	3.0	1.7
	OS (10%)-HA7	0.0	22.5	31.1	29.5	20.2
Lipid content of feces (%, db)[f]	NCS	6.2 ± 0.4	6.3 ± 0.4	9.9 ± 0.5	10.4 ± 0.2	12.5 ± 0.6
	HA7	7.3 ± 0.2	4.7 ± 0.8	5.2 ± 0.1	6.8 ± 0.4	6.4 ± 0.6
	OS (10%)-HA7	6.5 ± 0.4	13.8 ± 0.2	11.3 ± 0.4	11.9 ± 1.0	12.7 ± 1.1

[a] Values with the same letter in a column are not significantly different at $p < 0.05$.
[b] NCS: normal corn starch, HA7: high-amylose corn starch, OS (10%)-HA7: HA7 modified with 10% (w/w, dsb) OSA.
[c] Rats in different groups were all fed with NCS-diet in Week 0.
[d] Analyzed using a Total Starch Assay Kit with modifications.
[e] % Starch not being utilized = 100% × (starch content of feces, db × average daily feces weight, db)/(starch content of diet, db × average daily feed-disappearance weight, db).
[f] Analyzed following the AOAC Method 996.06.

between different groups. Little starch (0.3–1.4%) was detected in the feces in Week 0, indicating that almost all the cooked NCS was digested *in vivo*. The results were in agreement with the very small RS content of the NCS-diet (1.1%, Table 4) determined using *in vitro* analysis.

Starting from Week 1, the rats were fed with different diets for a total of nine weeks. During the feeding period, it was observed that the feces weight of the rats fed with the OS-HA7-diet was 3–5 times larger than that of the rats fed with the other two diets (Table 5). The feces from the OS-HA7-fed rats also had consistently larger starch contents (48.9–57.5%, w/w, db). These data indicated that the OS-HA7 was substantially more resistant to *in vivo* digestion compared with the NCS and HA7. On the basis of the feces weight and the starch content, it was calculated that 20.2–31.1% starch in the OS-HA7-diet was not utilized *in vivo* (Table 5). After the rats were sacrificed, it was observed that the rats fed with the OS-HA7-diet had larger cecum tissue weight (3.00 g/rat) and cecal content weight (4.25 g/rat, db) than that fed with the NCS-diet (0.92 g/rat and 1.05 g/rat, respectively) and HA7-diet (1.90 g/rat and 3.55 g/rat, respectively) (Table 6). Despite the large quantity of OS-HA7 starch not being utilized during the feeding period, the body-weight gain of the rats fed with the OS-HA7 was not significantly different from that of the rats fed with the other diets (Table 5).

The starch content of the feces from the rats fed with the HA7-diet decreased from 44.1% in Week 1 to 23.2% in Week 9 (Table 5), indicating increased proportion of the HA7 starch was utilized *in vivo* as the rats grew older and bigger. The results could be attributed to two factors: (1) the digestive tract grew longer and produced more amylolytic enzymes as the rats grew bigger and more mature (Marounek, Vovk, & Skrivanova, 1995); (2) the population of the gut microflora that could utilize the HA7 increased with the increasing feeding time and was able to ferment HA7 more efficiently. The fermentation of the HA7 was evidenced by the significantly lower ($p < 0.05$) cecal content pH (5.33) than that of the NCS-fed group (7.53) (Table 6) (Kishida et al., 2001; Zhao et al., 2011). The slightly higher pH of cecal content (5.79) and consistently larger starch contents of the feces from the rats fed with the OS-HA7-diet suggested that the OS-HA7 was less fermentable than the HA7 (Tables 5 and 6).

Table 6
Cecum tissue weights, cecal content weights and pH values of rats fed with different diets.[a]

Group	Cecum tissue weight (g/rat)	Cecal content weight (g/rat, db)	Cecal content pH
NCS[b]	0.92 ± 0.08 c	1.05 ± 0.15 b	7.53 ± 0.22 a
HA7	1.90 ± 0.33 b	3.55 ± 0.75 a	5.33 ± 0.10 c
OS (10%)-HA7	3.00 ± 0.60 a	4.25 ± 0.20 a	5.79 ± 0.09 b

[a] Values with the same letter in a column are not significantly different at $p < 0.05$.
[b] NCS: normal corn starch, HA7: high-amylose corn starch, OS (10%)-HA7: HA7 modified with 10% (w/w, dsb) OSA.

The calculated percentage of the OS-HA7 starch that was not utilized by the rats and their gut microflora (20.2–31.1%, dsb, excluding the weight of OS group) on the basis of feed-disappearance weight was substantially larger than that of the HA7-diet (≤4.9%) and NCS-diet (≤0.2%) (Table 5). The percentage of starch in the OS-HA7-diet not being utilized *in vivo* was much larger than the RS content analyzed *in vitro* using the Englyst Method (14.6%, Table 4). The differences between the *in vivo* and *in vitro* results could be attributed to the glomeration formed between the hydrophobic octenyl groups and other hydrophobic components in a concentrated matrix in the digestive tract (~20% solid content compared with 4% used for *in vitro* analysis, the Englyst Method), which made the starch physically less accessible to enzymatic hydrolysis. This was supported by the substantially larger lipid content of the feces from the OS-HA7 group (Table 5). It was also plausible that the octenyl groups of the OS-HA7 had a greater affinity interacting with the hydrophobic surface of the digestive tract and further hampered its susceptibility to enzymatic hydrolysis. The hydrophobic interactions between the octenyl groups of the OS-HA7 and other hydrophobic compounds in the digestive tract could facilitate the removal of those compounds, including toxins, out of the digestive tract and improve the gut health. It was intriguing that the rats fed with OS-HA7-diet maintained

similar body-weight gain, despite 20.2–31.1% starch not being utilized. The rats fed with the OS-HA7-diet appeared to be calmer by casual observation than rats fed with the other two diets. It has been reported that other types of dietary fiber (*e.g.*, sugar beet pulp and wheat bran) also reduce physical activities of animals (Ramonet, Robert, Aumaitre, Dourmad, & Meunier-Salaun, 2000; Rijnen, Verstegen, Heetkamp, Haaksma, & Schrama, 2003). More studies are needed to understand what caused the metabolic change for the rats fed with the OS-HA7-diet.

4. Conclusions

In this study, effects of OSA modification of the NCS and HA7 on their enzymatic hydrolysis were investigated. After the reaction with 3% and 10% OSA, RS content of the cooked OS-NCS increased, whereas that of the cooked OS-HA7 decreased. In contrast to the RS contents obtained by the *in vitro* analysis, the rat feeding study showed that 20.2–31.1% of the cooked OS (10%)-HA7 was not utilized *in vivo* and was found in the feces, which was substantially larger than that of the cooked HA7 (≤4.9%) and NCS (≤0.2%). Different *in vivo* digestion of the starches, however, did not significantly affect the body-weight gain of the rats.

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