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ARTICLE

# *In Vitro* Digestibility and Emulsification Properties of Phytoglycogen Octenyl Succinate

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This paper reports our recent studies on the *in vitro* digestibility and emulsification properties of an amphiphilic carbohydrate nanoparticle, phytoglycogen octenyl succinate (PG-OS). Phytoglycogen (PG), a glycogen-like α-D-glucan isolated from *sugary-1* sweet corn endosperms, was subjected to octenyl succinate substitution to prepare PG-OS. Waxy corn starch octenyl succinate (WCS-OS) was also prepared as the reference. The degree of substitution (DS), molecular weight, particle size, dispersed molecular density, and zeta-potential of PG-OS and WCS-OS were determined. Transmission electron microscope (TEM) was used to image PG and its derivatives. *In vitro* digestibility and emulsification properties of PG-OS and WCS-OS were compared. The results showed that the dispersed molecular density of PG and PG-OS was much greater than that of WCS and WCS-OS. Zeta-potential of PG-OS decreased as the pH of dispersion increased. In general, the digestibility of PG and PG-OS was lower than that of WCS and WCS-OS at equivalent DS, suggesting the effect of glucan structure on glucan digestibility. At equivalent DS, PG-OS showed similar or even greater capability than WCS-OS to physically stabilize fish oil emulsions. This work revealed the potential of amphiphilic carbohydrate nanoparticles in the applications of emulsions.

**KEYWORDS: Phytoglycogen octenyl succinate; dispersed molecular density; digestibility; emulsification property**

## INTRODUCTION

Phytoglycogen is a water-soluble glycogen-like α-D-glucan present in plants. The largest source of phytoglycogen is the kernel of the corn mutant *sugary-1*, a major genotype of commercial sweet corn. The *sugary-1* mutation leads to the deficiency of SU1, an isoamylase-type starch debranching enzyme (DBE) (*1*). During starch biosynthesis, the suggested role of DBE is to trim abnormal branches that inhibit the formation of physically organized starch structure and granules (*2*, *3*). In the absence of DBE, highly branched phytoglycogen is formed to replace starch granules.

It has been reported that phytoglycogen particles range from 30 to 100 nm under transmission electron microscope (TEM) observations (*4*). The highly branched structure of phytoglycogen results in unusually high molecular density in dispersion. In rice, the dispersed molecular density of phytoglycogen is over 10 times that of starch (*5*). Each phytoglycogen particle may contain thousands of glucan chains which form the highly packed structure. Fundamental structural difference exists between amylopectin of starch and phytoglycogen (*6*), as depicted in **Figure 1**. In amylopectin, long chains are involved in connecting individual clusters (*7*). For phytoglycogen, there are only two short chain populations (*8*), suggesting a noncluster structure with chains tethered by branches.

Phytoglycogen contains both α-1,4- and 1,6-glucosidic linkages; therefore it is susceptible to hydrolysis by amylases such as α-amylase, β-amylase, amyloglucosidase, and debranching enzymes. In addition, phytoglycogen may be susceptible to chemical modifications which have been used for starch. Among these modifications, octenyl succinylation has been used to introduce negative charge and hydrophobicity, and the properties of product can be controlled by the degree of substitution (DS). In this study, the digestibility and emulsification property of phytoglycogen octenyl succinate and waxy corn starch octenyl succinate would be compared.

According to its digestion behavior, starch can be classified as rapidly digestible starch, slowly digestible starch, and resistant starch. In addition, resistant starch (RS) has been classified into RS type-1 (physically inaccessible starch), RS type-2 (granular starch), RS type-3 (retrograded starch), and RS type-4 (chemically modified starch). A number of approaches have been developed to prepare RS type-4. For example, Woo and Seib (*9*) conducted phosphorylation of starch. For waxy corn starch, cross-linking followed by stabilization via hydroxypropylation or acetylation appeared to be more efficient to produce RS than cross-linking alone (*10*). For starch, substitution by octenyl succinate reduced the postprandial glycemic excursion compared to an equivalent glucose challenge (*11*), and the lowered glycemic response was partially due to malabsorption of octenyl succinate groups by the small intestine (*12*). When subjected to multiple amylases, even small amounts of substitution may cause a considerable decrease in the extent of degradation (*13*). Among different chemical modifications, octenyl succinate substitution was the most effective for

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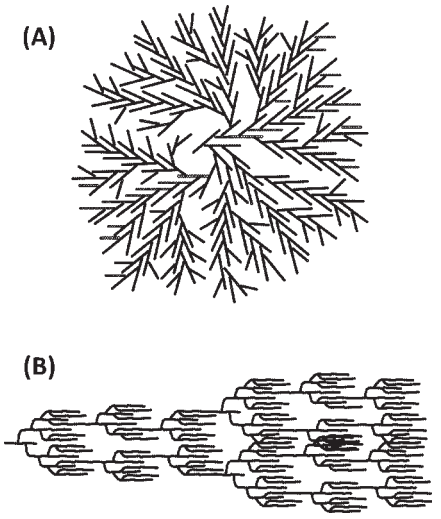


**Figure 1.** Two dimensional schematic of a phytoglycogen nanoparticle (**A**) and a segment of amylopectin (**B**) (*6*).

increasing RS (*10*), possibly due to the large size of octenyl succinate group.

Another strategy to reduce the digestibility of starch-related material is to increase the branch density, that is, to increase the percentage of α-1,6 linkages (*8*). Usually, these materials are water-soluble due to their highly branched structure, and the decrease of digestibility is caused by the lower susceptibility of α-1,6-glucosidic linkages to amyloglucosidase than α-1,4 linkages (*14*, *15*). Our recent work showed that both highly branched malto-oligosaccharides and phytoglycogen had lower digestibility than starch (*8*).

Starch octenyl succinate was invented over 50 years ago to create effective emulsifiers using starch (*16*). Since then, most works have been reported in patents. Recently, a number of works have been reported to characterize or optimize starch octenyl succinate (*17*−*20*). In emulsions, starch octenyl succinate distributed at the oil−water interface of droplets provides both steric hindrance and electrostatic repelling among individual oil droplets. Starch octenyl succinate has shown superior functionality compared to gum arabic for encapsulating flavors and fish oil, especially for improving oxidation stability (*21*). Due to its superior properties, starch octenyl succinate has been broadly used in the food industry.

The hypothesis of this study was that phytoglycogen octenyl succinate (PG-OS) provides equivalent, if not better, physical stability of emulsions than waxy corn starch octenyl succinate (WCS-OS), and that the digestibility of PG-OS is lower than that of WCS-OS at equivalent DS. Both phytoglycogen (PG) and waxy corn starch (WCS) were subjected to octenyl succinate (OS) substitution, and the *in vitro* digestibility and emulsion physical stability were compared between PG-OS and WCS-OS. Recently, we reported that PG-OS was superior to WCS-OS for maintaining the oxidative stability of fish oil emulsions (*6*). In contrast, the current report focuses on the physical stability of emulsions, and on the digestibility of PG-OS as compared to WCS-OS.

## MATERIALS AND METHODS

**Materials.** Waxy corn starch was kindly provided by National Starch Food Innovation (Bridgewater, NJ). Matured kernels of sweet corn Silver Queen *sugary* hybrid was purchased from Burpee Co. (Warminster, PA). 1-Octenyl succinate anhydride was obtained from Dixie Chemical Co. (Houston, TX). Fish oil from menhaden, pancreatin from porcine pancreas, and amyloglucosidase from *Aspergillus niger* were purchased from Sigma-Aldrich (St. Louis, MO). Glucose oxidase/peroxidase (GOPOD) kit was purchased from Megazyme (Wicklow, Ireland).

**Methods.** *Extraction of Phytoglycogen*. Sweet corn kernels were ground into grits and then mixed with 4 to 6 weights of deionized water. The suspension was homogenized using a high-speed blender (Waring Laboratory, Torrington, CT), and then centrifuged at 8000*g* for 20 min. The supernatant was collected whereas the solid was further extracted twice using deionized water. Thereafter, the supernatant at each batch was combined and passed through a 270-mesh sieve. The liquid was then added to 3 volumes of ethanol to precipitate the polysaccharide. After centrifugation and decanting the supernatant, repetitively the precipitate was dispersed by 3 volumes of ethanol and centrifuged. The suspension after the last ethanol addition was filtered to remove excess liquid. The solid material was placed in the fume hood to remove residual ethanol. The powder collected was the phytoglycogen material used for further treatments.

*Substitution Using 1-Octenyl Succinic Anhydride*. To the suspension of waxy corn starch (20% w/w) and dispersion of phytoglycogen (20%, w/w), 1-octenyl succinate anhydride was gradually added in 2 h at the levels of 3, 6, and 9% based on the dry weight of glucans. The pH was maintained between 8.5 and 9.0 using 2% NaOH. The reaction was conducted at room temperature (22 °C) and terminated after 24 h by reducing pH to 6.5 using 2% HCl. To collect substituted glucans, three volumes of ethanol were added to the reaction mixture. The precipitated materials were collected and further dehydrated using 3 cycles of ethanol suspension−centrifugation. The solid collected after filtration was placed in a fume hood to remove residual ethanol to prepare dry powder of phytoglycogen octenyl succinate and waxy corn starch octenyl succinate.

DS of phytoglycogen octenyl succinate and waxy corn starch octenyl succinate was determined using a method from the Joint FAO/WHO Expert Committee on Food Additives (*22*) with modifications. The glucan sample (0.5 g) was acidified with 3 mL of HCl (2.5 M) for 30 min. To each mixture, 10 mL of 90% isopropanol (v/v) was added, followed by centrifugation at 3000*g* for 10 min and supernatant decanting. To the precipitate, an additional 10 mL of 90% isopropanol was added to resuspend the glucan solid and then centrifugation was conducted. This procedure was repeated until the test of chloride ions using $AgNO_3$ showed negative. To test for chloride ions, one drop of 0.1 M $AgNO_3$ was added to the pooled supernatant to observe a white haze of AgCl. Once no noticeable AgCl haze was observed, 30 mL of deionized water was added to the glucan precipitate. The mixture was heated in a boiling water bath for 30 min, and titrated using 0.01 M NaOH. The DS was calculated by

$$\mathrm{DS} = \frac{162A}{1000 - 210A} \quad (1)$$

where $A$ (mmol/g) is the molar amount of octenyl succinate groups in one gram of derivative, and 162 and 210 are the molecular weights of the glucosyl unit and the octenyl succinate group, respectively. The value of $A$ was calculated as

$$A = \frac{(V - V_0) \times 0.01}{0.5} \quad (2)$$

In eq **2**, $V$ (mL) is the volume of NaOH solution consumed by the octenyl succinate derivative, $V_0$ (mL) is the volume of NaOH consumed by native phytoglycogen or starch, 0.5 is the weight of material in grams and 0.01 is the molar concentration of NaOH.

*Dispersed Molecular Density*. Weight-average molecular weight ($M_W$), $z$-average radius of gyration ($R_Z$) of phytoglycogen, waxy corn starch, and their octenyl succinate derivatives were determined using high performance size-exclusion chromatography (HPSEC) with multiangle laser light scattering (MALLS) and refractive index (RI) detectors (Wyatt Technology, Santa Barbara, CA) using two connected columns (PL Aquagel-OH 40 and 60, Polymer Laboratories, Varian Inc.) with a guard column. The flow rate was 1.0 mL/min using deionized water (pH 6.8, containing 0.02% $NaN_3$) as the mobile phase. Astra software (version 5.3.4.10, Wyatt Technology) was used to determine $M_W$ and $R_Z$. The dispersed molecular density ($\rho$, g/mol·nm$^3$) was calculated as $\rho = M_W/R_Z^3$ (*5*). ANOVA was conducted using Minitab 15 (Minitab Inc., State College, PA); Tukey test was utilized with a significant $F$ test ($P \leq 0.05$).

*Transmission Electron Microscope* (*TEM*). TEM imaging was conducted as described by Putaux et al. (*4*) with modifications. Carbon-coated 400 mesh grid was glow discharged before use. Droplets of around 0.01%

**Table 1.** Degree of Substitution (DS), Substitution Efficiency (SE), Weight-Average Molecular Weight ($M_W$), *z*-Average Radius of Gyration ($R_Z$), Dispersed Molecular Density ($\rho$), Glucose Yield for *in Vitro* Digestion, and Glucose Release Reduction Efficiency of Substitution for Phytoglycogen (PG), Waxy Corn Starch (WCS), and Their Derivatives Prepared Using 3, 6 and 9% Octenyl Succinic Anhydride (OSA)

| | | | | | | | glucose yield, %[d] | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | OSA used, % (w/w) of glucan | DS × 100[a] | SE,[b] % | MW, g/mol × $10^{-7}$ [c] | $R_Z$, nm[c] | $\rho$, g/mol·nm$^3$ [c] | 20 min | 40 min | 120 min | glucose release reduction efficiency of substitution,[e] % |
| PG | 0 (native) | | | 3.00 ± 0.02 a | 32.5 ± 0.1 a | 871 ± 8 a | 82.7 ± 1.9 b | 88.7 ± 1.1 a | 89.5 ± 1.4 a | |
| | 3 | 1.02 ± 0.08 | 44 | 3.23 ± 0.04 b | 37.5 ± 0.4 b | 613 ± 21 b | 77.5 ± 1.3 c | 82.1 ± 1.3 c | 82.8 ± 1.0 b | 6.6 |
| | 6 | 2.34 ± 0.03 | 51 | | | | 67.6 ± 0.6 e | 72.6 ± 1.0 e | 74.2 ± 2.5 c | 6.5 |
| | 9 | 4.98 ± 0.06 | 72 | 3.24 ± 0.09 b | 37.0 ± 0.5 b | 641 ± 15 b | 60.8 ± 1.1 f | 62.9 ± 2.1 f | 65.5 ± 3.0 d | 4.8 |
| WCS | 0 (native) | | | 2.79 ± 0.05 a | 60.5 ± 2.5 a | 127 ± 14 a | 88.6 ± 3.9 a | 91.6 ± 2.4 a | 92.7 ± 1.2 a | |
| | 3 | 1.21 ± 0.03 | 53 | 1.72 ± 0.06 b | 61.7 ± 1.5 a | 73 ± 4 b | 83.6 ± 1.2 b | 87.8 ± 0.8 b | 90.5 ± 1.0 a | 1.8 |
| | 6 | 2.60 ± 0.06 | 56 | | | | 75.3 ± 1.5 cd | 77.5 ± 1.3 d | 79.4 ± 2.9 bc | 5.1 |
| | 9 | 4.29 ± 0.07 | 62 | 1.39 ± 0.15 b | 60.0 ± 0.7 a | 65 ± 15 b | 71.0 ± 1.1 de | 73.5 ± 2.3 de | 75.1 ± 3.2 b | 4.1 |

[a] Data expressed as mean ± SD ($n$ = 3). [b] Calculated as actual DS/theoretical DS with complete substitution × 100. [c] Data expressed as mean ± SD ($n$ = 3). Significant differences within the group of PG or WCS are denoted by different letters ($p < 0.05$). [d] Data expressed as mean ± SD ($n$ = 4). Significant differences among all measurements for both PG and WCS groups at each specific digestion time are denoted by different letters ($p < 0.05$). The initial glucose amount was negligible. [e] Calculated as: [glucose yield of native PG (or native WCS) - glucose yield of PG (or WCS) derivative]/DS × 100. This index denotes the potential of 0.01 units of DS to reduce the glucose yield.

(w/v) PG and its derivatives in 0.02 M NaAc buffer (pH 5.5) was dried on this grid and stained by 2% aqueous uranyl acetate. Samples were imaged using a Philips CM-100 transmission electron microscope (FEI Company, Hillsboro OR) operated at 100 kV, 200 $\mu$m condenser aperture, and 70 $\mu$m objective aperture. Images were captured on a Kodak SO-163 film.

*Zeta-Potential of Phytoglycogen, Waxy Corn Starch, and Their Derivatives*. Phytoglycogen and its derivatives (0.01% w/v) were fully dissolved in 0.01 M NaAc buffer of pH 4.5, 5.5, 6.5, and 7.5. Waxy corn starch and its derivatives (0.01% w/v) were suspended in 0.01 M NaAc buffer (pH 6.5) and heated in a boiling water bath for 30 min to fully disperse the polysaccharide. A Zetasizer Nano (ZS90, Malvern Instruments) was used to measure the zeta-potential at room temperature.

*In Vitro Digestibility*. *In vitro* digestibility was measured according to the procedure described by Englyst et al. (*23*) with modifications. Dispersions of phytoglycogen and its derivatives, and suspensions of waxy corn starch and its derivatives, were combined with guar gum and heated in a boiling water bath for 10 min. After cooling and stabilizing in a 37 °C water bath, the enzyme preparation was added. The enzyme preparation contains pancreatin and amyloglucosidase prepared according the Englyst assay procedure (*23*). During the digestion, the reactant was aliquoted at 20, 40, and 120 min and mixed with 2 volumes of ethanol. The mixtures were used as the stock solutions for glucose assay. The GOPOD procedure was conducted using an assay kit (Megazyme). The initial glucose content for each sample was negligible detected using HPSEC analysis. The percentage of glucose release was calculated as (glucose amount after digestion/amount of initial glucan) × 0.9 × 100.

*Emulsion Preparation and Physical Stability Test*. To prepare fish oil-in-water emulsion, the emulsifier dispersions were prepared first. For phytoglycogen octenyl succinate, the solid was fully dispersed in deionized water. For waxy corn starch octenyl succinate, the solid was suspended in water followed by heating in a boiling water bath for 10 min to disperse the polysaccharides. To the dispersions, fish oil was added, and pH was adjusted to 5.5 using 2% HCl. The amount of emulsifier and oil in the final mixture was 10% and 5%, respectively. Preliminary mixing was conducted using a high-speed homogenizer (T25 ULTRA-TURRAX, IKA) for 2 min at 20,000 rpm. The mixture was then subjected to two cycles of high-pressure homogenization using Nano DeBEE homogenizer (BEE International Inc.) at 25,000 psi. The emulsion was transferred into a 50 mL tube and stored at 4 °C. To test the physical stability of emulsion, immediately after emulsification and after 2 and 4 weeks of storage, an aliquot was taken from each emulsion and diluted using 0.01 M NaAc buffer (pH 5.5) at a ratio of 1:125. The zeta-potential and particle size of the emulsions were analyzed using the Zetasizer Nano at room temperature.

## RESULTS AND DISCUSSION

**Octenyl Succinate Substitution.** As shown in **Table 1**, the DS was increased by the increased level of octenyl succinate anhydride (OSA). Theoretically, a complete substitution using 3, 6, and 9% OSA should yield DS 0.0231, 0.0462, and 0.0694, respectively. The actual DS values for individual OSA doses were 0.0121, 0.0260, and 0.0429, respectively, for waxy corn starch, and were 0.0102, 0.0234, and 0.0498, respectively, for phytoglycogen. At a low OSA dose (3%), the substitution efficiency (SE) was higher for starch than for phytoglycogen. At a medium OSA dose (6%) the SE value was similar between starch and phytoglycogen. At a high OSA dose (9%), the SE value was lower for starch than phytoglycogen. The cause of the different SE values between starch and phytoglycogen might be related to the proceeding of the reaction. Phytoglycogen was well dispersed in buffer, whereas starch was in the granular form during substitution reaction. Since OSA is an oily material not soluble in aqueous buffer, the substitution was a heterogeneous reaction. Therefore, the interaction between OSA oil droplets and the surface of dispersed phytoglycogen nanoparticles can be very different from that between OSA oil droplets and starch granule surface. In addition, the control of individual reactions might also contribute to variable SE values. Previous studies have reported the maximum efficiency (80% to 96%) for starch achieved at different substrate levels, temperature, reaction time, and pH range (*17*−*19*). In these works, the reaction was carried out with 25−35% starch slurry, pH 8.0−9.0, 30−35 °C. In this study, both the substrate concentration (20%) and the reaction temperature (22 °C) were lower than those reported by previous works, which might lead to relatively low SE.

**Transmission Electron Microscope (TEM) Image of Phytoglycogen and Its Derivatives.** As shown by TEM image in **Figure 2**, native phytoglycogen (**Figure 2A**) was shown as individual or clustered nanoparticles. The particle size ranges 30−100 nm, which was consistent with the observations by Putaux et al. (*4*). In the TEM image, native phytoglycogen (PG) nanoparticles tended to aggregate. In contrast, phytoglycogen octenyl succinate (PG-OS) with DS of 0.0102, 0.0234, and 0.0498 all showed less aggregation than native PG. Conceivably, repulsion among individual PG-OS nanoparticles caused by the negatively charged carboxylate groups reduced aggregation.

**Molecular Weight, Radius of Gyration, and Dispersed Molecular Density.** In waxy corn starch (WCS), the amount of amylose is negligible. Therefore, the properties of dispersed WCS were actually for those of dispersed amylopectin. As shown in **Table 1**, the weight-average molecular weight ($M_W$) of native PG and waxy corn starch amylopectin (WCS) was 3.00 and 2.79 × $10^7$ g/mol, respectively. The *z*-average radius of gyration ($R_Z$)

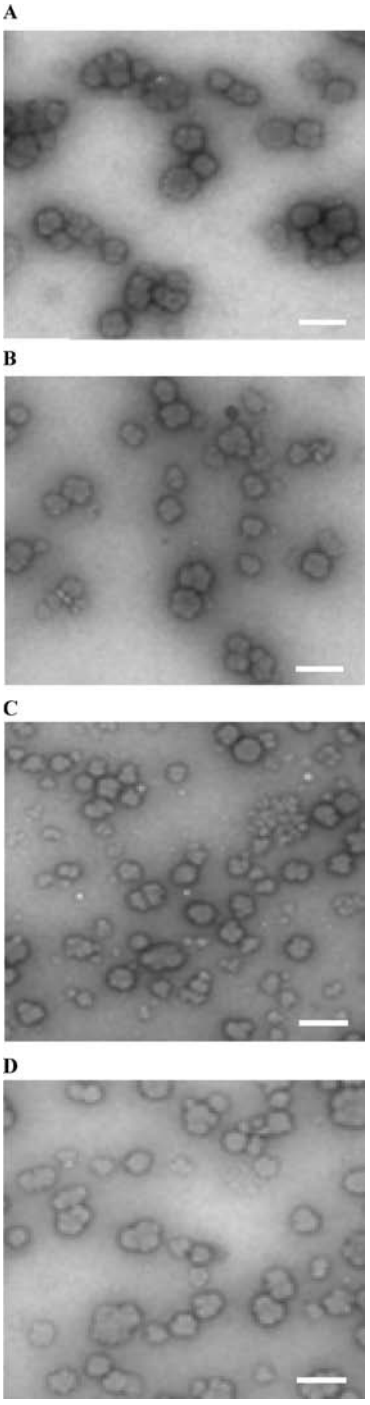


**Figure 2.** TEM images of 0.01% (w/v) solution of native PG (**A**), PG-OS (DS 0.0102) (**B**), PG-OS (DS 0.0234) (**C**), and PG-OS (DS 0.0498) (**D**). The bar indicates 100 nm.

of PG and WCS was 32.5 and 60.5 nm, respectively. The dispersed molecular density ($\rho$) of PG and WCS was 871 and 127 g/mol·nm$^3$, respectively. Apparently, there is a huge difference for the dispersed molecular density between PG and WCS. It has been established that amylopectin assumes a clustered branch structure (*7*). In aqueous dispersion, the organized structure of amylopectin chains is lost, and individual clusters are stretched due to the conformational flexibility associated with the long intercluster chains. In contrast, in a PG nanoparticle the chains are tightly tethered due to the lack of long internal chain segments and the overall dendritic structure. Therefore, the dispersed molecular density of PG was much higher than that of amylopectin.

After octenyl succinate substitution, the $M_W$ of PG was slightly increased from $3.00 \times 10^7$ g/mol for native PG to $3.23 \times 10^7$ g/mol for PG-OS (DS 0.0102) and to $3.24 \times 10^7$ g/mol for PG-OS (DS 0.0498). Such a minor increase of $M_W$ might be related to the grafting of octenyl succinate groups and a minor loss of the low-$M_W$ PG-OS population during the extraction of PG-OS after substitution reaction. In contrast, the $M_W$ of amylopectin was substantially reduced from $2.79 \times 10^7$ g/mol for native WCS to $1.72 \times 10^7$ g/mol for WCS-OS (DS 0.0121) and to $1.39 \times 10^7$ g/mol for WCS-OS (DS 0.0429). Such a reduction of $M_W$ might be related to the substitution reaction at a high pH (8.5 to 9.0). It is likely that the basic condition and the etherification promoted minor degradation in starch granules. However, such degradation did not appear to occur during the substitution of PG, suggesting a higher integrity of PG nanoparticles.

For PG, the substitution slightly increased the radius of gyration ($R_Z$) from 32.5 nm for native PG to 37.5 nm for PG-OS (DS 0.0102) and to 37.0 nm for PG-OS (DS 0.0498). The change of $R_Z$ could be related to a subtle stretching of outer chains caused by electrostatic repelling force among OS groups. However, such stretching was limited from causing a substantial change of nanoparticle size. Due to slight change of $R_Z$, the molecular density ($\rho$) was reduced from 871 g/mol·nm$^3$ for native PG to 613 and 641 g/mol·nm$^3$ for PG-OS with DS 0.0102 and 0.0498, respectively.

For amylopectin of WCS, no significant change of $R_Z$ was detected after OS substitution, even though the $M_W$ was substantially reduced. Accordingly, the $\rho$ value was reduced from 127 g/mol·nm$^3$ for native WCS to 73 and 65 g/mol·nm$^3$ for WCS-OS with DS 0.0121 and 0.0429, respectively. Conceivably, the reduction of $\rho$ value was caused by electrostatic repulsion among chains grafted with OS groups.

It should be indicated that the results of $M_W$, $R_Z$, and $\rho$ of this work were comparable with those of another study of our group (*6*). Both studies share the same native WCS material, but the extraction of native PG and the octenyl succinate substitution of both WCS and PG were conducted at different batches. Individual operations may contribute to some variability of material properties, which requires the characterization of each material. However, the general outcome remains the same.

**Zeta-Potential of Substituted Phytoglycogen and Starch.** Zeta-potential is the electric potential between the slipping plane and the bulk fluid away from the interface. It is a useful parameter for evaluating the colloidal stability and interactions among charged particles. In this study, zeta-potential was used to characterize the density of negative charges at the surface of particulates. **Figure 3** shows the zeta-potential of PG and PG-OS as affected by DS and the pH of dispersion. At the same pH, higher DS correlated to lower zeta-potential, suggesting higher charge density at the surface of nanoparticles. The zeta-potential of native PG ranged from −2 to −4 mV, indicating a slightly negatively charged surface. The origin of this low level charge is unknown; it might be related to trace amount of proteinaceous material or phosphates. In the presence of OS groups, higher DS led to lower zeta-potential. For example, at pH 6.5, an increase of DS from 0.0102 to 0.0498 led to an decease of zeta-potential from −15.8 to −25.2 mV. At the same DS, higher pH led to lower zeta-potential due to higher deprotonation of carboxylate groups.

**Figure 4** compares the zeta-potential of PG, WCS, and their OS derivatives at pH 6.5. In general, at similar DS values PG-OS had lower zeta-potential values than WCS-OS. For example, zeta-potential was −15.8 mV for PG-OS (DS 0.0102) and −7.7 mV for WCS-OS (DS 0.0121). Therefore, the surface charge density was higher for PG-OS than for WCS-OS. This could be related to the high dispersed molecular density of PG-OS and tethered chains at

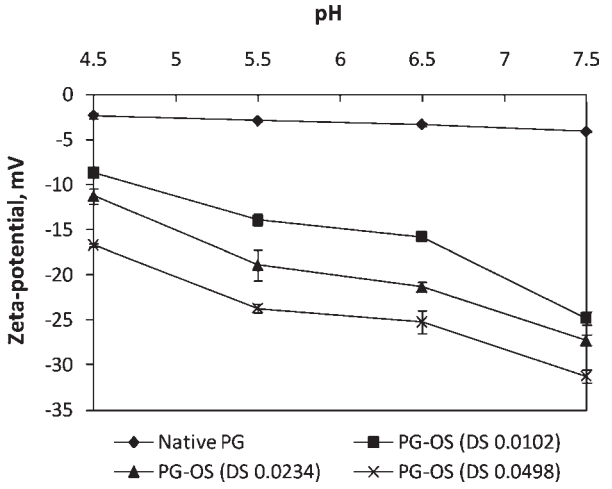


**Figure 3.** Zeta-potential of phytoglycogen (PG) and its derivatives (PG-OS) as affected by DS and dispersion pH (0.01 M NaAc Buffer). Each data point is the mean value of 3 measurements with error bar of standard deviation.

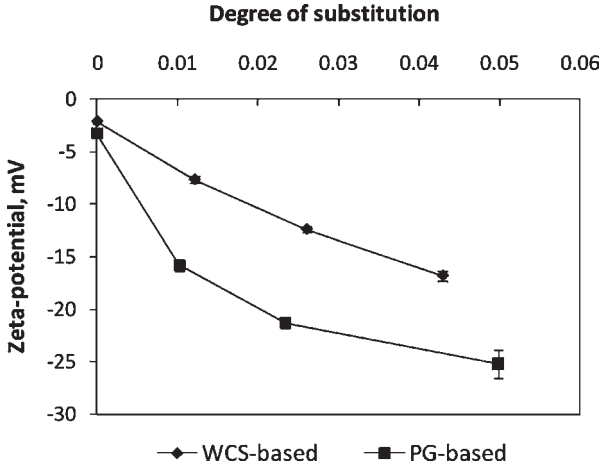


**Figure 4.** Zeta-potential of phytoglycogen (PG), waxy corn starch (WCS), and their derivatives (PG-OS and WCS-OS) with different DS in 0.01 M NaAc Buffer at pH 6.5. Each data point is the mean value of 3 measurements with error bar of standard deviation.

particulate surface. The relatively rigid structure of PG-OS restricted the migration of OS-grafted chains away from each other in a stronger way than the relatively flexible structure of WCS-OS.

***In Vitro* Digestibility. Table 1** shows the glucose yield of native PG, native WCS, and their derivatives when subjected to a combined hydrolysis by pancreatin and amyloglucosidase. For native glucans, the glucose release of PG (82.7%) was significantly lower than that of WCS (88.6%) at 20 min and slightly lower at 40 and 120 min. In general, the comparison showed a lower digestibility of PG than WCS, which is consistent with our previous study (*8*). In that work, PG materials from a number of sweet corns displayed lower digestibility than normal corn starch and waxy corn starch; however, these PG materials did not reach the low digestibility displayed by highly branched oligosaccharides extracted from starch. The branch density (i.e., the percentage of α-1,6-glucosidic linkages) was considered as a major factor for the difference of digestibility of α-D-glucans. Since α-1,6-glucosidic linkages were less susceptible to amyloglucosidase than α-1,4 linkages (*14*, *15*), higher amount of α-1,6 linkages in PG than WCS may lead to reduced rate of glucose release.

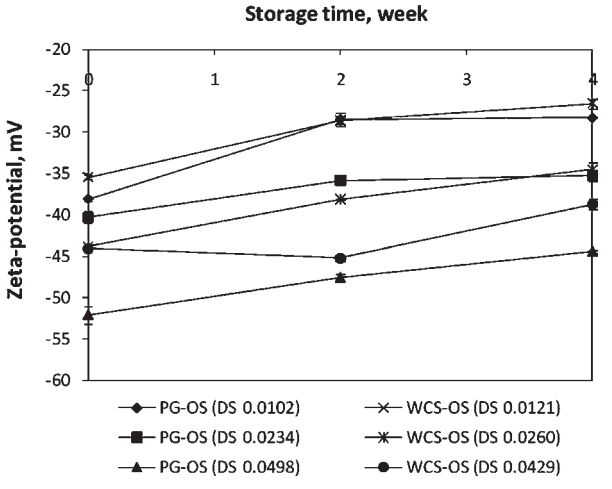


**Figure 5.** Zeta-potential of emulsions during 4 °C storage. These emulsions were prepared using phytoglycogen octenyl succinate (PG-OS) and waxy corn starch octenyl succinate (WCS-OS) with different DS. Each data point is the mean value of 3 measurements with error bar of standard deviation.

OS substitution resulted in substantial reduction of glucose yield for both PG and WCS-based materials. For PG, glucose yield was reduced from 89.5% for native PG to 82.8%, 74.2%, and 65.5% for PG-OS with DS 0.0102, 0.0234, and 0.0498, respectively. For WCS, the glucose yield was reduced from 92.7% for native WCS to 90.5%, 79.4%, and 75.1% for WCS-OS with DS 0.0121, 0.026, and 0.0429, respectively. In addition, the effect of OS substitution on the reduction of glucose yield was different among various DS values and glucans. For example, at low DS value (around 0.01) the glucose release reduction efficiency of substitution was 6.6% for PG-OS, much higher than that of WCS-OS (1.8%). At a much higher DS (approaching 0.05), the glucose release reduction efficiency of substitution for PG-OS (4.8%) was similar to that of WCS-OS (4.1%). In general, OS substitution of PG was more effective than that of WCS in reducing *in vitro* digestibility. Undoubtedly, such a difference was associated with the structural difference of PG and WCS. We consider that the distribution of OS groups in PG or WCS molecules could be a major factor affecting the overall susceptibility of glucosidic linkages to enzymes.

**Zeta-Potential of Emulsions. Figure 5** shows that, in general, PG-OS and WCS-OS with higher DS led to lower zeta-potential of emulsion. For PG-OS stabilized emulsion, the initial zeta-potential was −38.1, −40.3, and −52.1 mV for DS 0.0102, 0.0234, and 0.0498, respectively. For WCS-OS stabilized emulsion, zeta-potential was −35.5, −43.8, and −44.1 mV for DS 0.0121, 0.0260, and 0.0429, respectively. The surface charge of oil droplets originated from the coverage by negatively charged amphiphilic glucans (PG-OS or WCS-OS). Therefore, glucans with higher DS resulted in higher charge densities at droplet surface and thus lower zeta-potential. It should be noted that, at pH 5.5 (the pH of emulsions), the zeta-potential of each PG-OS material in nonemulsion dispersion (**Figure 3**) was higher than that of the corresponding emulsion. The reason for this difference is unclear. It might be related to a lower surface curvature for oil droplets in emulsion than that for individual PG-OS nanoparticles, or might be related to a synergistic effect among neighboring charged nanoparticles at the oil−water interface.

Regardless of the glucan type (PG or WCS) and DS value, zeta-potential of emulsions increased during the 4-week storage at 4 °C (**Figure 5**). It appears that most increment of zeta-potential occurred during the earlier period of storage (by 2 weeks) except

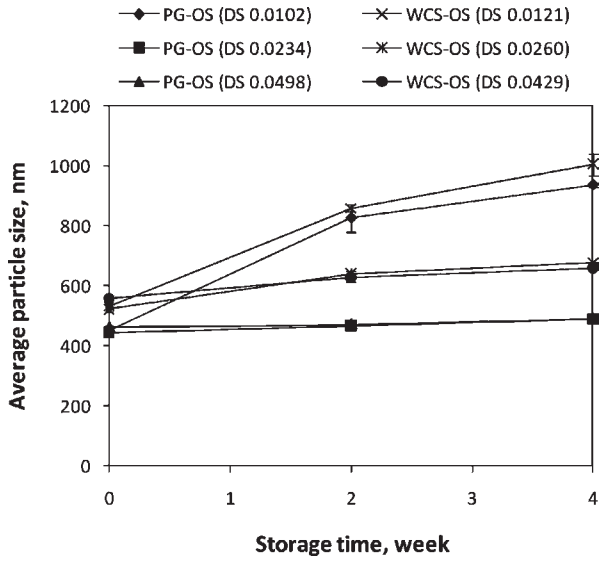


**Figure 6.** Average particle size of emulsions during 4 °C storage. These emulsions were prepared using phytoglycogen octenyl succinate (PG-OS) and waxy corn starch octenyl succinate (WCS-OS) with different DS. Each data point is the mean value of 3 measurements with error bar of standard deviation.

for WCS-OS with the highest DS (0.0429). We consider that the increment of zeta-potential was a result of partial desorption of negatively charged glucans from the interface. Since amphiphilic glucan molecules in the bulk of aqueous phase had lower zeta-potential, desorption of these molecules from oil−water interface may result in an overall increase of zeta-potential in emulsion system.

A high absolute value of zeta-potential is usually associated with a stable emulsion due to high electrostatic repelling force among individual oil droplets. For emulsion formed using high molecular weight bulky amphiphilic biopolymers, such as PG-OS and WCS-OS, zeta-potential should not be the sole factor governing emulsion stability. The steric hindrance caused by thick interfacial layer may play an important role in stabilizing emulsions.

**Particle Size of Emulsions. Figure 6** compares the progression of average particle size of emulsions formed using PG-OS and WCS-OS. In general, the emulsions prepared using PG-OS (DS 0.0102) and WCS-OS (DS 0.0121) were not very stable. The average particle size of PG-OS (DS 0.0102) increased by 487 nm, from 449 nm at the starting point of storage to 936 nm after 4 weeks. Similarly, for WCS-OS (DS 0.0121) the particle size increased by 473 nm, from initial 532 to 1005 nm after 4 weeks. When the DS values of PG-OS and WCS-OS were increased, the physical stability was substantially improved. For example, for PG-OS (DS 0.0234) the particle size increased by 44 nm, from the initial 445 nm to 489 after the 4-week storage. For WCS-OS (DS 0.0260) the particle size increased by 154 nm, from 523 to 677 nm after the 4-week storage. It is possible that an enhanced DS led to an enhanced hydrophobic binding of amphiphilic glucans at oil droplet surface and also an increased repulsion among oil droplets. Both factors may work together to stabilize oil droplets from coalescence.

However, the physical stability of emulsion was only slightly increased by an additional increase of DS (**Figure 6**). For PG-OS (DS 0.0498) the particle size increased by 29 nm, from the initial 462 to 491 nm after 4 weeks, showing only a slight difference compared to PG-OS (DS 0.0234). For WCS-OS (DS 0.0429) the particle size increased by 102 nm, from initial 558 to 660 nm after 4 weeks, showing a stability similar to that of WCS-OS (DS 0.0260). It appears that the effect of DS on emulsion stability can reach a limit. At high DS, high electrostatic repulsion among glucan particles (for PG-OS) or chains (for WCS-OS) may reduce their coverage on the surface of oil droplets and negatively affect emulsion stability. Conceivably, DS affect emulsion stability by a combined effect of hydrophobic binding, repulsion among oil droplets, and interfacial coverage.

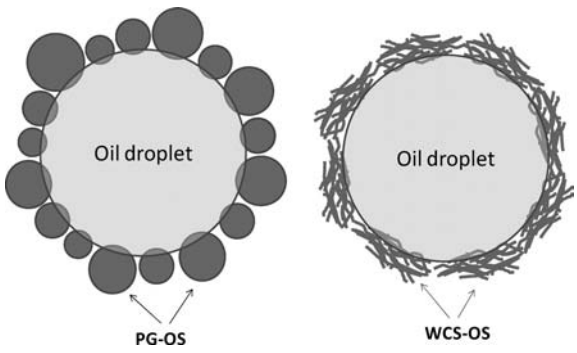


**Figure 7.** Schematic of PG-OS and WCS-OS coverage at the surface of an oil droplet.

**Figure 6** shows that, during the 4-week storage of emulsions, using PG-OS (DS 0.0234) and PG-OS (DS 0.0498) resulted in lower change of emulsion particle size than using WCS-OS (DS 0.0260) and WCS-OS (DS 0.0429). This indicates an appreciable capability of PG-OS nanoparticles to physically stabilize fish oil emulsion. Over a century ago, Pickering indicated that colloidal particles can be used to stabilize emulsions (*24*). Recently, there is a growing interest in the micro- and nanoparticle-stabilized emulsions. The unique properties of these emulsions are attributed to the large free energy of interfacial adsorption for particles. This adsorption usually leads to high stability of emulsions (*25*). In certain conditions, oil, water, and nanoparticles may even form thermodynamically stable oil-in-water emulsions (*26*). In a typical spherical particle-stabilized emulsion, the contact angle governs the type and stability of emulsion (*25*). For hydrophilic particles, contact angle is below 90° and emulsion is oil-in-water type. For hydrophobic particles, contact angle is over 90° and emulsion is water-in-oil type. In the current study, PG-OS is hydrophilic due to the presence of carboxylate and hydroxyl groups, and the oil-in-water emulsions formed were quite stable when the DS value of PG-OS was sufficient. Conceivably, the contact angle of PG-OS nanoparticles at the oil−water interface would be below 90°. However, work remains to be done to measure the contact angle of PG-OS nanoparticles to understand the impact of carbohydrate structure and environmental conditions on the hydrophobicity of nanoparticles and stability of emulsions.

**Figure 7** depicts the coverage of PG-OS nanoparticles or WCS-OS molecules on the surface of an oil droplet. The major structural differences of interfacial layer are the high molecular density for the PG-OS layer and high chain flexibility for the WCS-OS layer. From this study, we have found that the structural difference of interfacial layer may not result in a drastic difference of physical stability of emulsions. However, the interfacial layer functions as a barrier against lipid-degrading factors in the bulk of aqueous phase. These factors include pro-oxidative compounds such as oxygen, free radicals, and metal ions which may trigger or promote lipid oxidation and lead to deteriorated emulsion quality. By constructing a high-density interfacial layer using PG-OS, it is possible to reduce the susceptibility of lipids to lipid-degrading compounds and, thus, to improve lipid oxidative stability of emulsions (*6*).

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