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Structures of octenylsuccinylated starches: Effects on emulsions containing β-carotene

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ABSTRACT

Starches with different amylopectin contents and different molecular sizes prepared using acid hydrolysis were hydrophobically modified using octenylsuccinic anhydride (OSA). The OSA-modified starches were used as surfactants to stabilize emulsions of β-carotene and canola oil dispersed in water. The objective of this study is to investigate the relationship between starch molecular structure and the chemical stability of the emulsified β-carotene, as well as the colloidal stability of emulsion droplets during storage. The oil droplet size in emulsions was smaller when starch had (a) lower hydrodynamic volume (V_h) and (b) higher amylopectin content. The oxidative stability of β-carotene was similar across samples, with higher results at increased amylopectin content but higher V_h. Steric hindrance to coalescence provided by adsorbed OSA-modified starches appears to be improved by more rigid molecules of higher degree of branching.

1. Introduction

>Starch is used as a food additive and in non-food applications for stabilizers, emulsifiers, encapsulating agents, etc. To improve the functional properties of starch, various chemical modifications are performed; one such modification is the reaction with octenylsuccinic anhydride (OSA) to produce octenylsuccinylated (OS) starch, which has long been used as a texturizer and emulsion stabilizer (Caldwell & Wurzburg, 1953).

The emulsifying properties of OS starch are dependent not only on the chemical substitution, but also the structure of the parent starch molecules. It has been found that between the molecular weight range of 1.2 and 2.6×10^5, OS starches of higher molecular weights show better colloidal stability (Dokic, Dokic, Dapcevic, & Krstonosic, 2008), although there may be some trade-off in terms of viscosity and paste clarity above a certain molecular weight. The botanical source of the parent starch is the first criterion in determining the structure of the end product, particularly the ratio of amylose and amylopectin (He, Song, Ruan, & Chen, 2006) and the chain-length distributions of starch molecules. The structure of the parent starch may be further modified before, during or after OSA modification to enhance the emulsifying properties of OS starch. The effects of starch structure on the critical aggregation concentration of OS starch have been recently investigated (Tizzotti, Sweedman, Schäfer, & Gilbert, 2013); however, no study has yet linked starch structural architecture directly with colloidal stability, or the chemical stability of encapsulated molecules in the oil phase, despite growing interest in the field (Sweedman, Tizzotti, Schäfer, & Gilbert, 2013b).

The primary mechanism of oil droplets by OS starches has been described as steric hindrance (Tesch, Gerhards, & Schubert, 2002). Expressing this in terms of colloid science (Napper, 1983), this means they function as electrosteric stabilizer, with thermodynamic resistance to pushing colloidal particles together provided by the entropic effect of higher free energy by crowding solvated polymer molecules together (steric stabilization) plus the enthalpic effect of electrostatic repulsion (electrostatic stabilization), if the pH is high, due to the carboxylate group, following OSA modification. This suggests that colloidal stability should be improved (all other things being equal) by larger sized molecules. Certain metrics for molecular size, such as molecular weight, might be less suitable for predicting colloidal stability because they do not account for the structure of the molecules in their dissolved state (as appropriate for colloidal stabilization) and can therefore vary

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with the branching architecture and branch chain length. Most commercial products and academic studies have focused on waxy starches (~100% amylopectin), because the large molecular size and the highly branched structure of amylopectin provide good steric stabilization, in addition to their high solubility and high paste clarity (Baranauskiene, Bylaite, Zukauskaite, & Venskutonis, 2007; Bruckner, Bade, & Kunz, 2007; de Paz et al., 2012).

The ability of OS starches to stabilize emulsions containing β-carotene and other lipid-soluble compounds is important in the formulation of colorings, antioxidants and pro-vitamins in food products and beverages (de Paz et al., 2012); however, the emulsified β-carotene needs to be chemically stable, not only for the shelf-life and sensory quality of the product, but also for avoiding oxidation breakdown products like aldehydes and ketones (Mordi, 1993) that may be harmful in high doses (Siems et al., 2005). OS starches have been shown to provide protective effects from degradation when used as an encapsulating agent for peppermint oil (Baranauskiene et al., 2007) and lycopene (Choudhari, Bajaj, Singhal, & Karwe, 2012), a similar compound to β-carotene. Bruckner et al. (2007) reported that OS starches have better emulsion performance than whey protein isolate and unmodified maltodextrin. On the other hand, Mao et al. (2009) reported that OS starch did not protect β-carotene from degradation as well as some other food stabilizers.

The previous studies of emulsions stabilized by OS starches reviewed in the preceding paragraph have used different emulsion processes or conditions and different structures of the OS starches. The present study is to our knowledge the first to provide comparative data to understand how starch structure (molecular size distribution, degree of branching (DB) and amylose content) affects the colloidal stability of the emulsion (and in turn the chemical stability of functional components in the oil phase). We also investigate starch degradation during homogenization and the physicochemical properties of the starches used. Three maize starches of different amylopectin contents were used (waxy, normal and high-amylose varieties). Their molecular structures were modified using HCl in alcohol solution to give a series of molecular sizes. All starches were then substituted with OSA and used to make emulsions of β-carotene in canola oil in water under the same processing conditions. The outcomes provide evidence of the relationship between starch structure and chemical stability of emulsified β-carotene as well as characterizing the starch degradation during homogenization, an understanding of which is important for product development in the food emulsifier industry.

While there have been some advances of theory for simple branched electrostatic stabilizers, this is not the case for OS starches, which are primarily steric (Tesch et al., 2002), are polydisperse in structure, and probably adsorb in multiple layers rather than the monolayer assumed by all current models for stabilizers. Good stabilization by steric hindrance relies on high free energy requirements in the hydrophilic part of the polymer stabilizers to prevent droplets being pushed together (Napper, 1983). Semi-empirical concepts such as the HLB (hydrophilic-lipophilic balance), which is also related to DS, do not seem to be useful in trying to determine causal relations between structure and properties (Graciaa, Barakat, Schechter, Wade, & Yiv, 1982; Witthayapanyanon, Harwell, & Sabatini, 2008).

2. Materials and methods

2.1. Materials

Waxy maize (W), normal maize (N) and high-amylose maize (Gelose 80, G) starches were obtained from Penford Australia Pty. Ltd. (now Ingredion ANZ Pty. Ltd., Lane Cove, NSW, Australia). OSA was a *cis* and *trans* mixture of 2-octen-1-ylsuccinic anhydride (97%) and β-carotene was Type I synthetic (≥93% powder) purchased from Sigma–Aldrich Pty. Ltd. (Castle Hill, NSW, Australia). All water was processed through Milli-Q^{TM} ultra-pure system (Millipore Australia Pty. Ltd., Kilsyth, VIC, Australia). Canola oil was food-grade obtained from a local grocery store. Methanol, ethanol and isopropanol were purchased from Merck & Co., Inc. (Kilsyth, VIC, Australia). Hydrochloric acid (37%, analytical reagent) was from Lab Scan Analytical Sciences (Patumwan, Bangkok, Thailand).

2.2. Acid hydrolysis of starches

Acid hydrolysis of starches was based on the methods of Robyt, Choe, Fox, Hahn, and Fuchs (1996a) and Robyt, Choe, Hahn, and Fuchs (1996b). Native starch granules (200 g) were suspended in a solution containing 198 mL methanol, 297 mL isopropanol and 5 mL of concentrated HCl. The suspension was thoroughly mixed and then stored with stirring for a period of 5 d at either 23 or 45 °C. The supernatant was discarded after centrifugation at 3000 × *g* for 2 min and the solid was resuspended in 0.1 M tricine buffer at pH 7.5, which was then neutralized with 1 M NaOH or 1 M HCl. The acid-modified starch was recovered by centrifugation as precipitate and then washed 3 times by repeated suspension in absolute ethanol followed by centrifugation. The starch sample was air dried at 60 °C overnight. To denote the acid hydrolysis treatments, subscript N, H23 and H45 are used for native starch and starch after acid hydrolysis at 23 and 45 °C, respectively. For example, G_{H45} is high-amylose maize starch after acid hydrolysis at 45 °C. The method of acid hydrolysis in alcohol solution cannot be used to prepare a food grade starch, but is used in the present study to provide more narrow SEC weight distributions than those obtained using acid hydrolysis in aqueous solution, allowing improved correlations between hydrodynamic size and properties.

2.3. Octenylsuccinylation of starches

Native and acid-modified starch granules were modified with OSA using the method of Song, He, Ruan, and Chen (2006). OSA (1.5 g) was first dissolved in ethanol (7.5 g) as this mixture has been found optimal for starch modification (Shi & He, 2012). The OSA solution was added drop-wise and continuously over 2 h to 50 g starch in 200 mL water at 35 °C, and the mixture allowed to react for an additional hour at 35 °C. The pH was continuously adjusted to 8.5 with 0.2 M NaOH. After 3 h, the OS starch was neutralized with 0.02 M HCl, washed twice with ethanol and dried at 65 °C.

2.4. Size-exclusion chromatography

The SEC weight distributions of native, acid-modified, and OS starches as well as OS starches after homogenization were analyzed using size-exclusion chromatography (SEC) following a method used elsewhere (Vilaplana & Gilbert, 2010). The SEC system was an Agilent 1100 series (Agilent Technologies, Waldbronn, Germany) consisting of an isocratic pump, an autosampler injecting from a 100 μL piston without temperature control, and a column oven set at 80 °C. The separation of starch molecules was performed using a combination of GRAM Pre-Column, 30 and 3000 columns (Polymer Standards Service, Mainz, Germany) with dimethyl sulfoxide (DMSO) containing 0.5% w/w LiBr as eluent set at 0.3 mL min^{-1} and with a differential refractive index detector (RID-10A, Shimadzu, Kyoto, Japan), operating at 635 nm and thermostated at 45 °C. A series of pullulan standards with peak molar masses of 3.42×10^2 to 2.35×10^6 (representing R_h between 0.41 and 58 nm) were used to fit a third order polynomial calibration of SEC elution volume to hydrodynamic volume (V_h) or equivalent hydrodynamic radius

(R_h), and the SEC weight distributions are presented as $w(\log V_h)$ plotted against R_h.

2.5. Nuclear magnetic resonance spectroscopy

The ^{1}H nuclear magnetic resonance (NMR) spectra of OS starch samples were obtained using the method of Tizzotti, Sweedman, Tang, Schaefer, and Gilbert (2011) with modifications to limit disturbance of the OSA signal, as follows. A 1:1 mixture of deuterated trifluoroacetic acid (TFA-*d*) in deuterated dimethyl sulfoxide (DMSO-d_6) (30 μL) was added directly into the 5 mm NMR tube containing the sample medium (650 μL) immediately before the analysis. The NMR spectra were recorded at 50 °C using a Bruker Avance NMR spectrometer operating at an observation frequency of 500.15 MHz for ^{1}H, equipped with a TXI5z probe (Bruker Biospin, Alexandria, NSW, Australia) (8.80 μs 90° pulse, a repetition time of 15.98 s composed of an acquisition time of 3.98 s and a relaxation delay of 12 s, 64 scans, DMSO-d_6). The chemical shift scale was calibrated using the residual DMSO signal at 2.549 ppm (Schmitz, Dona, Castignolles, Gilbert, & Gaborieau, 2009).

Data were processed using Bruker TOPSPIN software (v2.1; Bruker Biospin). All spectra were manually phased and baseline-corrected. A Lorentzian fit was used for spectral deconvolution. DB and DS were calculated from the ^{1}H-NMR spectra using the method of Tizzotti et al. (2011).

The reaction efficiency (RE) is the ratio of measured DS of OS starch to the maximum (theoretical) DS that can be achieved from the moles of OSA per mole of starch used for the reaction, as follows:

$$\mathrm{RE} = \frac{210 \times \mathrm{DS} \times m_{\mathrm{starch}}}{162 \times m_{\mathrm{OSA}}} \times 100 \quad (1)$$

where 210 is the molecular weight of OSA and 162 is the molecular weight of the anhydroglucose unit (the monomer of starch), and m_{starch} and m_{OSA} are the masses of starch and OSA used in the reaction, respectively.

2.6. Differential scanning calorimetry

Onset temperature (T_o), peak temperature (T_p) and conclusion temperature (T_c) of starch gelatinization were determined using a method similar to that of Li, Hasjim, Dhital, Godwin, and Gilbert (2011). Granular OS starch (4–6 mg, dry weight basis) was precisely weighed into a high-pressure crucible for differential scanning calorimetry (DSC) analysis and moistened with water of about three times the starch mass. The thermal properties were analyzed in duplicate using a DSC 1 STAR^e system running STAR^e Software v9.10a (Mettler-Toledo Inc., Tennyson, QLD, Australia). The analysis used an initial temperature of 10 °C for 1 min, rising to 180 °C at 10 °C min^{-1}.

2.7. Rapid visco analyzer

The pasting properties of OS starches were analyzed in duplicate from 2 g starch (dry weight basis) suspended in water (23 g) using a Rapid Visco Analyser (RVA, Newport Scientific, Warriewood, NSW, Australia), operated by Thermocline for Windows v2.2. The heating profile consisted of holding at 50 °C for 1 min, rising to 95 °C at 6 °C min^{-1}, holding at 95 °C for 5 min, cooling to 50 °C at 6 °C min^{-1}, before finally holding at 50 °C for 2 min.

2.8. Paste clarity

Paste clarity was measured using the method of Bello-Pérez, Agama-Acevedo, Sánchez-Hernández, and Paredes-López (1999). OS starch (1%) was suspended in water and the suspension was heated in a boiling water bath for 30 min with shaking. The resulting gel was then analyzed in duplicate for its transmission (%T) at 650 nm using a PharmaSpec UV-1700 spectrophotometer (Shimadzu).

2.9. Preparation of β-carotene emulsions

Emulsions were prepared in duplicate as follows. Canola oil was used to dissolve β-carotene at 2% w/w concentration by heating in a boiling water bath for 10 min with agitation. Each OS (native or acid-modified) starch (1.0% w/w of final emulsion) was suspended in water and heated in a boiling water bath for 30 min with agitation. The starch suspension (pH 6.5–7.0) was allowed to cool, and sodium azide was added to a final concentration of 0.02% w/w. The β-carotene in canola oil solution was added to a final concentration of 1.0% w/w, giving a total β-carotene content of 200 mg L^{-1}. The entire mixture was shaken, coarsely homogenized using an ultra-turrax T25 (IKA-Werke GmbH & Co. KG, Staufen, Germany) for 20 min at 9500 min^{-1}, and finally subjected to high pressure homogenization (HPH) with a TwinPanda400 two-stage valve homogenizer (GEA Niro-Soavi, Parma, Italy) using 6 cycles (34 ± 1 s cycle^{-1}) of a first stage pressure of 200 bar and a second stage of 50 bar. The temperature of all samples throughout the homogenization processing did not exceed 50 °C.

Part of each emulsion (5 mL) was precipitated with 40 mL acetone, followed by centrifugation at 3000 × *g* for 2 min. The pellet was washed repeatedly with 30 mL acetone until it became white, which is the OS starch after the removal of β-carotene and canola oil. The starch pellets were then dried at room temperature overnight.

2.10. Colloidal stability

The colloidal stability during storage was characterized from the color change of the emulsion, degradation of emulsified β-carotene, change of oil droplet size, phase separation and sedimentation of the starch material. From each emulsion, 1 mL aliquots were placed into 15 mL centrifuge tubes for β-carotene analysis in triplicate, whereas the samples for droplet size analysis comprised 10 mL emulsion stored in 15 mL tubes. These were sealed after being flushed with nitrogen gas. Some were stored at room temperature (23 °C) and some in an incubator (IG550, Clayson, Australia) at 55 °C up to 20 d, except for initial (time-point "0") samples, which were stored overnight at 4 °C. This is an improvement upon similar experiments by Mao et al. (2009), which were performed by removing aliquots of the stock solution at different sampling times and may have resulted in sampling errors due to the heterogeneity of stock solution.

2.10.1. Droplet size of emulsion

The z-average droplet sizes in β-carotene emulsions were determined using a Zetasizer Nano-ZS (Malvern Instruments, Worcestershire, UK) at a fixed detector angle of 90°. Samples were consistently taken from approximately 20 mm below the emulsion surface after gentle inversion, diluted to 10% of the original concentration to minimize multiple scattering effects and analyzed in 4 mL cuvettes.

2.10.2. Phase separation of emulsion and sedimentation of OS starch

The 10 mL aliquots of emulsion samples were left undisturbed at room temperature and at 55 °C. The level of sediment or coagulation layer was examined at 13 and 20 d. Where a sediment layer and uneven texture were clearly visible, the emulsion was deemed to have broken.

At the conclusion of the colloidal stability test at 20 d, sediment was collected from emulsions containing OS-G_N and OS-G_{H23}, which were the only samples with substantial amounts of sediment. Each collected sediment was washed at least seven times with two volumes of 1:3:3 water:ethanol:*n*-hexane solution, followed by centrifugation at 4000 × *g* for 5 min, to remove oil and β-carotene, and then once with three volumes of pure ethanol and air dried at 60 °C. The isolated starch sediment was analyzed using ^{1}H NMR and SEC, and the results were compared with those from the whole starch material in the emulsion sample.

2.10.3. Color change of emulsion

The color change of each emulsion during storage was determined using standard evaluation values of L^* (overall lightness), a^* (redness and greenness) and b^* (yellowness and blueness). To ensure color change values reflected the conditions of β-carotene degradation analysis, 1 mL stored portions were combined before each test. The color of 5 mL of emulsion was determined using Chroma Meter CR-400 (Konica Minolta Sensing, Inc., Osaka, Japan) calibrated with standard white tile and using the average from three measurements. Each emulsion sample was placed in a 6 mL glass beaker, which was shielded from ambient light, giving a sample depth of 17 mm.

2.10.4. Degradation of emulsified β-carotene

The emulsified β-carotene was extracted from stored emulsion samples using the method of Mao et al. (2009) with slight modifications. The extraction used 3 mL ethanol and 4 mL cold (4 °C) *n*-hexane, followed by thorough agitation and centrifugation at 3000 × *g* for 1 min at 4 °C. Then the *n*-hexane (upper) phase was diluted to concentrations within the standard calibration range of 0, 1 and 2 mg L^{-1} β-carotene in *n*-hexane containing 1 g L^{-1} oil, for a linear relationship between concentration and absorbance and to minimize interactions that can alter the color of β-carotene at higher concentrations (Karabudak, Wohlleben, & Cölfen, 2010). The absorbance was measured immediately while the sample was still cold, to avoid any changes in concentration due to the evaporation of *n*-hexane solvent. The absorbance of extracted β-carotene was analyzed in triplicate using PharmaSpec UV-1700 spectrophotometer (Shimadzu) at a wavelength of 453 nm in 4 mL PMMA cuvettes.

2.11. Statistical analysis

Data were collected in replicates as stated and processed using Excel (Microsoft, 2010) and Minitab 16 (State College, PA, USA). Analysis of variance (ANOVA) used Fisher 95% individual confidence intervals from the mean values of replicate measurements. Correlations among starch structural parameters, properties of OS starches and stability of emulsified β-carotene were also determined.

3. Results and discussion

3.1. Starch structural parameters

3.1.1. Hydrodynamic size of starches at different processing steps

SEC weight distributions of waxy, normal and high-amylose maize starches and their OS counterparts before and after homogenization are presented in Supplementary data Fig. S1. Analyses of these larger, native structured starches were performed for comparison to the more degraded forms present in the final emulsions. Some samples contain significant amounts of molecules above the size range where shear scission can be neglected (Cave, Seabrook, Gidley, & Gilbert, 2009). However, starch in the final emulsions after HPH are mostly below the limitations of shear scission, allowing reliable conclusions to be drawn from these analyses. Furthermore, although shear scission depends on the particular SEC set-up (including sometimes the day on which the samples were run), the apparent SEC distribution obtained for samples above the shear-scission range is reproducible on a given day and set-up, and hence any trends (such as larger sizes in a given sample) are meaningful. The three native starches of different amylopectin contents and their acid-modified forms provide a series of starches with a wide range of SEC weight distributions. Of the three native starches, waxy maize starch showed the greatest size reduction by acid hydrolysis, whereas high-amylose maize (Gelose 80) starch showed the least change. Acid hydrolysis at 23 °C resulted in a decrease in the components with R_h between 200 and 1000 nm, mostly amylopectin, to R_h below 100 nm. Acid hydrolysis at 45 °C resulted in smaller molecular size where the peak R_h of starch molecules was between 5 and 7 nm regardless of the botanical sources or amylopectin content.

Esterification with OSA resulted in slight changes to the SEC weight distributions of starch molecules (Supplementary data Fig. S1), especially those derived from native waxy maize starch. However, the effect of OSA modification on SEC weight distribution is inconsistent, and indeed in a previous study no apparent changes to SEC weight distributions were observed as a result of this method of OSA modification (Sweedman, Hasjim, Tizzotti, Schäfer, & Gilbert, 2013a). Such changes in SEC weight distribution may be the result of starch hydrolysis during the esterification procedure, which uses strong acids and bases, or may also be the result of specific molecular populations being selectively removed during the washing steps.

Shear scission during the HPH process causes molecular degradation of large starch molecules such as amylopectin, and is known to cause starch pastes to thin after HPH (Che et al., 2009). Shear scission has also been observed after SEC separation, extrusion, and dry grinding (Cave et al., 2009; Liu, Halley, & Gilbert, 2010; Tran et al., 2011). Shear scission of OS starches by HPH has been investigated previously (Nilsson, Leeman, Wahlund, & Bergenståhl, 2006) and in Modig, Nilsson, Bergenstahl, and Wahlund (2006), which used three OS starches based on normal potato starch at different DS. In the current study, after HPH treatment, the SEC weight distributions of OS derivatives of native starches and starches acid-modified at 23 °C (both have max R_h > 100 nm) were severely reduced to an R_h range of 1–100 nm (Supplementary data Fig. S1a, b, e, d, g and h), while the SEC weight distributions of OS starches from the those acid-modified at 45 °C (R_h range of 0.1–50 nm) remained superimposable (Supplementary data Fig. S1c, f and i). The molecules are normally sheared to a maximum stable size (peak $R_h \sim 25$ nm), and no further reduction in molecular size occurs below this size, which is in accordance with the evidence presented in Modig et al. (2006). Amylose and some acid-hydrolyzed starch molecules are near or smaller than the maximum stable size, and hence the shear degradation is less apparent on OS starches from high-amylose maize starch and the starches acid-modified at 45 °C (R_h range of 0.1–50 nm). In addition, the SEC weight distributions of replicate starches after HPH treatment show almost no variation between the replicate emulsions (Supplementary data Fig. S1, *z* values). Although the large molecules in several of the OS starches used in this study were reduced to much smaller sizes, it is important that different SEC weight distributions were maintained between the different samples, allowing the comparisons to be made between them (Fig. 1). This process resulted in samples with peak R_h ranging from 6 to 29 nm (Table 1).

3.1.2. Degree of branching and degree of substitution of octenylsuccinylated starches

The results of NMR analysis for DB and DS are summarized in Table 1. There are distinct differences in the DBs among the three

Table 1
Physicochemical properties of OS starches[a].

Sample	Peak R_h (nm)	DB (%)	DS	RE (%)	T_o (°C)	T_p (°C)	T_c (°C)	Peak viscosity (cP)	Final viscosity (cP)	Paste clarity (%T)
OS-W_N	28.6 ± 0.6	2.86 ± 0.14	0.0174 ± 0.0011	75.1 ± 5.0	70.97 ± 1.05	80.21 ± 0.93	94.95 ± 1.26	6098.5 ± 4.9	3658.0 ± 5.7	32.1 ± 1.4
OS-W_{H23}	23.9 ± 1.8	3.13 ± 0.08	0.0149 ± 0.0003	64.5 ± 1.4	66.61 ± 0.64	78.70 ± 0.28	94.20 ± 0.53	217.0 ± 1.4	74.0 ± 9.9	93.8 ± 0.3
OS-W_{H45}	6.9 ± 0.0	3.46 ± 0.53	0.0103 ± 0.0001	44.7 ± 0.5	58.95 ± 5.04	75.22 ± 1.63	101.13 ± 2.24	17.0 ± 11.3	4.0 ± 4.2	96.6 ± 0.6
OS-M_N	26.5 ± 0.8	1.92 ± 0.19	0.0156 ± 0.0022	67.5 ± 9.6	67.51 ± 1.10	75.26 ± 0.52	87.94 ± 0.33	3784.5 ± 33.2	3211.5 ± 68.6	30.1 ± 0.4
OS-M_{H23}	25.8 ± 1.3	2.16 ± 0.10	0.0152 ± 0.0022	65.7 ± 9.6	65.18 ± 2.14	75.52 ± 0.01	89.34 ± 3.10	837.0 ± 4.2	339.5 ± 0.7	58.3 ± 3.1
OS-M_{H45}	5.5 ± 0.1	2.39 ± 0.02	0.0141 ± 0.0006	60.7 ± 2.6	59.00 ± 3.03	77.98 ± 0.45	102.39 ± 4.54	13.5 ± 6.4	−3.5 ± 6.4	93.6 ± 0.5
OS-G_N	11.1 ± 0.1	0.69 ± 0.02	0.0131 ± 0.0013	56.5 ± 5.4	74.26 ± 0.15	87.11 ± 0.87	111.91 ± 1.16	147.5 ± 12.0	139.5 ± 10.6	2.0 ± 0.1
OS-G_{H23}	10.5 ± 0.4	0.80 ± 0.06	0.0144 ± 0.0013	62.2 ± 5.7	70.90 ± 0.33	87.46 ± 0.13	111.95 ± 1.24	66.0 ± 2.8	67.0 ± 1.4	2.4 ± 0.2
OS-G_{H45}	6.1 ± 0.1	0.90 ± 0.01	0.0152 ± 0.0010	65.5 ± 4.3	70.39 ± 2.92	89.41 ± 4.70	108.80 ± 6.26	4.5 ± 7.8	−4.0 ± 5.7	4.1 ± 0.4

Peak R_h refers to samples taken after HPH. All other measurements used sample prior to emulsion preparation.
[a] Means ± standard deviations.

native starches, and the DB increases in line with the level of acid hydrolysis for each starch.

The DS of OS starch is slightly affected by the type and structure of the parent starch, and the REs of all samples are lower than those of Song et al. (2006); however, this does not affect the conclusions of the current study. There is significant positive correlation between RE and the peak R_h of OS starch after HPH ($p < 0.05$, Supplementary data Table S1). Keeping in mind that the OSA modification process was performed prior to HPH, though the sequential order of R_h values is maintained; the results from the present study disagree with that reported by He et al. (2006), who found increases in DS and RE with amylose content. This difference could be the result of the presence of pores on the surface of native waxy and normal maize starch granules, increasing the surface area for chemical reaction. The presence of the pores has been reported as less apparent for native high-amylose maize starch granules (Dhital, Shrestha, & Gidley, 2010). On the other hand, He et al. (2006) used rice starches with a narrower range of amylose contents (0–40%), which had similar surface structure of starch granules. These differences with the literature, however, do not affect the outcome of the current study.

3.2. Properties of octenylsuccinylated starches

3.2.1. Gelatinization temperatures

The gelatinization temperatures of OS starches are summarized in Table 1. Among the three OS native starches, normal maize starch has the lowest gelatinization temperature and high-amylose maize starch has the highest ($T_c > 110$ °C), showing that T_o and T_p are significantly and negatively correlated with DB ($p < 0.05$ and 001, respectively, Supplementary data Table S1). This is consistent with common knowledge that gelatinization temperatures are correlated to chain length distribution (Jane et al., 1999), and because the chain length distribution gives the DB of OS starch, which is determined by the native structure of starch prior to acid hydrolysis. After acid hydrolysis, all OS starches have lower T_o, and OS waxy and normal maize starches have higher T_c. As acid hydrolyses the amorphous layer more readily than the crystalline layer of starch granules (BeMiller, 1965), the crystallites are increased as a proportion of constitutive structures in the granule; however, the loss of the amorphous material also weakens the structural integrity of starch granules. As a result, there is a significant negative correlation between T_c and the peak R_h of OS starch after HPH ($p < 0.05$, Supplementary data Table S1).

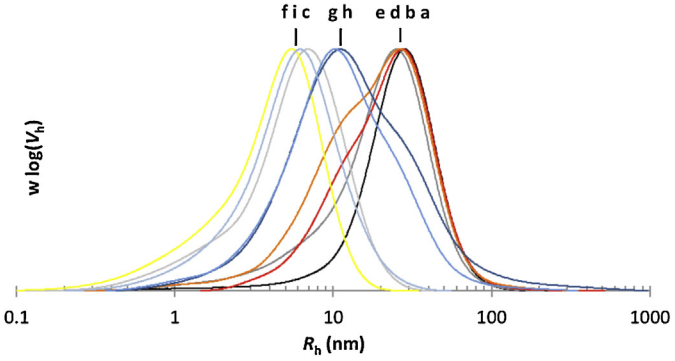

Fig. 1. SEC weight distributions of OS starches after HPH. (a) OS-W_N, (b) OS-W_{H23}, (c) OS-W_{H45}, (d) OS-M_N, (e) OS-M_{H23}, (f) OS-M_{H45}, (g) OS-G_N, (h) OS-G_{H23} and (i) OS-G_{H45}. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.2.2. Pasting properties

For beverage colorant applications, it is important that OS starch based emulsion stabilizers provide low viscosity formulations. Furthermore, highly viscous starch materials require greater pressure or energy to flow during processing. For this purpose, only peak and final viscosities from RVA analysis are compared among different OS starches. Both viscosities dramatically decrease with higher levels of acid hydrolysis (Table 1) as is consistent with common knowledge (Wang, Truong, & Wang, 2003) and there are significant positive correlations between both viscosities and the peak R_h of OS starch after HPH ($p < 0.05$, Supplementary data Table S1), since the peak R_h after HPH follows a similar sequence to that of the peak R_h of the parent starches prior to HPH treatment. This is simply because smaller molecules have less interaction among themselves in solution, resulting in lower viscosity. Furthermore, peak viscosity also decreases in order of increasing amylose content, whereas this trend is less apparent for the final viscosity. The development of peak viscosity is due to the swelling of starch granules during heating or gelatinization, which is dominated by amylopectin structure (Tester & Morrison, 1990). On the other hand, the final viscosity is due to the network formation of amylose molecules during cooling (Blazek & Copeland, 2008). The incomplete gelatinization of high-amylose maize starch at 95 °C is due to its high gelatinization temperature (Table 1) also contributes to the low viscosity of the starch paste (Jane et al., 1999).

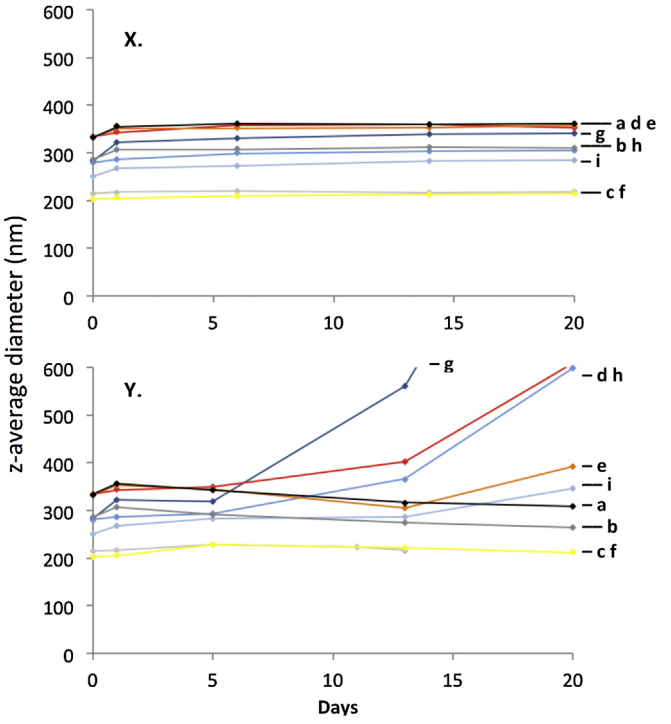

Fig. 2. *z*-Average droplet sizes in emulsions stored at (X) room temperature and (Y) 55 °C. (a) OS-W_N, (b) OS-W_{H23}, (c) OS-W_{H45}, (d) OS-M_N, (e) OS-M_{H23}, (f) OS-M_{H45}, (g) OS-G_N, (h) OS-G_{H23} and (i) OS-G_{H45}. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.2.3. Paste clarity

Measurements of paste clarity are related to amylose content and starch SEC weight distributions, in agreement with evidence previously reported by Visser, Suurs, Bruinenberg, Bleeker, and Jacobsen (1997) (Table 1). The paste clarity of OS starch increases with lower amylose content, as well as greater reduction in size (V_h) of the amylopectin molecules. However, the increases in paste clarity after acid hydrolysis are greater in OS waxy maize starch than in OS normal maize starch, and the increase is less apparent for the OS high-amylose maize starch than the others. The higher paste clarity of OS native and acid-modified waxy maize starches compared with that of OS native and acid-modified normal maize starches is because amylopectin has higher solubility in dispersed form than amylose (Miles, Morris, Orford, & Ring, 1985), as reinforced by the significant positive correlation between DB and paste clarity ($p < 0.01$, Supplementary data Table S1). The T_o and T_p of OS starch are also significantly and negatively correlated with paste clarity ($p < 0.05$). The low paste clarity of OS starches from high-amylose maize can also be attributed to their high T_c (>110 °C) (Table 1) not allowing a complete gelatinization during heating in a boiling water bath. The remaining crystallites that survive the heating process diffract light and contribute to the opacity of the solution.

3.2.4. Droplet size of emulsions

It must be noted that dynamic light scattering employed by zetasizer assumes homogeneity of the density of particles, which is clearly not the case in this instance, so the results reported here in nm do not refer to any true physical dimension. The droplet sizes of emulsion samples made from OS starches were monitored over 20 d during storage both at room temperature (22 ± 3 °C) and at elevated temperature (55 ± 2 °C) (Fig. 2X and Y, respectively). All emulsion samples display an initial increase in droplet size within the first 24 h at both storage temperatures. The droplet sizes of all emulsion samples remained relatively stable thereafter up to 20 d at room temperature, but those stored at elevated temperature were less stable and dependent on the amylose content and the acid hydrolysis treatment. The droplet sizes of OS native maize starches with higher amylose content increased more rapidly during storage at elevated temperature, whereas those of OS waxy maize starches (including the native and acid-modified forms) kept decreasing up to 20 d after the first 24 h. In this case it is likely that the multi-layered configuration of OSA-starch molecules on droplet surfaces tends to reconfigure during the hot storage process without a change in oil content of individual droplets, thus gradually forming thinner interfacial layers. Under this scenario, the heterogeneity of the overall droplet is changed along with the changes in surfactant configuration, which means the results from dynamic light scattering are inconclusive in determining the mechanisms of a sample's droplet size evolution over time.

Acid modification of substrates of OS normal and high-amylose maize starches increased the stability of the droplet sizes in emulsions stored at elevated storage temperature, with those acid-modified at 45 °C more stable than their counterparts acid-modified at 23 °C. Furthermore, for each starch, the OS starch from substrate that had been acid-modified at 45 °C displayed the smallest droplet size, because smaller molecules more readily conform to the droplet surface, are easier to dissolve and are more mobile in the aqueous phase, allowing lower radii of curvature (smaller droplets) to be achieved. Droplet sizes of emulsions after 20 d storage at room temperature (Fig. 2Y and Supplementary data Table S1) show significant positive correlations with peak R_h of OS starch after HPH, DS and RE ($p < 0.05$). Dokic et al. (2008) also reported that OS starch of larger molecular mass-produced larger droplet size in emulsions. Starch molecules of larger hydrodynamic size are less able to diffuse and to flex around the curved oil–water interface of smaller oil droplets, forming a thicker layer at the interface and larger droplet size. On the other hand, the droplet size of emulsion after 20 d storage at 55 °C has a significant negative correlation with DB ($p < 0.05$). Significant positive correlations ($p < 0.05$) occur between droplet size (both after 20 d storage at room temperature and 55 °C) and T_o as well as between the droplet size after 20 d storage at 55 °C and paste clarity, indicating that droplet size is related to the readiness of starch to gelatinize, as well as the dissolution of the starch after heating. The slight difference in DS between the samples in this study (as a result of variable RE) was not significant enough to determine any correlations with droplet size. This may be further investigated in variable DS studies to affect the free energy of solvation.

3.2.5. Phase separation of emulsions

Emulsion samples from OS-G_N, OS-G_{H23}, OS-M_N and OS-M_{H23} showed some separation (a layer of oil on the top of a layer of aqueous emulsion) before 13 d storage at 55 °C and hence were deemed to have broken, whereas other samples did not show visible phase separation. After 20 d storage, no further changes on the separation of the emulsion were observed. In the case of OS-G_N, the final subnatant was almost completely transparent and colorless. The same emulsion samples stored at room temperature did not break as noticeably; however, OS-G_N and OS-G_{H23} showed some degrees of separation and a distinguishable gradient of color with β-carotene oil increasing toward the top of the vessel. This is probably due to the higher temperatures increasing interactions resulting in coalescence of the suspended oil droplets.

3.2.6. Molecular structure of starch sediment

The high-amylose starches with high T_c (>110 °C) (Table 1), also displayed significant sedimentation of undissolved components, which were analyzed for comparative purposes. The sediment materials from OS-G_N and OS-G_{H23} were found to have DS levels

of 0.0069 ± 0.0002 and 0.0066 ± 0.0003, equivalent to 53 and 46% of the DS levels of their whole starch counterparts, respectively. Their DB levels were slightly less affected at 0.573 ± 0.061 and 0.634 ± 0.090%, equivalent to 83 and 79% of the DB levels of their whole starch counterparts, respectively. The results indicate that the sediment materials are less substituted and less branched, and hence they have poorer surfactant activity than starch molecules with higher DB and DS levels. These insoluble materials are also responsible for the lower paste clarity measurements, and arise from the high T_c (>110 °C) (Table 1).

The sediment materials, however, have similar SEC weight distributions of starch molecules to its whole sample counterparts (Supplementary data Fig. S1)—giving no clear indication of the influence of size on poorer solubility of the sediment material.

3.3. Stability of emulsified β-carotene

Because all OS starches produced emulsion with high stability (small changes during storage) at room temperature (Supplementary data), only the changes after storage at elevated temperature (55 °C) will be discussed for the stability of emulsified β-carotene.

Degradation of β-carotene is primarily the result of oxidation reactions and results in a loss of pigment in emulsions (Mordi, 1993). This study determined the pigment content by the color of intact emulsions (colorimetrically) and after isolation of the remaining β-carotene in *n*-hexane (photospectrometrically). Other effects on coloration, such as concentration and solvent qualities were kept consistent throughout and had no effect on this experiment.

The color of emulsions during the first 24 h storage at 55 °C changed from cloudy orange to pink, then gradually fading to almost white on the eighth day. These changes to color occurred regardless of any breaking or creaming in the emulsion. This is quantified by colorimetry in Fig. 3.

The changes in colorimetric values between starches were minimal. The L^* value increased slightly above initial levels during 8 d storage at elevated temperature, while b^* decreased rapidly in the first 24 h followed by a gradual decrease. The value of a^* showed the greatest changes during storage at elevated temperature, showing an initial increase (concurrent with the decrease in b^*), peaking at around 24 h, which decreased at about the same rate thereafter. The L^* value after 8 d storage at 55 °C was significantly and positively correlated with DB and paste clarity and negatively with T_o, T_p and droplet size of emulsion stored for 20 d at 55 °C ($p < 0.05$, Supplementary data Table S1), consistent with common understanding that more soluble OS starch produces more opaque emulsion. The emulsions containing OS starches from waxy and normal maize showed indications that reduction in the starch molecular size leads to greater color loss, as a^* and b^* values after 8 d storage at 55 °C are significantly and positively correlated with the peak R_h after HPH and RVA peak viscosity. Furthermore, a^* positively correlates with the droplet size of emulsion stored 20 d at room temperature and the b^* value correlates negatively with T_c and positively with final viscosity ($p < 0.05$, Supplementary data Table S1). The results suggest that the color of emulsion in the present study resulted from small amounts of breakdown products of β-carotene being within the continuous aqueous phase of the emulsion after HPH. Larger starch molecules give higher paste viscosity and greater steric hindrance to stabilize the emulsion droplets. Being free (not emulsified) in the aqueous phase means that these β-carotene molecules are readily oxidized or otherwise degraded, hence they lose their pigment characteristics more rapidly. The a^* and b^* values after 8 d storage at 55 °C are also significantly and positively correlated with DS and RE ($p < 0.05$), indicating that OS starch with higher DS or more hydrophobic groups can better stabilize the oil droplet and retain the β-carotene. However, those containing OS native high-amylose maize starch as surfactant showed greater color loss than those containing its acid-modified counterparts. As such, the results indicate no straightforward relationship between starch structure and reflected color loss, but may implicate a strong role of full starch gelatinization as a requirement of emulsion, thereby explaining the importance of amylose content to these formulations.

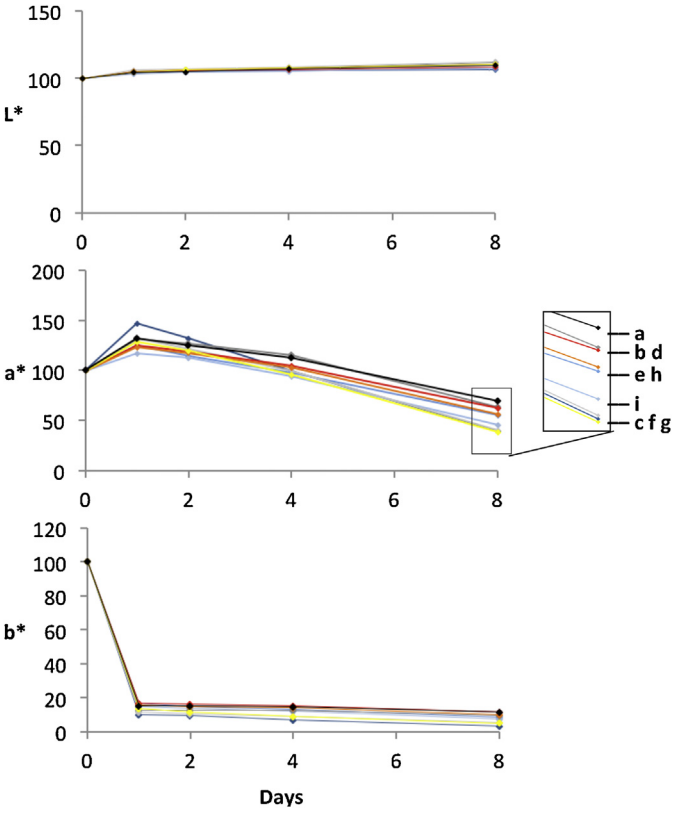

Fig. 3. Color changes of emulsions stored at 55 °C as a percentage of initial levels at Day 0. (a) OS-W_N, (b) OS-W_{H23}, (c) OS-W_{H45}, (d) OS-M_N, (e) OS-M_{H23}, (f) OS-M_{H45}, (g) OS-G_N, (h) OS-G_{H23} and (i) OS-G_{H45}. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

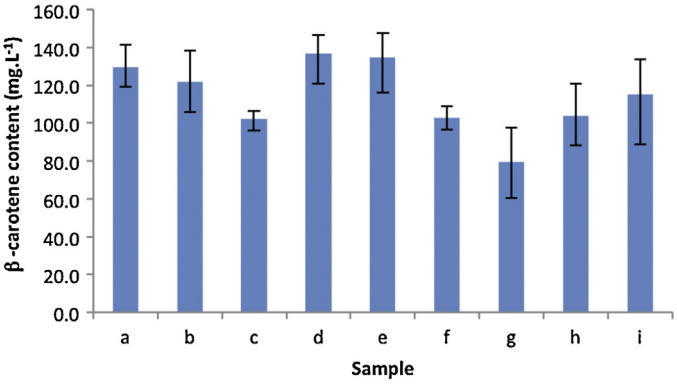

Fig. 4. Initial concentrations of β-carotene (Day 0) in emulsions after HPH; (a) OS-WN, (b)OS-WH23, (c) OS-WH45, (d) OS-MN, (e) OS-MH23, (f) OS-MH45, (g) OS-GN, (h) OS-GH23, (i) and OS-GH45. Different letters on the top of the bars indicate significant difference at $p < 0.05$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The total β-carotene contents of the emulsions stored at 55 °C were determined five times in a period of 13 d. Initial β-carotene contents (Day 0) (Fig. 4) were compared in absolute concentrations as an indication of the successful uptake of oil-phase in the

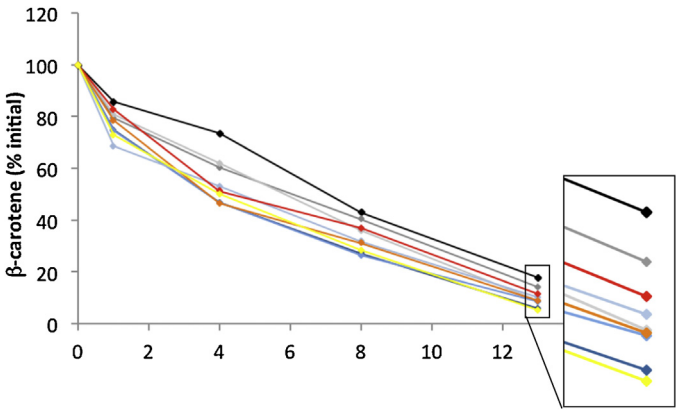

Fig. 5. Percentage of remaining β-carotene in emulsions during storage at 55 °C. (a) OS-W_N, (b) OS-W_{H23}, (c) OS-W_{H45}, (d) OS-M_N, (e) OS-M_{H23}, (f) OS-M_{H45}, (g) OS-G_N, (h) OS-G_{H23} and (i) OS-G_{H45}. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

emulsions, whereas comparisons of the degradation over time were measured as a % of initial levels. This method accounts for any β-carotene lost during the preparation process, which is mostly the result of the un-homogenized oil layer. In all OS starch samples the initial β-carotene contents vary between 70 and 140 mg L^{-1}, corresponding to 35–70% of the original amount of β-carotene used in preparing the emulsions. Using an excess of oil in preparation of these emulsions allows this method to determine the true upper limit of the capabilities of each starch as stabilizers. Even so, there are no clear correlations with structural attributes of the stabilizer used. For the OS starches from native starches and those acid-modified at 23 °C, the emulsions of high-amylose maize showed the lowest amounts of emulsified β-carotene at Day 0, which indicates the lowest uptake of oil and therefore weakest emulsion capacity, possibly due to incomplete starch gelatinization when heated in a boiling water bath (Table 1) and/or lower protective capacity of amylose molecules, such as diminished steric hindrance.

The emulsions obtained using OS starches from waxy and normal maize exhibited lower oil uptake at smaller starch molecular sizes (droplets were also smaller, as peak V_h correlated to droplet size ($p < 0.05$) so perhaps this is a function of systems where surfactant is saturated by the oil phase, thereby limiting total oil stabilized to the surface area covered by surfactant) whereas those obtained using OS starches from high-amylose maize showed increased oil uptake with smaller starch molecular size. In the case of the high-amylose maize starch the acid hydrolysis did not have a great impact on starch molecular size, thus indicating that other factors, such as solubility and degree of gelatinization, are more important for oil uptake in the emulsions.

The time dependent β-carotene degradation during storage at 55 °C (% of Day 0 concentration, Fig. 5) indicates that OS starches from waxy maize provide the best protection to β-carotene against degradation, with the OS native waxy starch performing the best of all. This indicates that hydrolysis before HPH is unnecessary and possibly detrimental in producing OS starch with high performance; though under consideration it may be necessary in terms of lowering the energy needed for processing by reducing the starch paste viscosity (Table 1).

For OS starches derived from native starches and those acid-modified at 23 °C, normal and high-amylose maize displayed higher degrees of degradation of emulsified β-carotene after 13 d storage at 55 °C than waxy maize (Supplementary data Table S2). The percentage of remaining β-carotene after 13 d storage at 55 °C is significantly and positively correlated with peak R_h of OS starch after HPH, RVA peak viscosity, RVA final viscosity and the changes in a^* and b^* after 8 d storage at 55 °C ($p < 0.05$, Supplementary data Table S1). As the stability of β-carotene agrees with the changes of a^* and b^* values with R_h, the results suggest that larger molecules are important in creating a stronger network that increases starch paste viscosity and steric hindrance, preventing the leaching of β-carotene into the aqueous phase, where it is more susceptible to oxidative degradation.

In terms β-carotene degradation, the results of this study are in line with the findings of (Mao et al., 2009), which compared just one OS starch against several non-starch surfactants. While it is clear that structural changes can improve the protective properties of OS starch stabilizers for β-carotene emulsions, the degree of variability seen in this study is probably less than that seen with different classes of surfactants.

Absorption spectra from wavelengths of 350 to 550 nm (Supplementary data Fig. S2) were obtained to determine any pigment changes to the intact β-carotene in emulsion sample (OS-G_{H45} was arbitrarily chosen as a representative) stored at 55 °C for 0, 1 and 13 d.

4. Conclusions

In this work, structurally controlled hydrophobically modified starch was prepared by selective amylopectin content and acid hydrolysis. These OSA-starches were used to stabilize emulsions containing β-carotene, which was monitored as degradation occurred over a period of 13 days. High amylopectin starches with a higher DB and more rigid structure showed the best colloidal stability, and thus protection of β-carotene against oxidation. Steric effects, specifically the effectiveness of larger and more rigid (high-amylopectin) molecules as stabilizers (in conformity with the theory of steric colloidal stabilizers) are of greatest impact in protecting β-carotene from degradation. Thus higher size had a protective effect, though this effect is counter to the ability of starch molecules to conform to the surface of oil droplets.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2014.05.067.

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