Research Article

Effect of hydroxypropylation and beta-amylase treatment on complexation of debranched starch with naringenin[†]

Ana Gonzalez[1], Ya-Jane Wang[1], Hanna Staroszczyk[2], Cindi Brownmiller[1], Sun-Ok Lee[1]

[1]University of Arkansas, Department of Food Science, 2650 N. Young Avenue, Fayetteville, AR 72704, U.S.A.

[2]Department of Food Chemistry, Technology and Biotechnology, Chemical Faculty, Gdansk University of Technology, G. Narutowicza 11/12, 80-952 Gdansk, Poland

Correspondence: Dr. Ya-Jane Wang, Department of Food Science, University of Arkansas, Fayetteville, AR 72704, U.S.A.

E-mail: yjwang@uark.edu

Fax: +1 479 575 3871.

Abbreviations: DP, degree of polymerization; **DS**, degree of substitution; **HPSEC**, high-performance size exclusion chromatography; **DSC**, differential scanning calorimetry; **ATR FT-IR**, attenuated total reflectance Fourier transform infrared

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ABSTRACT

Naringenin exhibits many health benefits but it has limited water solubility and consequently low bioavailability. The objective of this study was to investigate the effect of hydroxypropylation and enzymatic treatments on starch complexation with naringenin. Potato starch and Hylon VII were hydroxypropylated to two substitution degrees and then debranched or debranched/β-amylase treated prior to complexing with naringenin. Both soluble and insoluble complexes were recovered and characterized. An increase in hydroxypropylation level improved recovery of soluble complexes, while total recovery remained unchanged; the β-amylase treatment further increased soluble complex recovery. For the same treatment, the naringenin content was greater in Hylon VII complexes (6.72-15.15mg/g) than in potato starch complexes (2.45-11.18 mg/g). Insoluble complexes comprised greater naringenin contents (3.91-15.15 mg/g) compared to soluble counterparts (2.45-9.43 mg/g). All complexes exhibited a mixture of B+V X-ray diffraction pattern. This work is the first one to demonstrate that hydroxypropylated starch formed complexes with naringenin, and an appropriate level of beta-amylase hydrolysis further improved their complexation.

Keywords: hydroxypropylated starch / β-amylase treatment / insoluble complex / naringenin / soluble complex

1 Introduction

The formation of starch inclusion complexes is mainly attributed to amylose that is capable of adopting a single left-handed helical conformation to complex with hydrophobic molecules [1]. The extent of complexation is primarily affected by amylose degree of polymerization (DP) and guest structure, which has been extensively researched using fatty acids as model compounds [2-5]. Rutschmann et al. [6] reported that the thermal stability of amylose and menthone complex increased with an increase in amylose DP. Godet et al. [7] prepared amylose of different DPs (20, 30, 40, 100 and 900 anhydroglucose units) to form complexes with caprylic, lauric, and palmitic acid, and found that as the amylose DP increased the melting temperature of the resulting complexes increased. Later, Godet et al. [8] demonstrated that amylose of DP 20 was too short to form complex with fatty acids, and the yield of complexes increased with increasing amylose DP. Recently, Arijaje et al. [9] and Arijaje and Wang [10, 11] modified potato starch with acetylation followed by debranching and β-amylase hydrolysis and found that linear starch chains of DPs ~50-80 was involved in the formation of complexes with stearic, oleic, and linoleic acids. Wulff and Kubik [12] first reported the formation of soluble complexes of sodium dodecyl sulfate with potato amylose of hydroxypropylation at a degree of substitution (DS) 0.075 and DPs ~9-250. Arijaje et al. [9], and Arijaje and Wang [10, 11] also showed the formation soluble complexes, and soluble complex was increased by 154-245% for stearic acid, 233-375% for oleic acid, and 327-490% for linoleic acid when potato starch was acetylated and then reduced in DP by debranching and β-amylase hydrolysis.

The complexation between starch and bioactive compounds is not well researched. The complex of starch with tea polyphenols (TPLs) was suggested to contribute to changes in starch rheological properties as well as *in vitro* starch digestion rate when TPLs were mixed with common

corn, waxy maize, and high amylose corn starches [13, 14]. Different amounts of quercetin were complexed with ungelatinized and pregelatinized common corn starch, and the thermal stability of the resulting complex was governed by the quercetin content in the complex [15]. Lorentz et al. [16] studied the inclusion complex of potato amylose with chlorogenic acid and concluded that grafting 4-*O*-palmitoyl to the acid increased the complex formation. Genistein, the major isoflavone in soybean, was complexed to a greater extent with potato amylose (DP ~900) than with high amylose corn starch, implying that amylopectin long branches was also involved in the complexation [17]. Van Hung et al. [18] showed that debranched cassava starch complexed with ferulic acid, resulting in insoluble complexes of B-type X-ray diffraction pattern with improved solubility and antioxidant capacity. Recently, β-carotene exhibited improved stability and water solubility after complexed with common corn starch [19].

Naringenin is a phenolic compound belonging to the flavanone class, and its three-ring structure effects a low water solubility [20]. Although naringenin has been shown to display anti-cancer [21], anti-inflammatory [22], and anti-microbial activities [23], the low water solubility limits its pharmaceutical application. The solubility of naringenin was improved 365 and 400-fold when complexed dimethyl β-cyclodextrin (β-CD) [24] and hydroxypropyl-β-CD [25], respectively. However, concerns remain on the toxicity and high cost of CD derivatives. In a separate study [26], potato and high amylose corn (Hylon VII) starches were acetylated and then debranched without or with DP reduction by β-amylase before complexing with naringenin. The acetylation study results showed that both soluble and insoluble starch-naringenin complexes were formed and the naringenin content in both complexes increased as result of acetylation and the enzymatic treatment. Although the solubility of naringenin has been improved by hydroxypropyl-β-CD [25], hydroxypropylated starch has not been used as a complexing agent for bioactive

molecules like naringenin [25]. Therefore, the objective of this study was to investigate the complexation of naringenin with starch that was hydroxypropylated and debranched without and with β-amylase hydrolysis, and the resultant soluble and insoluble complexes were recovered and characterized for their physicochemical properties.

2 Materials and methods

2.1 Materials

Potato starch and high amylose corn starch (~70 % amylose, Hylon VII) were kindly provided by Ingredion (Bridgewater, NJ). Potato starch was used without further treatment, and Hylon VII was defatted by extraction with 85% (v/v) methanol for 24 h, dried at 40°C for 24 h, and milled using a UDY Cyclone Mill (UDY Corporation, Ft. Collins, CO) fitted with a 0.5-mm sieve. Isoamylase from *Pseudomonas sp.* (specific activity 240 U/mg), pullulanase from *Klebsiella planticola* (specific activity 34 U/mg), and β-amylase from *Bacillus cereus* (specific activity 2,182 U/mg) were purchased from Megazyme Ltd. (Wicklow, Ireland) and used without further purification. Naringenin was obtained from Sigma-Aldrich (St. Louis, MO). All other chemicals and reagents were of analytical grade.

2.2 Hydroxypropylation of Starch

Hydroxypropylation of potato starch and Hylon VII were carried out as described by Wang and Wang [27]. Starch (100 g, db) was used to prepare a 35% (w/w) slurry in a 1-L reaction vessel and added with Na_2SO_4 (15%, starch db). Hydration was allowed for 30 min with stirring prior to pH adjustment to 11.5 with 1 M NaOH. The vessel was carefully sealed, 4 (low) or 8% (high) propylene oxide (starch db) was added through an opening, and then the temperature was gradually increased to 45°C. After 18 h of reaction, the pH was adjusted to 5.5 with 0.1 M HCl, and the

slurry was washed three times with two-fold volume of deionized water, vacuum filtered, and dried at 40°C for 24 h. The hydroxypropyl content was determined by following the colorimetric method of Johnson [28], and the corresponding DS was calculated according to Wurzburg [29].

2.3 Debranching of Starch and β-Amylase Treatment

The debranching of native and hydroxypropylated starches followed the procedure of Arijaje et al. [9] with modifications. A mixture of 7.5 g starch (wet basis) and 200 mL of deionized water (3.75% w/w) was gelatinized in boiling water for 1 h. The solution temperature was equilibrated to 40°C and adjusted to pH 5.0 with 0.5 M HCl. A mixture of isoamylase and pullulanase (1.33% each, starch db) was added, and the reaction was carried at 40°C for 48 h with constant stirring. The resulting debranched starch was recovered by precipitation with pure ethanol (four-fold volume), centrifuged at 7000 ×g for 15 min, dried at 40°C for 48 h, and milled to powder using the UDY Cyclone Mill. The additional β-amylase treatment followed the method described by Arijaje et al. [9].

2.4 Preparation of Starch-Naringenin Complex

Starch-naringenin complexes were prepared by following the method described by Arijaje et al. [9] with modifications. The pH of the debranched starch slurry (3.75% w/v) was adjusted to pH 7.0, and the temperature was gradually increased from 40 to 80°C. Naringenin (20% of starch db) that was previously dissolved in warm ethanol (6% w/v) was slowly added into the starch slurry and incubated at 80 °C for 30 min. The temperature of the mixture was adjusted to and maintained at 45°C with constant stirring for 24 h. The resulting solution was centrifuged at 7,000 ×g for 15 min to obtain the precipitate as the insoluble complex, whereas the soluble complex remained in the supernatant. Both the supernatant and the precipitate, i.e. the soluble and insoluble complexes,

respectively, were dried at 40°C for 48 h and then ground using the UDY Cyclone Mill. Uncomplexed naringenin was removed from the soluble and insoluble complexes by rinsing the complexes with 95% ethanol (10 % w/v). The mixture was vortexed for 30 s and then rotated using a labquake shaker (Barnstead/ thermolyne, Dubuque, IA) for 15 min. The slurry was centrifuged at 20,000 ×g for 10 min, and then the supernatant was discarded. A second wash was carried out under the same conditions to ensure a complete removal of uncomplexed naringenin. The resulting precipitate was dried at 40°C overnight and ground using mortar and pestle. The complex recovery was expressed as the complex weight recovered over the initial material weight.

2.5 Characterization of Inclusion Complexes

2.5.1 Starch Molecular-Size Distribution

The molecular-size distribution of the enzyme-treated starches was characterized by high-performance size-exclusion chromatography (HPSEC) according to Arijaje et al. [9]. The HPSEC system was equipped with an inline degasser, a model 515 HPLC pump with a 200-µL injector valve (model 7725i, Rheodyne, Cotati, CA), a model 2414 refractive index detector, a guard column (OHpak SB-G, 6.0 × 50 mm i.d. × length), and two Shodex columns (OHpak SB-804 HQ and KB-802, 8.0 × 300 mm i.d. × length). The molecular size distribution was calculated by comparing against standards of molecular weight 180.16, 828.72, 1,153, 5,200, 148,000, 872,300, and 1,100,000 g/mol (Waters Corp., Milford, MA), and 1,100,000 g/mol (Sigma-Aldrich, St. Louis, MO).

2.5.2 Naringenin Content

Naringenin was released from the complex by acid hydrolysis. Twenty-five mg of the complex was added with 2.5 mL of 1 M HCl, heated in boiling water for 1 h, added with 12.5 mL of

methanol, and then rotated overnight with the labquake shaker. An aliquot of 1.5 mL was transferred to a 2-mL micro-centrifuge tube and centrifuged at 9,300 ×g for 10 min. From the resulting supernatant, an aliquot of 0.5 mL was transferred to 1.5 mL screw-capped glass vials and placed in an autosampler before injection into an HPLC system (Beckman-Coulter, Fullerton, CA) for analysis [26].

2.5.3 Thermal Properties

The thermal properties of the complexes were analyzed using a differential scanning calorimeter (DSC, Diamond, Perkin-Elmer, Shelton, CT). Approximately 3 mg of the complex was weighed into a stainless steel pan, and then 10 μL of distilled water was added. The pan was hermetically sealed and allowed to equilibrate at room temperature for 18 h before scanning from 25 to 200°C, cooling from 200 to 25°C, and re-scanning from 25°C to 200°C. The scanning speed was set at 10°C/min. The onset temperature, peak temperature, end temperature, and enthalpy of any transition were calculated by Pyris data analysis software.

2.5.4 Wide Angle X-ray Diffraction Pattern

The X-ray diffraction pattern of the complexes was determined using a Philips PW 1830 MPD diffractometer (Almelo, the Netherlands). The X-ray generator was set at 45 kV and the current tube at 40 mA. The scanning 2θ angle went from 5° to 35° with a step size of 0.02° at 1 s per step.

2.5.5 Attenuated Total Reflectance Fourier Transform Infrared (ATR FT-IR) Spectroscopy

The ATR FT-IR spectra of the complexes was recorded after scanning with a Nicolet 8700 spectrometer (Thermo Electron Scientific Inc., Waltham, MA) using a Golden Gate ATR accessory (Specac) equipped with a single-reflection diamond crystal. A constant temperature (25°C ± 0.1°C) was maintained during the scanning, and for each sample, 64 scans were collected with a resolution of 4 cm^{-1}. The spectrometer's EverGlo source was on turbo mode during measurements. The complexes were conditioned for 7 days in a desiccator containing silica gel prior to analysis.

2.6 Statistical Analysis

The experiment was replicated two times. For each analysis, at least two duplicates were conducted. The data was analyzed using JMP Pro13 Software (SAS Institute Inc., Cary, NC, USA), and the means compared using Tukey's honestly significant differences (HDS) test.

3 Results and discussion

3.1 Molecular Size Distribution

Figure 1 illustrates the molecular size distribution of native and hydroxypropylated (HP) potato starch and Hylon VII that were debranched or debranched/β-amylase treated. In general, debranched potato and Hylon VII starches displayed four and three peaks, respectively, and three and two peaks, respectively, when the β-amylase treatment was incorporated. Hydroxypropylation resulted in an increase in the proportion of large molecular size peaks, which was ascribed to an increase in hydrodynamic volume from the substituted hydroxypropyl groups. When the β-amylase treatment was included, the molecular size distributions became narrower and peak DP of all peaks shifted to shorter retention times. A similar trend was observed in our separate study with acetylated starches [26]. Hylon VII starches with or without β-amylase hydrolysis displayed

a larger proportion of longer DP chains ~40-950 (Figure 1B and 1D); potato starches showed a larger proportion of shorter DP chains ~40-140 (Figure 1A and 1C) after the same treatments. For both starches, the fraction of peak DP 35-45 decreased with increasing hydroxypropylation level.

3.2 Degree of Substitution (DS)

The initial DS values of low and high HP DS of potato starch were 0.051 and 0.129, respectively, and those of low and high HP Hylon VII were 0.063 and 0.122, respectively. Table 1 summarizes the DS values of soluble and insoluble complexes of potato starch and Hylon VII that were debranched or debranched/β-amylase treated. For the same treatment, soluble complex displayed a similar or greater DS compared to that of insoluble complex, agreeing with our separate study of acetylated potato and Hylon VII starches [26]. The DS values of high HP potato starch complexes were significantly greater than those of high HP Hylon VII, which was attributed to the presence of a greater proportion of shorter chains in potato starch. The incorporation of the β-amylase treatment did not alter the DS for both complexes of both starches. The trend was different from our study with acetylated starches, where the DS of all complexes increased with the introduction of β-amylase treatment. It is hypothesized that acetyl groups were predominantly introduced in the amorphous lamella close to the branching points, whereas the hydroxypropyl groups may be present in the amorphous lamella close to the branching points as well as the amorphous regions close to the non-reducing ends due to their different reaction conditions [27, 30-31]. Because β-amylase cannot bypass the hydroxypropyl groups in the non-reducing ends, the resultant starch chains comprised a greater amount of hydroxypropyl groups compared with acetylated starch in a separate study [26].

3.3 Complex Recovery and Naringenin Content

Table 1 presents the recovery, which was expressed as recovered complex weight (g) over initial materials weight (g), and the naringenin content in both soluble and insoluble complexes for all treatments. The total recovery ranged 0.91-0.99 g/g and similarly to previous studies [9-11, 26] the combination of hydroxypropylation with the enzymatic treatment had no significant impact on the total recovery of both complexes. Hydroxypropylation significantly increased the formation of soluble complex, whereas the β-amylase treatment did not change the recovery of soluble or insoluble complex in both starches.

For the same treatment, the naringenin content was greater in the insoluble complex than in the soluble complex, and was greater in Hylon VII complexes (6.72-15.15mg/g) than in potato starch complexes (2.45-11.18 mg/g), agreeing with our study of acetylated Hylon VII and potato starch [26]. The naringenin content generally increased with increasing DS of HP starches, and increased for low HP starches when the β-amylase treatment was incorporated for both complexes. The bulky hydroxypropyl groups at a high hydroxypropylation level may interfere with the conformation of helical structure, thus resulting in reduced complexation capability. Furthermore, hydroxypropyl groups are less hydrophobic than acetyl groups. Thereby, it is suggested that a combination of decreased hydrophobicity and increased stearic hindrance was responsible for the lower complexation yield of HP starches compared with acetylated starches [26, 32]. Wulff et al. [33] showed that the soluble complex of HP potato amylose (DS 0.13) complexed a greater amount of iodine than that of acetylated counterpart of a similar DS (0.14). The insoluble complex of acetylated Hylon VII was reported to comprise more α-naphthol than that of HP Hylon VII [34]. These discrepancies suggest that guest molecule structure and DS of starch also impacted complexation yield besides substitution type.

Starch DP involved in complexing with fatty acids was suggested to be greater than 20 but less than 400 [8, 35]. Starch chains greater than DP 400 were believed to be too long to form an ordered helical structure, but starch chains less than DP 20 were too short to induce helical structure. When considering all treatments, the majority of starch chains were present approximately between DP 40 and 200 for potato starch and between 40 and 600 for Hylon VII (Figure 1). Because of a greater naringenin content in Hylon VII complexes than in potato starch complexes, starch chains longer than DP 200 were suggested to be also involved in complexing with naringenin, similar to the DP ranges found in acetylated starches [26].

The ferulic acid in the complex with debranched cassava starch ranged 6.8-31.5 mg/g [18]; the genistein content in the complex with unmodified potato amylose ranged 14-113 mg/g [17]. It should be noted that both studies only reported the bioactive compounds in the insoluble complex. The greatest complexed naringenin contents in the insoluble complexes were 11.18 and 15.15 mg/g for potato starch and Hylon VII, respectively, and the naringenin contents in the soluble complexes ranged 2.45-9.43 mg/g in the present study. The differences among these studies confirm that the structure of the guest molecule was of great importance in determining the extent of complexation. The non-planar conformation of naringenin B-ring compared with genistein is suggested to contribute to the lower complexation of naringenin since changes on its orientation might prevent naringenin from properly positioning inside the helical structure, thus forming unstable complexes.

3.4 Wide Angel X-ray Diffraction Pattern

Figures 2 and 3 display the X-ray diffraction pattern of both complexes from potato starch and Hylon VII, respectively. For all soluble and insoluble complexes, hydroxypropylation resulted in an increased amorphous structure compared to the native counterpart. The V-type X-ray diffraction

pattern, with a weak diffraction angle 2θ at 19.9°, was greatly affected by hydroxypropyl groups that might prevent the alignment and formation of the helices for a more defined V-type structure. Unlike acetylated potato starches [26], the β-amylase treatment did not increase the V-type structure but generally increased the amorphous structure. In contrast, the β-amylase treatment slightly decreased the amorphous structure of Hylon VII complexes, which was attributed to an increase in the proportion of longer DP chains (Figure 1).

Except for the soluble complex of native potato starch displaying the A-type pattern, all soluble and insoluble complexes showed a mixture of B+V type crystalline structure. Most complexes of acetylated potato and Hylon VII also showed a mixture of B+V patterns, except the soluble complexes of all treated potato starch displaying A+V-type [26]. Zhang et al. [36] studied the complexation of lauric acid with debranched high amylose corn starch (Hylon V), and observed that as the debranching time increased from 0 to 24 h, the X-ray diffraction pattern of the insoluble complexes shifted from the V-type to a mixture of B+V-type. Van Hung et al. [18] only observed the B-type crystalline structure for the insoluble complex of ferulic acid with debranched cassava starch. These results indicate the importance of starch DP and the introduction of substituents on the structure of the resultant complexes.

3.5 Attenuated total reflectance Fourier Transform Infrared (ATR FT-IR) Spectroscopy

The ATR FT-IR spectra of soluble and insoluble complexes along with the physical mixture of naringenin with potato starch and Hylon VII are shown in Figure 4. Forrest [37] suggested that the absorption of methyl group of hydroxypropyl substituents occurred between ~2850-3000 cm^{-1} (CH-stretching) and ~1350-1475 cm^{-1} (CH deformation), which is the same range where native starch naturally present groups, i.e. C-O-C, display strong absorption, hence the characteristic bands overlap. The FT-IR spectra of HP starches and their complexes displayed an increase in the intensity of the bands at ~2850-3000 cm^{-1} and ~1350-1475 cm^{-1} with increasing DS of hydroxypropylation, which was attributed to the incorporation of hydroxypropyl groups into starch chains. Although no appreciable difference was observed between the spectra of parent starches and that of the complexes, the spectra of the physical mixture was different and displayed a superimposition of the individual spectra of starch and naringenin.

Analysis of the differential spectra (data not shown) revealed that the bands ~2850-3000 cm^{-1} and ~1350-1475 cm^{-1} remained for all the complexes, and therefore it is suggested that hydroxypropyl groups were involved in the complex. The intensity of the band was greater for the soluble complexes than the insoluble complexes, which is agreement with the DS results (Table 1) where soluble complexes generally had a greater DS. In agreement with a separte study with acetylated starches [26] the bands between ~3600-3000 cm^{-1} (hydroxyl groups) were present after the subtraction of the spectrum of starch and naringenin, suggesting that some molecular interaction between naringenin and starch was involved in the complex formation. It should be noted that the intensity of the bands between ~3600-3000 cm^{-1} was greater for Hylon VII than for potato starch, confirming a greater involvement of Hylon VII chains in complexing with naringenin. The differential spectrum (data not shown) also showed that the band ~988 cm^{-1} (CO

stretching of ring B) [38] was involved in both complexes, indicating that the B ring of naringenin was involved in complexing with starch chains. When the β-amylase treatment was incorporated, a slight increase in the band ~988 cm^{-1} was observed, which might be related to the amount of naringenin complexed. This effect was more evident in Hylon VII, presumably because of its greater complexing capability. These results corroborate the X-ray diffraction pattern results and confirm that some molecular interaction took place between starch and naringenin in forming complexes of different crystalline structures instead of physical mixtures.

3.6 Thermal Properties by Differential Scanning Calorimetry

The complexes from native starches displayed a typical endothermic transition at the first scan; however, the complexes from HP starches exhibited two exothermic peaks during the first scan and a single endothermic peak at the second scan, which was not observed in a separate study of acetylated starches [26]. Therefore, the data reported in Table 2 corresponds to the thermal transitions observed during the second scan for HP starches. The bulky hydroxypropyl groups may effect a less organized packing of the helices [31, 39-40], which rearranged to become more ordered during the first scan. The soluble complexes did not exhibit any thermal transition, whereas the insoluble complexes displayed a transition between 100.7 and 122.2°C (Table 2) for both native starches.

When HP starches were used, the onset melting temperature decreased, the melting temperature range reduced, and the enthalpy decreased, except that the melting temperature range increased for high HP potato starch. The high enthalpy values of complexes from native starches relative to other complexes indicate the role of hydroxypropyl groups in destabilizing the complexes. The complexes of high HP potato starches exhibited the lowest melting temperatures

and a large melting temperature range similar to those of native starches. For Hylon VII, hydroxypropylation resulted in decreased melting temperatures and enthalpy, but melting temperature range remained unchanged with a further increase in DS. It is proposed that the crystalline structure in high HP potato starch complexes comprised a mixture of crystallites with varied thermal stability due to the presence of less homogeneous DP distribution compared with Hylon VII, which produced complexes of overlapping endotherms. The β-amylase treatment increased the melting temperature of some complexes of HP potato starch, but decreased melting temperatures of some complexes of HP Hylon VII.

4 Conclusion

In conclusion, hydroxypropylated starches formed both soluble and insoluble complexes with naringenin, and the introduction of hydroxypropyl groups enhanced the recovery of the soluble complexes. An increase in complexed naringenin content was attributed to larger proportions of linear starch chains with longer DP. The naringenin content was greater in the insoluble complex than in the soluble complexes, and generally increased with increasing DS of hydroxypropylation. When the β-amylase treatment was included, a further increase in naringenin content was observed for low HP starches. Hylon VII comprised a greater amount of naringenin than potato starch, which was ascribed to a larger proportion of long DP chains in Hylon VII. ATR FT-IR results confirmed the molecular interaction between starch and naringenin in both complexes. A combination of favored DP range and hydroxypropylation level of starch was important in maximizing its complexation with naringenin.

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Figure legends

Figure 1. Normalized size-exclusion chromatograms of native and hydroxypropylated (HP) debranched and debranched/β-amylase treated potato and Hylon VII starches.

Figure 2. X-ray diffractograms of soluble and insoluble starch-naringenin complexes of native and hydroxypropylated debranched and debranched/β-amylase treated potato starch.

Figure 3. X-ray diffractograms of soluble and insoluble starch-naringenin complexes of native and hydroxypropylated debranched and debranched/β-amylase treated Hylon VII.

Figure 4. Attenuated Total reflectance Fourier Transform infrared (ATR FT-IR) spectra of soluble and insoluble starch-naringenin complexes of native and hydroxypropylated debranched and debranched/β-amylase treated potato starch and Hylon VII. The spectra of native potato starch and physical mixture of native potato starch and naringenin are included for comparison.

Table 1. Degree of substitution, complex recovery and percentage of naringenin content recovered from all complexes[1]

Debranched starch	Hydroxypropylation	β-amylase treatment	Type of complex	DS of hydroxypropylation	Recovery (g/g)	Naringenin in individual complex (mg/g)
Potato	No	No	Soluble	NA[2]	0.30±0.01C	2.45±0.01D
		No	Insoluble	NA	0.64±0.00b	4.42±0.10g
		Yes	Soluble	NA	0.33±0.02C	2.67±0.31D
		Yes	Insoluble	NA	0.62±0.01b	3.91±0.26g
	Low	No	Soluble	0.063±0.001C	0.60±0.01B	4.35±0.40CD
		No	Insoluble	0.047±0.000d	0.32±0.00d	5.34±0.28fg
		Yes	Soluble	0.068±0.002C	0.65±0.01B	5.09±0.47B-D
		Yes	Insoluble	0.060±0.000cd	0.29±0.01d	11.18±2.15b-d
	High	No	Soluble	0.153±0.000A	0.74±0.02A	6.75±2.77A-C
		No	Insoluble	0.150±0.002a	0.19±0.01e	9.29±0.04c-e
		Yes	Soluble	0.154±0.001A	0.73±0.04A	4.34±0.75CD
		Yes	Insoluble	0.151±0.002a	0.18±0.00e	7.73±0.55ef
Hylon VII	No	No	Soluble	NA	0.12±0.00D	6.72±1.12A-C
		No	Insoluble	NA	0.87±0.00a	8.06±1.20d-f
		Yes	Soluble	NA	0.14±0.00D	8.82±0.07AB
		Yes	Insoluble	NA	0.83±0.00a	13.61±0.52a-b
	Low	No	Soluble	0.066±0.000C	0.65±0.04B	6.87±0.51A-C
		No	Insoluble	0.067±0.000c	0.31±0.03d	11.33±0.10bc
		Yes	Soluble	0.066±0.004C	0.60±0.00B	7.73±0.02A-C
		Yes	Insoluble	0.059±0.001cd	0.37±0.00c	15.15±0.80a
	High	No	Soluble	0.115±0.008B	0.79±0.01A	9.43±0.41A
		No	Insoluble	0.092±0.001b	0.18±0.00e	10.97±0.17b-d
		Yes	Soluble	0.107±0.008B	0.78±0.01A	9.21±1.15A
		Yes	Insoluble	0.082±0.008b	0.18±0.00e	10.31±0.04c-e

[1]Mean of two replicates with standard deviation. Mean values in the same column followed by different uppercase or lowercase letters are significantly different based on Tukey's HSD test. [2]NA: Not applicable.

Table 2. Melting temperatures and enthalpies[1] of recovered soluble and insoluble fractions of native and hydroxypropylated potato starch and hylon VII naringenin complexes.

Debranched starch	Hydroxypropylation	β-amylase treatment	Type of complex	Temperature (°C)			Enthalpy (J/g)
				Onset	Peak	End	
Potato	No	No	Soluble	ND[2]	ND	ND	ND
		No	Insoluble	100.7±0.2a	110.1±0.9b	118.3±0.20b	8.9±0.2a
		Yes	Soluble	ND	ND	ND	ND
		Yes	Insoluble	101.0±0.2a	109.7±0.1b	118.1±0.02b	9.7±0.2a
	Low	No	Soluble	84.0±0.1B	86.5±0.1CD	91.9±0.3B-D	2.6±0.1A-C
		No	Insoluble	83.1±0.1c	88.9±0.0cd	93.6±0.1d	2.6±0.1cd
		Yes	Soluble	85.5±0.0A	91.1±0.2A	97.1±0.1A	2.5±0.5A-C
		Yes	Insoluble	85.1±0.3b	90.7±0.3c	96.7±0.3c	2.3±0.3cd
	High	No	Soluble	76.7±0.1F	88.5±0.5B	94.6±0.6AB	1.6±0.5CD
		No	Insoluble	76.9±0.2d	87.4±0.7de	93.4±0.4d	2.5±0.5cd
		Yes	Soluble	77.5±0.5E	88.6±0.0B	94.0±0.1A-C	1.7±0.0B-D
		Yes	Insoluble	76.9±0.2d	87.4±0.7de	93.4±0.4d	2.5±0.5cd
Hylon VII	No	No	Soluble	ND	ND	ND	ND
		No	Insoluble	101.8±0.9a	112.5±0.5a	122.2±0.8a	4.1±1.0bc
		Yes	Soluble	ND	ND	ND	ND
		Yes	Insoluble	102.2±0.9a	114.4±0.3ab	119.7±0.9b	5.4±0.9b
	Low	No	Soluble	82.7±0.2C	85.5±0.0DE	91.3±2.0CD	2.9±0.4AB
		No	Insoluble	82.7±0.2c	85.6±0.1ef	92.6±0.1de	2.6±0.6cd
		Yes	Soluble	81.8±0.2D	84.4±0.4E	87.8±0.2E	3.0±0.2A
		Yes	Insoluble	82.7±0.0c	86.0±0.7ef	92.6±0.5de	2.5±0.2cd
	High	No	Soluble	82.3±0.1CD	86.9±0.4C	90.3±0.7DE	2.5±0.0A-C
		No	Insoluble	82.3±0.5c	85.2±0.1f	91.8±0.1de	1.6±0.2d
		Yes	Soluble	83.0±0.0C	86.0±0.4CD	91.2±0.7CD	1.2±0.0D
		Yes	Insoluble	82.8±0.4c	85.9±0.1ef	90.7±1.6e	1.4±0.2d

[1]Mean of at least two replicates with standard deviation. Mean values in the same column followed by different uppercase or lowercase letters are significantly different based on Tukey's HSD test. [2]ND: not detected.

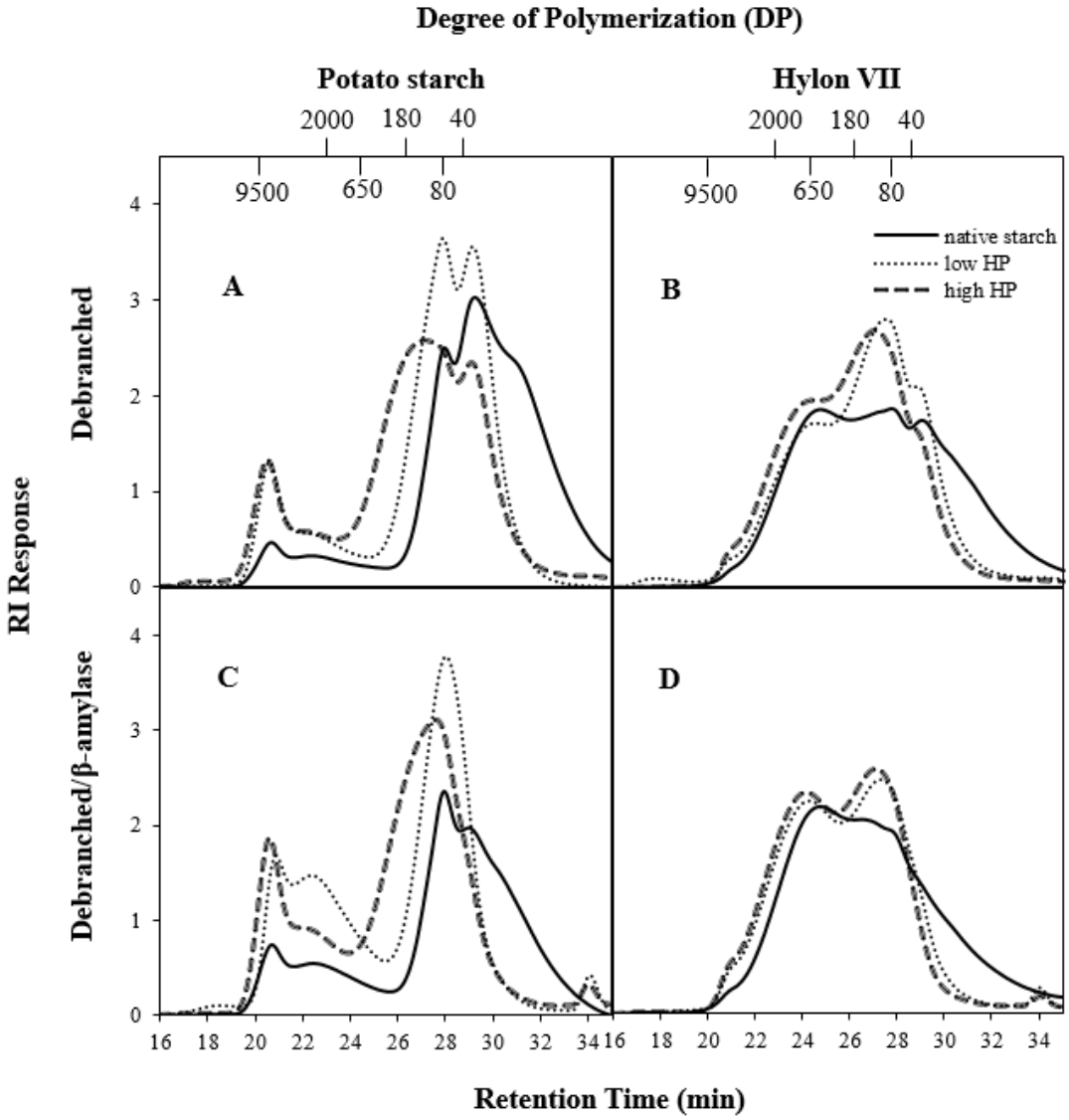


Figure 1.

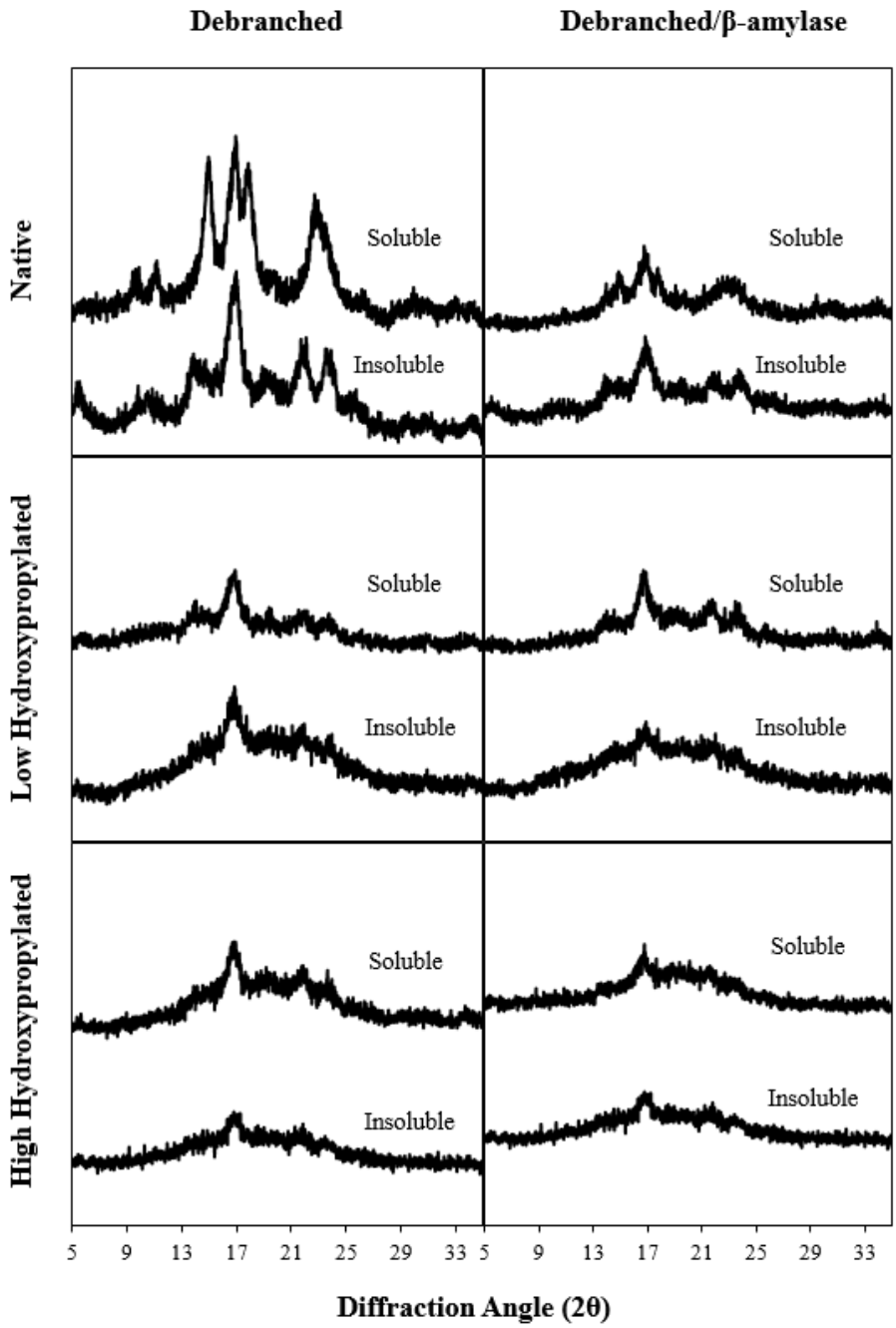
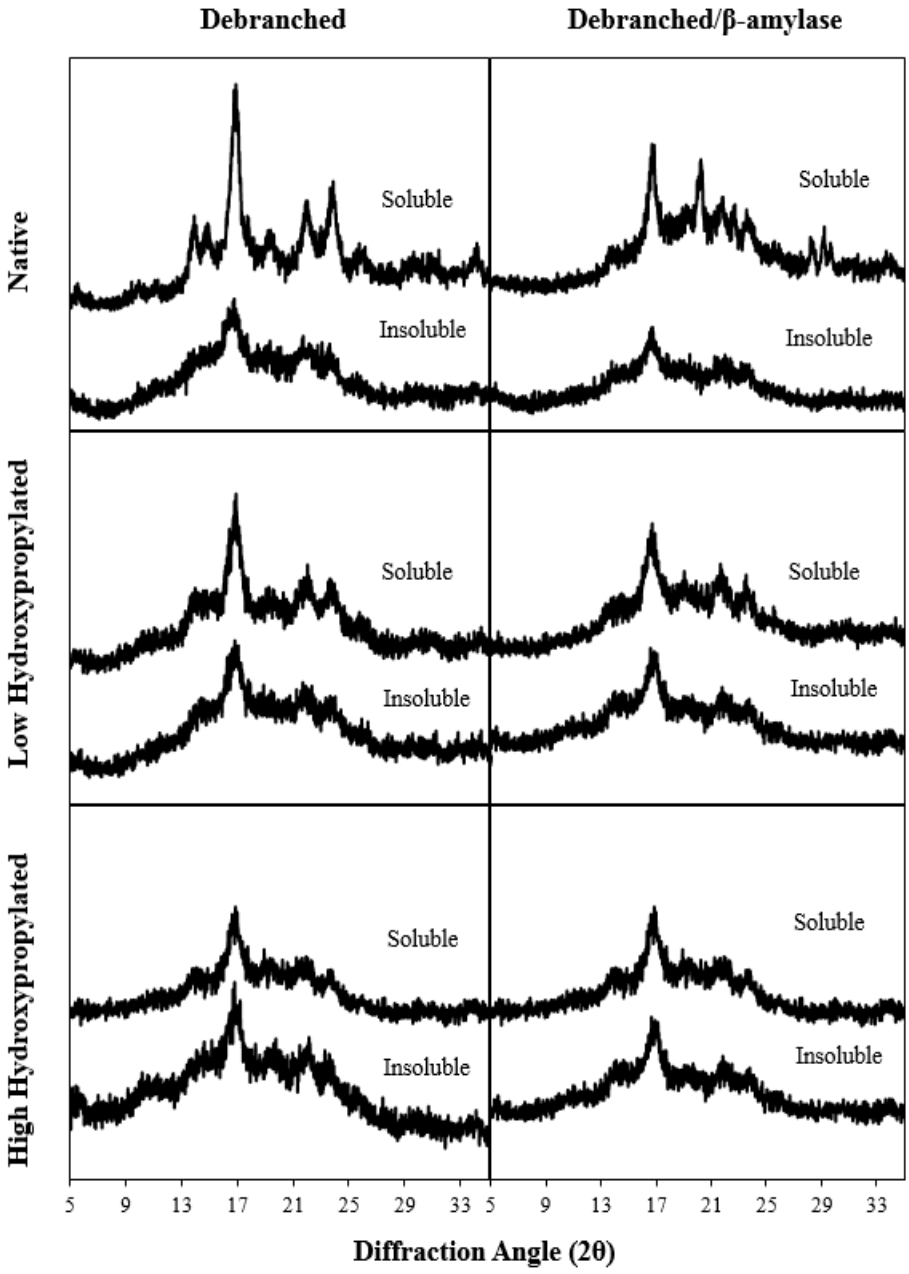


Figure 2.



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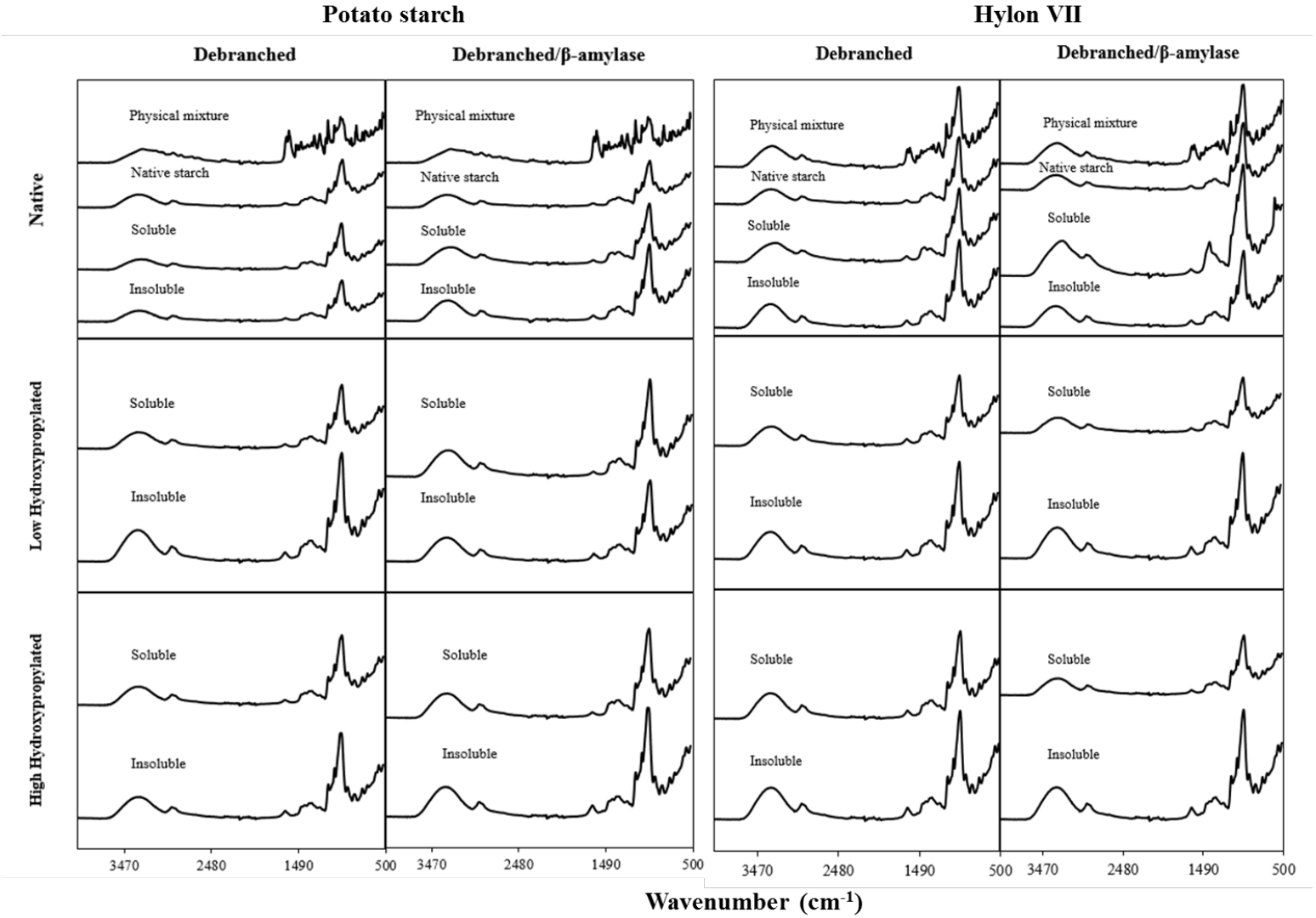

Figure 4.

Supplemental Material

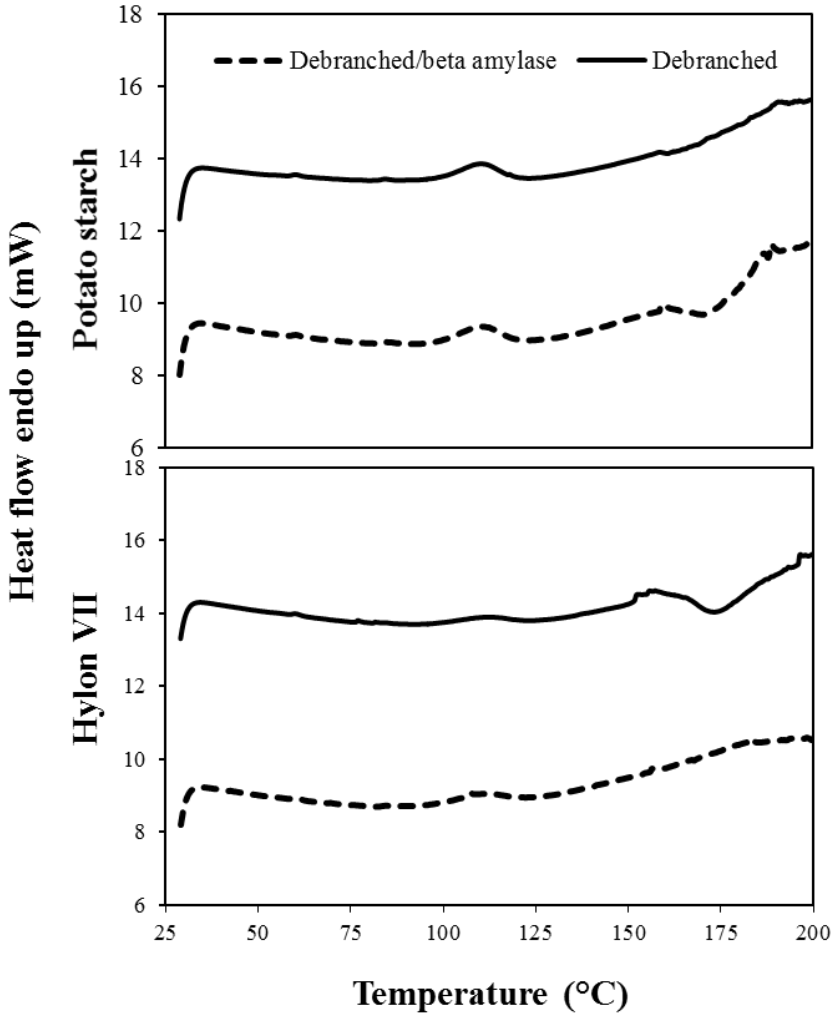

Figure. Relative thermal curves of insoluble complexes of potato starch and Hylon VII debranched or debranched/beta amylase treated