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# Preparation and characterization of the inclusion complexes between amylosucrase-treated waxy starch and palmitic acid

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**Abstract** Amylosucrase-treated waxy corn starch (AS) was produced to extend the chain length of amylopectin to a great extent in comparison to its native chain length. An amylopectin–palmitic acid (PA) complex was prepared by heat-treating (121°C) a starch/PA mixture and its subsequent further incubation (95°C, 24 h); moreover, its structure and digestibility were studied. Unmodified waxy starch could not complex at all, whereas elongation due to amylosucrase modification allowed amylopectin to form a complex with PA to a small extent. Complexation between AS and PA caused a decrease in relative crystallinity. The AS–PA complex displayed an endothermic peak representing type I inclusion complexes rather than type II complexes. The formation of complexes did not significantly affect the *in vitro* digestibility maintaining the low digestibility of AS resulting from extremely small amounts of complexes and the type of complex.

**Keywords:** starch-lipid complex, waxy corn starch, amylosucrase, palmitic acid, amylopectin

## Introduction

Starch, an important source of carbohydrate, comprises amylose and amylopectin. Amylose is a linear polymer composed of (1→4)-α-D-glucose and a few (1→6)-α-D-glucose units. Amylopectin also contains (1→4)-α-D-glucose that is extensively branched with (1→6)-α-D-glucose. Amylose can form complexes with ligands such as fatty acids, iodine, monoacylglycerols, flavor compounds, and other hydrophobic organic polymers (1). When amylose exists as a single helix, guest molecules enter the central cavities of the amylose helix during complex formation (1). Amylose–lipid complexes decrease starch's swelling capacity, solubility, and granule disruption (2). Holm *et al*. (3) found that the amylose–lysolecithin complex is more slowly degraded by α-amylase as compared with free amylose. Besides, complex formation restricts the formation of double helices and recrystallization of amylose by reducing its solubility and mobility and reduces the retrogradation of starch, thereby producing slowly digestible and/or resistant starches (4,5).

Complex formation is affected by the type of solvent used with amylose, chain length of amylose and/or fatty acid, and thermal treatment conditions for complexation (1,6). Based on these factors, two types of complexes with different thermal properties are formed in the mixture of starch and fatty acid. Type I complex has a dissociation temperature lower than 100°C and is considered as an amorphous form, whereas type II complex is semi-crystalline in crystalline regions and dissociated at temperatures above 100°C (1,7). Generally, type II complexes, which are thermally more stable than type I complexes, can be obtained by heating the mixture of amylose and fatty acid at a high temperature (at least 90°C) (6). Unlike amylose, amylopectin has a limited ability to form complexes with fatty acid because of steric hindrance and its short branch chain length (8).

Amylosucrase (EC 2.4.1.4) catalyzes the transglycosylation reaction to produce an insoluble α-1,4-glucan using sucrose and consequently releases fructose (9). In particular, amylosucrase accelerates the elongation of some external chains at their non-reducing ends in the presence of an acceptor such as a glucosyl unit (10).

In this study, amylosucrase from *Neisseria polysaccharea* was treated onto waxy corn starch mostly comprising amylopectin to elongate its branch chains to a certain extent. The objectives of the study were to prepare fatty acid-inclusion complexes of amylopectin using amylosucrase-treated waxy corn starch and palmitic acid (PA) and to investigate their structural characteristics and digestibility.

## Materials and Methods

**Materials** Waxy corn starch was obtained from Samyang Genex Co. (Incheon, Korea). PA was purchased from Sigma-Aldrich (St. Louis, MO, USA). Pancreatin (activity 8×USP/g) and amyloglucosidase (AMG 300L, activity 300 AGU/mL) were purchased from Sigma-Aldrich and Novozymes (Bagsværd, Denmark), respectively. Isoamylase (activity 1,000 U/mL) was obtained from Megazyme (Bray, Ireland). The gene of amylosucrase from *Neisseria polysaccharea* was cloned and expressed in *Escherichia coli* BL21, and the purification and assay of the enzyme were performed according to the method reported by Jung *et al*. (11). Based on a previous study (12), one unit (U) of amylosucrase was defined as the amount of enzyme that catalyzes the release of 1 μM of fructose per min under the assay conditions.

**Preparation of amylosucrase-treated starch** Starch suspension (2%, w/v) was prepared by mixing starch and 100 mM sucrose in a 100 mM sodium acetate buffer (pH 7.0) to obtain the final volume of 150 mL. The starch suspension was boiled for 30 min and cooled to 30°C. Amylosucrase (10,000 U/30 mL) was added to the starch suspension and then incubated in a water bath at 30°C for 6 h. The enzyme reaction was stopped by adding 450 mL of ethanol to the suspension. The amylosucrase-treated waxy corn starch (AS) was precipitated by centrifugation at 10,000×*g* for 10 min. The pellet was repeatedly washed with 450 mL of distilled water via centrifugation at 10,000×*g* for 10 min, freeze-dried, ground, and passed through a 100-mesh sieve. The control starch (AC) for amylosucrase treatment was prepared via the method used for AS preparation but without enzyme addition.

**Preparation of amylopectin–palmitic acid complex** The starch sample (5%, w/v) suspended in distilled water was boiled for 30 min with vortexing and autoclaved at 121°C for 30 min to fully gelatinize the starch. PA solution (10%, w/v), which was prepared by dissolving 0.2 g of PA in 2 mL of ethanol, was added to the gelatinized starch dispersion. The mixture was continually boiled for 30 min and re-autoclaved at 121°C for 30 min, followed by incubation at 95°C for 24 h. The sample was cooled to room temperature and recovered via centrifugation (10,000×*g*, 20 min); it was then washed with 50% ethanol and distilled water. The final precipitates were freeze-dried, ground, and passed through a 100-mesh sieve. Samples without the addition of PA were prepared for comparison.

**Determination of the branch chain length distribution in amylopectin** The branch chain length distribution of starch was determined after debranching. Starch (15 mg) was dispersed in 90% dimethylsulfoxide (3 mL) and boiled for 20 min. Ethanol (15 mL) was added to the starch suspension and centrifuged at 10,000×*g* for 10 min to precipitate the starch. Distilled water (1.5 mL) was added to the pellet and boiled for 10 min. After boiling, 1.5 mL of a 50 mM sodium acetate buffer (pH 4.3) and 30 U of isoamylase were added; then, the sample was reacted in a water bath at 45°C and 50 rpm for 2 h. The debranched sample was boiled for 10 min, filtered through a 0.45-μm membrane filter, and then analyzed using high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) on a Carbo-pack PA1 anion-exchange column (4×250 mm, Dionex, Sunnyvale, CA, USA). This analysis was performed by using a gradient increase of 600 mM sodium acetate in a 150 mM NaOH solution against 150 mM NaOH for sample elution as follows: 0-20% for 0-5 min, 20-45% for 5-30 min, 45-55% for 30-60 min, 55-60% for 60-80 min, 60-65% for 80-90 min, 65-80% for 90-95 min, and 80-100% for 95-100 min. The degree of polymerization (DP) values were determined using a mixture of maltooligosaccharides (DP 1-7; Sigma-Aldrich) as the standard.

**Measurement of complex content** The complex content in each sample was determined via the method proposed by Kim *et al*. (13) with slight modification. The starch–PA complex sample (20 mg) was dispersed in ethanol (0.2 mL) and then mixed with 1 M NaOH (1.8 mL); this was followed by boiling for 10 min. The sample was cooled to room temperature and then diluted five times with distilled water. A 0.5 mL aliquot of the diluted sample solution was mixed with 0.1 mL of 1 M acetic acid and 9.2 mL of distilled water. Iodine solution (0.2 mL, 0.2% $I_2$ and 2.0% KI) was added to the sample solution and immediately vortexed. After 20 min of the iodine binding reaction, the absorbance was measured at 620 nm. The apparent amylose content (AAC) of the sample was calculated using the standard curve that was plotted based on different ratios of the amylose/amylopectin mixture. The complex content was computed using the following equation:

$$\text{Complex content (\%)} = \text{AAC}_{\text{control}} - \text{AAC}_{\text{complex}}$$

**X-ray diffraction patterns** X-ray diffraction was analyzed using a powder X-ray diffractometer (New D8 Advance; Bruker, Karlsruhe, Germany) at 40 kV and 40 mA. A sample scan was performed in the $2\theta$ range from 3° to 33° with a 0.02° step size and a count time of 2 s. The relative crystallinity was determined by the following equation:

$$\text{Degree of crystallinity (\%)} = \left(\frac{\text{Area of the peaks}}{\text{Total curve area}}\right) \times 100$$

**Starch digestibility** Starch digestibility was determined via the method proposed by Brumovsky and Thompson (14) with slight modification. Pancreatin (2 g) was dissolved in distilled water (24 mL) and stirred well for 10 min. It was precipitated via centrifugation at 1,500×*g* for 10 min. The supernatant (20 mL) was mixed with 0.4 mL of amyloglucosidase and 3.6 mL of distilled water and incubated at 37°C for 10 min. A starch sample (30 mg) was dispersed in a 2 mL microtube containing sodium acetate buffer (0.75 mL, 0.1 M, pH 5.2) with one glass bead. The sample was equilibrated in a shaking incubator (240 rpm at 37°C) for 10 min. Then, 0.75 mL of the prepared enzyme solution was added to the tube, and the starch

sample was incubated in a shaking incubator (240 rpm at 37°C) for 10 and 240 min, respectively. The reaction was stopped by boiling for 10 min. The glucose released by the hydrolysis of starch was obtained in the supernatant after centrifugation at 5,000×*g* for 10 min. The glucose content was measured using a glucose oxidase-peroxidase (GOD-POD) kit (BCS Co., Anyang, Korea). Starch fractions were classified as follows (15): rapidly digestible starch (RDS) was measured as the quantity of glucose after reaction for 10 min, slowly digestible starch (SDS) was the fraction digested between 10 and 240 min, and resistant starch (RS) was the unhydrolyzed fraction after 240 min.

**Measurement of thermal properties** The thermal properties of the samples were investigated using a differential scanning calorimeter (Diamond DSC; Perkin-Elmer, Waltham, MA, USA). Each sample (8 mg) was weighed in a hermetic pan to which distilled water (24 μL) was added. The sample pan was sealed and kept overnight at room temperature for equilibrium. An empty pan was used as a reference. DSC scan was performed from 30 to 180°C at 10°C/min.

**Statistical analysis** All the experiments were performed in triplicate, and the data were expressed as the mean±standard deviation. The significant differences between the samples were evaluated via *t*-test or analysis of variance with Duncan's multiple-range test ($p<0.05$) using IBM SPSS Statistics (version 21.0.0, IBM, Armonk, NY, USA).

## Results and Discussion

**Changes in branch chain length after amylosucrase treatment** The branch chain length distributions of the starch samples are given in Table 1. AC showed large proportions of short chains with a DP≤24, typical of an A-type starch. AS showed decreased proportions of short chains but increased proportions of longer chains, thereby resulting in an increase in the average DP. Potocki De Montalk *et al.* (16) reported that amylosucrase catalyzes the attachment of 12–18 glucose units at the non-reducing ends of amylose and amylopectin. According to Godet *et al.* (17), chain length affects the structural properties of amylose–lipid complexes, e.g. the melting temperature and crystallinity. Through this elongation of branch chains, amylopectin could possess a chain length that is sufficiently long to form V-type complexes with fatty acids (18); in addition, this may alter its complexation property in comparison to that of unmodified amylopectin.

**Complex content and relative crystallinity of the amylopectin–PA complex** Amylose has the ability to form a complex with iodine when it exists as a single helix, and the long branch chains of amylopectin also bind iodine like amylose (19). This suggests that if the linear branch chains of amylopectin get elongated to a structure similar to that of linear amylose, they could complex with lipid molecules. It is known that the longer the linear chains, the easier they form complex with fatty acid molecules (17). Especially, according to the hypothesis regarding the minimum helical segment length of amylose required to form complexes with various fatty acids, linear chains comprising more than 28 glucose units have the ability to form stable inclusion complexes with PA (20). Therefore, it was expected that the larger number of branch chains with DP≥28 in AS than in AC would contribute to complexing with PA. AC did not possess the ability to complex with PA, whereas there was a slight increase in the complex content of the AS–PA complex (2.01%). Despite the large number of branch chains with a length sufficient to form a complex with PA, the complex content in AS–PA was not very high. Presumably, most of the long branch chains in AS might exist in the double helical form: this is in agreement with the obtained relative crystallinity values (16.9 and 28.7% of AC and AS, respectively). This means that only a limited number of branch chains exist in the single helix conformation that can participate in the complexation with PA. This could be a possible reason for a small increase in the complex content of AS–PA.

X-ray diffraction and the relative crystallinity values supported the obtained complex content results for AC and AS. In contrast to an amorphous curve of AC, as displayed in Fig. 1, AS showed a weak B-type X-ray pattern similar to that previously reported for amylosucrase-treated waxy starches (10,12,21). The relative crystallinity of AS was higher than that of AC (Table 2). The branches of amylopectin in AS, which contain amylose-like long linear chains, might reassociate and organize into a crystalline structure more easily than those of AC. AC–PA basically showed an amorphous diffractogram but presented sharp peaks at 4.9°, 7.4°, 12.3°, 20.5°, 21.5°, and 24° corresponding to the crystalline structure of free PA (22). This suggests that uncomplexed free PA was not completely removed during the

**Table 1.** Branch chain length distributions of waxy corn starch before and after amylosucrase treatment

| Sample[1)] | Percent distribution (%) | | | | | Average DP |
|---|---|---|---|---|---|---|
| | DP[2)]≤5 | DP 6-12 | DP 13-24 | DP 25-36 | DP≥37 | |
| AC | 1.6±0.2 | 32.5±0.4 | 47.5±0.1 | 12.6±0.2 | 5.9±0.1 | 17.8±0.0 |
| AS | 0.5±0.1 | 7.2±1.5 | 51.1±1.8 | 32.8±1.5 | 8.4±1.6 | 23.8±0.5 |
| *p* | **[3)] | *** | * | *** | * | *** |

[1)]AC, control starch for amylosucrase treatment; AS, amylosucrase-treated starch.
[2)]DP, degree of polymerization.
[3)]Significant differences between samples are presented as *, **, and *** for $p<0.05$, 0.01, and 0.001, respectively.

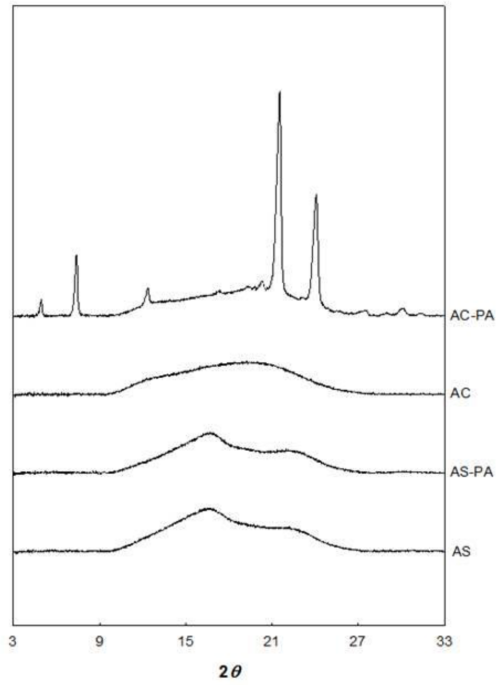


**Fig. 1.** X-ray diffraction patterns of starches complexed with palmitic acid. AC, control starch for amylosucrase treatment; AS, amylosucrase-treated starch; PA, palmitic acid

preparation of the sample despite performing the washing process. The absence of V-type peaks from the inclusion complex between AC and PA indicated that AC had no ability to form an inclusion complex with PA. An indirect evidence supporting this result could be that the relative crystallinity between AC and AC–PA showed no significant differences ($p$>0.05). A V-type X-ray diffraction pattern derived from type II complexes was expected for AS–PA based on the fact that complex formation could be presumed from the complex content result. However, instead of a V-type pattern, AS–PA maintained a B-type pattern like AS with negligible peaks from free PA. The complexes showing the typical V-type peaks can be derived from type II complexes, which form a semi-crystalline structure, whereas type I complexes are considered to be amorphous (7). Therefore, despite the high complexation temperature (95°C), there was a possibility of the predominant creation of type I complexes in AS–PA during complexation. Another possible reason for this result could be a too low complex content (approximately 2%) in AS–PA; therefore its inclusion complexes did not have sufficient crystallinity to be reflected as a peak in X-ray diffraction.

**Table 2.** Complex contents and relative crystallinities of the amylopectin–palmitic acid complexes

| Sample[1)] | Complex content (%) | Relative crystallinity (%) | |
|---|---|---|---|
| | | Without PA | With PA |
| AC–PA | 0.00±0.05 | 16.9±0.6 | 16.1±0.5 |
| AS–PA | 2.01±0.20 | 28.7±1.3 | 23.7±1.0 |
| $p$ | *[2)] | *** | *** |

[1)]AC, control starch for amylosucrase treatment; AS, amylosucrase-treated starch; PA, palmitic acid.
[2)]Significant differences between samples are presented as *, **, and *** for $p$<0.05, 0.01, and 0.001, respectively.

Because type I complex is amorphous and therefore does not contribute to the crystallinity of starch–fatty acid complex (1,7), the relative crystallinity of AS–PA was lower than that of its original counterpart. In addition, PA molecules could disrupt a part of the existing double helical structure of AS by incorporation into the crystalline regions, as reported by Cheetham and Tao (23) for the complexation of iodine with waxy corn starch.

**Thermal transition properties of the amylopectin–PA complex**

The thermal transition properties of the amylopectin–PA samples were determined by measuring their melting temperature ($T_m$) and melting enthalpy ($\Delta H$) (Table 3). AC did not show any endothermic peaks owing to the disruption of most of the crystalline structures by gelatinization before the complexation process. AS showed two endothermic peaks. The first peak with $T_m$ of ~79.2°C indicates the melting of double helices formed with short chains (mainly with DP 6-24) of amylopectin (12). The melting range of the second peak with a peak temperature above 100°C generally corresponds to the melting of double helices induced by the retrogradation of amylose (24). During retrogradation, long chains make double helices and enhance the hydrogen bonding between chains, thereby contributing to the formation of the crystalline region (25,26). However, because the waxy corn starch used in this study barely contained amylose

**Table 3.** Thermal transition properties of the amylopectin–palmitic acid complexes

| Sample[1)] | First endothermic peak[2)] | | | | | Second endothermic peak | | | |
|---|---|---|---|---|---|---|---|---|---|
| | $T_{o1}$ (°C) | $T_{m1\text{-}1}$ (°C) | $T_{m1\text{-}2}$ (°C) | $T_{f1}$ (°C) | $\Delta H_1$ (J/g) | $T_{o2}$ (°C) | $T_{m2}$ (°C) | $T_{f2}$ (°C) | $\Delta H_2$ (J/g) |
| AC | | | | | ND[3)] | | | | |
| AC–PA | | | | | ND | | | | |
| AS | 69.5±3.5 | 79.2±4.4 | ND | 88.5±1.9 | 2.6±1.0 | 124.0±2.6 | 130.8±1.7 | 143.0±2.3 | 4.8±0.5 |
| AS–PA | 64.3±1.8 | 75.3±0.0 | 82.0±0.7 | 94.1±2.0 | 7.8±0.7 | 139.8±0.4 | 140.6±0.3 | 144.8±2.9 | 1.8±0.1 |
| $p$ | | | | *[4)] | * | *** | *** | | *** |

[1)]AC, control starch for amylosucrase treatment; AS, amylosucrase-treated starch; PA, palmitic acid.
[2)]$T_o$, onset temperature; $T_m$, melting temperature; $T_f$, offset temperature; $\Delta H$, melting enthalpy.
[3)]ND, not detected.
[4)]Significant differences between samples are presented as *, **, and *** for $p$<0.05, 0.01, and 0.001, respectively.

**Table 4.** *In vitro* digestibility of the amylopectin–palmitic acid complexes

| Sample[1)] | Starch fractionation (%)[2)] | | |
|---|---|---|---|
| | RDS | SDS | RS |
| AC | 77.0±1.6[b 3)] | 14.5±1.7[c] | 8.5±0.6[b] |
| AC–PA | 72.9±2.8[b] | 17.0±0.5[bc] | 10.0±3.1[b] |
| AS | 48.6±1.5[a] | 27.7±4.3[a] | 23.7±3.8[a] |
| AS–PA | 50.6±2.8[a] | 22.3±4.5[ab] | 27.1±3.1[a] |

[1)]AC, control starch for amylosucrase treatment; AS, amylosucrase-treated starch; PA, palmitic acid.
[2)]RDS, rapidly digestible starch; SDS, slowly digestible starch; RS, resistant starch.
[3)]The values with different superscripts in the same column are significantly different ($p$<0.05).

(<1%), the second peak of AS could be considered to represent the melting of double helices comprising amylopectin chains that are sufficiently long to form crystalline structures similar to those formed by amylose. The elongated chains in AS could yield crystalline structures via easier and more effective recrystallization compared with native waxy corn starch, which resulted in the emergence of the melting peak after the modification of AC into AS (12,27).

AC–PA underwent a thermal transition at ~60°C because of the melting of the uncomplexed free PA. However, any endothermic peak corresponding to the inclusion complexes was not observed, indicating that AC hardly formed a complex with PA. The complexation between AS and PA showed two endothermic peaks similar to those of AS. However, their $T_m$ values were not exactly the same as those of AS. AS–PA showed broad and unresolved endothermic peaks at 75.3°C ($T_{m1\text{-}1}$) and 82.0°C ($T_{m1\text{-}2}$), and the shoulder peak at 82.0°C was difficult to discern. Generally, the type I complex in normal starch has a melting temperature higher than that of the aggregates of amylopectin branch chains (28) but lower than that of the double helices of amylose chains (18). Therefore, it is logical to believe that $T_{m1\text{-}2}$ of the first endothermic peak in AS–PA indicates the melting of type I complexes, as presumed from the decrease in the relative crystallinity observed in the X-ray diffraction result. The second endothermic peak ($T_{m2}$) corresponding to the double helices of long chains shifted toward a high temperature when AS complexed with PA. Although this phenomenon may be understood by the possibility of the formation of type II complexes, the unclear melting peak for the type II complex and the absence of V-type peaks in the X-ray diffraction of AS–PA indicated the existence of only a negligible amount of type II complexes.

$\Delta H$ reflects the content of double helical strucuture and the degree of crystalline order and represents the overall crystallinity (29). The total $\Delta H$ (sum of $\Delta H_1$ and $\Delta H_2$ values) of AS–PA (9.6 J/g) was higher than that of AS (7.4 J/g), indicating that the interchain packing energy of AS–PA was greater than that of AS (2). The increase in $\Delta H$ after complexation has also been reported (20,28). Interestingly, after complexation with PA, $\Delta H_1$ increased but $\Delta H_2$ decreased. Since the $\Delta H$ values for type I complexes are generally lower than those for type II complexes, the reduction in $\Delta H_2$ in AS–PA could be an evidence for the lack of type II complexes. As stated above, the second endothermic peak could reflect the melting of double helices formed with the long chains of amylosucrase-treated amylopectin and type II complexes between the long amylopectin chains of AS and PA. Type I complexes are in an amorphous form and melt at temperatures much lower than 100°C, whereas type II complexes have a high melting temperature because of their organized crystalline structure (30). Thus, the decrease in $\Delta H$ for the second endothermic peak could indicate that type II complexes might not exist in AS–PA, as revealed by the X-ray diffraction results.

In addition, the complexation of AS with PA affected the heterogeneity (in other words, homogeneity) of crystalline structures, because it affected the number of double helices and the degree of crystalline order. After complexation, because $T_{f1}$ and $T_{o2}$ increased, the melting temperature range ($T_f$–$T_o$) of the first endothermic peak increased from 19.0°C to 29.8°C, whereas that of the second endothermic peak decreased from 19.0°C to 5.0°C. It is suggested that the relatively weak part of the double helices comprising long chains becomes loosened and then forms type I complexes with PA instead of forming double helices. This could explain the increase in $\Delta H$ in the first endothermic peak and the decrease in $\Delta H$ in the second endothermic peak after complexation in AS–PA. The consistency between the results obtained from X-ray diffraction and DSC analyses strongly supports the assumption. A previous study reported that complexation uses single helices to form complexes with ligands interrupting the formation of double helices and thereby hinders starch retrogradation (31).

**Starch digestibility of the amylopectin–PA complex** The *in vitro* digestibility of the amylopectin–PA complexes is shown in Table 4. AC exhibited a high RDS content (77.0%) and a low RS content (8.5%). This was caused by the gelatinization process, which induced the disruption of the semi-crystalline structure of its original starch granules (27). Compared with AC, the RDS and RS contents in AS were 28.4% lower and 15.2% higher, respectively. In addition, the SDS content in AS was almost twice as high as that of AC; this result is in agreement with those of previous studies (21,27). SDS is affected by the amylopectin structure and chain length; in particular, the association of long chains in amylopectin could be related to the high SDS content (32). The lower *in vitro* digestibility of AS in comparison to that of AC could be explained by the changes in the branch chain length distributions caused by the unique chain elongation behavior of amylosucrase. Compared with the branch chains of AC, the longer branch chains of AS are favored to be crystallized, and starches with a higher crystallinity show resistance to the hydrolysis enzyme because of the reduced accessibility of the enzyme to the reaction site in the starch (21,27).

The digestibility of amylose–lipid complexes is affected by their complex types, e.g. type I complexes get hydrolyzed more slowly than free amylose and their final digestibility is almost the same as

their respective free amylose (3). Conversely, type II complexes have been reported to contribute to the elevation of RS content, and the length of amylose and/or fatty acid is inversely proportional to the digestibility of amylose–lipid complexes (2,31). In addition, it has been reported that the complexation between lipid and amylose molecules decreases RS owing to the competition with the retrogradation of amylose (31).

In this study, no significant change ($p$>0.05) was observed in digestibility before and after complexation of AS and PA. The reason why the inclusion complexes in AS–PA did not affect the digestibility of AS could be the formation of extremely small amounts of complexes (less than 5%) and the difficulty in the formation of type II complexes in AS (as revealed by the DSC and X-ray diffraction results). Soong *et al.* (33) reported similar results that there were no significant differences in the hydrolysis rate between waxy starches complexed with fatty acid and their respective raw starches because the complexes were not formed in sufficient amounts.

In conclusion, this study presented a possibility of amylopectin complexing with fatty acid, notwithstanding a scant amount. Via elongation of the branch chains, amylosucrase modification of waxy corn starch enabled amylopectin to form a complex with fatty acid despite the presence of a highly branched structure with steric hindrance. The amylopectin–PA complex formed using AS was type I rather than type II, and it may have induced the partial collapse of the relatively weak double helical structure of long chains, as revealed in the X-ray diffraction and DSC analyses. The low digestibility of AS, which was maintained after complexation without any significant change, might be due to the insufficient degree of complexation. Considering the specific chain length of amylopectin and/or fatty acids, further studies to increase the level of complexation between amylopectin and fatty acids are required to utilize the amylopectin–fatty acid complex as a potential ingredient in processed foods.

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## References

1. Biliaderis CG, Galloway G. Crystallization behavior of amylose-V complexes: Structure-property relationships. Carbohyd. Res. 189: 31-48 (1989)
2. Biliaderis CG, Senevirante HD. On the supermolecular structure and metastability of glycerol monostearate-amylose complex. Carbohyd. Polym. 13: 185-206 (1990)
3. Holm J, Björck I, Ostrowska S, Eliasson AC, Asp NG, Larsson K, Lundquist I. Digestibility of amylose-lipid complexes *in-vitro* and *in-vivo*. Starch-Stärke 35: 294-297 (1983)
4. Zhou Z, Robards K, Helliwell S, Blanchard C. Effect of the addition of fatty acids on rice starch properties. Food Res. Int. 40: 209-214 (2007)
5. Hasjim J, Lee S-O, Hendrich S, Setiawan S, Ai Y, Jane J. Characterization of a novel resistant-starch and its effects on postprandial plasma-glucose and insulin responses. Cereal Chem. 87: 257-262 (2010)
6. Karkalas J, Ma S, Morrison WR, Pethrick RA. Some factors determining the thermal properties of amylose inclusion complexes with fatty acids. Carbohyd. Res. 268: 233-247 (1995)
7. Lesmes U, Cohen SH, Shener Y, Shimoni E. Effects of long chain fatty acid unsaturation on the structure and controlled release properties of amylose complexes. Food Hydrocolloid. 23: 667-675 (2009)
8. Hirai M, Hirai T, Ueki T. Effect of branching of amylopectin on complexation with iodine as steric hindrance. Polymer 35: 2222-2225 (1994)
9. Potocki de Montalk G, Remaud-Simeon M, Willemot R-M, Sarçabal P, Planchot V, Monsan P. Amylosucrase from *Neisseria polysaccharea*: Novel catalytic properties. FEBS Lett. 471: 219-223 (2000)
10. Rolland-Sabaté A, Colonna P, Potocki-Véronèse G, Monsan P, Planchot V. Elongation and insolubilisation of $\alpha$-glucans by the action of *Neisseria polysaccharea* amylosucrase. J. Cereal Sci. 40: 17-30 (2004)
11. Jung J-H, Seo D-H, Ha S-J, Song M-C, Cha J, Yoo S-H, Kim T-J, Baek N-I, Baik M-Y, Park C-S. Enzymatic synthesis of salicin glycosides through transglycosylation catalyzed by amylosucrases from *Deinococcus geothermalis* and *Neisseria polysaccharea*. Carbohyd. Res. 344: 1612-1619 (2009)
12. Kim BK, Kim HI, Moon TW, Choi SJ. Branch chain elongation by amylosucrase: production of waxy corn starch with a slow digestion property. Food Chem. 152: 113-120 (2014)
13. Kim B-S, Kim H-S, Hong J-S, Huber KC, Shim J-H, Yoo S-H. Effects of amylosucrase treatment on molecular structure and digestion resistance of pre-gelatinised rice and barley starches. Food Chem. 138: 966-975 (2013)
14. Brumovsky JO, Thompson DB. Production of boiling-stable granular resistant starch by partial acid hydrolysis and hydrothermal treatments of high-amylose maize starch. Cereal Chem. 78: 680-689 (2001)
15. Shin SI, Choi HJ, Chung KM, Hamaker BR, Park KH, Moon TW. Slowly digestible starch from debranched waxy sorghum starch: preparation and properties. Cereal Chem. 81: 404-408 (2004)
16. Potocki De Montalk G, Remaud-Simeon M, Willemot RM, Planchot V, Monsan P. Sequence analysis of the gene encoding amylosucrase from *Neisseria polysaccharea* and characterization of the recombinant enzyme. J. Bacteriol. 181: 375-381 (1999)
17. Godet MC, Tran V, Colonna P, Buleon A, Pezolet M. Inclusion/exclusion of fatty acids in amylose complexes as a function of the fatty acid chain length. Int. J. Biol. Macromol. 17: 405-408 (1995)
18. Zhou X, Wang R, Zhang Y, Yoo S-H, Lim S-T. Effects of amylose chain length and heat treatment on amylose–glycerol monocaprate complex formation. Carbohyd. Polym. 95: 227-232 (2013)
19. Yasui T, Ashida K, Sasaki T. Chain-length distribution profiles of amylopectin isolated from endosperm starch of waxy and low-amylose bread wheat (*Triticum aestivum* L.) lines with common genetic background. Starch-Stärke 61: 677-686 (2009)
20. Gelders GG, Vanderstukken TC, Goesaert H, Delcour JA. Amylose-lipid complexation: A new fractionation method. Carbohyd. Polym. 56: 447-458 (2004)
21. Kim EJ, Kim HR, Choi SJ, Park C-S, Moon TW. Low digestion property of amylosucrase-modified waxy adlay starch. Food Sci. Biotechnol. 25: 457-460 (2016)
22. Exarhopoulos S, Raphaelides SN. Morphological and structural studies of thermally treated starch-fatty acid systems. J. Cereal Sci. 55: 139-152 (2012)
23. Cheetham NWH, Tao L. Variation in crystalline type with amylose content in maize starch granules: An X-ray powder diffraction study. Carbohyd. Polym. 36: 277-284 (1998)
24. Chung H-J, Jeong H-Y, Lim S-T. Effects of acid hydrolysis and defatting on crystallinity and pasting properties of freeze-thawed high amylose corn starch. Carbohyd. Polym. 54: 449-455 (2003)
25. Jane J, Chen YY, Fee LF, McPherson AE, Wong KS, Rodosavljevic M, Kasemsuwan T. Effects of amylopectin branch chain length and amylose content on the gelatinization and pasting properties of starch. Cereal Chem. 75: 629-637 (1999)
26. Srichuwong S, Sunarti TC, Mishima T, Isono N, Hisamatsu M. Starches from different botanical sources I: Contribution of amylopectin fine structure to thermal properties and enzyme digestibility. Carbohyd. Polym. 60: 529-538 (2005)
27. Shin HJ, Choi SJ, Park CS, Moon TW. Preparation of starches with low glycaemic response using amylosucrase and their physicochemical properties. Carbohyd. Polym. 82: 489-497 (2010)
28. Chang F, He X, Huang Q. The physicochemical properties of swelled maize starch granules complexed with lauric acid. Food Hydrocolloid. 32: 365-372 (2013)
29. Tester RF, Morrison WR. Swelling and gelatinization of cereal starches. I. Effects of amylopectin, amylose and lipids. Cereal Chem. 67: 551-557 (1990)
30. Tufvesson F, Wahlgren M, Eliasson A-C. Formation of amylose-lipid complexes and effects of temperature treatment. Part 2. fatty acids. Starch-Stärke 55:

138-149 (2003)
31. Tufvesson F, Skrabanja V, Björck I, Elmståhl HL, Eliasson A-C. Digestibility of starch systems containing amylose–glycerol monopalmitin complexes. LWT-Food Sci. Technol. 34: 131-139 (2001)
32. Zhang G, Ao Z, Hamaker BR. Nutritional property of endosperm starches from maize mutants: a parabolic relationship between slowly digestible starch and amylopectin fine structure. J. Agr. Food Chem. 56: 4686 (2008)
33. Soong YY, Goh HJ, Henry CJ. The influence of saturated fatty acids on complex index and *in vitro* digestibility of rice starch. Int. J. Food Sci. Nutr. 64: 641-647 (2013)