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A Thesis for the Degree of Master of Science

**Blends of native and amylosucrase treated waxy
starches complexed with fatty acid: digestibility and
physicochemical properties**

지방산과 복합체를 이룬 생찰전분과 아밀로수크레이스
처리 찰전분 혼합물의 소화율과 이화학적 특성

February 2014

Lee, Suk Young

Department of Agricultural Biotechnology

Seoul National University

농학석사학위논문

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이 논문을 석사학위 논문으로 제출함

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by

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**Submitted in Partial Fulfillment of the Requirement
for the Degree of Master of Science**

February 2014

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ABSTRACT

Preparation of waxy starch-lipid complex is hampered by steric hindrance and short branch chains of amylopectin (AP). In the current study, starch-lipid complexes were prepared by using the blends of native and amylosucrase (AS) treated waxy corn starches and fatty acid (0.5 mM/g-starch) i.e. lauric acid (LA, C12:0) and palmitic acid (PA, C16:0). This study aimed to investigate the effects of branch chain length of starch and kinds of fatty acid on the complex content, X-ray diffraction pattern, thermal properties, digestibility, and rheological properties of the products.

With increasing proportion of the AS-treated starch, branch chain length increased which led to an increase of apparent amylose content. The ability of starch to complex with fatty acids increased with branch chain length of AP and lipid chain length. The X-ray diffraction patterns of starch blends and starch-LA complexes showed a B-type polymorph, while that of starch-PA complexes revealed a B+V-type. The relative crystallinity, melting enthalpy, and viscoelasticity were increased and the digestibility was decreased owing to double helical structure formed by elongated branch chains. However, because the complexed fatty acid disrupted the double helical structure, relative crystallinity, melting enthalpy, and viscoelasticity

were decreased and digestibility was increased with an increase in complex content. Meanwhile, though melting temperature of starch blends was decreased with increasing amount of AS-treated starch because of disordered arrangement of elongated branch chains, melting temperature of AS-treated starch increased after complexation with fatty acid due to ordered crystallinity of starch-lipid complex.

In conclusion, this study demonstrated that the complexed fatty acid impairs the formation of double helical structure of elongated branch chains by AS treatment, which induces the changes in functional properties of waxy starch-lipid complexes.

Keywords: amylosucrase, waxy corn starch, branch chain length, starch blends, starch-fatty acid complex, *in vitro* digestibility, physicochemical properties

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ABBREVIATIONS

AM: amylose

AP: amylopectin

RDS: rapidly digestible starch

SDS: slowly digestible starch

RS: resistant starch

AS: amylsucrase from *Neisseria polysaccharia*

DP: degree of polymerization

LA: lauric acid

PA: palmitic acid

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INTRODUCTION

Starch is a primary carbohydrate, the most important source of human nutrition that is digested by pancreatic α -amylase in the gastrointestinal (GI) tract. Native starches, consisting of linear amylose (AM) and highly branched amylopectin (AP) are used as ingredients for processed foods, exhibiting unique rheological behavior. Starch granules are stored as partially crystalline structure consisting mainly of double helices of AP (Jiang et al., 2013). For nutritional purposes, starch is generally classified into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) depending on the rate and extent of digestion (Englyst et al., 1992). RDS is digested rapidly in the mouth and the small intestine, leading to a rapid increase followed by an equally rapid drop in blood glucose level. In contrast, SDS which is digested slowly but completely in the small intestine might consist of less perfect crystalline regions containing small portions of double helices and amorphous region (Shin et al., 2004). The principal health effect of SDS is a slow increase of postprandial blood glucose level, thus SDS may be beneficial in products used by athletes that can provide a more consistent source of exogenous glucose to the body. Also, SDS can have implications for physical and mental performance, satiety, and diabetes

management (Wolf et al., 1999). RS cannot be digested in the small intestine, but is fermented in the large intestine (Topping & Clifton, 2001). SDS and RS are both correlated with a low glycemic index (Englyst et al., 1996). Native starch digestibility is greatly influenced by the interplay of many factors, including starch sources, AM/AP ratio, amount of AM-lipid complexes, AP chain length distribution, extent of molecular association between starch components, degree of crystallinity, and type of crystalline polymorphic forms (Singh et al., 2010).

Native starches do not meet industrial needs for a wide range of application purposes due to various disadvantages including high viscosity of starch gel at room temperature; poor thermal shear; and a high rate and extent of retrogradation (Jayakody & Hoover, 2008), but can be chemically or physically modified to obtain desired properties. From a health standpoint, due to consumers dislike of chemical derivatives of starch, a recent trend is to avoid use of chemically-modified starch products in the processed foods (Kim & BeMiller, 2012; Ortega-Ojeda & Eliasson, 2001). It is therefore of interest to find new ways to improve the properties of native starches without chemical modification. Some possibilities are the enzymatical modification, the mixing of starches from different sources, and complexation.

Recently, modification of gelatinized starches with recombinant amylosucrase (E.C. 2.4.1.4., AS) from *Neisseria polysaccharea* to lower

digestibility was reported (Kim et al., 2013; Park et al., 2013; Ryu et al., 2010; Shin et al., 2010). AS catalyzes a transglycosylation reaction to produce an insoluble AM-like polymer using sucrose as a substrate while releasing fructose (Hehre, 1949; Potocki-Veronese et al., 2005; Potocki de Montalk et al., 2000; Rolland-Sabaté et al., 2004). Overall, AS is the only known enzymes that catalyze the elongation of some external chains of the acceptor such as AM and AP at their non-reducing ends from a cheap agroresource without any primer (Hehre, 1949; Potocki-Veronese et al., 2005; Potocki de Montalk et al., 2000; Rolland-Sabaté et al., 2004). Because of its action, the proportion of the branch chains with short and/or medium length decreases, and the proportion of long branched chains increases (Park et al., 2013). Shin et al. (2010) who conducted modification of waxy and normal starches with AS suggested that the enhanced SDS fractions were due to formation of crystallites using elongated AP branch chains and gel-forming ability at low concentration could be provided by the AS treatment on starch. In other study, the RS contents of AS-treated starches were directly associated with the enhanced intermediate AP branch chains by AS (Kim et al., 2013). In the study of Park et al. (2013), the AS-treated starches contained more SDS and RS, and these starches were the most resistant to starch degradation under the conditions that mimic the human GI tract.

In certain cases such as a new product development, novel properties of starch system may be achieved through blending native and modified starches (Zhu & Corke, 2011). Properties of blended starches have been proposed to be associated with mixing ratio (Chen et al., 2003), AM/AP ratio (Ortega-Ojeda et al., 2004), and interactions between the granules (Obanni & BeMiller, 1996). Most recently, starch blends, instead of pure starches, can be used to impart desired physicochemical quality such as improved texture or promote health potentials of food (Chen et al., 2003; Hong & Yoo, 2012; Karam et al., 2005).

Lipids have long been known to form inclusion compounds with AM, where the hydrocarbon portion of the lipid is located within the helical cavity of AM (Banks & Greenwood, 1972; Zobel, 1988). The hydrophobic complexing agents can complex within the AM single helix stabilized through van der Waals forces with the adjacent hydrogen atoms of AM (Godet et al., 1993). Although there is report that linear outer branches of AP may interact with surfactants (Gudmundsson & Eliasson, 1990), AP has a limited ability to form complexes due to steric hindrance and short length of branch chains (Conde-Petit et al., 2006). The AM inclusion complexes, or V-AM, are used to retard gelatinization and retrogradation of starchy food, to produce novel starches (Chang et al., 2013a; Guraya et al., 1997; Zhang et al., 2012), and even to create a new delivery system for controlled release of

various hydrophobic bioactive compounds (Kim & Lim, 2009). The AM-lipid inclusion complex which is resistant to α -amylase leads to prolonged enzymatic hydrolysis as compared with amorphous AM (Guraya et al., 1997; Seneviratne & Biliaderis, 1991; Tufvesson et al., 2001). On the other hand, AM-lipid complexation can impede the AM-AM double helix formation, which would lead to generation of RS, and crystallization during cooling of a heated starch, reducing RS formation and increasing the starch hydrolysis rate (Kulp et al., 1981; Sajilata et al., 2006). Tufvesson et al. (2001) showed that the addition of glycerol monolaurate increased the hydrolysis rate of autoclaved high-AM maize starch. Autoclaving at a high temperature makes AM more available to form complex with monoacyl glycerols, and thereby decrease the AM retrogradation. Putseys et al. (2010a) also demonstrated the addition of glycerol monostearate into starch reduces the RS content and increases the hydrolysis rate. Therefore, the complexation between AM and lipids to lower the degradability of starch should be conducted by using AM chains freed from recrystallization. Meanwhile, the gelling properties of V-AM suggested practical applications as thickeners and as dispersants for lipids in foods, lotions, and water-based lubricants (Byars et al., 2013). V-AM formation is affected by several factors including starch type, degree of polymerization (DP), and the structure of the included molecule (Obiro et al.,

2012). Also, complex formation and its crystalline properties are influenced by the size of aliphatic lipid (Zhou et al., 2013).

Because AP fine structure determines the crystalline type and its perfection in native starch granules, it is the key to understand the structure-digestion relationship (Zhang et al., 2006a). Moreover, the enhanced SDS and RS fractions were due to elongation of AP branch chains by AS treatment (Kim et al., 2013; Shin et al., 2010). Waxy corn starch is composed of 100% AP, and endogenous lipid content of them is very low (0.15%) (Swinkels, 1985). Little work has been done in using waxy starch with different branch chain length distribution to prepare V-AM complex. Thus, waxy corn starch was used in this work. The objectives of this study were to prepare AS-treated waxy corn starch-lipids complex and to investigate the effects of branch chain length of starch and kind of fatty acids on complex content, digestibility, and physicochemical properties of the products, which may have potential applications in food industry. For this purpose, AS-treated waxy corn starch was mixed with native waxy corn starch, and those starch blends were complexed with lauric acid (LA) or palmitic acid (PA).

MATERIALS AND METHODS

1. Materials

1-1. Starch

Waxy corn starch was obtained from Samyang Genex Corp. (Incheon, Korea).

1-2. Enzymes

Amylosucrase from *Neisseria polysaccharea* was provided by Food Microbiology and Bioengineering Laboratory of Kyunghee University.

Isoamylase (activity 1000U) was obtained from Megazyme (Bray, Ireland). Pancreatin (P7545, activity $8 \times$ USP/g) was from Sigma Chemical Co. (St. Louis, MO, USA) and amyloglucosidase (AMG 300L, activity 300 AGU/mL) from Novozymes (Bagsvaerd, Denmark).

1-3. Fatty acids

Lauric (C12:0) and palmitic (C16:0) acids were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

2. Methods

2-1. Enzyme assay of AS activity

The AS was purified by affinity chromatography with Ni-NTA (nickel-nitrilotriacetic acid) resin according to the method of Jung et al. (2009). Enzyme activity was determined using the method of Van der Veen et al. (2004) with a modification. The mixture of 0.1 mL of 4% sucrose, 0.1 mL of 1% glycogen, 0.25 mL of 0.1 mM sodium citrate buffer (pH 7.0) and 0.05 mL of diluted enzyme was reacted in a water bath at 30°C for 10 min. The released fructose was quantified using the dinitrosalicylic acid method of Miller (1959). One unit of amylosucrase was defined as the amount of enzyme that catalyzes the release of 1 μ M of fructose per min under the assay conditions.

2-2. Preparation of AS-treated starch

Starch suspension (2%, w/v) was prepared by mixing waxy corn starch, 100 mM sucrose, and 100 mM sodium acetate buffer (pH 7.0). The starch suspension was boiled for 30 min to increase enzyme accessibility. After the solution was cooled to 30 °C, AS (20000 U/30 mL) was added, and incubated in a water bath at 30°C for 24 h. The enzyme reaction was stopped by adding two-fold ethanol to the suspension. The AS-treated starch was

precipitated by centrifugation at 10,000g for 10 min, and the supernatant was removed. The pellet was washed off three times with distilled water by centrifugation at 10,000g for 10 min. The precipitate was freeze-dried, ground, and passed through a 100-mesh sieve.

Fructose concentration of the soluble fraction after enzyme reaction was determined by the dinitrosalicylic acid method. After incubating for 24 h, 2 mL of suspension was boiled for 10 min, cooled to room temperature, and centrifuged at 7000g for 20 min. Then, released fructose of 50-fold diluted supernatant (0.5 mL) was quantified using the method of Miller (1959). The AS-treated/raw starch ratio was determined using the weight of freeze-dried precipitate after the reaction to raw starch before the reaction.

2-3. Preparation of starch blends complexed with fatty acids

Native waxy corn starch and AS-treated waxy corn starch were mixed at ratios of 100:0, 75:25, 50:50, 25:75, and 0:100. The starch blend slurry (5%, w/v) was cooked with vigorous vortexing for 30 min and autoclaved at 121 °C for 30 min to gelatinize completely. Fatty acid (LA and PA) (0.5 mM/g-starch), dissolved in ethanol (10%, w/v), was added to the starch dispersion, and the mixture was continuously cooked for an additional 30 min with vigorous vortexing to facilitate the interaction between starch and fatty acid. After autoclaving at 121 °C for 30 min, the mixture was incubated

at 95 °C and 120 rpm for 24 h to form stable complex. Then, the mixtures were cooled to room temperature and washed four times with 50% ethanol solution and two times with distilled water by centrifugation at 10,000g for 10 min to remove uncomplexed fatty acids. The final resulting precipitates were freeze-dried, ground, and passed through a 100-mesh sieve. The control samples were prepared using the same procedure without adding fatty acid.

2-4. Determination of branch chain length distribution by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD)

The branch chain length distributions of native and AS treated starch mixtures were determined by debranching the starches with isoamylase. Starch (15 mg) was dispersed in 90% DMSO (3 mL) and boiled for 30 min. Ethanol (15 mL) was added to the starch suspension to precipitate starch and centrifuged at 10,000g for 10 min. Then distilled water (1.5 mL) was added to the pellet and boiled for 15 min. After boiling, 1.5 mL of 50 mM sodium acetate buffer (pH 4.3) was added and boiled for 20 min. Isoamylase (30 μ L, 1000 U/mL) was added to the starch dispersion and the sample was incubated at 45 °C and 30 rpm for 2 h in a water bath. Enzyme reaction was stopped by boiling for 10 min. Debranched sample was filtered through a

0.45 µm membrane filter and analyzed using HPAEC-PAD on a Carbo-pack PA1 anion-exchange column (4x250 mm, Dionex, Sunnyvale, CA, USA) with a pulsed amperometric detector. The sample was eluted with a gradient of 600 mM sodium acetate in 150 mM NaOH with a flow rate 1 mL/min. The gradients of sodium acetate used were as follows: increasing from 0-20 % for 0-5 min, 20-45 % for 6-30 min, 45-55 % for 31-60 min, 56-60 % for 61-80 min, 61-65 % for 81-90 min, 66-80 % for 91-95 min, and 81-100 % for 96-100 min. The values of DP were designated using a mixture of maltooligosaccharides (DP 1-7, Sigma Chemical) as standard. PeakNet software (version 5.11, Dionex) was used for calculation of peak areas. Number-based average DP (DP_n) was determined by following equation.

$$DP_n = (\%A_i \times DP_i) / 100$$

A_i : peak area / total area (i: 1, 2, 3 ...)

2-5. Determination of apparent AM content and complex content

Apparent AM contents were determined according to the colorimetric method outlined by AACC Approved Method 61-03 (AACC, 2000). Starch (20 mg) was dispersed in absolute ethanol (0.2 mL), and then mixed with 1 M NaOH (1.8 mL) with vigorous vortexing. The starch suspension was cooked for 10 min, and cooled to room temperature. The resultant starch solution (1 mL) was diluted to 10 mL with distilled water. An aliquot (0.5

mL) of the diluted starch solution was combined with 1 M acetic acid (0.1 mL) and Lugol's solution (0.2 mL; 0.2% I₂ + 2.0% KI), and diluted again to 10 mL with distilled water, followed by holding for 20 min in the dark. The absorbance of the color-developed starch solution was measured at 620 nm. The apparent AM content of the starch sample was determined from a standard curve prepared with amylose from potato and amylopectin from maize (Sigma–Aldrich Chemical Co., St. Louis, MO, USA).

Complex content was calculated as the difference between apparent AM content of control sample and that of starch sample complexed with fatty acid.

2-6. X-ray diffraction patterns and relative crystallinity

X-ray diffraction was analyzed using a powder X-ray diffractometer (New D8 Advance, Bruker, Karlsruhe, Germany) at 40 kV and 40 mA. Starch sample scan was performed through 2θ range from 3° to 30° with a 0.02° step size and a count time of 2 sec. The relative crystallinity was determined by the following equation according to the method of Nara and Komiya (1983). The area was calculated using the software developed by the instrument manufacturer (EVA, 2.0).

$$\text{Relative crystallinity (\%)} = \left(\frac{\text{Crystalline area}}{\text{Total curve area}} \right) \times 100$$

2-7. Measurement of thermal properties

Thermal properties of the samples were investigated using a differential scanning calorimeter (DSC, Diamond DSC, Perkin-Elmer, Waltham, MA, USA). Each sample (10 mg) was weighed in a hermetic aluminum pan (Seiko, Tokyo, Japan), and 30 μL of distilled water was added. The sample pan was sealed and kept at room temperature overnight for equilibrium. An empty aluminum pan was used as a reference, and indium was used for calibration. DSC scan was performed from 30 $^{\circ}\text{C}$ to 160 $^{\circ}\text{C}$ at 10 $^{\circ}\text{C}/\text{min}$. The onset temperature (T_o), the peak temperature (T_p), the conclusion temperature (T_c), and the melting enthalpy (ΔH) were recorded.

2-8. Starch digestibility

The digestibility of uncooked and cooked blends of native and AS-treated starches complexed with fatty acid was determined by the method of Englyst et al. (1992) with slight modification. Pancreatin (6 g) was dissolved in distilled water (72 mL) and stirred well for 10 min. It was precipitated by centrifugation at 1,500g for 10 min. A 60 mL aliquot of supernatant was mixed with 1.2 mL of amyloglucosidase and 10.8 mL of distilled water, and incubated at 37 $^{\circ}\text{C}$ for 10 min.

A starch sample (30 mg) was dispersed in a 2 mL-microtube with sodium

acetate buffer (0.75 mL, 0.1 M, pH 5.2) with one glass bead. After mixing each microtube, it was equilibrated in a shaking incubator (240 rpm, 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, 37 °C). A microtube was removed each time at 10, 20, 30, 60, 120, and 240 min and boiled in a cooker for 10 min to stop reaction.

The glucose released under hydrolysis of starch was obtained in supernatant after the centrifugation at 5,000g for 10 min. The glucose content was determined by the glucose oxidase method (Karkalas, 1985) using a commercially available kit (Embiel Co., Gunpo, Korea). To measure the content of released glucose, 11-fold diluted supernatant (0.1 mL) was added to a 2 mL-microtube containing 1.5 mL of glucose oxidase and peroxidase reagent. The microtube was incubated in a water bath at 37 °C for 20 min. The absorbance of the sample was then read at 505nm.

Cooked samples were prepared by boiling starch dispersions with sodium acetate buffer for 10 min before equilibrating to 37 °C in a shaking incubator.

Starch fractions were classified based on the rate and degree of hydrolysis. RDS was measured by the quantity of glucose after reaction for 10 min. SDS was the fraction digested between 10 and 240 min. RS was the unhydrolyzed fraction after 240 min.

2-9. Measurement of rheological properties

The rheological properties of starch gel were measured using an oscillatory rheometer (Rheostress 1, Thermo HAAKE, Karlsruhe, Germany) with a cone-plate system (35 mm diameter, cone angle 1°, gap size 0.052 mm). A starch sample (15 mg) was dispersed with 0.3 mL of distilled water. The starch dispersion (5%, w/v) was boiled for 5 min and placed between the cone and plate in a rheometer. And then it was cooled to 25 °C for 10 min to form starch gel. After expelled materials were trimmed off, the sample at the edge of the system was covered by thin layer of silicon oil to prevent drying. The frequency sweep measurement was carried out in a frequency range of 0.1-10 Hz at 25 °C. All measurement was performed in the linear viscoelastic region.

2-10. Statistical analysis

All the experiments were done in triplicate, and data were expressed as mean±standard deviation. Analysis of variance (ANOVA) was conducted and the mean separations were done by the Duncan's multiple range tests at a significance level of 0.05. Pearson correlations (bivariate correlations algorithms, $p < 0.01$ and $p < 0.05$) of selected physicochemical properties were obtained to investigate the effects of branch chain length of starch and complex content according to the mixing ratio of AS-treated starch. All the

statistical analyses described above were conducted using PASW statistic 18 (SPSS corp., Chicago, IL, USA).

RESULTS AND DISCUSSION

1. Fructose concentration after AS treatment and AS-treated/raw starch ratio

Released fructose concentration of soluble fraction after the AS reaction and the AS-treated/raw starch ratio are shown in Table 1. Because AS forms glucose by sucrose hydrolysis releasing fructose, consumption of sucrose increased along with production of fructose (Shin et al., 2010). The fructose concentration after the enzyme reaction was 75.2 mM. The branch chain length of AP was elongated by using about 75% of added sucrose. The ratio of AS-treated and raw starch was 1.3. Because of elongated branch chain after AS treatment, it is greater than 1.

Table 1

Fructose concentration after AS treatment and AS-treated/raw starch ratio

Sample	Fructose concentration (mM)	AS-treated/raw starch
AS with 20000 U/30 mL for 24 h	75.2±1.4 ¹⁾	1.3±0.0

¹⁾ Data are expressed as average value and standard deviation.

2. Branch chain length distributions of starch blends

The branch chain length distributions and the relative percentages of peak area with DP of starches are shown in Table 2 and Figure 1, respectively. Amylopectin branch chains are classified into A chain (DP 6-12), B₁ chain (DP 13-24), B₂ chain (DP 25-36), and B₃ chain (DP ≥ 37) depending on DP (Hanashiro et al., 1996). The waxy corn starch, one of A type starches, had a larger proportion of short chains such as A chain but smaller proportions of longer chains (Zhang et al., 2006b). Shin et al. (2010) reported that AS treatment induced the increase in the chain length of amylopectin, and accordingly the decrease in the proportions of short chains. After the AS treatment, A chains (DP ≤ 12) decreased while both B₂ chains (DP 25-36) and B₃ chains (DP ≥ 37) increased with the increase of mixing ratio of AS-treated starch (Table 2). Also, DP_n of the starches significantly increased from 17.0 to 26.3. These results were due to the elongation of external chains by AS. Bimodal distributions of branch chain length were observed for A25, A50, and A75. The first peak was due to native waxy corn starch, and second peak was due to AS-treated waxy corn starch. The highest peak was shifted to the right with the increase of mixing ratio of AS-treated starch from the A50 (Figure 1).

In the work of Kim et al. (2013), who conducted enzymatic modification

of pre-gelatinized starches (e.g., rice and barley) using AS of 10 U/mL for 3% starch suspension, A chains decreased, whereas B₁ and B₂ chains increased. However, in the present study, B₁ chains were not changed. This was due to the discrepancy of loading levels of AS and acceptor (starch) and donor (sucrose) concentration between two studies.

Branch chain length distribution plays an important role in determining functional properties in food systems (Zhang & Hamaker, 2012). Higher number of short branch chains contributes to lower crystallinity, viscosity, and degree of retrogradation (Jane et al., 1999). In contrast, starch molecules with longer branch chain length contribute to the higher degree of retrogradation, gel firmness, and lower degree of digestibility (Zhang & Hamaker, 2012).

Table 2
Branch chain length distributions of starch blends

Samples	Percent distribution (%)					DP _n ²⁾
	DP ¹⁾ ≤ 5	DP 6-12	DP 13-24	DP 25-36	DP ≥ 37	
A0 ³⁾	1.9±0.8 ^{a, 4), 5)}	34.3±1.9 ^a	47.9±1.4 ^a	11.8±0.4 ^e	4.2±1.1 ^d	17.0±0.5 ^e
A25	1.3±0.3 ^{ab}	29.3±0.8 ^b	45.8±1.5 ^{ab}	17.5±1.0 ^d	6.1±0.5 ^d	18.8±0.3 ^d
A50	1.1±0.1 ^{bc}	23.9±1.0 ^c	43.6±2.0 ^b	23.0±0.9 ^c	8.3±0.9 ^c	20.6±0.3 ^c
A75	0.7±0.1 ^{bc}	14.5±0.6 ^d	44.6±1.1 ^b	28.9±0.5 ^b	11.3±1.6 ^b	23.3±0.6 ^b
A100	0.5±0.1 ^c	3.5±0.7 ^e	44.5±1.4 ^b	36.8±0.7 ^a	14.6±1.5 ^a	26.3±0.4 ^a

¹⁾ DP, degree of polymerization.

²⁾ Number-based average degree of polymerization.

³⁾ The starch mixture containing 0% of AS-treated starch.

⁴⁾ Data are expressed as average value and standard deviation.

⁵⁾ The values with different superscripts in the same column are significantly different ($p < 0.05$).

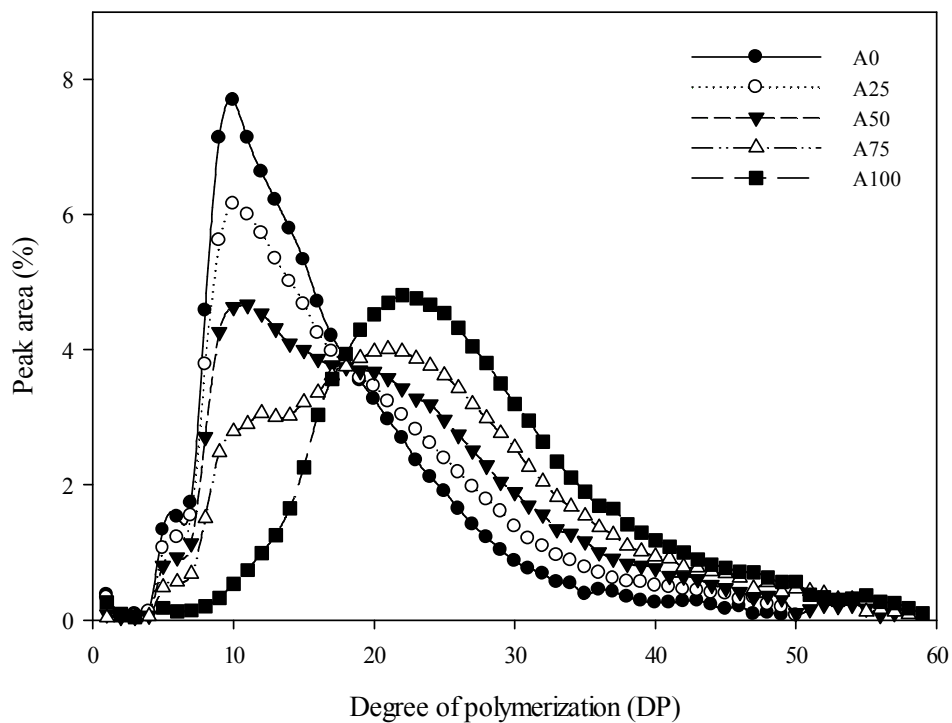


Figure 1. Branch chain length distributions of starch blends.

3. Apparent AM content and complex content

AM forms single-helical complexes with iodine to give a blue color (Conde-Petit et al., 1998). The A0 was almost AM-free (Table 3). As the mixing ratio of AS-treated starch increased, apparent AM contents also increased. The AM content of A100 was 22.1%, showing an increase by about 21.8% in the AS-treated starch compared with native starch (Table 3). Pearson correlation coefficients between branch chain length and apparent AM content are presented in Table 7. Apparent AM content was negatively correlated to $DP \leq 24$ and positively correlated to $DP \geq 25$ and DP_n . It might be due to the elongated branch chains of AP acting like AM. Because long chains of AM are easily combined with iodine, AM gives high iodine absorption values, while AP with short linear chain has low values (Bates et al., 1943). In other words, the possibility of forming single helical structure increased with increasing branch chain length.

Complex content in the blends of native and AS-treated starches complexed with fatty acid are summarized in Table 3 and Figure 2. In the case of V-AM, it was stained blue with iodine depending on the degree of complexation (Exarhopoulos & Raphaelides, 2012; Tang & Copeland, 2007). Inclusion of fatty acids reduced the iodine binding capacity of starch (Tang

& Copeland, 2007) leading to an increase in complex content. In this study, there was a small amount of complex content in A0-L and A0-P. This result is consistent with Chang et al. (2013b)'s report that there was a small amount of complexed lipids in waxy maize starch-LA complex due to lack of AM and short branch chain length of AP. The ability of starch to complex with LA and PA was enhanced with increasing mixing ratio of AS-treated starch as shown in Figure 2. $DP \geq 25$ and DP_n positively correlated to complex content (Table 7). In the case of fatty acids, AM is believed to create left handed helices with six units of glucose per helix turn with the fatty acid situated within the helices, with the polar head group localized outside the cavity (Conde-Petit et al., 2006; Godet et al., 1995; Godet et al., 1993). The minimum AM size is around 30-40 glucosyl residues to complex PA and 20-30 glycosyl residues to complex LA (Godet et al., 1995). In the study of Chang et al. (2013b), there was a strong positive correlation between apparent AM content and complexed lipid content. Judging from this, elongated branch chains of AP by AS treatment would act like AM. Previous studies reported that long branch chains of AP might mimic AM to form helical complexes with lipids (Jane et al., 1999; Rolland-Sabaté et al., 2004). Although helical crystalline formation between the linear branch chains of AP may be hindered by α -(1,6)-linked branch points, elongated branch

chains by AS could overcome the hindrance and make stable recrystallized structure (Ryu et al., 2010).

Native and AS-treated starch mixtures exhibited a higher complexing ability with PA, and a lower with LA. Pearson correlation coefficient between $DP \geq 25$ and starch-PA complex content was greater than that between $DP \geq 25$ and starch-LA complex content (Table 7). Also, Pearson correlation coefficient between DP_n and starch-PA complex content was higher than that between DP_n and starch-LA complex content (Table 7). In previous studies, an inverse relationship between complex index values and the number of carbons in saturated fatty acids was reported on potato, wheat, and rice starch (Kawai et al., 2012; Soong et al., 2013; Tang & Copeland, 2007). The reason is that the fatty acids with a longer carbon chain has low water solubility, and thus has poor dispersitivity in the gelatinized starch (Tufvesson et al., 2003). However, the result of the current study that PA with 16 carbon atoms was the preferred lipid chain length for native and AS-treated waxy corn starch blends than LA containing 12 carbon atoms was different to those findings. Presumably, LA was too soluble in the aqueous environment to be maintained properly in the hydrophobic cavity of AP chains. Additionally, the longer hydrocarbon chain of PA which has the lower hydrophilicity had a stronger interaction with the hydrophobic cavity of the AP helix, whereas the shorter hydrocarbon chain of LA had weaker

interactions with AP helix. There is, however, argument on the identity of the best complexing lipid. Some suggest that a lipid chain length with 14 carbon atoms is best for complex formation (Bhatnagar & Hanna, 1994; Krog, 1971; Soong et al., 2013). And lipid chain lengths of 12, 16, and 18 carbon atoms have been reported as the best complexing lipid with potato starch, amylose, and wheat starch, respectively (Hoover & Hadziyev, 1981; Kawai et al., 2012; Krog, 1971; Lagendijk & Pennings, 1970). Accordingly, it is still unknown which lipid shapes a stronger complex.

Table 3

Apparent amylose content (AAC) and complex content of the blends of native and AS-treated starches complexed with fatty acid

Ratio of AS-treated starch (%)	Type of fatty acids added	Sample codes	AAC (%)	Complex content (%)
0	-	A0	0.3±0.5 ^{k, 1), 2)}	-
	LA	A0-L	0.1±0.4 ^k	0.1±0.1 ^h
	PA	A0-P	-0.4±0.6 ^k	0.7±0.3 ^g
25	-	A25	5.6±0.8 ^{hi}	-
	LA	A25-L	5.1±0.8 ⁱ	0.5±0.6 ^{gh}
	PA	A25-P	3.6±0.3 ^j	2.0±0.6 ^f
50	-	A50	11.7±0.5 ^e	-
	LA	A50-L	9.0±0.3 ^g	2.7±0.2 ^e
	PA	A50-P	6.1±0.2 ^h	5.6±0.4 ^c
75	-	A75	17.5±0.4 ^c	-
	LA	A75-L	13.8±0.2 ^d	3.6±0.3 ^d
	PA	A75-P	10.6±0.4 ^f	6.8±0.2 ^b
100	-	A100	22.1±0.2 ^a	-
	LA	A100-L	18.4±0.3 ^b	3.7±0.1 ^d
	PA	A100-P	12.3±0.1 ^e	9.8±0.1 ^a

¹⁾ Data are expressed as average value and standard deviation.

²⁾ The values with different superscripts in the same column are significantly different ($p < 0.05$).

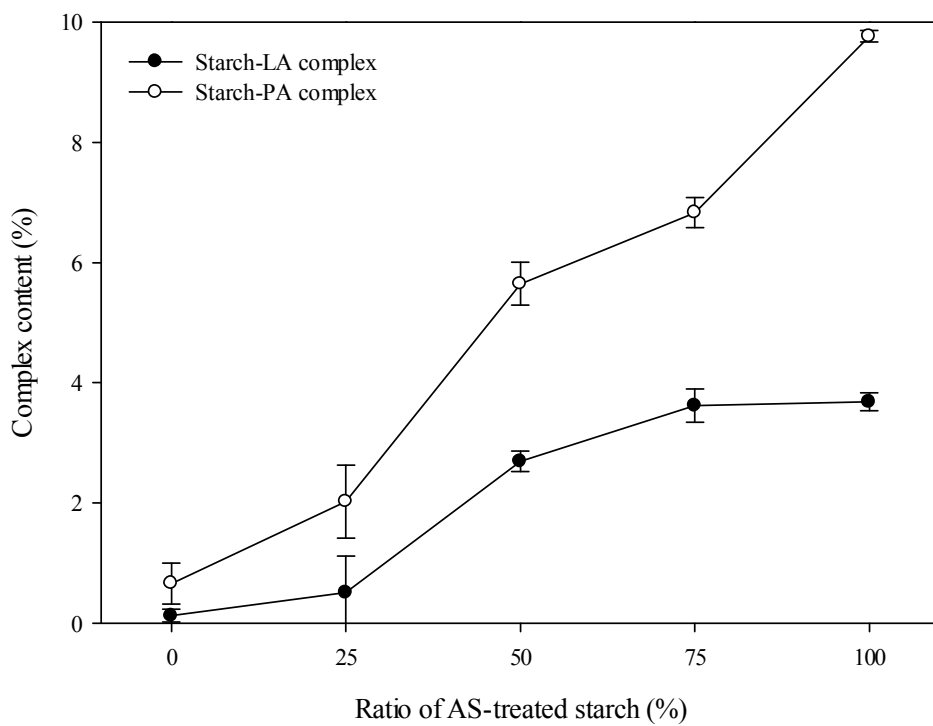


Figure 2. Complex content according to mixing ratio of AS-treated starch.

4. X-ray diffraction patterns and relative crystallinity

The crystalline packing arrangements of the blends of native and AS treated starches complexed with fatty acid were investigated with X-ray diffraction analysis and shown in Figure 3. The diffraction patterns and crystallinity were influenced by mixing ratio of AS-treated starch and type of fatty acid added. The raw waxy corn starch showed major peaks at 15°, 17°, 18°, and 23°, presenting a typical A type pattern (Hizukuri et al., 1980). In this study, A0 mainly composed of the amorphous regions because of gelatinization during sample preparation, and so did not show any pattern. The AS-treated starch showed a B-type X-ray pattern with the peaks at 5.5°, 14.5°, 17°, 19.3°, 22°, and 24°. With increasing the proportion of AS-treated starch, the peak intensity increased. Branch chain elongation resulting from AS action facilitated and solidified the inter-chain association, which in turn led to stable B-type polymorph (Ryu et al., 2010). Generally, B-type crystallites have a cluster with higher DP as compared to A-type amorphous lamellae (Gerard et al., 2000).

Type I V-AM is considered to be amorphous, while the semi-crystalline type II V-AM displays three peaks at Bragg angles of 7.4°, 13.1°, and 19.8° in its X-ray diffraction pattern (Bhatnagar & Hanna, 1994; Godet et al., 1995;

Lesmes et al., 2009; Tang & Copeland, 2007). Starch blend-LA complexes exhibited the similar crystal pattern as its starch blend counterpart (Figure 3). While the absence of the V-AM pattern would imply that the endogenous complexes lack crystalline order, the predominance of B-type crystals might hide the presence of a V-AM contribution in terms of starch blend-LA complexes. On the other hand, starch blend-PA complexes except A0-P displayed a B+V-type pattern (reflections at around 13°, 17°, and 20°) (Figure 3). And the V-type pattern (reflections at around 13° and 20°) became dominant when the mixing ratio of AS-treated starch was increased. It suggested that as the mixing ratio of AS-treated starch increased, more AP would be in position to interact with PA molecules. The results of scattering intensity analysis of starch-PA complexes were consistent with the results of complex content (Figure 2). Uncomplexed lipids with lower water solubility, rather than forming V-AM complexes, may self-associate to form micellar bodies (Tang & Copeland, 2007) and be physically trapped in the lamella and not molecularly included (Biais et al., 2006). Peaks observed at 2θ of 7.4°, 21.4°, and 23.8° were identified as the ones corresponding to the crystalline pattern of PA aggregates which were not used to form complexes (Fanta et al., 1999). In the case of A25-P, A50-P, A75-P, and A100-P, the scattering intensity of free PA increased with increasing mixing ratio of AS-treated starch. The PA would complex with AP and penetrate into the void

space slightly entangling with inner AP. Free PA also moves into the inner AP. However, 50% ethanol could wash off a small amount of fatty acid on the surface of the starch granules (Chang et al., 2013a). Therefore, free PA in the inner AP could not be washed off by 50% ethanol. Meantime, there was no peak of free LA in X-ray diffraction pattern of starch blend-LA complex. Because LA has a high solubility in ethanol, it makes uncomplexed LA to be removed easily by 50% ethanol solution.

Table 4 shows the relative crystallinities of starches. The relative crystallinity was mainly attributed to the residue of AP double helices, the formation of starch-fatty acid complex, and the amount of free fatty acids (Chang et al., 2013a). The relative crystallinity of A0 was the lowest due to the gelatinization during the sample preparation. The relative crystallinity of native and AS-treated starch mixture gradually increased from 18.9 to 34.2 % with increasing mixing ratio of AS-treated starch. The relative crystallinity showed positive correlation with DP 25-36, $DP \geq 37$, and DP_n and negative correlation with $DP \leq 5$, DP 6-12, and DP 13-24 (Table 7). This indicated that the elongated branch chains favored to form double helices, which could lead to the formation of crystalline structures densely packed in the AS-treated starch. Generally, starch crystallites are due to sequential packing of double helices that are found between the flexible external chains of AP (Miao et al., 2010). Thus, the crystallinity of starch could be influenced by

AP content, average AP chain length, and orientation of double helices within the crystallites to the X-ray beam, and crystallite size (Chung et al., 2009). Although AM disrupts the crystalline packing of AP (Cheetham & Tao, 1998), the crystallinity of branch chains of the AS-treated starch acting like AM increased because of the properties of AP forming double helices.

In case of A0 and A25, the crystallinity of starch-fatty acid complex increased compared with the starch blends without fatty acid (Table 4), giving rise to residual free PA and the formation of more crystalline starch-fatty acid complex. Also, complex content was positively correlated to relative crystallinity (Table 8). The molecular association of AM with the ligands can produce crystalline structures (Fanta et al., 2008). Conversely, in case of A50, A75, and A100, the crystallinity of starch-fatty acid complex decreased compared with the control samples containing absolute starch (Table 4). It suggested that semi-crystalline structure consisting of elongated AP double helices was disrupted by complexed fatty acid. Thus, the B+V-type showed a less degree of crystallinity than B-type crystallinity.

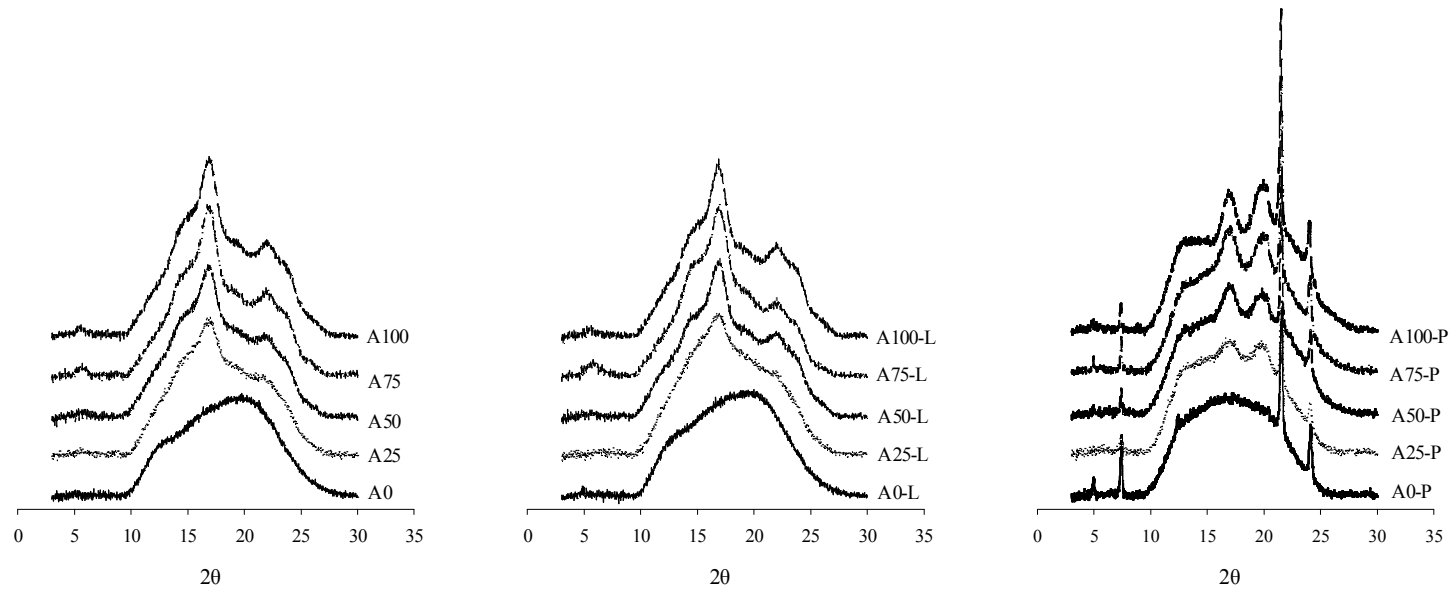


Figure 3. X-ray diffraction patterns of the blends of native and AS treated starches complexed with fatty acid.

Table 4

Relative crystallinity of the blends of native and AS-treated starches complexed with fatty acid

Samples	Relative crystallinity (%)
A0	18.9±0.2 ^{1), 2)}
A0-L	19.5±0.3 ^k
A0-P	23.0±0.2 ^j
A25	25.2±0.2 ⁱ
A25-L	26.8±0.2 ^h
A25-P	27.9±0.2 ^g
A50	31.6±0.4 ^c
A50-L	30.3±0.3 ^e
A50-P	29.5±0.1 ^f
A75	32.9±0.3 ^b
A75-L	31.1±0.2 ^d
A75-P	30.8±0.1 ^d
A100	34.2±0.4 ^a
A100-L	33.9±0.3 ^a
A100-P	33.0±0.2 ^b

¹⁾ Data are expressed as average value and standard deviation.

²⁾ The values with different superscripts in the same column are significantly different ($p < 0.05$).

5. Thermal properties

The thermal properties of the blends of native and AS-treated starches complexed with fatty acid are represented in Table 5. Changes in T_o , T_p , T_c , and ΔH of endotherms reflect the crystallinity, structure, and composition of starches (Chang et al., 2013b). In the present study, starch blend-fatty acid complexes exhibited three peaks. The first peak (49 °C - 73 °C) was due to the melting of native waxy corn starch and free PA. The second peak (76 °C - 110 °C) presumably represented the melting of AP double helices of AS-treated starch and type I starch-lipid complex, and the third peak (112 °C - 145 °C) was attributed to the melting of crystallites formed by long branch chains of AP acting like AM and the type II complex (Table 5). Type I complex is the less ordered form that melts at a lower temperature, whereas type II complex is more crystalline and melts at a higher temperature (Zhang et al., 2012).

5.1. Peak I

Previous studies on the mixtures of two starches suggested that the gelatinization of each individual starch happens independently, and that each starch contributes to the gelatinization enthalpy based on its proportion (Liu

& Lelievre, 1992; Ortega-Ojeda & Eliasson, 2001; Zhu & Corke, 2011). All the tested starch blends exhibited distinctive peaks during gelatinization, suggesting that each starch component gelatinized independently instead of spontaneously and together (Zhu & Corke, 2011). In the present study, the first peaks of A25, A50, and A75 would correspond to the gelatinization of native waxy corn starch which pre-gelatinized during the sample preparation, and the second peaks of them would correspond to the melting endotherm of AS-treated waxy corn starch. ΔH of peak I showed a positive correlation to $DP \leq 12$ and negative correlation to DP 25-36 and DP_n (Table 7). Low T_o , T_p , and T_c reflect the presence of abundant short AP chains (Noda et al., 1998). Based on this result, the first peak was due to the native waxy corn starch consisting of shorter branch chains than that of the AS-treated starch (Table 2). On the contrary, there was no first peak in A0 which consists of absolute native waxy corn starch. This result might be attributed to water competition between two different starch sources, making water molecules less available for gelatinizing each individual granule by lowering water activity of the system (Gunaratne et al., 2007). Therefore, A0, which could fully absorb water, gelatinized completely, leading to the destruction of crystalline structure. However, in A25, A50, and A75, native waxy corn starch did not gelatinize completely due to lowered water activity. Because of gelatinization during the sample preparation, ΔH of native waxy corn

starch was lower than that of general waxy corn starch which is about 8-15 J/g (Miao et al., 2009; Wongsagonsup et al., 2008).

In the case of starch-PA complexes, the melting range of native waxy corn starches overlapped with that of free PA because of larger enthalpy of free PA. The free PA showed a significant increase of ΔH when the mixing ratio of AS-treated starch increased in A25-P, A50-P, A75-P, and A100-P, which was in accordance with the X-ray diffraction patterns (Figure 3). Complex content of A50 and A75 was positively correlated to ΔH . These observations indicated that higher apparent AM content caused a large amount of free PA. Fifty percent ethanol could wash off a small amount of fatty acid on the surface of the starch granules (Chang et al., 2013a). Thus, free PA in the inner AP could not be washed off by 50% ethanol. Presumably, the amount of free PA in the inner AP could increase with increasing AS-treated starch proportion.

5.2. Peak II

In the second peak, melting endotherm of crystallized AP by AS treatment is shown on the DSC thermogram (Table 5). T_o , T_p , and T_c are influenced by the molecular architecture of the crystalline region corresponding to the structure of AP chains, but not the proportion of crystalline regions or AM-AP ratio (Miao et al., 2011). In particular, T_o and T_p are associated with

melting of the weakest crystallites and the structural stability, respectively (Barichello et al., 1990). In addition, the gelatinization temperature may also be attributed to the internal arrangement of starch fractions within the granules (Miao et al., 2010). Endotherm peaks were not observed for A0, while those were shown in the samples containing AS-treated starch. A100 showed a value of 6.2 J/g for melting enthalpy and 87.5 °C for T_p , higher than those reported for retrograded unmodified starches (Kohyama et al., 2004). The recrystallization was accelerated by the AP with longer average chain length (Kalichevsky et al., 1990). This suggests that the crystallites of elongated branch chains of the AS-treated starches in this study were more stable than those of the A0 and other retrograded starches. Noda et al. (2003) found a negative correlation between the proportion of AP short chains (DP 6-12) and gelatinization temperature. However, in the present study, T_o and T_p was positively correlated to DP 6-12, and negatively correlated to $DP \geq 25$ and DP_n (Table 7). Thus, the structures of A100 are presumably less ordered than those of A25, A50, and A75. Consequently, the more amounts of the elongated branch chains in AP, the more amounts of the randomly ordered crystals. As previously mentioned, the crystal size was found to increase with decreasing average chain length, which is consistent with a higher ability of short chains to reorganize into larger crystals (Potocki-Veronese et al., 2005). In addition, if the AM chains are too long, it leads to

conformational disorders, resulting in faults in the crystal structure (Gelders et al., 2004). In case of waxy starch, this phenomenon might occur when there are many long branch chains like A100. Meanwhile, Cooke and Gidley (1992) demonstrated that ΔH is mainly due to the loss of double helical order rather than loss of crystalline order. Thus, ΔH increased with increasing mixing ratio of AS-treated starch (Table 5) due to the formation of new double helices consisting of elongated branch chains by the AS treatment. The higher ΔH suggested that the thermal energy required for disrupting long AP double helices during gelatinization was more pronounced. Therefore, thermal data confirmed that the crystals formed by AS-treated starch had great thermal stability because they dissociated by a large enthalpy change.

Also, it was noteworthy that the broad endotherms of starches were attributed to the melting of starch complexes with lipids as well as AP double helices. The X-ray diffraction patterns of A25-P, A50-P, A75-P, and A100-P displayed the characteristics of B+V-type crystal structure (Figure 3). These results revealed the formation of V-AM complex and the residues of AP double helices in them. However, A25-P and A50-P did not show the second endotherm peak. Also, A75-P and A100-P showed a substantial loss of ΔH in the second endotherm peak compared with A25, A50, A75, and A100. This was probably due to that a large amount of AP double helices was disrupted

with a small amount of type I complex formed. Based on these results, the contribution of the degree of order to the ΔH value was thought to be significantly higher than that of the amount of complex. Thus, the major factor in the ΔH value was the amount of double helices. It is actually confirmed that the ΔH value was a positive linear function of the branch chain length (Table 7) and a negative linear function of the complex content of A100 (Table 8).

In the A100-fatty acid complexes, the gelatinization temperatures (T_o and T_p) increased compared with A100 (Table 5). Complex content of A100 was positively correlated to T_o and T_p (Table 8). These results suggested that the addition of fatty acid played a role in retarding starch gelatinization. An increase in T_o and T_p is a reflection of more ordered crystallinity (Wongsagonsup et al., 2008). It was inferred that the ability of V-AM that makes ordered crystalline arrangement is shown in starch that has randomly ordered crystallites as in A100.

5.3. Peak III

In general, AM crystallites are more stable than AP crystallites (Zobel, 1988). During recrystallization, the behavior of AP with very long chains in the AS-modified starches was similar to that of long linear AM. The

thermograms of third peak exhibited the melting of double helices formed by long branch chains of AP. The endotherm from 114.6 °C to 150.4 °C could be the melting of the non-complexed recrystallized AM (Cai & Shi, 2010; Li et al., 2008). In other words, the long branch chains of AP like AM formed double helices. The T_o of A25 was lower than that of A50, A75, and A100, which suggested that long branch chains was beneficial to the formation of crystallites like AM-AM double helices.

There were no endothermic peaks in starch-PA complexes. It was because crystallites consisting of long branch chains like AM was disrupted by PA. That was attributed to the competitive mechanism existing between AM retrogradation and formation of starch-lipid complexes (Putseys et al., 2010a). As far as we can judge, the interaction between the long branch chains of AP and PA is faster than the intermolecular interaction of long branch chains of AP in the current experimental conditions.

Table 5
Thermal properties of the blends of native and AS-treated starches complexed with fatty acid

Samples	Peak I				Peak II				Peak III			
	T_o (°C) ¹⁾	T_p (°C)	T_c (°C)	ΔH (J/g)	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
A0	N.D. ²⁾											
A0-L	N.D.				N.D.				N.D.			
A0-P	60.2±0.4 ^{ab3)}	63.4±0.7 ^a	66.7±0.4 ^d	6.6±0.5 ^a								
A25	53.4±0.3 ^c	60.7±0.4 ^b	73.0±0.6 ^a	1.2±0.3 ^e	91.1±3.0 ^a	95.5±1.3 ^a	104.7±5.3 ^{bc}	1.4±1.0 ^{cd}	116.2±4.1 ^{bc}	124.8±1.6 ^e	131.7±1.6 ^f	2.7±2.5 ^{ab}
A25-L	49.9±1.9 ^d	56.6±2.3 ^d	66.9±0.0 ^d	0.9±0.2 ^e	83.4±2.3 ^b	90.7±6.2 ^{bc}	105.6±3.4 ^a	1.1±1.0 ^{cd}	112.7±0.9 ^c	121.5±1.7 ^f	128.7±1.0 ^f	3.5±1.0 ^{ab}
A25-P	50.4±0.4 ^d	60.7±0.7 ^b	63.6±1.1 ^g	1.2±0.3 ^e	N.D.				N.D.			
A50	54.1±2.9 ^{bc}	61.1±1.5 ^b	71.2±1.0 ^b	0.9±0.3 ^e	78.0±3.4 ^d	95.5±0.3 ^a	110.0±0.8 ^a	1.8±0.6 ^c	124.4±3.7 ^a	136.8±4.8 ^a	145.1±2.9 ^a	2.0±2.4 ^{bc}
A50-L	54.5±2.4 ^{bc}	60.8±1.1 ^b	66.6±0.2 ^d	0.9±0.0 ^e	82.5±0.8 ^b	93.7±1.1 ^{ab}	103.5±3.0 ^{bc}	1.9±0.4 ^{bc}	116.8±1.3 ^b	128.6±1.1 ^d	139.3±3.0 ^{cd}	2.5±0.8 ^b
A50-P	60.5±0.1 ^a	62.8±0.0 ^a	65.2±0.1 ^c	4.1±0.0 ^d	N.D.				N.D.			
A75	55.6±0.5 ^b	60.6±0.1 ^b	68.2±1.5 ^c	0.5±0.3 ^f	83.2±2.1 ^b	91.8±2.4 ^{ab}	104.5±0.6 ^{bc}	3.3±1.9 ^b	123.5±3.8 ^a	137.2±0.3 ^a	143.5±0.8 ^{ab}	3.9±0.3 ^{ab}
A75-L	50.6±0.5 ^d	58.5±0.3 ^c	63.8±0.5 ^{fg}	0.5±0.2 ^f	76.2±3.5 ^d	86.8±3.1 ^c	99.9±0.6 ^{cd}	3.2±1.0 ^b	121.7±3.2 ^a	131.2±2.8 ^{cd}	136.7±4.7 ^d	3.1±0.6 ^{ab}
A75-P	60.6±0.0 ^a	62.7±0.1 ^a	64.7±0.0 ^{ef}	5.1±0.1 ^c	83.9±3.8 ^b	91.8±3.0 ^{ab}	96.8±4.7 ^d	1.4±1.1 ^{cd}	N.D.			
A100	N.D.				76.8±2.0 ^d	87.5±1.3 ^c	102.0±2.4 ^{bc}	6.2±1.2 ^a	123.4±2.4 ^a	133.5±2.7 ^{bc}	141.2±0.6 ^{bc}	4.6±1.9 ^a
A100-L	N.D.				78.5±1.3 ^{cd}	89.6±0.1 ^{bc}	106.9±3.7 ^a	5.4±0.2 ^a	124.7±2.5 ^a	134.5±0.9 ^{ab}	139.8±2.1 ^{cd}	2.8±0.2 ^{ab}
A100-P	60.6±0.2 ^a	62.7±0.1 ^a	65.1±0.2 ^e	5.7±0.2 ^b	81.8±1.3 ^{bc}	90.6±2.2 ^{bc}	102.4±4.6 ^{bc}	0.9±0.1 ^{cd}	N.D.			

¹⁾ T_o , T_p , T_c , and ΔH indicate the onset temperature, peak temperature, conclusion temperature, and enthalpy change of melting, respectively.

²⁾ Not detected.

³⁾ The values with different superscripts in the same column are significantly different ($p < 0.05$).

6. Starch digestibility

Figure 4 displays the enzymatic digestion profiles of uncooked and cooked samples. The digestion rate was decreased with increasing mixing ratio of AS-treated starch (Figure 4(A)). The A0 was digested 74.9% at 10 min, and at 240 min, the digestion value was 79.5%. In contrast, the A100 at 10, 20, 30, 60, 120, and 240 min showed digestibility of 18.2, 25.1, 30.9, 35.4, 46.0, and 58.3%, respectively. Table 6 shows the content changes in the starch fractions of blends of native and AS-treated starches complexed with fatty acid. The A0 had the highest RDS content, because the gelatinization process during the sample preparation destroyed the semicrystalline structure of raw starch granules (Cousin et al., 1996; Zhang et al., 2006b). The amorphous regions because of gelatinization are easier to access by enzyme (Zhang et al., 2006b). The contents of RDS were decreased with increasing the mixing ratio of AS-treated starch. After the AS treatment, the SDS and RS contents in the A100 increased by 35.5 % and 21.2 %, respectively, while the RDS content reduced by 56.7 % (Table 6). The resistance to enzymatic hydrolysis by the reaction of AS with starch was greatly influenced by AP structural modification. Hydrolysis of starch granules by α -amylase have been reported to be correlated with unit-chain length distribution of AP

(Srichuwong et al., 2005). The RDS contents of uncooked and cooked samples were positively correlated to the proportion of $DP \leq 12$. However, they were negatively correlated to the proportions $DP \geq 25$ and DP_n . On the contrary, SDS and RS contents of uncooked and cooked samples were negatively correlated to the proportion of DP 6-12, and positively correlated to the proportions of $DP \geq 25$ and DP_n (Table 7). Overall, a significant increase in the proportion of intermediate AP B₃-like branch chains (DP 25-36) with AS appeared to enhance the SDS and RS contents, even though the starch already lost its original crystalline structure or granular form. This result is consistent with the previous study in terms of RS contents (Kim et al., 2013). Previous studies postulated that the short double helices formed from the short A chains (DP 6-12) in the crystalline region cause weak points in starch crystalline structure, resulting in greater susceptibility to enzymatic hydrolysis (Chung et al., 2011; Jane et al., 1997; Zhang et al., 2006a). However, Zhang et al. (2008a) using cooked maize starch revealed a positive relationship between SDS content and AP short chains or long chains, showing that there are two mechanisms to the slow digestion of gelatinized starches. The first one, SDS as a chemical entity where the starch molecular structure is the origin for its slow digestion, and the second, SDS as a physical entity is produced through retrogradation of long linear chains of starch. In this study, because of the elongated outer short chains acting as

anchor points to slow the digestion of branch chains and insufficient retrogradation by them, the SDS content was increased (Zhang et al., 2008b) by the AS treatment. It was consistent with Pearson correlation analysis between branch chain length and contents of starch fraction (Table 7). The branch chain elongation permits the formation of ordered structural crystallites which may be hindered by α -(1,6)-linked branch points in waxy type starches (Ryu et al., 2010; Shin et al., 2010). The double helices could be formed by elongated branch chains, which could contribute to the crystalline structure in the AS-treated starch. The crystalline regions have decreased susceptibility to enzymatic hydrolysis (Zhang et al., 2006b). Jane et al. (1999) stated that the long branched chains of starch contribute to retrogradation which reduces the enzyme susceptibility, while the short chains form short or weak double helices that would produce imperfect crystalline structures. Conclusively, the elongation of branch chain length and recrystallization during the sample preparation might increase the resistance to enzymatic hydrolysis and increase both SDS and RS contents.

However, after the AS-treated waxy corn starches were cooked, the SDS fractions decreased from 40.1% to 10.9% accompanied by a corresponding increase of RDS, whereas the RS content was kept fairly constant (Table 6). When starch is used in food preparation, cooking increases the rate of hydrolysis by gelatinizing the starch and making it more easily available for

enzymatic attack (Singh et al., 2010). During gelatinization of starch, the crystalline structures of AP disintegrate and the polysaccharide chains take up a random configuration thus causing the swelling and rupturing of the starch granules (Singh et al., 2007). The structure of SDS was disrupted during cooking because the SDS fraction consists of less perfect crystallites and amorphous components, which can be thermally unstable (Shin et al., 2004). The little change of RS levels reflects unaffected interactions by cooking. Thus, cooked AS-treated starch showed promise as a starch additive with relatively balanced energy release, and an increased RS. This suggested that targeted and reduced digestibility could be achieved via blending AS-treated starch into regular starch/food systems at optimized ratio for diverse food application.

When compared with the digestibility of uncooked A0, *in vitro* digestibility was significantly reduced when uncooked A0 complexed with LA and PA (Table 6). The rate and extent of enzymatic degradation of starch-lipid complexes decreased with increasing crystallinity (Seneviratne & Biliaderis, 1991) resulting from the presence of crystalline V-AM complex. Formation of AM-lipid complexes was shown to reduce the susceptibility of AM to enzymatic hydrolysis *in vitro* by Holm et al. (1983). Crowe et al. (2000) reported that LA, myristic acid, and PA inhibited the enzymatic hydrolysis of amylose by about 35%. Kawai et al. (2012) showed a

significant reduction in the hydrolyzed starch content by inclusion with LA, myristic acid, PA, and stearic acid. There is a considerable number of *in vivo* studies substantiating the addition of fat to a carbohydrate food which reduces the glycemic response (Henry et al., 2008; Thomsen et al., 1999; Thomsen et al., 2003). On the other hand, when compared with the digestibility of uncooked A25, A50, A75, and A100, *in vitro* digestibility was increased when they complexed with PA. The RDS contents of cooked A50, A75, and A100 complexed with PA were larger than those of cooked A50, A75, and A100 without PA, and this led to a significant reduction of RS contents when they complexed with PA. It could be inferred that the decrease in melting enthalpy change of starch-lipid complex was directly correlated with a decrease in SDS and RS (Table 5 and Table 6). For uncooked and cooked A50, A75, and A100, complex content is positively correlation to the RDS content (Table 8). This contradicts the literature data from the previous studies mentioned above that formation of AM-lipid complexes could reduce the degradability of starches. On the contrary, it is known that starch digestibility is affected by the quantity and quality of the single helix AM-lipid complexes (Soong et al., 2013) and double helices formed by branch chains of AP. In this study, though the lower hydrophilicity of longer lipid chains of PA resulted in a stronger preference to reside within the hydrophobic helix cavity, the more hydrophobic interactions between the PA

and helix cavity could not take a part in the resistance to enzymatic hydrolysis, because starch-PA complexes disrupted double helical structure of AP. Clegg et al. (2011) reported that the addition of fat to pancakes increased the digestibility of starch and release of sugars. On the other hand, the LA-included A50, A75, and A100 gave relatively higher complex content values compared with A25-P, but the amount of starch hydrolyzed during *in vitro* digestion was similar to A50, A75, and A100, indicating the weak physical interactions between LA and starch blends. It is in accordance with the results of X-ray diffraction analysis (Figure 3) and thermal properties (Table 5).

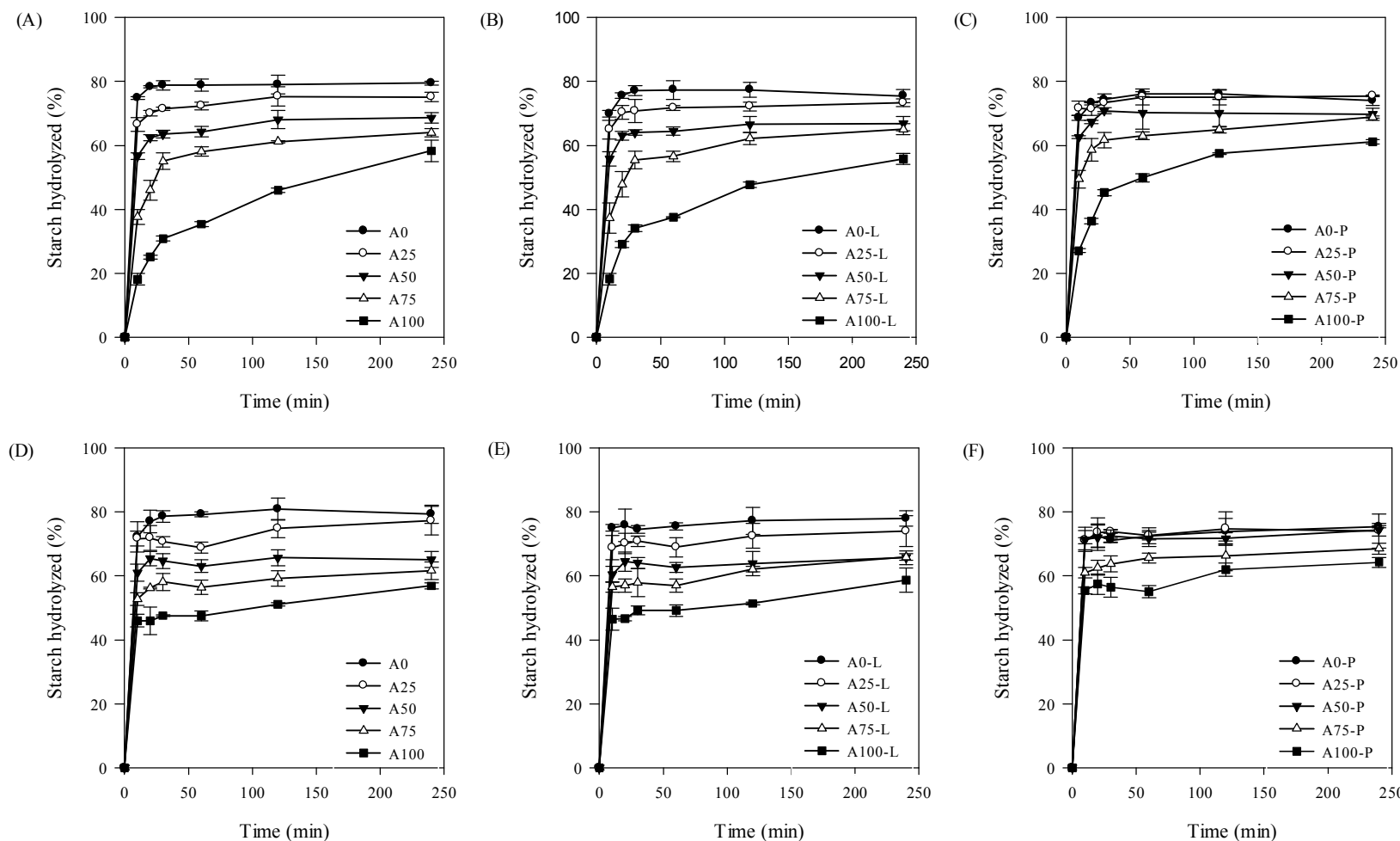


Figure 4. Hydrolysis patterns of the blends of native and AS-treated starches complexed with fatty acid. (A), (B), and (C) are uncooked samples; (D), (E), and (F) are cooked samples.

Table 6

Contents of RDS, SDS, and RS of the blends of native and AS-treated starches complexed with fatty acid

Samples	Uncooked samples			Cooked samples		
	RDS (%)	SDS (%)	RS (%)	RDS (%)	SDS (%)	RS (%)
A0	74.9±0.5 ^{a, 1), 2)}	4.6±0.8 ^{gh}	20.5±0.6 ^f	72.2±4.8 ^{ab}	7.1±3.2 ^{cde}	20.7±2.8 ^d
A0-L	69.9±1.0 ^{bc}	5.6±2.5 ^{gh}	24.5±0.6 ^e	75.0±1.0 ^a	2.9±1.4 ^f	22.1±2.4 ^d
A0-P	68.6±0.8 ^{bcd}	5.4±1.9 ^{gh}	26.0±1.5 ^e	71.0±0.9 ^{ab}	4.4±0.7 ^{def}	24.7±0.3 ^d
A25	66.6±2.1 ^{cd}	8.5±1.4 ^{efg}	24.9±1.5 ^e	71.7±2.4 ^{ab}	5.6±3.4 ^{def}	22.8±4.4 ^d
A25-L	65.0±2.9 ^{de}	8.4±3.5 ^{efg}	26.7±1.2 ^e	68.8±3.8 ^b	5.2±1.1 ^{def}	26.0±4.9 ^d
A25-P	71.6±2.3 ^{ab}	3.9±2.6 ^h	24.5±0.3 ^e	71.1±3.0 ^{ab}	2.9±2.7 ^f	25.9±5.3 ^d
A50	56.7±1.1 ^f	12.0±1.7 ^c	31.4±1.6 ^d	61.0±2.7 ^c	4.1±0.3 ^{ef}	34.9±2.5 ^{bc}
A50-L	55.8±2.3 ^f	11.0±0.2 ^{ef}	33.2±2.2 ^{cd}	60.5±2.6 ^c	5.2±1.8 ^{def}	34.3±2.1 ^{bc}
A50-P	61.6±2.0 ^e	7.5±1.7 ^{fgh}	30.9±2.0 ^d	71.3±3.8 ^{ab}	3.1±1.8 ^f	25.6±2.0 ^d
A75	37.7±2.3 ^h	26.3±1.1 ^c	36.0±1.2 ^c	52.8±2.0 ^d	8.8±1.2 ^{bc}	38.4±2.7 ^{ab}
A75-L	37.3±4.8 ^h	27.7±4.5 ^c	35.0±1.7 ^c	56.6±1.6 ^{cd}	9.3±0.7 ^{abc}	34.1±1.1 ^{bc}
A75-P	49.5±2.8 ^g	19.3±3.1 ^d	31.1±0.3 ^d	61.0±1.7 ^c	7.6±0.4 ^{cd}	31.5±1.7 ^c
A100	18.2±1.8 ^j	40.1±3.2 ^a	41.7±3.3 ^a	46.0±2.0 ^e	10.9±1.5 ^{ab}	43.1±0.9 ^a
A100-L	18.3±1.8 ^j	37.5±2.3 ^{ab}	44.2±1.8 ^a	46.5±3.5 ^e	12.2±0.7 ^a	41.4±3.7 ^a
A100-P	27.1±0.6 ⁱ	34.0±1.2 ^b	38.9±0.7 ^b	55.4±1.1 ^d	8.8±0.5 ^{bc}	35.8±1.6 ^{bc}

¹⁾ Data are expressed as average value with standard deviation.

²⁾ The values with different superscripts in a column are significantly different ($p < 0.05$).

7. Rheological properties

The determination and understanding of a gel's rheological properties are useful for understanding changes in microstructure and gel formation, which is essential for the development of new products. Intermolecular associations can lead to the subsequent formation of junction zones and molecular aggregation in the developing gel networks. A well-known example of this gelation phenomenon is the retrogradation (Byars et al., 2013). Retrogradation is the reassociation of AM and AP to form double helices and crystalline structures, which is an important process that occurs during cooling storage of gelatinized starch (Hoover, 1995). Dynamic rheometry is useful information for understanding the textural change of retrogradation potency (Lin et al., 2013). This report examined the viscoelastic nature of gels formed after heating and cooling. The parameters of storage modulus (G' ; solid component of the network, related to elastic energy) and loss modulus (G'' ; liquid component, related to viscous flow) were obtained. The mechanical spectra of the blends of native and AS-treated starches complexed with fatty acid are presented in Figure 5. All the starches except for A0, A0-L, and A0-P showed solid-like behavior (G' larger than G''). And all the starches showed only slight frequency dependence in the entire

frequency range. This result is consistent with the previous study of Shin et al. (2010). The value of G' and G'' increased with the mixing ratio of AS-treated starch, indicating the increase in gel strength after the AS treatment. $G'_{1\text{Hz}}$ and $G''_{1\text{Hz}}$ was negatively correlated to $DP \leq 12$, and positively correlated to $DP \geq 25$ and DP_n (Table 7). One possible reason for this result was that the elongated branch chains of AP by the AS treatment behave like linear chains of AM (Rolland-Sabaté et al., 2004). In general, chemical structure ($DP \geq 110$) and physicochemical properties of AM enable its macromolecules to form a gel network on cooling, whereas AP is not a part of the gel network but just acts as an agent of aggregation and a network filler (Byars et al., 2013; German et al., 1992; Hoover, 1995; Jane et al., 1999). The ability of macromolecules to form gel depends on their DP (Gidley & Bulpin, 1989). Jane et al. (1999) found that the gels formed by longer branch chains of AP were stronger than the ones prepared by shorter AP. The elongated branch chains of AP by the AS treatment had a strong tendency to interact and entangle between chains because they had many possible contact points (Shin et al., 2010). The results here clearly demonstrated that interactions existed between different starch ingredients and they were dependent on the mixing ratio. It can be used as a paste of high viscosity and its ability to set a firm gel, and provides good handling properties and improves texture in the final product (Yue & Waring, 1998).

Meanwhile, G' and G'' of starch-fatty acid complex were lower than those of the samples containing absolute starch (Figure 5). Especially, the A100-fatty acid complex which revealed the highest complex content (Figure 2) had the largest reduction of G' and G'' from the A100 in comparison with the phenomenon of other starch blend-fatty acid complexes. In case of A25, A50, A75, and A100, complex content showed negative correlation to $G'_{1\text{Hz}}$ and $G''_{1\text{Hz}}$ (Table 8). Some theories have been put forward to explain the reduction in paste viscosity when starches are pasted with AM complexing agents (Obiro et al., 2012). It was suggested that the weak starch gels resulted from a restricted crystallization during cooling due to AM-lipid complexation (Putseys et al., 2010b). LA complexed with AM to form V-AM complex instead of AM/AP network, which resulted in a low paste viscosity (Chang et al., 2013b). In the study of Fanta et al. (2010), complexed sodium palmitate stabilized the AM in solution and inhibited intermolecular interactions, resulting in non-turbid and low-viscosity liquids. Additionally, Richardson et al. (2004) found that potato AM dispersions formed open structured gels with many pores with addition of low levels of an emulsifier to produce weaker gels. Gel formation is largely dependent on short-term starch retrogradation, due to formation of AM double helices, and long-term retrogradation due to AP (Lii et al., 2004). It has been suggested that the formation of V-AM complexes and AP-lipid complexes compete with the

retrogradation process of not only AM but also AP in the starch system (Putseys et al., 2010b). Thus, there is little formations of double helices, junction zones, and gel network in the AP-fatty acid complex. These non-gelling properties of starch-fatty acid complex can open new avenues in food application, for example, better mouthfeel with lower starch concentration.

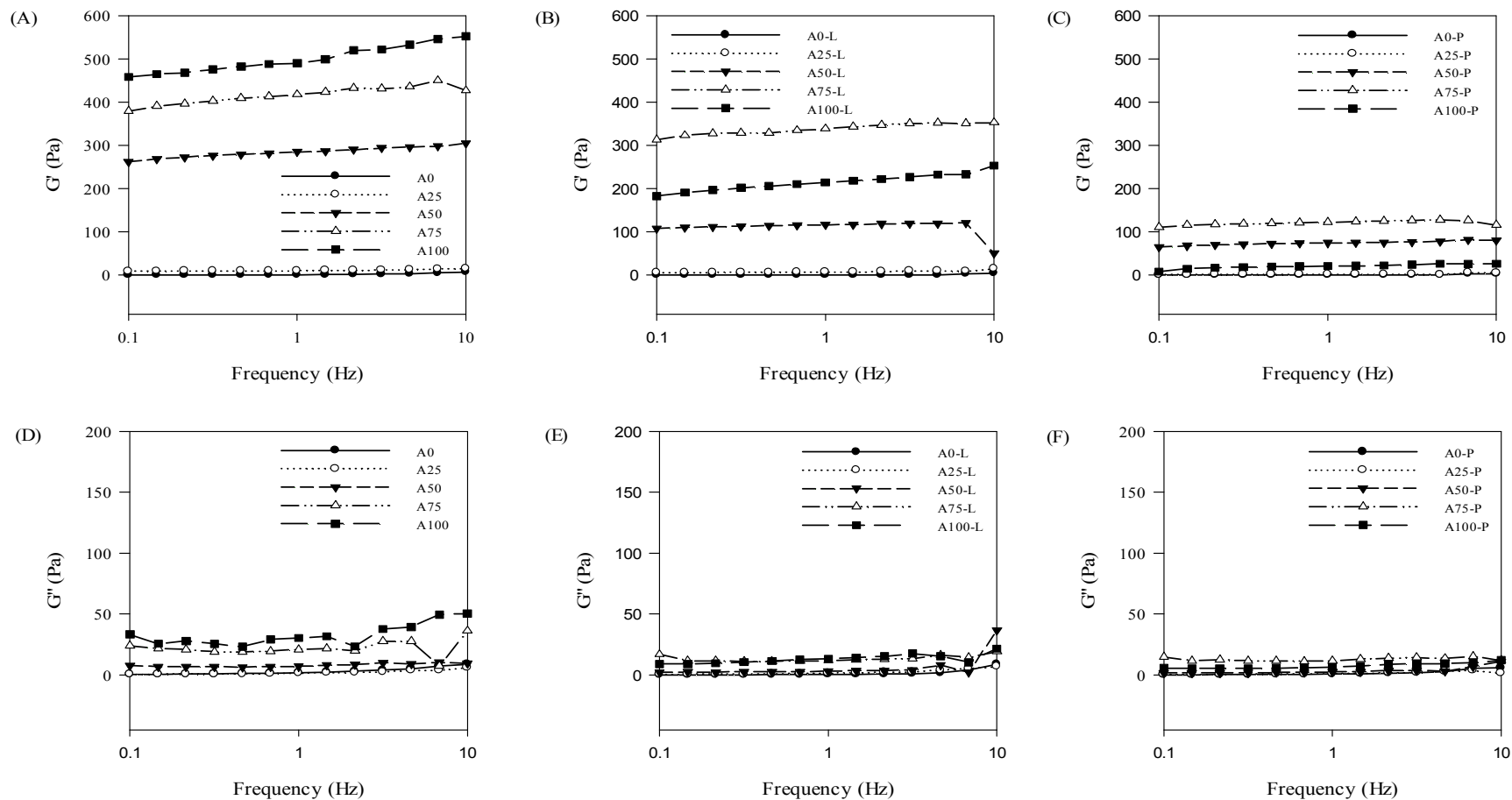


Figure 5. Frequency dependence curves for gels of the blends of native and AS-treated starches complexed with fatty acid. (A), (B), and (C) are the values of storage moduli, G' ; (D), (E), and (F) are the values of loss moduli, G'' .

Table 7

Pearson correlation coefficients for the relationship between branch chain length and characteristics of samples

Parameters ¹⁾	AAC	CC-L	CC-P	RC	$\Delta H1$	T_o2	T_p2	$\Delta H2$	uRDS	uSDS	uRS	cRDS	cSDS	cRS	G'_{1Hz}	G''_{1Hz}
DP \leq 5	-0.824** ¹⁾	-0.805**	-0.800**	-0.806**	0.702*	-0.621*	-0.637*	-0.725**	0.777**	-0.750**	-0.780**	0.819**	-0.466	-0.797**	-0.733**	-0.713**
DP6-12	-0.972**	-0.888**	-0.967**	-0.869**	0.802**	-0.507	-0.534*	-0.914**	0.991**	-0.982**	-0.953**	0.954**	-0.575*	-0.917**	-0.849**	-0.909**
DP13-24	-0.587*	-0.572*	-0.565*	-0.722**	-0.034	-0.599*	-0.661**	-0.479	0.485	-0.404	-0.596*	0.494	0.065	-0.593*	-0.504	-0.409
DP25-36	0.991**	0.911**	0.982**	0.920**	-0.763*	0.584*	0.617*	0.903**	-0.987**	0.965**	0.970**	-0.948**	0.514*	0.929**	0.857**	0.895**
DP \leq 37	0.950**	0.880**	0.943**	0.881**	-0.648	0.520*	0.553*	0.916**	-0.957**	0.935**	0.943**	-0.949**	0.515*	0.931**	0.848**	0.882**
DP _n	0.978**	0.900**	0.970**	0.894**	-0.749*	0.540*	0.570*	0.919**	-0.985**	0.968**	0.961**	-0.959**	0.544*	0.933**	0.858**	0.903**

¹⁾ Parameters, RC = relative crystallinity; CC-L = starch-lauric acid complex content; CC-P = starch-palmitic acid complex content; $\Delta H1$ = melting enthalpy of peak I; T_o2 , = onset temperature of peak II; uRDS, cRDS = RDS of uncooked samples and cooked samples, respectively; G'_{1Hz} = G' when the frequency was 1 Hz

²⁾ Significance level: * and ** = $p < 0.05$ and 0.01 , respectively.

Table 8

Pearson correlation coefficients for the relationship between complex content and characteristics of samples according to the mixing ratio of AS-treated starch

Parameters ¹⁾	RC	$\Delta H1$	T_o2	T_p2	$\Delta H2$	uRDS	uSDS	uRS	cRDS	cSDS	cRS	G'_{1Hz}	G''_{1Hz}	
A0	0.880** ²⁾	-0.963		N.D.		-0.615	0.379	0.421	-0.360	-0.107	0.532	-0.303	-0.304	
Complex content	A25	0.882**	0.117	-0.268	0.006	-0.425	0.551	-0.445	-0.418	-0.205	-0.555	0.448	-0.784*	-0.793*
	A50	-0.966**	0.873**	0.743	-0.779	0.210	0.755*	-0.843**	-0.208	0.779*	-0.300	-0.845**	-0.884**	-0.949**
	A75	-0.929**	0.846**	0.055	-0.028	-0.541	0.730*	-0.595	-0.829**	0.910**	-0.507	-0.858**	-0.844**	-0.488
	A100	-0.888**	0.971	0.850**	0.689*	-0.939**	0.891**	-0.785*	-0.490	0.856**	-0.596	-0.838**	-0.920**	-0.910**

¹⁾ Parameters, RC = relative crystallinity; $\Delta H1$ = melting enthalpy of peak I; T_o2 = onset temperature of peak II; uRDS, cRDS = RDS of uncooked samples and cooked samples, respectively; G'_{1Hz} = G' when the frequency was 1 Hz

²⁾ Significance level: * and ** = $p < 0.05$ and 0.01 , respectively.

³⁾ Not detected.

CONCLUSION

With increasing mixing ratio of AS-treated starch, short chains decreased, while long chains increased due to the elongation of external chains in AP molecules. Elongated branch chains had higher iodine binding capacity and ability of complex with fatty acid owing to like-AM properties. Particularly, elongated branch chains of AP better combined with PA than LA which has less carbon, because of stronger interaction formed by PA. With an increase in the branch chain length, the relative crystallinity, melting enthalpy, and viscoelasticity increased and *in vitro* digestibility decreased. It is due to the recrystallization between double helices formed by elongated branch chains. In the meantime, melting temperature decreased with increasing AS-treated starch proportion because of disordered crystalline arrangement.

Starch blend-PA complexes displayed a mixture of B+V-type X-ray diffraction pattern. On the contrary, starch blend-LA complexes did not change the pattern compared with the starch blends due to weaker interaction between starch and LA. With increasing complex content, the formation of double helices was disturbed by complexed fatty acid, which led to the decrease in relative crystallinity, melting enthalpy, and viscoelasticity but the increase *in vitro* digestibility. However, in case of A100, it was revealed that

fatty acids were able to make ordered crystalline arrangement leading to the retardation of gelatinization.

These results suggest that AS treatment affects the waxy corn starch as a prospective delivery system for fatty acids. They also provided a basis to utilize the AS-treated starch complexed with fatty acid for novel and healthy food products. It is suggested that the blends of native and AS-treated starches complexed with fatty acid have a potential to be used as low-glycemic response food ingredients and texture-controlling additives. Further studies are required to assess the effects of lipid structure (mono-glyceride or fatty acid), chain length, and degree of saturation on the physicochemical properties, and *in vitro* digestibility of AS-treated waxy starch-lipid complexes.

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국문 초록

찰전분은 아밀로펙틴의 입체적 장애와 짧은 가지 사슬 길이 때문에 지방질과의 복합체 형성이 어렵다. 이 연구에서는 생찰옥수수 전분과 amylosucrase(AS)를 처리한 찰옥수수 전분을 혼합한 후 지방산(라우르산(C12:0)과 팔미트산(C16:0))을 0.5mM/g-전분의 농도로 첨가하여 복합체를 형성하였다. 이 연구의 목적은 전분의 사슬길이와 지방산의 종류가 복합체의 전분-지방산 복합체 함량, X-선 회절 및 상대적 결정화도, 열특성, 소화율, 그리고 유변학적 특성에 미치는 영향을 알아보는 것이었다.

AS 처리한 전분의 함량이 증가함에 따라 가지 사슬 길이와 상대적 아밀로오스 함량은 증가하였다. 전분의 지방산과의 복합체 형성능은 아밀로펙틴의 가지 사슬 길이와 지방산의 사슬 길이가 길수록 증가하였다. 전분 혼합물과 전분-라우르산 복합체의 X-선 회절도형은 B 형을 나타낸 반면, 전분-팔미트산 복합체의 X-선 회절도형은 B+V 형을 나타냈다. 연장된 가지 사슬이 형성한 이중 나선 구조에 의해 상대적 결정화도, 용융 엔탈피, 점탄성율은 증가하였고 소화율은 감소하였다. 그러나 복합체를 이룬 지방산이

이중 나선 구조의 형성을 방해하기 때문에, 전분-지방산 복합체 함량이 증가함에 따라 상대적 결정화도, 용융 엔탈피, 점탄성율은 감소하였고 소화율은 증가하였다. 한편, 연장된 가지 사슬의 불규칙한 배열에 의해 전분 혼합물의 용융 온도는 감소하였으나, 전분-지방질 복합체의 규칙적인 결정형 영역 형성에 의해 AS 처리한 전분의 용융 온도는 지방산과 복합체를 이룬 후 증가하였다.

이 실험을 통해, 복합체를 이룬 지방산은 AS 처리에 의해 연장된 가지 사슬의 이중 나선 구조 형성을 방해함을 확인하였고, 나아가 찰전분-지방질 복합체가 나타내는 기능적 특성의 변화를 야기함을 증명하였다.

주요어 : 아밀로수크레이스, 찰옥수수 전분, 가지 사슬 길이, 전분 혼합, 전분-지방산 복합체, 소화율, 이화학적 특성

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