A Thesis for the Degree of Master of Science

Comparison of starch-lipid complex between normal corn starch and amylosucrase-treated waxy corn starch

메옥수수 녹말과 아밀로수크레이스 처리
찰옥수수 녹말-지방산 복합체의 이화학적 특성 비교

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농학석사학위논문

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Comparison of starch-lipid complex between normal corn starch and amylosucrase-treated waxy corn starch

by

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ABSTRACT

In the presence of a ligand such as fatty acid, amylose undergoes conformational changes to form a amylose-fatty acid inclusion complex. However, complexation of waxy starch with a fatty acid is restricted by steric hindrance and short branch chains of amylopectin (AP). In this study, starch-lipid complexes were prepared by using normal corn starch (NC) and amylosucrase (AS)-treated waxy corn starch (ASWC) with fatty acid (5% on the starch basis) i.e. myristic acid (MA, C14:0) and palmitic acid (PA, C16:0). This study aimed to investigate the differences in characteristics of starch-lipid complex of normal starch and waxy starch with elongated branch chain length of AP by enzymatic modification. The property of AS-treatment was determined by high-performance anion-exchange chromatography system. The complex index and X-ray diffraction analysis were utilized to evaluate the formation of complexes. Thermal property and digestibility were investigated by differential scanning calorimeter and log of slope (LOS) method. LOS plot method can demonstrate the change of k in the first order kinetics. Rate constants for digestion could be named k_{RDS} and k_{SDS} which means rate of rapid digestible starch and slow digestible starch.

The AS-treatment increased the branch chain length of WC which led to a raise of apparent amylose content (29.7%) similar to that of normal corn starch (29.0%). The ability of the ASWC to complex with fatty acids was higher than that of NC. This result suggested that elongated branch chain length of ASWC induced more favorable lipid complexation as compared with NC. The X-ray diffraction analysis of starch-lipid complexes revealed a V-type pattern, a clear indication of complex formation. The relative crystallinity, peak temperature (T_p) and melting enthalpy (ΔH) of NC complexes increased owing to crystalline structure formed by complexation. As the complexed fatty acids of ASWC disrupted the double helical structure of elongated branch chain, relative crystallinity, T_p, and ΔH decreased. Therefore, digestibility and k_{RDS} of NC complexes decreased and those of ASWC increased.

In contrast with previous studies, this study found that ASWC has higher ability to combine with fatty acids and lower digestibility than those of NC. These findings strengthen the hypothesis that the structure of ASWC attributes to formation of starch-fatty acid complexes. Hence, the ASWC can be preferred over NC in delivery system.

Keywords: normal corn starch, amylosucrase, waxy corn starch, starch-fatty acid complex, *in vitro* digestibility, LOS plot, physicochemical properties

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INTRODUCTION

Starch is one of the most abundant polysaccharide in the nature and is composed of two types of macromolecules, amylose and amylopectin. Amylose (AM) is considered as an essentially linear polymer which is almost entirely composed of α-1, 4-linked $_{D}$-glucopyranose. Amylopectin (AP) consists of α-1, 4-linked glucose segments connected by α-1, 6-linked branch points.

For nutritional purposes, starch in food products can be classified into three fractions depending on the time and extent of digestion as follows: rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (Englyst et al., 1992). RDS is digested rapidly in the mouth and the small intestine and represents the fraction hydrolyzed *in vitro* within 20 min. SDS is hydrolyzed between 20 and 120 min and considered to be digested slowly but completely in the small intestine. According to Shin et al. (2004), SDS consists of less perfect crystallite and amorphous regions. RS has resistance from hydrolysis by the enzymes in the small intestine after 120 min (Englyst et al., 1986). There are nutritional benefits from SDS and RS. One of the health effects of SDS is a slow increase of postprandial blood

glucose level, thus products which are used by athletes can provide a carbohydrate source by being gradually released 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 (Toppings and Clifton, 2001). For these reasons, SDS and RS both contribute to a low glycemic index (Englyst et al., 1996). Native starch digestibility is greatly influenced by the interplay of many factors, such as 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 polymorphism (Singh et al., 2010).

However, there are some arguments about assigning labels of RDS and SDS, because a time frame of a digestibility curve does not give an accurate explanation of the enzymatic hydrolysis of starch. The digestibility data of cooked or gelatinized starch proceed as a first-order reaction described by a single rate constant, irrespective of the 'Englyst' classification (Dhital et al., 2010; Goñi et al., 1997). It has been stated that all digestible fractions of starch have the same intrinsic reactivity. Further, it is not proper to describe the digestion behavior of starch granules as RDS and SDS.

Meanwhile, a 'logarithm of slope' (LOS) plot based on the first-order

kinetics was introduced recently for the analysis of starch hydrolysis (Butterworth et al., 2012; Patel et al., 2014). The basic digestion kinetic parameters can be estimated from this analysis as follows: the rate constant (k) is represented by the negative slope of the linear plot, and the total starch digested (C∞) can be calculated from the y-axis intercept. The digestion process is described by two separate first-order reactions that differ in their rate constant. Therefore, the LOS plot method would be a useful analytical tool for accurate determination of RDS and SDS starch fractions, if present, from discontinuities in the linear plot (Patel et al., 2014).

Lipid has 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 and Greenwood, 1972; Zobel, 1988). When AM molecules accommodate a guest molecule, they form a single left-handed helix structure, called V-amylose. These complexes are based on a non-covalent interaction between AM and hydrophobic molecules which create inclusion compounds (Lalush et al., 2005; Shogren et al., 2006). The AM inclusion complexes can modify the properties and functionalities of starches, including the retardation of gelatinization and retrogradation of starchy food (Chang et al., 2013; Guraya et al., 1997; Zhang et al., 2012). The AM inclusion complexes can be divided into type I and type II complexes. Type I

complexes form amorphous complexes at a low temperature (approximately 60°C), while randomly oriented type I complexes form more ordered structure as lamellae which are named type II complexes (at temperatures above 90°C) (Gelders et al., 2004; Meng et al., 2014; Putseys et al. 2010). The AM-lipid inclusion complex shows a resistance to α-amylase which leads to a prolonged enzymatic hydrolysis as compared with amorphous AM (Guraya et al., 1997; Seneviratne and Biliaderis, 1991; Tufvesson et al., 2001).

Meanwhile, there are some researches about the using of AM inclusion complexes in food and biotechnology industries. Shimoni et al. (2007) suggested that starch molecular complexes can prospectively be used as carriers for targeted delivery of nutraceuticals and drugs to gastrointestinal tract. They prepared the AM-guest complexes with genistin, which is a phytoestrogen from dietary plants, and confirmed the complex formation and release of genistin molecules. Also, conjugated linoleic acid (CLA) complexed with AM (Lalush et al., 2005) can protect and deliver the CLA as molecular nanocapsules. Besides, Ma et al. (2011) described the formation of starch inclusion complexes with ascorbyl palmitate, retinyl palmitate, and phytosterol esters as bioactive compounds. The vanillin as a flavor compound complexed to starches and its flavor release was monitored by an

electronic nose (Rodriguez and Bernik, 2014). Singh et al. (2014) referred to AM-potassium oleate inclusion complex as a fat replacer in skim milk yogurt.

However, AP cannot form a complex with a fatty acid due to the steric hindrance caused by its short chain length of it (Bhatnagar and Hanna, 1994; Conde-Petit et al., 2006; Huang and White, 1993; Kugimiya et al., 1980). Thus, there are no changes in physicochemical properties and functionalities after formation of complexes. Recently, amylosucrase (E.C. 2.4.1.4.) from *Neisseria polysaccharea* has been used for the modification of gelatinized starches (Kim et al., 2013; Kim et al., 2014; Ryu et al., 2010). Amylosucrase (AS) catalyzes a transglycosylation reaction to produce an insoluble AM-like polymer using sucrose as a substrate while releasing fructose (Hehre, 1949; Potocki-Verones et al., 2005; Potocki de Montalk et al., 2000; Rolland-Sabaté et al., 2004). In sum, AS is the only known enzyme that catalyzes the elongation of some external chains of the acceptor such as AM and AP at their non-reducing ends from cheap agricultural resources without any primer. By the AS treatment, the proportion of short branch chains decreases and that of long chains increases (Kim et al., 2014). The elongated branch chains can attribute to the cavity for formation of inclusion complexes with fatty acids. Regarding the digestibility properties of starch, Kim et al. (2014) who conducted modification of waxy corn starch with AS suggested that the

enhanced SDS fractions and relative crystallinity were due to formation of crystallites caused by elongated AP branch chains. In other study, the RS contents of AS-treated starches were directly associated with the enhanced AP branch chains (Kim et al., 2013). Park et al. (2014) stated that to the AS-treated starches contained more SDS and RS, and that these starches were the most resistant to hydrolysis of starch under the mimicker of the human gastrointestinal tract conditions.

Little work has been done by using AP to prepare V-amylose complexes. Waxy corn starch is composed of almost 100% AP, and its endogenous lipid content is very low (0.15%) (Swinkels, 1985). Thus, waxy corn starch was used in this work. The objectives of this study were to prepare AS-treated waxy corn starch-lipid complexes and to compare their complex index, digestibility, and physicochemical properties with those of normal corn starch-lipid complexes. It is expected that AS-treated waxy corn starch may have potential applications in food, pharmaceutical, and biotechnology industries as a useful tool for targeted delivery.

MATERIALS AND METHODS

1. Materials

1.1. Starch

Normal and waxy corn starches were obtained from Ingredion (Westchester, IL, USA).

1.2. Enzymes

Amylosucrase (AS) from *Neisseria polysaccharea* was obtained from the Food Microbiology and Biotechnology Laboratory of KyungHee University.

α-Amylase from porcine pancreatin (type VI-B, A3176) was purchased from Sigma Chemical Co. (St. Louis, MO, USA), and its activity was 30 U/mg solid. Amyloglucosidase (AMG 300L, activity 300 AGU/mL) was obtained from Novozymes (Bagsvaerd, Denmark). Isoamylase (activity 1,000 U) was from Megazyme (Bray, Ireland), and GOD-POD assay kit from Embiel Co. (Gunpo, Korea).

1.3. Fatty acids

Myristic (C14:0, MA) and palmitic acid (C16:0, PA) were purchased from TCI (Tokyo, Japan).

2. Method

2.1. Analysis of AS activity

By the use of affinity chromatography with Ni-NTA (nickel-nitrilotriacetic acid) resin, AS was purified as described by Jung et al. (2009). Enzyme activity was analyzed according to the method of Van der Veen et al. (2004) with a modification. The mixture of 0.1 mL 4% sucrose, 0.1 mL 1% glycogen, 0.25 mL 0.1 mM sodium citrate buffer (pH 7.0) and 0.05 mL diluted enzyme was kept in a shaking water bath at 30°C and 80rpm for 10 min. The dinitrosalicylic acid method (Miller, 1959) was used for quantifying released fructose. One unit of amylosucrase was the amount of enzyme catalyzing the release of 1 μM of fructose from sucrose per min under the assay conditions.

2.2. Preparation of AS-treated starch

Waxy corn starch and 100 mM sucrose were suspended in 100 mM sodium acetate buffer (pH 7.0) to make a 2% suspension (150 mL, w/v). To increase enzyme accessibility, the starch suspension was boiled with vortex

mixing for 30 min. After that, the solution was cooled to 30°C for 30 min to reach the temperature of enzyme activity measurement. AS was added to the starch suspension (20000 U/30 mL) and incubated in a water bath at 30°C for 24 h. The reaction was stopped by adding three-fold ethanol to the suspension. The AS-treated starch was recovered by centrifugation (10,000 xg, 10 min), washed three times with distilled water. The precipitate was freeze-dried, ground, and passed through a 100-mesh sieve.

2.3. Determination of branch chain length distribution

Starch (15 mg) was dispersed in 90% DMSO (3 mL) and boiled for 30 min. Ethanol (15 mL) was added to the starch dispersion and centrifuged at 10,000 xg for 10 min. The pellet was boiled in distilled water (1.5 mL) for 15 min and 50 mM sodium acetate buffer (pH 4.3, 1.5 mL) was added and boiled for 20 min. After being cooled to 45° C, isoamylase (30 μL) was added to the starch dispersion and incubated at 45°C and 30 rpm for 2 h in a shaking water bath for debranching the starches. The sample was boiled for 10 min to inactivate the enzyme.

The debranched starch sample was passed through a 0.45 μm membrane filter (DISMIC-13CP, Advantec, Tokyo, Japan) and analyzed with high-

performance anion-exchange chromatography (HPAEC) system to determine branch chain length distribution. The HPAEC system was equipped with a Carbo-pack PA1 anion-exchange column (4x250 mm, Dionex, Sunnyvale, CA, USA) and with a pulsed amperometric detector (PAD; ED40 electrochemical detector, Dionex, CA, USA). This analysis was performed by eluting the sample with 600 mM sodium acetate in 150 mM NaOH with a flow rate of 1 mL/min with gradients 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 DP values were designated using a mixture of maltooligosaccharides (DP 1-7, Sigma-Aldrich Chemical Co., St. Louis, MO, USA) as standard. The peak areas were calculated by PeakNet software (version 5.11, Dionex, CA, USA). Number-based average DP (DP_n) values were obtained according to the following equation.

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

$$A_i: peak\ area/total\ peak\ area (i: 1, 2, 3 \cdots)$$

2.4. Determination of apparent amylose content and complex index

Apparent amylose contents were measured 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 1 M NaOH (1.8 mL) was added to the mixture and boiled with vigorous vortex mixing (10 min). After boiling, the dispersion was cooled to room temperature for 30 min. The cooled starch dispersion (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 diluted again to 10 mL with distilled water. Lugol's solution (0.2 mL; 0.2% I_2 + 2.0% KI) was added and 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 index (CI) was calculated using the following equation.

$$CI\ (\%) = \left(\frac{absorbance_{control} - absorbance_{sample}}{absorbance_{control}}\right) \times 100$$

2.5. Preparation of starch complexed with fatty acids

The sample preparation followed the method of Meng et al. (2014) with a modification. A 1g portion of normal corn starch (NC) and AS-treated waxy corn starch (ASWC) were dispersed in 7.2 mL of distilled water and cooked with vigorous vortex mixing for 30 min. To facilitate the complexation between starch and lipid, the starch suspension was autoclaved for 30 min at 121°C. Different fatty acids (5% on the starch basis, MA and PA), dissolved in ethanol (1%, w/v), were added to the starch suspension and autoclaved again (121°C, 30 min). After autoclaving, the mixture was incubated at 95°C and 120 rpm for 30 min and cooled to 70°C for 30 min in a shaking water bath to form a stable complex. An aliquot of 50% ethanol was added immediately to wash off free fatty acids twice and the complex was precipitated by centrifugation at 3,000 xg for 30 min. The pellet was freeze-dried, ground, and passed through a 100-mesh sieve and used as a sample. Control was prepared with the same procedure without fatty acids.

2.6. X-ray diffraction patterns and relative crystallinity

X-ray diffraction was carried out using a powder X-ray diffractometer (Model New D8 Advance, Bruker, Karlsruhe, Germany) at 40 kV and 40 mA.

Starch sample was scanned through 2θ range from 3° to 30° with a 0.02° step size and a count time of 2 sec. The relative crystallinity was calculated according to the method of Nara and Komiya (1983). The area was calculated by the following equation using the software developed by the instrument manufacturer (EVA, 2.0; Bruker, Karlsruhe, Germany).

$$\text{Relative crystallinity (\%)} = \frac{A_c}{A_a + A_c} \times 100$$

A_a: area of amorphous region, A_c: area of crystalline region

2.7. Evaluation of thermal properties

Thermal properties of the samples were analyzed using a differential scanning calorimeter (DSC, Diamond DSC, Perkin-Elmer, Waltham, MA, USA). A sample of complex (10 mg) was weighed in a stainless steel pan (03190029, Perkin-Elmer, Waltham, MA, USA), and 40 μL and 30 μL of distilled water were added to NCs and ASs samples, respectively. The sample pan was sealed and kept at room temperature overnight for moisture equilibrium. A stainless steel pan containing distilled water without sample was used as a reference. Samples were heated from 30°C to 160°C at a rate of 10°C/min. The onset temperature (T_o), the peak temperature (T_p), the conclusion temperature (T_c), and the melting enthalpy (ΔH) were obtained.

2.8. Starch digestibility

2.8.1. Degree of starch hydrolysis

The degree of hydrolysis was measured at various points following the method of Shin et al. (2007) with slight modification. Pancreatic α-amylase (6.09 g) was added into distilled water (23 mL) and stirred for 10 min. After the stirring, the suspension was centrifuged at 1,500 xg for 10 min. For enzyme preparation, an aliquot of supernatant (20 mL) was mixed with 0.4 mL of amyloglucosidase and 3.6 mL of distilled water and kept in a water bath at 37°C for 10 min.

A starch sample (30 mg) was dispersed in a 2 mL-microtube containing 0.75 mL of 0.1 M sodium acetate buffer (pH 5.2) with one glass bead. After vortexing, the microtubes were equilibriated in a shaking incubator (240 rpm, 37°C) for 10 min. The prepared enzyme solution (0.75 mL) was added to each microtube and incubated in a shaking incubator (240 rpm, 37°C). A microtube was removed at certain times and boiled in a cooker (DW-5600, Daewon, Bucheon, Korea) for 10 min to stop the reaction.

The supernatant was taken after the centrifugation at 5,000 xg for 10 min (4°C) and GOD-POD kit (Embiel Co., Gunpo, Korea) was used for analysis of the glucose released by hydrolysis of starch.

2.8.2. Determination of starch fraction according to log of slope (LOS) method

Starch fractions were estimated based on a previous study (Butterworth et al., 2012) with slight modification. According to Goñi et al. (1997), the first-order equation can be shown in the digestibility curves of starch. After differentiation of first-order equation, it can be expressed in logarithmic form as follows:

$$\ln(\frac{dC}{dt}) = \ln(C_\infty k) - kt$$

Poulsen et al. (2003) referred to this plot as 'logarithm of the slope' or 'log of slope'. A plot of $\ln(dC/dt)$ against t is a linear graph with a slope of $-k$. The intercept on the y axis is the value of $\ln(C_\infty k)$, and the value of k can be calculated from the slope of the plot. The slope was estimated from the fraction ΔC, i.e., $(C_{n+1} - C_n / t_{n+1} - t_n)$, $(n: 1, 2, 3, \cdots)$ and the natural logarithms plotted against the mean of time, i.e., $(t_{n+1} - t_n)/2$, $(\mathrm{n}: 1, 2, 3, \cdots)$. The slope is sensitive to the change of k that occurs during a reaction, which would be shown by two discontinuous lines in the linear plot. The point of intersection was the distinction point between RDS and SDS: the former region with steeper slope was considered as RDS, and the latter part was determined as SDS.

2.9. Statistical analysis

All experiments were performed in triplicate, and analysis of variance (ANOVA) was used for analyzing the data. The data were expressed as mean ± standard deviation, and the mean separations were done by the Duncan's multiple range tests at a significance level of 0.05. All the statistical analyses were conducted by IBM SPSS statistics version 22.0 (IBM, New York, NY, USA).

RESULTS AND DISCUSSION

1. Branch-chain length distribution of starch samples

The branch-chain length distribution of starches determined by HPAEC-PAD is shown in Figure 1 and Table1. Branch-chains of amylopectin are classified into four groups depending on degree of polymerization (DP); A chain (DP 6-12), B_1 chain (DP 13-24), B_2 chain (DP 25-36), and B_3 chain (DP≥37) (Hanashiro et al., 1996). Hizukuri et al. (1983) categorized starch based on the chain length of amylopectin. The amylopectin average chain length of normal corn starch is shorter than 19.7 and it belongs to A-type cereal starches such as rice, wheat and waxy corn starches. In the current study, statistical analysis showed that A, B_1, B_3 and the average degree of polymerization (DP_n) of NC were the same as those of WC, indicating that both starches have the identical proportions of amylopectin branch chain length. The waxy corn starch has a larger proportion of short chains such as A chain and B_1 chain than that of long chains (B_2 and B_3) (Zhang et al., 2006). According to Kim et al. (2014), AS-treatment induces an increase in the chain length of amylopectin and a decrease in the proportion of short chains. After AS-treatment, the proportion of A chain decreased roughly

thirteen fold, and those of B_2 and B_3 chains increased more than threefold. At the same time, DP_n increased from 17.7 to 27.3. These results were caused by those changes that resulted from the elongation of external chains by AS-treatment. The AS-treatment catalyzes the elongation of branch chains length by attachment of 12 to 18 glucosyl units to the nonreducing ends (Potocki et al., 1999). It has been suggested that short chains such as A chain and B_1 chain are readily accessed by AS because they are located outside of the cluster structure (Kim et al., 2014). Previous studies conducted for A, B, and C-type of starches showed the highest proportion of branch-chain with DP 13-24, which corresponded to this study (Jane et al., 1999; Kim et al., 2013; Kim et al., 2014; Ryu et al., 2010). Ryu et al. (2010) reported that the weight-average degree of polymerization (DP_w) increased from 17.9 to 24.9 for waxy corn, from 17.8 to 23.7 for normal corn, and from 20.6 to 23.5 for amylomaize VII starches. It has also has been reported that waxy and normal rice and potato starches representing A-type and B-type, respectively, were elongated in DP≥25 and that the proportion of A and B_1 chains decreased from 24.6%-38.0% to 1.2%-3.6% and from 50.6%-55.9% to 28.4%-38.6%, respectively.(Shin et al., 2010).

Table 1. Branch chain length distribution of normal corn, waxy corn and AS-treated waxy corn starches

Sample[1)]	Percent distribution (%)				DPn[3)]
	DP[2)] 6-12	DP 13-24	DP 25-36	DP ≥ 37	
NC	32.8±2.5[a 4) 5)]	46.3±1.7[a]	13.6±0.6[b]	7.4±0.2[b]	18.5±0.3[b]
WC	34.5±1.1[a]	47.8±1.4[a]	12.1±0.3[c]	5.9±1.2[b]	17.7±0.4[b]
ASWC	2.7±0.2[b]	44.7±2.9[a]	36.3±0.1[a]	16.3±3.2[a]	27.3±1.0[a]

[1)] NC = native normal corn starch; WC = native waxy corn starch; ASWC = Amylosucrase (20,000 U/30 mL)-treated waxy corn starch

[2)] DP, degree of polymerization.

[3)] Number-based average degree of polymerization

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

[5)] The values with different superscripts in the same column are significantly different ($p < 0.05$) by Duncan's multiple range test.

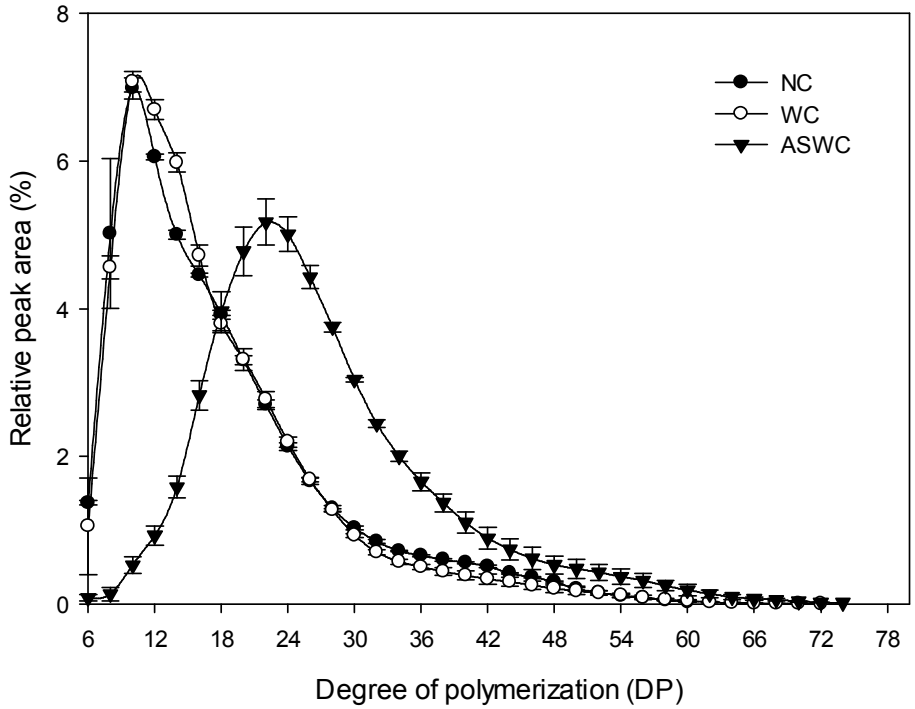

Figure 1. Branch chain length distributions of starch samples. NC = native normal corn starch; WC = native waxy corn starch; ASWC = Amylosucrase (20,000 U/30 mL)-treated waxy corn starch.

2. Apparent amylose content and complex index

Amylose developes blue color when it forms a single helical complex with iodine, and amylopectin-iodine complex is purple (Conde-Petit et al.. 1998; McGrance et al., 1998). Apparent amylose content of NC corresponded to previous researches, and that of WC was almost zero since waxy starches contains approximately 95% or more of amylopectin and no amylose (Chinnaswamy and Hanna, 1988; Jane et al., 1999; Mercier, 1973). Interestingly, apparent amylose content of AS-treated waxy corn starch (ASWC) was not significantly different from that of NC ($p>0.05$). It could be due to the elongated amylopectin which was modified by the enzyme obtains the capacity of formation complex with ligands such as iodine, fatty acids, and etc.. Amylose combines well with iodine because of its long chains, and shows a high iodine absorption value, whereas amylopectin with short chains has a low iodine value (Bates et al., 1943). In other words, elongated chain length of amylopectin can increase the possibility of forming single helical structure. In a preliminary experiment, WC formed a complex less than 5% of complex index. Due to the judgement of a difficulty in expecting an effect form the modification, the further comparison of properties was not conducted.

Starch-fatty acid inclusion complex reduce the cavity of iodine combine with starch. The difference of absorbance between starch-iodine complex and starch-lipid-iodine complex can be used for calculating of complex index (Guraya et al., 1997; Soong et al., 2013; Tang et al., 2007). There are some factors which impact the degree of complexation. Firstly, the chain length of amylose affects amount of complexes. Long amylose chains can form more complexes with lipid molecules having higher melting temperatures (Godet et al., 1993). The melting temperature, crystal thickness and yield of complexes increase with the chain length of amylose (Godet et al., 1995, 1996). Second factors are aliphatic chain length of fatty acid and degree of unsaturation. The chain length of lipids lesser than 10 carbon atoms are too short to forms starch-lipid complexes since they are hard to keep in the hydrophobic helix cavity (Godet et al., 1995; Karkalas and Raphaelides, 1986; Lebail et al., 2000). However, there is disagreement on the identity of the best complexing lipid. Some researchers suggest that a lipid chain length of 14 carbon atoms is the best for complex formation (Bhatnagar and Hanna, 1994; Krog, 1971; Soong et al., 2013). On the other hand, it has been suggested that that lipid chain lengths of 12, 16, and 18 carbon atoms are preferred for complexation in amylose, potato starch, and wheat starch, respectively (Hoover and Hadziyev, 1981; Kawai et al., 2012; Krog, 1971; Lagendijk and Pennings, 1970). Accordingly, the correlation between lipid

chain length and complex index is still in controversy. The number of double bonds in the aliphatic chain also influences on starch-lipid complexation. The higher degree of unsaturation shows the lower stability and yield of the complex due to steric hindrance inside the helix cavity (Eliasson and Krog, 1985; Karkalas and Raphaelides, 1988; Krog, 1971; Lagendijk and Pennings, 1970; Zabar et al., 2009). The complex indices of NC and ASWC complexes are give in Table 2. In the complexes of NC, palmitic acid (C16:0) combined than myristic acid (C14:0) did. It might be that myristic acid formed a less stable complex in hydrophobic helix cavity than palmitic acid and was washed off easily during 50% EtOH washing in the sample preperation process. The complex index of ASWC+MA and ASWC+PA were 26.0% and 27.4%, respectively, which were not much difference between them but higher than those of NC complexes. The amount of ASWC complexes were a 1.5-2 fold increase compared with those of NC complexes, which could be caused by the different structural properties of starches.

For the formation of a stable amylose-lipid complex, the length of helical segment should be approximately twice that of a fatty acid without carboxyl group. Thus, the approximate amylose DP which makes an amylose-lipid inclusion complex can be calculated with a pitch of 0.805 nm and six glucose molecule per a helix turn (Gelders et al., 2004). The length of C14 and C16

fatty acids without the carboxyl group are 1.63 and 1.88 nm, respectively (Karkalas and Raphaelides, 1986). The helical segment lengths of them are be approximately 3.26 and 3.76 nm, respectively. The DP required for complex formation with C14 and C16 can be calculated to DP 25 and 28, respectively. The percent distribution of ASWC in DP 25-36 is threefold to NC. Also amylose and amylopectin proportions can contribute to complex formation and complex index. The normal corn starch is composed of 30% of amylose and 70% of amylopectin (Table 2), and the amylopectin chain length distributions of NC and WC are not significantly different ($p>0.05$). Also the amylopectin of NC does not affect the complexation with fatty acids. Thus, the 30% amylose of NC can only form a helical inclusion complex. However, ASWC had almost 100% amylopectin which was elongated by enzyme modification, and the probability of combining fatty acid to amylopectin portion was higher than that of NC.

Table 2. Apparent amylose contents of starches

Sample[1)]	Amylose contents (%)
NC	29.0±1.1[a 2) 3)]
WC	0.7±0.3[b]
ASWC	29.7±0.9[a]

[1)] NC = normal corn starch; WC = waxy corn starch; ASWC = Amylosucrase (20,000 U/30 mL)-treated waxy corn starch.

[2)] Data are expressed as average value and standard deviation.

[3)] The values with different superscripts in the same column are significantly different ($p < 0.05$) by Duncan's multiple range test.

Table 3. Complex index of starch-lipid complexes

Sample[1)]	Complex index (%)
NC+MA	13.7 ± 1.1^{c} [2) 3)]
NC+PA	19.7 ± 1.3^{b}
ASWC+MA	26.0 ± 1.9^{a}
ASWC+PA	27.4 ± 0.4^{a}

[1)] NC+MA = NC with myristic acid; NC+PA = NC with palmitate acid; ASWC+MA = ASWC with myristic acid; ASWC+PA = ASWC with palmitate acid.

[2)] Data are expressed as average value and standard deviation.

[3)] The values with different superscripts in the same column are significantly different ($p < 0.05$) by Duncan;s multiple range test..

3. X-ray diffraction patterns and relative crystallinity

The crystalline packing arrangements of the normal corn and AS-treated waxy corn starches complexed with fatty acid were investigated with X-ray diffraction analysis and the diffractograms are shown in Figure 2. The diffraction patterns and crystallinity were influenced by type of starch and fatty acid added. The normal and waxy corn starch showed major peaks at 15°, 17°, 18°, and 23° 2θ (Hizukuri et al., 1980) which indicated a typical A type starch. However, there were no significant diffraction peaks in the XRD patterns of NC+non sample (Fig. 2), demonstrating the complete disruption of starch crystallites during sample preparation but slight rises were observed near 13° and 22°, which was presumed to be amylose caused by amylose complexed with lipid contained in normal starch (Karkalas and Raphaelides, 1986; Tester and Morrison, 1990). The diffraction pattern of ASWC+non sample was similar to B-type with major peaks at 17° and 24°, and a minor peak at 5°. During AS-treatment in the process of sample preparation, the crystallinity of waxy corn starch was completely disrupted and reordered to a B-type crystalline structure (Kim et al., 2014; Ryu et al., 2010). As shown in Figure 2, after addition of fatty acids, the diffraction patterns of complexed samples changed to V-type polymorph displaying characteristic major peaks

at 7.4°, 12.9°, and 19.8° (Bhatnagar and Hanna, 1994; Godet et al., 1995; Lesmes et al., 2009; Tang and Copeland, 2007). The presence of V-type diffraction pattern, suggested that amylose formed inclusion complexes with fatty acids. The normal corn starch complexed with palmitic acid sample showed an additional minor peak between 19° and 21° which corresponded to a pure crystalline of free fatty acids in some researches (Fanta et al., 1999; Lebail et al., 2000; Tang and Copeland, 2007). The exist of free fatty acids are also detected by the endotherm in the DSC spectrum as melting of free fatty acids at 62°C, corresponding to the melting temperature of palmitic acid (data not shown). The diffraction peaks of normal corn starch complex samples were sharper compared to those of AS-treated waxy corn starch. The formation of type II complexes in normal corn starch were conducted by the DSC measurements and it might be attributed to the sharper peak. Gelders et al. (2004) reported that the lipid complex with higher DP of amylose showed sharper peaks than short DP of that.

Table 4 shows the relative crystallinities of starch-lipid complexes. The relative crystallinity was mainly attributed to the elongated residues of AP double helices and the formation of starch-fatty acid complex (Chang et al., 2013). The difference in relative crystallinity might be caused by such factors as crystal size, amount of crystalline regions, orientation of the

double helices within the crystalline domains, and extent of interaction between double helices (Miao et al., 2009). The relative crystallinity increased in the order +non, +MA, +PA in normal corn starch and decreases as +non, +MA, +PA in AS-treated waxy corn starch. In case of NC+non sample, the disruption of starch crystallite resulted in the lowest relative crystallinity. NC+PA sample, showed the highest relative crystallinity, twice as more than NC+non sample among normal corn starch complexes. The longer hydrocarbon chain can form a more stable complex since its hydrophobicity contributes to the strong hydrophobic interactions with the inner side of the helix (Eliasson and Krog, 1985; Gelders et al., 2004; Godet et al., 1995; Karkalas and Raphaelides, 1988). It is well known that the stable complexes form less ordered type I complex which can contribute to the formation of semicrystalline type II amylose-inclusion complexes. The % crystallinity depends on the ability of individual complexed amylose helices to pack into a crystallite (Ma et al., 2011). Thus, the NC+PA induced more compact packing of single helices resulting in higher relative crystallinity than NC+MA did.

The addition of fatty acids makes the relative crystallinity decrease in ASWC starch compared with ASWC+non to which no fatty acid was added, showed the highest relative crystallinity (Table 4). A previous study

suggested that the elongated AP chains by AS-treatment favored to form double helices, which could lead to the formation of crystalline structure densely packed and had higher relative crystallinities (24.0-29.7%) compared with the control starch (21.8%) (Kim et al., 2014).

During the preparation of ASWC lipid complexes, the AP double helices were dissociated to single chains and combined with a fatty acid to form a helix inclusion complex. Thus, the fatty acid disrupted the double helices of elongated AP-chains. Kawai et al. (2012) suggested that crystallites of amylose double helices have a compact double helical order while lipid-amylose complex has loosen single helical order. The palmitic acid forms a more stable helical inclusion complex than myristic acid does and interferes with reassociation of AP-chains to double helices, leading to less ordered crystallinity.

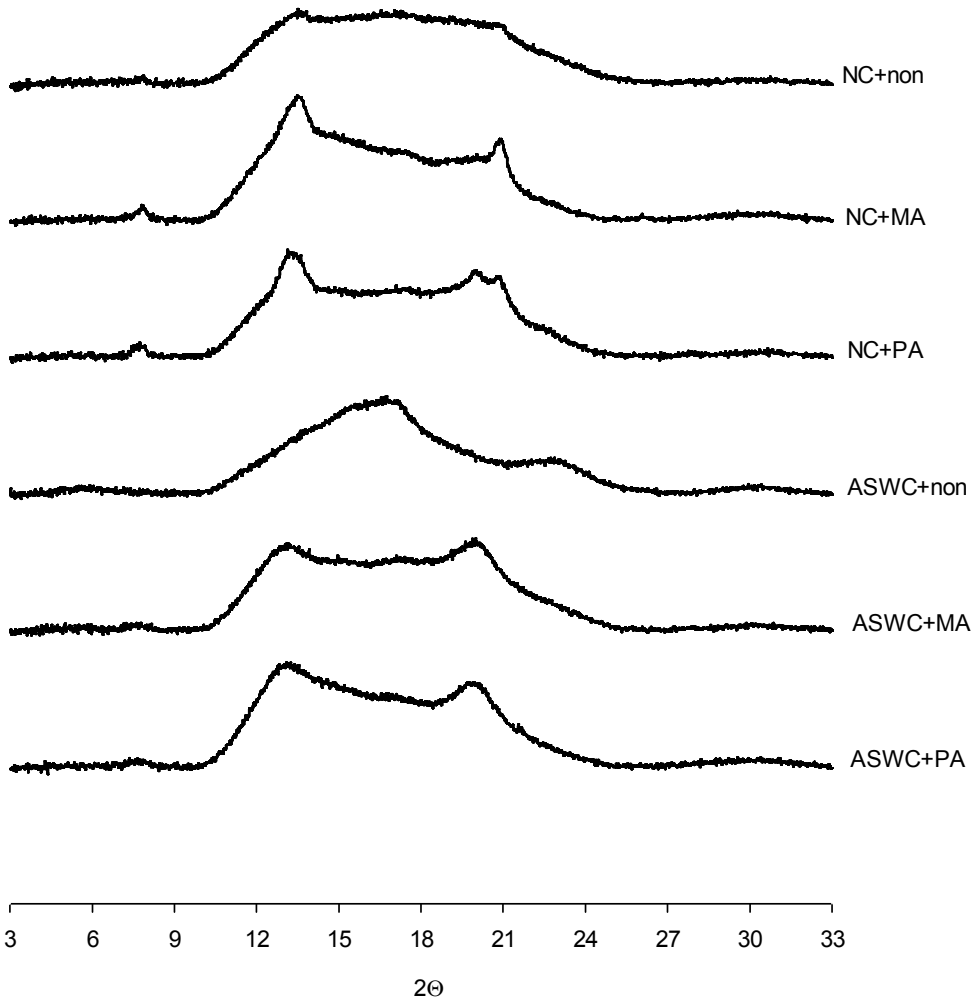

Figure 2. X-ray diffraction patterns of starch-lipid complex samples. NC+non = NC without fatty acid; NC+MA = NC with myristic acid; NC+PA = NC with palmitic acid; ASWC+non = ASWC without fatty acid; ASWC+MA = ASWC with myristic acid; ASWC+PA = ASWC with palmitic acid.

Table 4. Relative crystallinity of the starch-lipid complex samples

Sample[1)]	Relative crystallinity (%)
NC+non	11.2±0.3[e2)]
NC+MA	19.4±0.8[c]
NC+PA	22.4±0.3[b]
ASWC+non	25.1±0.7[a]
ASWC+MA	20.2±0.6[c]
ASWC+PA	18.3±0.1[d]

[1)] NC+non = NC without fatty acid; NC+MA = NC with myristic acid; NC+PA = NC with palmitic acid; ASWC+non = ASWC without fatty acid; ASWC+MA = ASWC with myristic acid; ASWC+PA = ASWC with palmitic acid.

[2)] The values with different superscripts in the same column are significantly different ($p<0.05$) by Duncan's multiple range test.

4. Thermal properties

The thermal properties of the normal corn starch and AS-treated waxy corn starch complexed with fatty acids are represented in Table 5. Changes in T_o, T_p, T_c, and ΔH of endotherms reflect the crystallinity and structure of starches (Chang et al., 2013). In the present study, normal corn starch-fatty acid complexes and AS-treated waxy corn starch-fatty acid complexes exhibited two and three peaks, respectively. In case of normal corn starch complexes, the first peak (95°C - 101°C), named peak II, indicated melting of type I starch-lipid complex, and the second peak (116°C - 117°C), named peak III, presumably represented the melting of type II starch-lipid complex. On the other hand, the first peak (77°C - 90°C) of AS-treated waxy corn starch-fatty acid complex corresponded to the melting of AP double helices of AS-treated waxy corn starch, and the second peak showed at 90°C - 95°C which indicates the melting of type I starch-lipid complex. The third peak (132°C - 147°C) was attributed to the melting of crystallites formed by long branch chains of AP acting like AM (Table 5).

4.1. Complexes of normal corn starch with fatty acid

No peak was observed in normal corn starch without addition of fatty acid (NC+non) because the starch was fully gelatinized and all crystalline regions were disrupted. However, there were two peaks (peak II and peak III) in NC-fatty acid complexes. Starch-lipid complexes are divided into two categories, the less ordered type I and the semicrystalline type II (Putseys et al., 2010). It has been suggested that type I starch-lipid complex forms at low temperatures (roughly 60-90°C) which is the less ordered crystallites that melts between 90-105°C whereas type II starch-lipid complex form at least 90°C lead to well-defined crystalline region which melts at a higher temperature (115-120°C) (Biliaderis et al., 1985; Karkalas et al., 1995; Putseys et al., 2010). In peak II (Table 5), NC+PA was dissociated at a higher temperature and its enthalpy was twofold than NC+MA. These phenomena suggest that hydrocarbon chain of PA causes a stronger hydrophobic interaction with amylose and need more energy to dissociate the amylose-PA complex than MA does. In peak III (Table 5), the NC-fatty acid complexes formed a type II complex and peak temperature and enthalpy are same in statistically. This means that the energy for melting of semicrystalline region

is same between NC+MA and NC+PA.

4.2. Complexes of AS-treated waxy corn starch with fatty acid

ASWC showed three peaks in DSC measurements which indicate melting of AP-double helices, type I complex, and crystallite of elongated AP-double helices, respectively. AP-double helices were melted between 77.0°C and 89.6°C decreasing with the addition of fatty acids, and the temperature range is corresponding to the reported by Kim et al. (2014) . The addition of fatty acid interferes in the formation of AP-double helices which can contribute to a low level of crystallinity. The loosened amylose packing of AP-double helices lead to a decrease in enthalpy of ASWC-lipid complexes. In fact, crystalline amylose has a high T_p at 126-165°C (Sievert and Pomeranz, 1989) and the amylose-fatty acids complex melts at 78-97°C which is much lower than crystalline amylose does (Kawai et al., 2012). The peak II of ASWC-fatty acid complexes indicated the formation of type I complex, and peak temperature of PA was lower than MA. The MA combined with more stable than PA did, different to NC-fatty acid complexes. It indicates that the branch-chain length of ASWC was more favorable to MA than PA for the

formation of ASWC-lipid complexes. On the other hand, the peak III of ASWC samples exhibited a much higher peak temperature than that of NC indicating the melting of crystalline region of elongated AP-double helices. The melting temperature and enthalpy of crystalline region decrease with the addition of fatty acids because fatty acids disrupted the crystallites and double helices of elongated AP failed to form a compact semicrystalline structure. Szezodrak et al. (1992) suggested that the amylose-amylose association is hindered by the addition of lipids due to the competitive interaction between amylose association and amylose-lipid complexation.

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Table 5. Thermal properties of normal corn starch and amylosucrase-treated waxy corn starch complexed with a fatty acid

Sample	Peak I				Peak II				Peak II			
	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)
NC +non	N.D[2)]				N.D.				N.D.			
NC +MA	N.D.				92.9 ±0.8[b]	94.9 ±1.1[b]	97.3 ±1.9[b]	3.8 ±0.4[b]	113.9 ±0.3[a]	117.0 ±1.0[a]	120.5 ±0.9[a]	8.2 ±0.7[a]
NC +PA	N.D.				98.3 ±0.6[a]	101.0 ±1.2[a]	103.0 ±1.0[a]	7.2 ±1.2[a]	112.3 ±2.1[a]	116.5 ±2.0[a]	118.6 ±3.6[a]	8.0 ±0.3[a]
ASWC +non	83.2 ±3.0[a3)]	89.6 ±0.2[a]	97.4 ±3.8[a]	10.2 ±0.2[a]	N.D.				142.2 ±1.5[a]	147.1 ±2.4[a]	150.7 ±4.6[a]	2.7 ±0.6[a]
ASWC +MA	87.1 ±2.2[a]	87.7 ±1.3[b]	88.7 ±1.0[b]	1.6 ±0.3[b]	91.6 ±3.5[a]	94.6 ±1.9[a]	96.6 ±1.6[a]	2.7 ±0.5[b]	135.5 ±3.2[b]	137.6 ±1.9[b]	141.3 ±1.0[b]	0.5 ±0.2[b]
ASWC +PA	74.2 ±1.1[b]	77.0 ±0.9[c]	80.0 ±0.5[c]	2.4 ±0.4[c]	89.3 ±1.2[a]	90.7 ±1.3[b]	92.1 ±1.0[b]	3.8 ±0.3[a]	130.5 ±2.9[b]	132.9 ±2.3[c]	135.2 ±2.2[c]	0.4 ±0.2[b]

[1)] *To*, *Tp*, *Tc* 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 starch and same column are significantly different ($p < 0.05$) by Duncan's multiple range test.

5. Digestibility

In general, the hydrolysis of amylose-inclusion complexes occurs by two following steps; a rapid hydrolysis of amorphous areas of the complexes, and a slow degradation of the amylose-inclusion complexes (Galloway et al., 1989; Godet et al., 1996; Jane and Robyt, 1984). Hydrolysis of complexes is influenced by both amylose and lipid chain length. Resistance to enzyme hydrolysis increases with amylose DP and lipid chain length (Gelders et al., 2005)

5.1. Digestion pattern of starch-lipid complexes

Figure 3 and 4 display the enzymatic digestion profiles of starch-lipid complexes. The degree of digestion was measured for NC-lipid complexes over the 4-h period (0-240 min) and the ASWC-lipid complexes during 0-600min reaction time to clarify the emergence of a plateau.

Normal corn starch (NC) without fatty acid reached a plateau at 20 min of digestion, and the observed maximum degree of hydrolysis (C_∞) was approximately 85% (average of the hydrolysis degree values after 20 min).

The plateau of NC+MA and NC+PA emerged at 30 min, and the observed C_∞ were 77.37% and 73.65%, respectively. The complexation attributes to the formation of the crystalline structure that hinder the enzyme activity. Thus, the time of equilibrium was delayed in both complex samples and some researches are corresponding with decrease of digestibility (Ai et al., 2013; Guraya et al., 1997; Hasjim et al., 2010; Kawai et al., 2012; Meng et al., 2014). Also, the observed C_∞ decreased to 73-77% that indicates the increase in the remained starch fraction after the digestion process (the hydrolysis is a reaction for 240 min). The carbohydrates which can be digested are reduced by crystallization of type I and type II complexes. The ordered structure led to lower susceptibility towards enzymes. Thus, the starch-lipid complexes were more resistant to enzyme digestion than non-complexed starch. Holm et al. (1983) and Nebesny et al. (2004) suggested that AM-inclusion complexes are considered to be less enzyme degradable than amorphous amylose due to their low solubility and steric hindrance.

As compared with NC complex, ASWC complexes measured 56-67% of the observed C_∞, which was lower, and the time of plateau increased dramatically to 480 min. The longer time was required to hydrolyze a smaller amount of starch (C_∞). It demonstrated that the AS-treated starch resistants to starch hydrolysis. In the present study, the modification of AP

chains by AS-treatment revealed an increased proportion of long chains, and the long branch chains formed the strong, stable and long double helices that contribute to retrogratdation. During the AS-treatment, the starches were gelatinized and recrystallized to form a retrograded structure (Kim et al., 2014). In general, the retrograded starch has resistance to enzymatic hydrolysis, and the crystalline structure formed by retrogradation could reduce the hydrolytic enzyme susceptibility. The long linear chains can be retrograded, and it is one of the mechanisms for slow digestion property of starches (Zhang et al., 2008). Further, the elongated AP chains would cause the formation of ordered crystallites (Ryu et al., 2010; Shin et al., 2010), and the crystallites have low susceptibility to enzymatic hydrolysis (Zhang et al., 2006). The gelatinization process during the preparation of ASWC-fatty acid complexes, the control (ASWC+non) could form the compact structure of crystallites and showed 58% of the maximum degree of hydrolysis. After the addition of fatty acids, the observed C_∞ was higher than the control. The fatty acid forms single inclusion helix with elongated AP chains. Thus the formation of AP-double helices is interrupted. In general, starch-fatty acid complexes retard the starch retrogradation. The fatty acids contribute to less ordered structures by interfering the crystallization of AP double helices. As a results, addition of fatty acids

increased the starch susceptibility to enzymatic hydrolysis.

5.2. LOS plot of starch-lipid complexes

Figure 5 and 6 display LOS plots of NC and ASWC complexes with fatty acids, respectively. The kinetic parameters of complex samples estimated by LOS plot are summarized in Table 6. Furthermore, the contents of RDS, SDS and RS estimated using the parameters from the LOS plot are shown in Table 7.

LOS plot of NC+non showed a single line (Figure 5A), supported by the high coefficient of determination (R^2=0.9944). It implied that this sample hydrolyzed at the same rate during the hydrolysis. Calculated C_∞ obtained according to the LOS linear equation closely agreed with the measured degree of hydrolysis value (Table 6). Gallant et al. (1992) suggested that the hydrolysis of starch predominantly occurs in the amorphous regions of the granule. Most of NC+non was the digestible amorphous region. Thus, its rapid and singular digestion rate can be understood by the high amount of RDS estimated using the LOS plotting (Table 7). However, the intersecting lines was observed in the LOS plots of NC+MA and NC+PA indicating a discontinuity around 20-24 min of digestion time (Figure 5B and 5C,

respectively). This observation demonstrated that normal corn starch-fatty acid complexes are digested in two separate phases. These lines indicated rapid and slow phases of hydrolysis with the considerably different rate constants. For the NC+MA, the rate constant was 0.2266 for the rapid phase and 0.1691 for the slow phase. Similarly, that of NC+PA indicated 0.1691 and 0.0227 for the rapid and slow phase, respectively. The formation of crystallites caused by type I and type II complexes results a starch resistant to enzymatic hydrolysis. Thus, the digestion rate decreased. The final C_∞ of NC+MA and NC+PA calculated by LOS plot were 81.75% and 71.45% agreed well with the measured data (77.37% and 73.65%, respectively) despite the low determination coefficient of slow phase (R2=0.6938 and 0.7245, respectively). The estimated content of RDS decreased and that of SDS and RS increased (Table 7). The amorphous region which attributes to the first step hydrolysis of starch-lipid complex decreased and crystalline region increased by complexation (Guraya et al., 1997;Putseys et al., 2010).

LOS plots of all ASWC samples displayed two distinct lines with different rate constants. ASWC+non showed different rate constants around 75 min of digestion time (Figure 6A): k of rapid phase was 0.0343, and that of slow phase was 0.0047 with a high determination coefficient (R^2=0.8075 and R^2=0.9041, respectively). The rate constant of rapid phase

(0.0343) was significantly lower than other samples, which was reflected in low contents of RDS (Table 7). The AP-double helices and that of the crystalline region (Table 5) contributed to slow rate of digestion. However, the addition of fatty acid affected the rate of rapid phase hydrolysis. The rate constant of a rapid phase is increased by 10-fold from the control, and the time of intersecting was shortened by 1 h.

Digestion process of NC+non was described by a single rate constant, and the other samples (NC+fatty acid samples and ASWC samples) showed two rate constants that were significantly different from each other. The addition of fatty acids mainly affected the changes in rapid phase constant, while that of slow phase changed slightly. The decreased rate constant indicates slower digestion rate with a gentle slope, whereas increasing digestion rate involves a steep slope formed by the increases in rate constant increases (Patel et al., 2014). The rate constants categorized into two groups; k_{RDS}, and k_{SDS}. k_{RDS} reflects the first phase of rapid digestion while the k_{SDS} follows the slow digestion phase. These groups were clearly distinguished because k_{RDS} and k_{SDS} had the value from the first and second decimal place, respectively.

In the case of NC samples, the content of RDS estimated using the LOS plot decreased approximately 15% by the addition of fatty acids, and that of

SDS and RS increased (5% and 10% respectively) with 10% decrease of C_∞. It was observed that the time of plateau was delayed 10 min from that of control (NC+non). It means that more time required for hydrolysis of a lower amount of starch. The addition of fatty acids to ASWC increased RDS and SDS fractions from 46.03% to 56.72% and from 12.45% to 15.55%, respectively, while the content of RS was reduced from 41.52% to 29.26%. The C_∞ increased with addition of fatty acids while the time of plateau was same each other. Under the same time, increasing of C_∞ indicate that the digestibility of ASWC increased after addition of fatty acids. A decreasing of RS was observed in contrast to the increases in the contents of RDS and SDS. On the other hand, the amounts of amorphous region and single inclusion complexes increased.

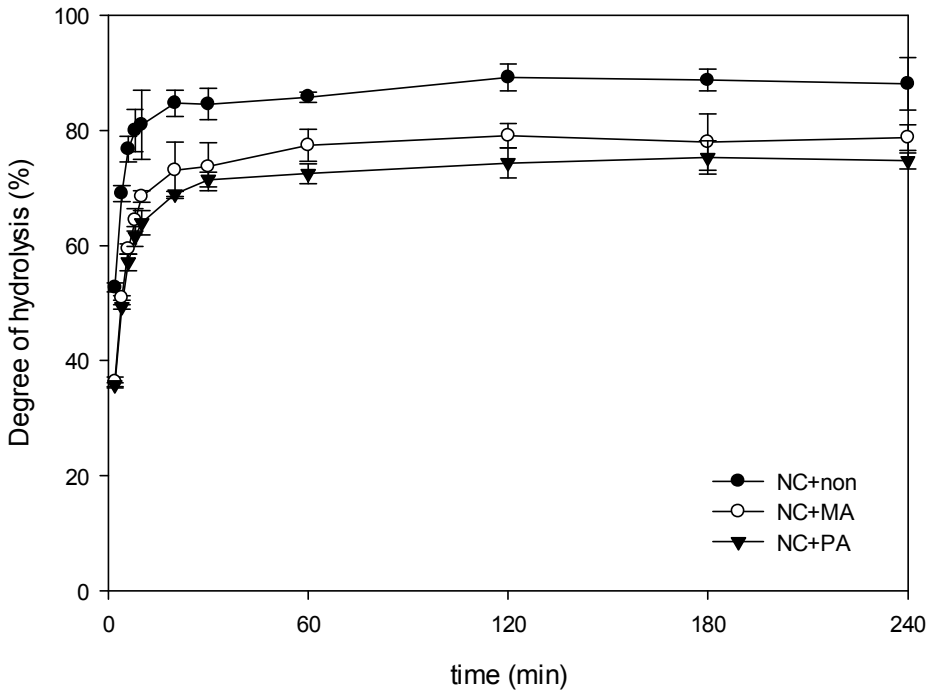

Figure 3. Degree of hydrolysis pattern of normal corn starch (NC) -fatty acid complexes. NC+non = NC without fatty acid; NC+MA = NC with myristic acid; NC+PA = NC with palmitic acid.

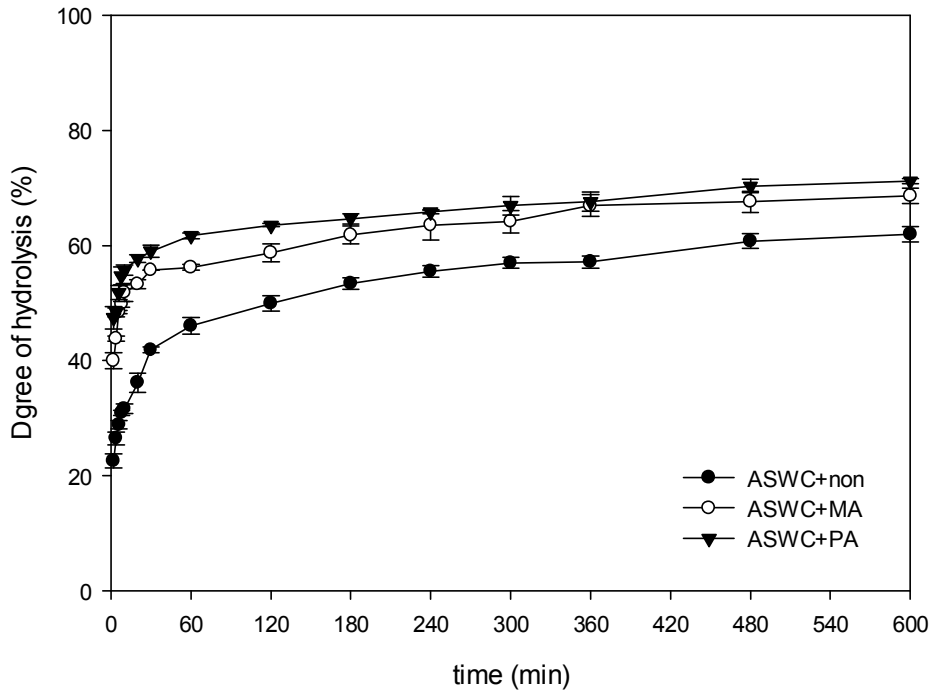

Figure 4. Degree of hydrolysis pattern of amylosucrase-treated waxy corn starch (ASWC- fatty acid compelxes. ASWC+non = ASWC without fatty acid; ASWC+MA = ASWC with myristic acid; ASWC+PA = ASWC with palmitic acid.

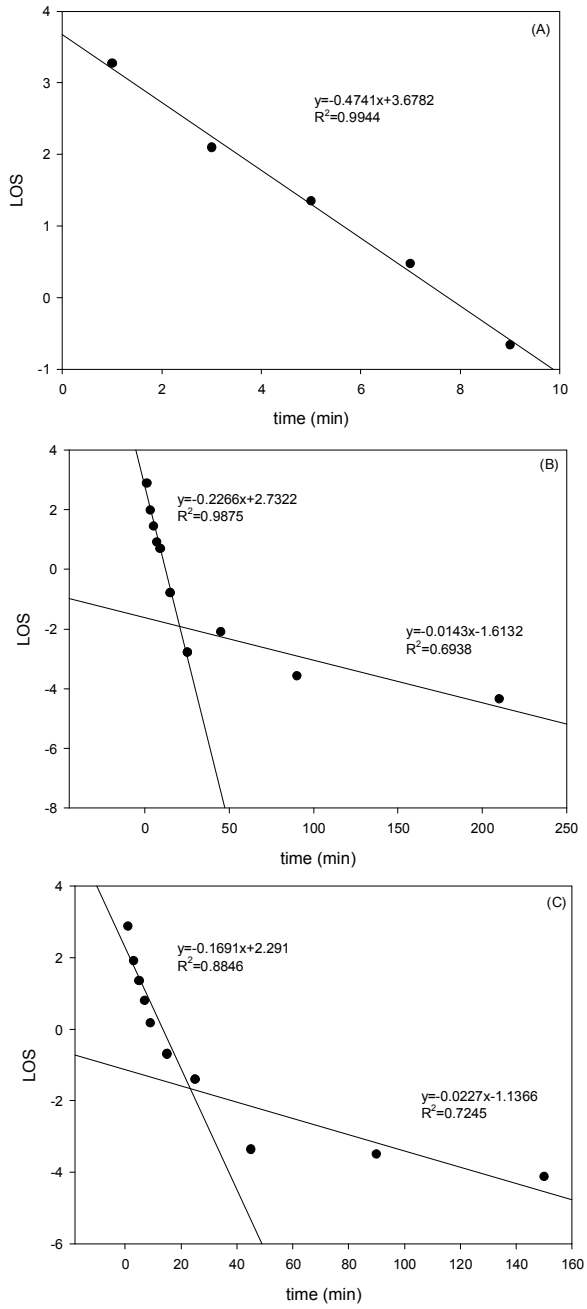

Figure 5. Log of slope plot of normal corn starch (NC)-fatty acid complexes. (A) NC+non = NC without fatty acid, (B) NC+MA = NC with myristic acid, (C) NC+PA = NC with palmitic acid.

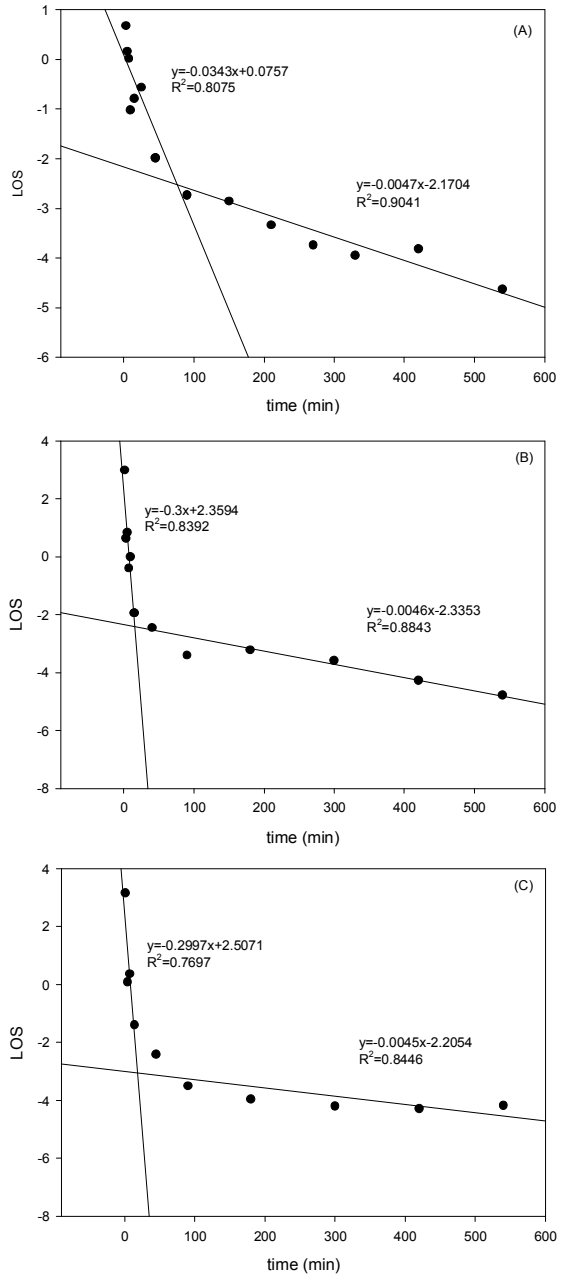

Figure 6. Log of slope plot of amylosucrase-treated waxy corn starch (ASWC)-fatty acid complexes. (A) ASWC+non = ASWC without fatty acid, (B) ASWC+MA = ASWC with myristic acid, (C) ASWC+PA = ASWC with palmitic acid.

Table 6. Hydrolysis kinetics parameters of starch samples estimated from LOS plot

Sample[1)]	k_{RDS}[2)](min^{-1})	k_{SDS}(min^{-1})	Time of intersect[3)] (min)	Time of plateau[4)] (min)	Calculated C_∞[5)] (%)	Measured C_∞ (%)
NC+non	0.4741	N.D.[6)]	N.D.	20	83.47	85.24
NC+MA	0.2266	0.0143	20.47	30	81.75	77.37
NC+PA	0.1691	0.0227	23.41	30	71.45	73.65
ASWC+non	0.0343	0.0047	75.88	480	55.73	58.48
ASWC+MA	0.3	0.0046	15.89	480	56.32	65.4
ASWC+PA	0.2997	0.0045	15.96	480	65.43	67.75

[1)] NC+non = normal corn starch without fatty acid; NC+MA = normal corn starch with myristic acid; NC+PA = normal corn starch with palmitic acid; ASWC+non = amylosucrase-treated waxy corn starch without fatty acid, ASWC+MA = amylosucrase-treated waxy corn starch with myristic acid; ASWC+PA = amylosucrase-treated waxy corn starch with palmitic acid.

[2)] k_{RDS} = rate constant for the RDS phase of starch hydrolysis; k_{SDS} = rate constant for the SDS phase of starch hydrolysis.

[3)] Time of intersect = the time when the two linear graphs of RDS and SDS intersect.

[4] Time of plateau = the time when the degree of hydrolysis reached plateau, revealing no more significant changes.

[5] C_∞ = the maximum degree of hydrolysis

[6] N.D. = not detected.

Table 7. Contents of RDS, SDS, and RS1) of starch samples estimated using the LOS plot

Sample[2)]	RDS (%)	SDS (%)	RS (%)	Measured C_∞[3)] (%)
NC+non	85.24	N.D.[4)]	14.76	85.24
NC+MA	73.06	4.31	22.63	77.37
NC+PA	68.97	4.68	26.35	73.65
ASWC+non	46.03	12.45	41.52	58.48
ASWC+MA	52.54	15.55	31.91	65.4
ASWC+PA	56.72	14.02	29.26	67.75

1) RDS = rapidly digestible starch; SDS = slowly digestible starch; RS = resistant starch.

2) NC+non = normal corn starch without fatty acid; NC+MA = normal corn starch with myristic acid; NC+PA = normal corn starch with palmitic acid; ASWC+non = amylosucrase-treated waxy corn starch without fatty acid, ASWC+MA = amylosucrase-treated waxy corn starch with myristic acid; ASWC+PA = amylosucrase-treated waxy corn starch with palmitic acid.

3) C_∞ = the maximum degree of hydrolysis.

4) N.D. = not detected.

CONCLUSION

With the AS-treatment, the proportion of short chains decreased, while that of long chains increased due to the elongation of external chains in AP molecules. Further, the apparent amylose content significantly increased similar to that of NC due to the elongation of AP branch-chains. Elongated branch chains had iodine binding capacity implying the ability of complexation with fatty acids in AP-rich starch owing to AM-like properties. Particularly, the ASWC formed more complexes with lipid than NC starch did. Because the elongated bundle of AP chains led to a higher chance to combine with fatty acids than amylose of NC did. In the case of NC, the relative crystallinity, melting temperature, and melting enthalpy increased with the addition of fatty acid. It is due to the ordered structure caused by the formation of type I and type II complexes. Thereby, the *in vitro* digestibility and k_{RDS} decreased with increasing SDS and RS contents indicating low digestibility of starch. After the formation of an ASWC-lipid complex, the X-ray diffraction pattern was changed to V-type from an amorphous pattern. With addition of lipid to ASWC, the formation of double helices was disturbed by complexed fatty acid, which led to the decreases in relative crystallinity, melting temperature, and melting enthalpy, but the increases *in*

vitro digestibility and k_{RDS}.

Therefore, AS treatment could make waxy corn starch as a prospective delivery system for fatty acids and provide a basis to utilize the ASWC complexed with fatty acid. It is suggested that the AS-treated starches complexed with fatty acids have a potential to be used as low-glycemic response food ingredient and a flavor delivery compound in novel and healthy food products. Further studies are required to assess the effects of ligand structure (monoacylglyceride or aromatic compound), rheological properties for food additives, and *in vitro* digestibility, and released properties for application of ASWC as delivery material.

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국문 초록

지방산과 같은 리간드 존재 시에 아밀로스는 그 형태를 변화해 아밀로스-지방산 내제 복합체를 형성한다. 그러나 찰 녹말은 아밀로펙틴의 짧은 가지 사슬과 입체적 장애 때문에 지방산 과의 복합체 형성이 어렵다. 이 연구에서는 메옥수수 녹말과 amylosucrase (AS) 를 처리한 찰옥수수 녹말에 지방산(미리스트산(C14:0)과 팔미트산(C16:0))을 녹말의 5%만큼 처리하여 복합체를 형성하였다. 이 연구의 목적은 기존에 알려진 메옥수수 녹말-지방산 복합체와 효소적 변형으로 얻은 찰 녹말-지방산 복합체의 함량, X-선 회절 및 상대적 결정화도, 열 특성, 그리고 소화율에 미치는 영향을 알아보는 것이었다.

AS 처리에 의해 찰옥수수 녹말의 아밀로펙틴 가지 사슬 길이가 증가하였고, 겉보기 아밀로스 함량은 메옥수수 녹말의 그 양과 비슷하게 증가하였다. AS 처리 찰옥수수 녹말은 메옥수수 녹말과 비교하여 복합체 함량이 더 많았다. 이 결과는 효소적 변형으로 연장된 찰 녹말의 아밀로펙틴 사슬이 다발로 존재 하기 때문에, 지방산과 복합체를 이루는데 더 유리함을 보여준다. 녹말-지방산 복합체

의 X-선 회절도형은 V 형을 나타내며 복합체가 형성되었음을 확인하였다. 메옥수수 녹말-지방산 복합체는 type I 과 type II 복합체가 형성 되었고 이에 따라 결정형 구조가 형성되었다. 따라서 상대적 결정화도, 용융 온도, 용융 엔탈피는 증가하였고 소화율은 감소하였다. 반면 AS 처리한 찰옥수수 녹말 복합체의 지방산은 연장된 가지 사슬이 형성한 이중 나선 구조를 해리시킴에 따라, 지방산이 첨가되지 않은 시료에 비해 상대적 결정화도, 용융 온도, 용융 엔탈피는 감소하였고 소화율은 증가하였다. Log of slope 법으로 소화성의 가수분해 곡선을 미분하여 1차식으로 나타내었고, 이를 이용하여 샘플의 소화속도를 분석하였다. 메옥수수 녹말 복합체의 소화 속도 상수 (k_{RDS}) 는 지방산 복합체 형성 시 감소하며 느린 소화 성을 보였고, AS 처리 찰옥수수 녹말 복합체는 지방산 복합체 형성시 그 값이 증가하며 지방산이 첨가되지 않은 시료와 비교했을 때 더 빠르게 가수분해 될 수 있음을 확인했다. 이 또한 복합체의 구조적 특성으로 설명 할 수 있으며, 메옥수수 녹말의 경우 결정형 영역이 증가하며 낮아진 소화 성을, AS 처리 찰 녹말은 지방산이 연장된 아밀로펙틴 사슬의 결정형 구조를 해리시키며 증가된 소화 성을 보인다. 메옥수수 녹말과 AS 찰 녹말을 비교해 볼 때,

AS 찰 녹말은 메옥수수 녹말 보다 지소화성 획분의 양이 많아 더 느리게 소화됨을 알 수 있다.

이 실험을 통해, 상대적 결정화도, 열 특성, 소화 특성에서 AS 처리 찰 녹말-지방산 복합체가 메옥수수 녹말-지방산 복합체와는 이화학적 특성이 반대 경향을 보임을 확인하였고, 소화 성 또한 메옥수수 녹말에 비해 낮음으로써 저 소화 성 복합체 소재로 이용될 수 있음을 제안 할 수 있다. 나아가 AS처리 찰 녹말-지방산 복합체는 그 형성률이 높음에 따라 유용 물질의 전달 매개체로 사용될 수 있음을 제안하였다.

주요어 : 메옥수수 녹말, 아밀로수크레이스, 찰옥수수 녹말, 녹말-지방산 복합체, 소화율, LOS plot, 이화학적 특성

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