Effect of Dual Modification with Succinylation and Annealing on Properties of Corn Starch

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(サクシニル化およびアニーリングの二重処理がコーンスターチの物性に及ぼす影響)

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Acknowledgment

ABSTRACT

Starch is one of the essential components in the food industry. However, the native starch has some disadvantages related to the functional properties. Native starches have some weaknesses, such as low stability on high temperature and shear stress, thermal decomposition, and high retrogradation tendency. The starch modification could overcome the weaknesses of native starch. Succinylation process can increase consistency and annealing process can increase stability on heating and shearing, but no research report about this dual combination process. Thus, the objective of this research is to investigate the effect of succinylation, annealing and dual modification on the physicochemical, thermal and morphological properties of corn starch. The study is divided into two parts.

The first part focused on the effects of different concentration of succinic anhydride on the physicochemical, pasting and morphological properties. Corn starch was modified with different concentration of succinic anhydride (2, 4 and 6% w/w). Physicochemical properties (water binding capacity, paste clarity, swelling power, and solubility) were investigated. RVA measurement was used to determined stability ratio. The change of granule shape was investigated with a light microscope. Succinylation process with 2% succinic anhydride increased the paste clarity and swelling power of the starch. Succinylation with over 2% decreased solubility, swelling power, and paste clarity, while it has no effect on water binding capacity, stability ratio, PV, and changed of granule shape compared with the native starch. These results suggest that the low paste clarity properties in native can be overcome through succinylation process with 2% succinic anhydride. However, it could not overcome the decrease in viscosity under heat and shear stress.

The second part of this study highlighted the effects of dual modification of succinylation and annealing on the properties of corn starch. The objective was to investigate effects of single annealing, single succinylation and dual modification process (succinylation-annealing) of corn starch on the physicochemical (water binding capacity, swelling power, paste clarity, solubility, pasting properties), thermal and morphological properties. Physicochemical properties (water binding capacity, swelling power, solubility, and paste clarity) were increased after dual modification process. Dual modification process also increased gelatinization temperature and enthalpy of gelatinization, but it is no effect on morphological properties. Dual modification process also increased gelatinization temperature and enthalpy of gelatinization, but it is no effect on morphological properties and X-Ray diffraction patterns. A comparison of samples made using each of the processes showed that dual modification increased the stability ratio (more stable viscosity under thermal and shear stress), which was 0.69 for dual modified starch, compared with 0.64, 0.58, and 0.44 for native, succinylated, and annealed starches, respectively. The findings of the present study are of potential use in the food industry.

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1. Ariyantoro, A.R., Katsuno, N., and Nishizu, T. (2018). Effects of Dual Modification with Succinylation and Annealing on Physicochemical, Thermal and Morphological Properties of Corn Starch. *Foods*. 7 (9), 133. https://doi.org/10.3390/foods7090133.

2. Ariyantoro, A. R., Katsuno, N., and Nishizu, T. (2018). Effect of annealing process on physicochemical, morphological and gelatinization properties of cereal starches. *Reviews in Agricultural Science* (Manuscript in press).

List of Conferences

1. <u>Ariyantoro, A. R.,</u> Katsuno, N., and Nishizu, T. "Effects of dual modification of succinylation and annealing on physicochemical properties of corn starch and potato starch" 64th JSFST - Japanese Society for Food Science and Technology Conference, Kanagawa, Japan 30th August 2017. (Oral Presenter)

2. <u>Ariyantoro, A. R.,</u> Katsuno, N., and Nishizu, T. "Effects of dual modification of succinylation and annealing on physicochemical properties of corn starch and potato starch" 64th JSFST - Japanese Society for Food Science and Technology Conference, Nihon University, Kanagawa, Japan 29th August 2017. (Poster Presenter)

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CHAPTER 1

General Introduction

1.1 Starch

Starch, complex molecules that store mass energy in plants has become an important material in the food industry (Sweedman et al., 2013). It is a substantial of the dietary source in energy for humans and serves as 70-80% of the regular daily caloric intake (Tetlow, 2006). Native starch in the form of white and semi-crystalline granules, which is produced in chloroplasts or amyloplasts of plant organs such as in seeds, stems, tubers, roots, leaves, and fruits (Robyt,1998). It is a one of kind of carbohydrate, which is very important for food industry and non-food industry. In food industry, starch was used as a thickener, making bread and confectionary. Starch is also used in many non-food industries such as textiles, paper, cosmetic and pharmaceutical (Lauro et al., 1999). Starch has characteristic aspects varying in structure, molecular organization, morphological, gelatinization and pasting properties, starch polymorphs, percentage crystallinity, and enzyme digestibility (Jane, 2004).

Starch granule is composed of amylose and amylopectin chain (Martin and Smith, 1995). Amylose is a linear polymer with 1,4-linked D-glucopyranose units (Takeda et al., 1993). In another hand, amylopectin is a highly branched structure, which involves 1,4-linked D-glucopyranose units, and it has branch-linked by ~5% (1,6) glycosidic bonds (French,1984). Amylose and amylopectin give different properties of the starch. Structure of amylose and amylopectin was presented in Figure 1.1.
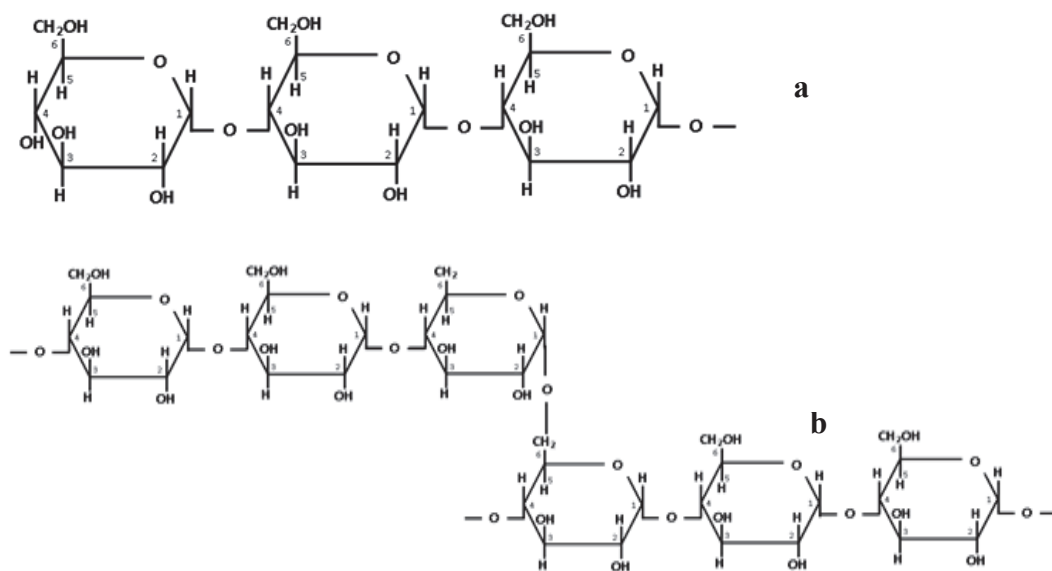
Figure 1.1. Structure of amylose (a) and amylopectin (b). Redrawn from Tester and Karkalas (2002).

1.2. Corn starch

Corn provides a huge amount to produce the starch in the world compare the another botanical sources. Moreover, Waterschoot et al. (2015) have stated that the the corn is the most important sources for producing starches compare with cassava, wheat and potato starch. Also, the starch production in the world is almost 80% come from corn.

Corn starch is one of the A-type cereal starches. It has amylose content approximately 29%, it has higher than rice starch and wheat starch, 25% and 28%, respectively. It has medium paste viscosity, high retrogradation rate and opaque in paste clarity (BeMiller and Whistler, 2009). The corn starch have some weaknesses, one of them is opaque in paste clarity. Starch modification was generally used to overcome the weakness of the native corn starch, especially opaque in paste clarity.

1.3 Starch Modification

Native starch could not be used widely in the industry because of some weaknesses, such as high opacity of cooked paste, high retrogradation tendency, low shear and thermal resistance, thermal decomposition (Jayakody and Hoover, 2008; Hui et al., 2009). The starch modification was used to overcome the undesirable properties of the native starch. The starch modification can change physical and chemical properties of the native starch to increase its functional properties (Hermansson and Svegmark, 1996). The various modification methods used to overcome the weaknesses of native starch. It can be divided into physical, chemical, and enzymatic modification (Zavareze and Dias, 2011). Physical modification leaves no chemical residue or chemical modification, so is safe for consumption (Wang et al., 2014), some examples being pre-gelatinization, heat-moisture treatment, and annealing. There are two hydrothermal treatments; annealing and heat moisture treatment are generally used, which the combination of the moisture with the heating for modifying the thermal and rheological properties of the starch (Stute, 1992; Jacobs and Delcour, 1998; Tester and Debon, 2000). Heat–moisture treatment and annealing process lead to a physical modification of starch without any gelatinization, damage to granular integrity, or loss of birefringence (Abbas et al., 2010).

Chemical modification process was used chemical reagent to react with starch in certain condition process. It can be conducted used by etherification, esterification, cross-linking and grafting of the starch (Hui et al., 2009). It uses a chemical reagent to modify the properties of the starch using processes such as succinylation, acetylation, octenyl succinic acid modification, and hydroxypropylation (Zavareze and Dias, 2011). Moreover, enzymatic modification process utilized enzyme for modifying the starch properties. It involves hydrolysis of any component of starch into a low molecular

weight of starch (maltodextrin or dextrin), which utilizing amylolytic enzymes (Miyazaki et al., 2006).

The annealing process is a physical modification related to moisture, temperature and heating time (Tester & Debon, 2000). It is generally conducted in excess of water (76% w/w) or an intermediate (40% w/w) (Jacobs and Delcour, 1998). Succinylation process is a starch modification method using a chemical reagent such as succinic anhydride, octenyl succinic anhydride and etc. These chemicals reagents react with starch to improve the properties of the starch.

Recently, the dual modification was chosen to modify starch because dual modification gives a better result than single modification. Many methods of dual modification were reported by many researchers. The combining method was used to modify the properties of the starch. The combination method of chemical modification with different types of modifications was used in the dual modification process (Alcá zar-Alay and Meireles, 2015). There are many dual modification process methods to improve the properties of the native starch.

1.4. Succinylation process

Succinylation process is starch modification methods using esterification method. Starch was contacted with a chemical reagent to produce the better properties of the starch. Modification starch with succinylation has been done by Lawal, 2012 on yam starch, Olayinka et al., 2011 on sorghum starch, Chi et al., 2007 on corn starch, Moin et al., 2016 on rice starch, and Bhandari and Singhal, 2002 on corn and amaranth starch.

Succinylation process is an esterification reaction of a hydroxyl groups in the starch molecules with succinic anhydride (Lawal, 2012). The succinylation process was chosen in this research because if it compare with other starch modification using chemical, succinylation process has potency to overcome the weaknesses of the native

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starch. It results in higher viscosity, greater thickening power, and lower retrogradation rate (Olayinka et al., 2011). Moreover, the succinylation process of starch modification provide many advantages such as high solubility, high viscosity, better thickening power, improved paste clarity, retarded retrogradation and freeze-thaw stability (Moin et al., 2016). However, it has the disadvantages of instability during shearing at high temperatures (Ačkar et al., 2015), thus it needs another starch modification to overcome the disadvantages of succinylation process.

1.5. Annealing process

The annealing process was chosen in this research because of its ability to increase the thermal and shear stability of starch (Hoover and Vasanthan, 1994b). Decreased in breakdown viscosity (BD) after annealing has been reported by Dias et al., 2010; Lan et al., 2008; Ali and Hasnain, 2016 and Singh et al., 2011. Low BD value mean that high shear resistance and temperature of the starch (Corke et al., 1997). From this reason, annealing process has potency to overcome the weakness of the succinylated starch regarding with instability viscosity during shearing at high temperatures.

Unlike succinylation, annealing is a method of physically modifying starch. This process is widely used because it does not leave a residue as does the chemical process (Wang et al., 2014). This process modifies the starch without damaging the granules (Dias et al., 2010, Rocha et al., 2012; Singh et al., 2011). Annealing process has been shown to decrease swelling power in a number of sources of starch (Wang et al., 2014; Waduge et al., 2006; Hoover and Vasanthan, 1994a; Dias et al., 2010; Hormdok and Noomhorn, 2007; Adebowale et al., 2005a; Gomes et al., 2004 and Ali and Hasnain, 2016).

The annealing process modifies starch physically to intermediate moisture content (40 55% w/w) or excess water (> 60%) at above the glass transition temperature (Tg)

but below the gelatinization temperature (Jacobs and Delcour, 1998). Studies on the various water to starch ratios ranged from those using an excess of water to water to starch ratios of 2:1, 3:1, and 10:1 and adjusting the moisture content of the samples to 75%. The annealing process with cereal starches using various temperatures: 55 °C, 50 °C, and 45 °C, below 45 °C, and above 55 °C. The process times on the annealing process in cereal starches ranged from 24 h (1 day) to 168 h (7 days) and also included 48 and 72 h, below 24 h and above 72 h.

Many studies have investigated the effect of the annealing process on modifying different types of cereal starch (Krueger et al., 1987a, 1987b; Knutson, 1990; Shi and Seib, 1992; Seow and Teo, 1993; Hoover and Vasanthan, 1994a, b; Jacobs et al., 1995, 1996; Jacobs and Delcour, 1998; Tester et al., 2000b; Qi et al., 2004; Adebowale et al., 2005a; Kiseleva et al., 2005; Kohyama and Sasaki, 2006; Waduge et al., 2006; Lan et al, 2008 O'Brien and Wang, 2008; Liu et al., 2009a, 2009b; Chung et al., 2009; Dias et al., 2010; Singh et al., 2011; Rocha et al., 2012; Vamadevan et al., 2013; Wang et al., 2014, 2017; Ali and Hasnain, 2016).

1.5.1. Effect of annealing on the amylose content

Starch consists of amylose and amylopectin, which have linear chains and highly-branched chains, respectively (Alcázar-Alay and Meireles, 2015). Some studies have shown that the annealing process did not change the amylose content of maize starch (Knutson, 1990; Liu et al., 2009 and Rocha et al., 2012; Wang et al., 2014). Wang et al. (2014) reported that the annealing process did not significantly change the amylose content of maize starch. Wang et al. (2014) suggested that the annealing process modified starch using a purely physical process, which did not lead to any leaching of amylose. Rocha et al. (2012) have also shown that gelatinization process did not occur during the annealing process.

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However, other studies have reported contrasting findings on the effect of the annealing process in decreasing the amylose leaching. It was determined with heating starch suspension in the above gelatinization temperature. Then, it was cooled to room temperature and centrifuged. The supernatant was used to measure the amylose content. Amylose leaching is the amount of amylose leached per 100 g of dry starch (Chung et al., 2009). Several studies have found that annealing led to amylose leaching rice starch (Jacobs et al., 1995), wheat starch (Hoover and Vasanthan, 1994a; sorghum starch (Singh et al., 2011), oat starch (Hoover and Vasanthan, 1994b; Jacobs et al., 1995), and barley starch (Waduge et al., 2006). It has been suggested that amylose leaching after annealing is caused by the greater interaction between amylose-amylose and/or amylose-amylopectin chains (Hoover and Vasanthan, 1994a). The restructuring of the starch molecules leading to a stable structure also causes a decrease in amylose leaching (Zavareze and Dias, 2011).

Another type of effect has been reported only by Chung et al. (2009) who found that the annealing process could slightly increase the amount of amylose leaching from maize starch. However, Chung et al. (2009) provided no explanation of this result. The effect of the annealing process on amylose content or amylose leaching in different types of cereal starches has been very controversial. Research studies show three different effects of the annealing process on amylose content or amylose leaching: no effect, and a decreased or increased amylose leaching. Thus, the annealing process can increase or decrease or not affect the amylose content or amylose leaching of different types of cereal starch depending on the conditions of the annealing process and the different sources of cereal starch.

1.5.2. Effect of annealing on swelling power

Measuring the swelling power indicates the level of interplay between starch chains

in the crystalline and amorphous regions of the native granule (Lan et al., 2008). Generally, the annealing process can decrease the swelling power in maize starch (Wang et al., 2014), barley starch (Waduge et al., 2006), wheat starch (Hoover and Vasanthan, 1994a), rice starch (Hormdok and Noomhorn, 2007; Dias et al., 2010), oat starch (Hoover and Vasanthan, 1994b), and sorghum starch (Adebowale et al., 2005a; Singh et al., 2011; Ali and Hasnain, 2016). The annealing treatment of maize starch resulted in a lower swelling power compared with that of native starch. Improving the long-range forces between amylopectin chains can lead to the rearrangement of amylose molecules. This prevents the free swelling of amylopectin molecules and results in a lower swelling power (Wang et al., 2014). Another study by Waduge et al. (2006) reported a decrease in the swelling power of barley starch after annealing, possibly because of the interactions of crystalline perfection and interplays between amylose-amylose during the annealing process.

Several factors influence swelling power: the structure of amylopectin (Shi and Seib, 1992; Sasaki and Matsuki, 1998), complexes of V-amylose lipid (Tester and Morrison, 1990), amylose content (Morrison et al., 1993; Tester et al., 2000a), an increase in crystalline perfection (Waduge et al., 2006; Singh et al., 2011), an increment in molecular organization (Gomes et al., 2004), and the level of interaction between amylose-amylose and/or amylose-amylopectin chains (Hoover and Vasanthan, 1994b; Hoover and Manuel, 1996; Eerlingen et al., 1997; Tester et al., 2000a; Gunaratne and Hoover, 2002; Waduge et al., 2006). Chung et al. (2009) found different results from others, reporting that the swelling factor increased slightly in maize starch after annealing, but no explanation was given.

1.5.3. Effect of annealing on pasting properties

Studies on the effect of the annealing process on pasting properties have included

several types of cereal starch: maize starch (Wang et al., 2014), wheat starch (Lan et al., 2008), sorghum starch (Adebowale et al., 2005a; Singh et al., 2011; Ali and Hasnain, 2016) and rice starch (Dias et al., 2010). According to Wang et al. (2014), the form of the amylograph curve did not change after annealing maize starch. There was a decrease in the viscosities (peak, through and final viscosity) of the maize starch after annealing. This could have been caused by the prevention of swelling of the annealed starch as a consequence of the improved reinforcement of the amylopectin groups by the realignment of amylose. Lan et al. (2008) also reported that the peak, breakdown, final, and setback viscosities of wheat starch decreased after the annealing process. The decrease in the swelling factor and amylose leaching were associated with the decreased peak and setback viscosities after the annealing process.

The annealing process decreased the peak viscosity in sorghum starch and was correlated with its swelling power (Singh et al., 2011). A reduction in the peak viscosity was caused by inter- and intra-molecular hydrogen bonding in the starch chains (Hoover and Vasanthan, 1994c). They also found that the annealing process could decrease the through and final viscosities of the starch. This was consistent with findings by Dias et al. (2010), who showed that annealing could decrease the final viscosity of rice starch.

The value of the breakdown viscosity indicates the stability of the starch during heating and agitation (Singh et al., 2011). Annealed starch exhibited a lower breakdown viscosity than native starch (Lan et al., 2008, Dias et al., 2010, Singh et al., 2011; Ali and Hasnain, 2016). This decrease in breakdown viscosity is associated with a decrease in granular swelling and amylose content (Lan et al., 2008; Singh et al., 2011). The annealing process reduced the setback viscosity in sorghum and rice starches (Singh et al., 2011; Dias et al., 2010) because of several factors such as the extent of amylose leaching, granule size, and length of the amylose chain (Singh et al., 2011).

However, some authors have postulated that the annealing process can increase the

viscosity of starch (Jacobs et al., 1995; Wang et al., 2013, 2017). In rice and wheat starch, the shape of the pasting profile exhibits a slightly increased viscosity after annealing (Jacobs et al., 1995). Wang et al. (2013, 2017) found an increase in the viscosity of wheat starch after annealing which can occur because of the enhanced rigidity and a reduction in deformability (Wang et al., 2017). This was also associated with an increment in the stability of the annealed starch during heat and shear condition (Jacobs et al., 1995). The higher stability has meant that starch more resistant to heat and shear stress. It is indicated by low breakdown viscosity value (Corke et al., 1997).

The different temperatures during annealing affected the pasting properties of wheat starch: annealing at 30 °C and 40 °C increased the overall paste viscosity. The annealing process at 50 °C also decreased the whole paste viscosity and increased the pasting temperature (Wang et al., 2017). This finding was consistent with that of Dias et al. (2010), who showed that annealing at a higher temperature (55 °C) decreased the peak viscosity of high-amylose starch. However, annealing at a lower temperature (45 °C and 50 °C) increased the peak viscosity of low-amylose starch. The increase in peak viscosity after annealing was not caused by limited granular swelling in starch. Annealing also increased the breakdown viscosity suggesting that a lower level of stability during heating (Adebowale et al., 2005a). Hormdok and Noomhorn (2007) also found that the annealing process increased the breakdown viscosity of rice starch.

Annealed starch exhibits a higher pasting temperature compared with native rice starch. It also strengthens the bonds, so requires a higher temperature to gelatinize (Gomes et al., 2004). Hormdok and Noomhorn (2007) found that the annealing process increased the setback viscosity of rice starch. The different effects of annealing on the pasting temperature have been related to the structural characteristics of the starch and the conditions of analysis (Jacobs et al., 1996; Dias et al., 2010).

1.5.4. Effect of annealing on retrogradation properties

Only a few studies have reported on the effect of the annealing process on retrogradation properties: Lan et al. (2008) on wheat starch; and Singh et al. (2011) and Ali and Hasnain (2016) on sorghum starch. Lan et al. (2008) postulated that the annealing process decreased the percentage transmittance of wheat starch, possibly attributed to a decrease in swelling power. Based on differential scanning calorimetry (DSC) data, they suggested that the annealing process could increase crystalline perfection and that the range of disconnection and unfolding of the double helices during gelatinization would be slower. Therefore, the formation and lateral association of the unfolded double helices would be faster in the annealed starch than in native starch during storage of the starch gel (Lan et al., 2008).

Retrogradation properties can also be determined using DSC measurements. Singh et al. (2011) showed that after the annealing process, the onset (To), peak (Tp), and conclusion temperatures (Tc), and enthalpy of retrogradation ($\Delta Hret$) were significantly higher compared with native starches. This implied that the crystalline structure of the annealed starches had been rearranged more than that of the native starch. During annealing, the rearrangement of the double helix between adjacent amylopectin is slower and less broad than in native starch (Singh et al., 2011).

Different results have been found for annealed sorghum starch, where annealing increased the paste clarity compared with native starch (Ali and Hasnain, 2016). This increased clarity was correlated with decreased values of amylose leaching and solubility. Amylose with higher levels of leaching and amylopectin interaction resulted in higher turbidity values of starch pastes caused by the arrangement of junction zones (Perrera and Hoover, 1999).

1.5.5. Effect of annealing on morphological properties

Many studies have investigated the effects of the annealing process on granule morphology including maize starch (O'Brien and Wang, 2008; Rocha et al., 2012; Wang et al., 2014), barley starch (Waduge et al., 2006), sorghum starch (Singh et al., 2011; Ali and Hasnain, 2016), wheat starch (Kiseleva et al., 2005; Lan et al., 2008; Wang et al., 2017) and rice starch (Dias et al., 2010). Generally, most of these studies found that the annealing process did not change the granule morphology of starch compared with that of native starch.

Wang et al. (2014) reported that the granule morphology of maize starch did not change significantly after annealing treatment. The granules showed a variety of shapes: small spherical granules and large polyhedral granules with an average particle size between 3 and 20 µm. Rocha et al. (2012) also observed no differences after annealing in the shape of maize starch granules which were round and polyhedral. Annealed starch has pores, and the pore size was greater than that of native starch. The presence of a small amount of reducing sugar on the annealed starch indicated the activity of endogenous amylase which produced a greater number of pores (Rocha et al., 2012). These findings agreed with those of Dias et al. (2010), who found that annealing caused pores on the surface of granules of rice starch.

Another study by Waduge et al. (2006) showed similar results where annealing did not change the shapes and pore size of barley starch granules which contained a mixture of large granules (rounded, spherical, and lenticular-shaped) and small irregularly-shaped granules. The annealing process also did not change the shape of wheat starch granules where both native and annealed wheat starch ranged in shape from oval to elliptical to spherical (Lan et al., 2008).

However, there are some contrasting findings. Wang et al. (2017) showed that the annealing process at 50 °C changed the granules of wheat starch: the annealed starch

was damaged and fused compared with granules of native wheat starch, agreeing with a previous study (Singh et al., 2011). After annealing, the starch surface appeared coarse and grooved compared with native granules. Dias et al. (2010) also noted that annealed rice starch granules were larger than the native granules.

1.5.6. Effect of annealing on starch crystallinity

In general, starch exhibits three kinds of X-ray diffraction pattern. Type-A is generally found in cereal starches and type-B in tuber and high-amylose starches. Type-C, a mixture of the A and B type is found in legume starches and some tuber starches such as yam (Jayakody and Hoover, 2008; Wang et al., 2017). Other studies on the effect of the annealing process on starch crystallinity have included maize starch (O'Brien and Wang, 2008; Liu et al., 2009; Rocha et al., 2012; Wang et al., 2014), wheat starch (Lan et al., 2008; Wang et al., 2017); barley starch (Waduge et al., 2006); rice starch (Dias et al., 2010) and sorghum starch (Singh et al., 2011). These studies mostly found that the annealing process did not change the type of crystallinity of cereal starches compared with the native starch. Native cereal starches and annealed cereal starches have A-type crystallinity (Waduge et al., 2006; O'Brien and Wang, 2008; Liu et al., 2009b; Dias et al., 2010; Singh et al., 2011; Rocha et al., 2012; Wang et al., 2014, 2017).

Although annealing does not change the type of crystallinity pattern compared with native cereal starch, it had a different effect on relative crystallinity. In general, relative crystallinity is calculated as the percentage total crystallinity area divided by the total area of the XRD graph data. Wang et al. (2013, 2014) found that the relative crystallinity of maize starch did not change significantly after annealing because the annealing process occurred only in the amorphous regions so had no effect on the crystalline region. Wang et al. (2017) also reported that annealing wheat starch at 30 °C

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or 40 °C did not change the relative crystallinity.

Another different effect of the annealing treatment on starch crystallinity, increasing the relative crystallinity, was reported by Waduge et al. (2006) in barley starch, Lan et al. (2008) in wheat starch, and Rocha et al. (2012) in maize starch. Annealing could increase the relative crystallinity compared with native starch because of several factors: the amount of amylopectin, changes in the size and reorientation of starch crystallites, crystallite perfection, the improved ordering of the crystalline V amylose-lipid complex, and the formation of new crystallites (Waduge et al., 2006; Lan et al., 2008).

In contrast, Wang et al. (2017) showed that annealing wheat starch at 50 °C could reduce the crystallinity from 22.6% to 14.3%. Dias et al. (2010) also showed that the annealing process could decrease the relative crystallinity. This reduction was related to the disruption of the starch crystallites (Wang et al., 2017 and Dias et al., 2010).

1.5.7. Effect of the annealing on the gelatinization properties

The gelatinization properties of starch can be determined using DSC measurements of To (onset temperature), Tp (peak temperature), Tc (conclusion temperature), and ΔH (gelatinization enthalpy). Many studies have investigated the effect of the annealing process on the gelatinization properties of various types of cereal starch such as maize starch (Krueger et al., 1987a, b; Shi and Seib, 1992; O'Brien and Wang, 2008; Chung et al., 2009; Liu et al., 2009; Wang et al., 2014), wheat starch (Hoover and Vasanthan 1994a; Jacobs et al., 1995, 1998a, b, Lan et al., 2008, Wang et al., 2017), barley starch (Shi and Seib, 1992; Qi et al., 2004; Waduge et al., 2006; Vamadevan et al., 2013), rice starch (Shi and Seib, 1992; Jacobs et al., 1995; Hormdok and Noomhorn, 2007), oat starch (Hoover and Vasanthan, 1994b ;Vamadevan et al., 2013), rye starch (Vamadevan et al., 2013), and sorghum starch (Singh et al., 2011). Generally, the annealing process increased the values of To, Tp and Tc in all sources of cereal starch because of the

perfection of the pre-existing crystallites (Knutson, 1990; Hoover and Vasanthan, 1994b; Jacobs and Delcour, 1998; Tester et al., 1998, 2000; Waduge et al., 2006).

Regarding the enthalpy of gelatinization *(ΔH)* properties, the studies provided varying results. Some studies found that the annealing process had no effect on the enthalpy of gelatinization of maize starch (Tester et al., 2000b; Chung et al., 2009; Wang et al., 2014), barley starch (Waduge et al., 2006), rice starch (Hormdok and Noomhorn, 2007), and wheat starch (Lan et al., 2008). No change in the enthalpy of gelatinization (ΔH) in the barley starch after annealing indicated that no new double helices had been formed (Waduge et al., 2006).

Other studies found that annealing increased the enthalpy of gelatinization *(ΔH)* of maize starch (Krueger et al., 1987a, b; O'Brien and Wang, 2008), wheat starch (Jacobs et al., 1995, 1998a; Liu et al., 2009), barley starch (Qi et al., 2004; Vamadevan et al., 2013), oat starch (Hoover and Vasanthan, 1994b, Vamadevan et al., 2013;), sorghum starch (Singh et al., 2011), rice starch (Shi and Seib, 1992) and rye starch (Vamadevan et al., 2013).

After annealing, the number of double helices remained unchanged but the crystalline order improved (Tester et al., 1998 and Qi et al., 2004). Annealing could improve crystalline order from less perfection order of double helices into an optimized crystalline registration without forming new double helices (Qi et al., 2004). Enhancing the crystalline order needs more heat energy to gelatinize the starch so that the enthalpy of gelatinization becomes high. In another study, Singh et al. (2011) found that the annealing process increased the enthalpy of gelatinization because the interaction between the double helices of adjacent amylopectin chains in the crystalline region produced a stable structure.

Contrasting results were found by Wang et al. (2017) on wheat starch and Shi and Seib (1992) on maize and barley starch. Wang et al. (2017) suggested that the reduction

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in the enthalpy of gelatinization for the annealing process at 50 °C could affect the ordered structure based on XRD and FTIR measurement, but no explanation of the mechanism by which the annealing process decreased the enthalpy of gelatinization was provided.

1.6. Properties of the starch

1.6.1 Physicochemical properties of the starch

The physical and chemical properties of starch granules are valuable in specifying their end use (Lindeboom et al., 2004). Properties of starch are influenced by different kind of starches. Physicochemical properties of the starch consist of swelling power, solubility, water binding capacity, paste clarity and etc. The swelling power determined as weight ratio of starch under heating with water to weight of starch sample. The swelling power of starch is related with the amylopectin content because of the amylose roles as an inhibitor of swelling (Singh et al., 2013). Solubility is an important aspect of the physicochemical properties of the starch. Heating process of the starch in hot water leads to the leaching out of swollen starch granules, which resulted in the solubility of the starch (Liu et al., 2014).

The transparency of cooked paste is useful for the certain food industries. The high transmittance value of the starch associated with high paste clarity of the starch. The transmittance of the starch pastes indicated the complete swell of granules and correlated with the starch solubility (Liu et al., 2014). The different paste clarity of the starch depends on the botanical origin of the starch.

The end quality of modified starches and their food application was determined by pasting properties of starch (Martinez et al., 2017). The pasting properties can be investigated using rapid visco analyzer (RVA). This machine can simulate starch under heating and shear stress with a certain condition. From this machine, we can get an

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amylograph curve with many parameters of the pasting properties of the starch. The peak viscosity value of the starch associated with swelling of the starch granule (Chen et al., 2014). The viscosity of the starch paste was influenced by modification method, reaction condition and sources of starch (Gonzalez and Perez, 2002).

1.6.2 Thermal properties of the starch

Thermal properties or gelatinization properties of starch was determined using differential scanning calorimeter (DSC). With the machine, we can get onset temperature (To), peak temperature (Tp), conclusion temperature (Tc) and the amount of energy to gelatinize (ΔH). The definition To is the temperature of the paste viscosity starts to increase; Tp is the maximum viscosity temperature, and Tc is the final temperature of viscosity (Alca´zar-Alay and Meireles, 2015). The different thermal properties of the starch can be influenced by the amylose/amylopectin ratio, the size and shape of starch granule, and lipids and proteins amount (Singh et al., 2003; Tan et al., 2006).

1.6.3 Morphological properties of the starch

Normal starch has semi-crystalline insoluble granules varying in size range from 0.5 to 100 μm and shape (spherical, elliptical, or polyhedral) (Martin and Smith, 1995). The starch granules have diameter size range from 0.1 to 200 μm, and different shapes, for example, oval, ellipse, spherical, smooth, angular and lenticular, depending on the kind of sources of starch (Buléon et al., 1998; Hoover, 2001; Singh et al., 2003). Granule morphology of the starch also has an influential effect on physiochemical properties of starch (Da Silva et al., 1997; Lindeboom et al., 2004).

1.7 Research objectives and organization of the thesis

1.7.1 Research objectives

Taking the above into consideration, the aim of this research is to investigate the effect of succinylation, annealing and dual modification on the properties of corn starch. We have therefore tried to study the effect of succinylation with varying concentration of succinic anhydride on the properties of corn starch. Physicochemical, pasting and morphological properties of corn starch were investigated. Moreover, the effect of dual modification with succinylation and annealing on the properties of corn starch was examined. Using this approach, we hope to provide a better understanding of the effect of dual modification and the role of succinylation and annealing in dual modification.

1.7.2 Organization of the thesis

In chapter 1, the starch structure, corn starch and weaknesses of the native starch have been discussed. Then, a brief discussion about the benefit of starch modification method, the kinds of starch modification process, the succinylation process, and the effect of annealing process on the physicochemical, morphological and gelatinization properties of the cereal starches and the objectives of this dissertation were explained.

In chapter 2, the effects of different concentration of succinic anhydride on physicochemical, pasting and morphological properties were examined. The experimental investigations have been performed on the physicochemical measurements, RVA measurement, and morphological observation.

In chapter 3, the effects of the succinylation, annealing and dual modification on the physicochemical, thermal and morphological properties were investigated. In this chapter, the experimental details regarding the effect of the dual modification on the starch properties have been studied.

In chapter 4, a brief summary of this research works was presented. It also focuses on the future prospects of these results.

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CHAPTER 2

Effects of Different Concentration of Succinic Anhydride on Physicochemical, Pasting and Morphological Properties on Corn Starch

2.1 Introduction

Starch is one essential material in the food industry. Many food industries use the starch in the processing the product. However, native starch is not widely used in the food industry because of the limited properties. Native starches do not have good transparency paste and instability under shear and high temperature. The starch modification was used to improve the native starch properties. Many starch modification method was used by researcher. Modification starch was consists of physical, enzymatic and biological modification. Modification method was affected by different sources of starch and kind of starch modification.

Succinylation process is one of the modification methods using esterification method. Starch was reacted with a chemical reagent to result in the better properties of the starch. Starch modification with succinylation has been done by Lawal, 2012 on yam starch, Olayinka et al., 2011 on sorghum starch, Chi et al., 2007 on corn starch, Moin et al., 2016 on rice starch, and Bhandari and Singhal, 2002 on corn and amaranth starch. The properties of the modified starch using chemical modification depend on kind of starch sources, type of chemical reagent, different condition of reaction (pH, concentration, time and presence of catalyst), degree of substitution and distribution of the reagent in the starch molecules (Hirsch and Kokini, 2002; Kavitha and BeMiller, 1998). The aim of this research is to investigate the effect of the varying of the concentration of succinic anhydride in the physicochemical, pasting and morphological properties of corn starch.

2.2 Materials and Methods

2.2.1 Materials

Corn starch (12.39% moisture content), succinic anhydride, and sodium carbonate were obtained from Nacalai Tesque, Inc. (Kyoto, Japan).

2.2.2 Succinylation

Succinylated starch was prepared by reacting native starch with succinic anhydride according to the method of Olayinka et al. (2011) with some modifications. Starch (50 g) was added to 125 mL of distilled water with 1 g of sodium carbonate and mixed with a different concentration of succinic anhydride (2, 4, 6 % w/w of starch). Succinylation was performed for 24 hours at room temperature with stirring using a magnetic stirrer. The mixture was filtered through filter paper (Whatman No. 1, GE Healthcare UK Ltd., Buckinghamshire, UK) and then washed with 20 mL of 95% ethanol. The precipitate was then washed with distilled water and dried in an incubator (Eyela LTI-100ISD, Tokyo Rikakikai Co. Ltd., Tokyo, Japan) at 25 °C for 24 h. The dry starch was ground and sieved (100 mesh) to obtain succinylated starch using a cyclone sample mill (model 3010-018, UDY Corporation, Fort Collins, CO, USA).

2.2.3 Physicochemical properties

2.2.3.1 Water binding capacity

The WBC of the starch was determined using the method described by Abbey and Ibeh (1998) with slight modification. One gram of starch sample was weighed (W1) and added to 10 mL of distilled water at room temperature. The starch mixture was mixed thoroughly using a vortex mixer for 30 s and centrifuged at 1665 × g for 15 min. The

supernatant and filtrate were separated, and the supernatant was weighed (W2). The WBC was expressed as g/g of starch.

$$WBC = \frac{W2 - W1}{W1}$$

2.2.3.2 Swelling power and solubility

Swelling power measurement was measured at 95°C according to the method described by Waliszewki et al. (2003) with slight modification. The starch sample (5 mg) was added to a tube (W1). Distilled water (10 mL) was added to the tube, and the starch mixture was combined using a vortex mixer for 30 s. The starch suspension was heated in a water bath at 95 °C for 30 min. The mixture was cooled at room temperature and centrifuged at 1665× g for 15 min. The precipitate and supernatant were separated, and the precipitate was weighed (W2). The swelling power was measured using the following formula:

Swelling of starch = (W2 − W1)/weight of starch

Solubility was measured using 5 mL of supernatant, which was dried in an oven at 110 °C for 24 h. The following formula was used to determine the solubility:

Solubility (%) = (weight of dry supernatant/weight of starch) × 100

2.2.3.3 Paste clarity

The paste clarity of native and modified starches was determined based on the method of Bhandari and Singhal (2002). Starch (5 mg) was added to a tube with 5 mL of distilled water. The starch suspension was heated at 95 °C for 30 min and shaken every 5 min. After cooling at room temperature, the percentage of transmittance of starch was measured using a spectrophotometer (Shimadzu model UV-3600, Shimadzu

Corp., Kyoto, Japan) at 650 nm against a blank of water. Paste clarity was expressed as the percentage transmittance.

2.2.3 Pasting properties

The pasting profiles were measured using a Rapid Visco Analyzer (RVA-Super 3, Newport Scientific, Warriewood, NSW, Australia) according to the procedure described by Wang et al. (2014) with some modification. Corn starch (3 g) was added into RVA canisters, and distilled water was added to make a total weight of 28 g. Peak viscosity (PV), Breakdown viscosity (BD), and Trough viscosity (TV) were obtained from the RVA data. The relative breakdown was measured as the ratio of BD to PV (Arocas et al., 2009), while the stability ratio was calculated as TV/PV (Shafie et al., 2006). All measurements were performed in triplicate.

2.2.4 Morphological properties

Morphology of starch granules was observed using the method of Thao and Noomhorn (2011). The shape of the starch granules was determined using a light microscope (BX53, Olympus, Tokyo, Japan) and cellSens Software, Version 2.1 (Olympus, Tokyo, Japan). Starch (0.1 g) was added to a tube containing 10 mL of distilled water and stirred thoroughly. A drop of starch suspension was placed onto a microscope slide and covered with a glass coverslip. The sample was observed at 1000× magnification.

2.2.5 Statistical analysis

Data were analyzed statistically using the Origin software (Version 2016, OriginLab Corp., Northampton, MA, USA). Analysis of variance (ANOVA) and Tukey

tests with a significance threshold of $p < 0.05$ was used to compare the differences between mean values.

2.3 Results and Discussion

2.3.1 Effect of different concentration of succinic anhydride on the physicochemical properties

2.3.1.1 Water binding capacity (WBC)

Water binding capacity (WBC) of native and succinylated starches was presented in Table 1. Water binding capacity can exhibit the hydrophilic tendency of starch (Ali and Hasnain, 2016). The WBC of SUC 2% and native starch were 0.84 and 0.69 g/g, respectively. Succinylation process enhanced water binding compared with that in the native counterpart. Succinylation results in bulky functional groups, and their electrostatic repulsion stimulated percolation and water into the starch matrices (Lawal, 2004 and Arueya and Oyewaye, 2015). Succinylation increased water binding capacity was reported by Arueya and Oyewaye, 2015, Lawal, 2004, and Emeje et al., 2002. WBC of the SUC 2% is not significance difference with SUC 4% and SUC 6%. It is indicated the different concentration of succinic anhydride has no effect on the water binding capacity.

2.3.1.2 Swelling power and solubility

Swelling power and solubility from native and succinylated starches were summarized in Table 2.1. Swelling power increased slightly after the succinylation process with 2%, from 16.4 g/g (native starch) to 17.3 g/g. Succinylated starch has more water into starch molecules; this is introduced more swelling granule compare to the

native counterpart. Some important factors influence swelling power, like as the structure of amylopectin (Sasaki and Matsuki, 1998; Shi and Seib, 1992), complexes of V-amylose lipid (Tester and Morrison, 1990), amylose content (Morrison et al., 1993; Tester et al., 2000), and the level of interaction between amylose–amylose and/or amylose–amylopectin chains (Hoover and Vasanthan, 1994 b). However, there was decreased swelling power after succinylation process with more than 2% compared with native. There was no significance difference between native and succinylated starches in the solubility.

Table 2.1. Effect of different concentration of succinic anhydride on the physicochemical properties

Sample	WBC* (g/g)	Paste Clarity (%)	Swelling power (g/g)	Solubility (%)
Native	0.69 ± 0.04^b	65.4 ± 0.5^b	16.4 ± 0.2^b	11.2 ± 0.2^{ab}
Suc 2%	0.84 ± 0.01^a	72.9 ± 1.3^a	17.3 ± 0.2^a	12.7 ± 1.2^a
Suc 4%	0.76 ± 0.08^{ab}	60.7 ± 2.0^c	13.0 ± 0.2^c	8.8 ± 0.3^b
Suc 6%	0.76 ± 0.05^{ab}	64 ± 0.6^{bc}	12.9 ± 0.1^c	7.6 ± 0.3^b

* : Water binding capacity
Values are mean of triplicate determinations $\pm$ SD. Means within columns with different letters are significantly different ($p < 0.05$).

2.3.1.3 Paste clarity

The paste clarity for native and succinylated starches is shown in Table 2.1. The clarity of the starch pastes was increased following succinylation with 2% succinic anhydride (72.9%) compared with the native counterpart (65.4%). The paste clarity of corn starch improved after succinylation compared with that of the native starch paste. The result was agreement with report of Bhandari and Singhal (2002), with improved paste clarity of corn and amaranth starches after succinylation. Hydroxyl groups in the

starch molecules were substituted by succinyl groups. This prevents the formation of ordered structures after gelatinization and inhibited retrogradation, and consequently leads to a more transparent paste (Lawal, 2004). However, using over 2% concentration of succinic anhydride decreased paste clarity compare with native and SUC 2%.

2.3.2 Effect of different concentration of succinic anhydride on the pasting properties

The pasting properties of these all starches are shown in Table 2.2. The all succinylated starches have higher peak viscosity (PV) and trough viscosity (TV) than the native counterpart. However, different concentration of succinic anhydride has no significant difference on the PV and TV. This finding is consistent with the results of Olayinka et al. (2011), Hui et al. (2009) and Moin et al. (2016). Hui et al. (2009) reported that PV was increased following octenyl succinic anhydride modification in the potato starch. Promotion of the bulky hydrophilic succinate groups, result in increased starch chain expansion and PV (Moin et al., 2016 and Olayinka et al., 2011).

The breakdown viscosity (BD) native, suc 2%, and suc 4% starches were 970 mPas, 1697 mPas, and 1996 mPas, respectively. The succinylation process increased BD compare with the native starch. Same trends were observed by Moin et al., 2016, Lawal, 2012, Awokoya et al., 2011 and Chen et al., 2014 in rice, yam, cocoyam and corn starch, respectively. Succinylation of the starch cause partial degradation, because of which the integrity of the starch granule cannot be maintained. The decreasing of granule integrity leads to increase of BD when heat and shear stress were applied (Awokoya et al., 2011). These results also indicated that higher concentration of succinic anhydride leads to higher breakdown viscosity.

The resistance of starch to heat and shear stress could be determined with a

stability ratio showed (Shafie et al., 2016). The stability ratio is determined by trough viscosity (TV) divided by peak viscosity (PV). The stability ratio of succinylated was lower than the native counterpart. The introduced of succinyl group which its more hydrophilic properties lead to enhanced PV (Lawal, 2004, Arueya and Oyewaye, 2015, Moin et al., 2016 and Olayinka et al., 2011). These findings also showed that the different concentration of succinic anhydride has no effect in BD.

Table 2.2. Effect of different concentration of succinic anhydride on pasting properties

Sample	Peak Viscosity (mPas)	Trough Viscosity (mPas)	Breakdown Viscosity (mPas)	Relative Breakdown	Stability Ratio
Native	2707 ± 43^b	1737 ± 37^b	970 ± 33^c	0.36 ± 0.01^c	0.64 ± 0.01^a
Suc 2%	4111 ± 258^a	2414 ± 42^a	1697 ± 165^b	0.42 ± 0.02^b	0.58 ± 0.02^b
Suc 4%	4337 ± 73^a	2341 ± 69^a	1996 ± 69^{ab}	0.46 ± 0.01^{ab}	0.54 ± 0.01^c
Suc 6%	4366 ± 97^a	2329 ± 47^a	2036 ± 140^a	0.47 ± 0.02^a	0.53 ± 0.02^c

Values are mean of triplicate determinations $\pm$ SD. Means within columns with different letters are significantly different ($p < 0.05$).

2.3.3 Effect of different concentration of succinic anhydride on the morphological properties

Figure 2.1 showed that corn starch granules were observed under a light microscope. Corn starch granules have polyhedral and rounded shapes. These results are in agreement with the previous report of Wang et al., 2014 and Rocha et al., 2012. The findings showed that succinylation with different concentration of succinic anhydride had no effect changes in granule shaped compared with native. These results are similar to those reported by Arueya and Oyewaye, 2015, Emeje et al., 2012 and Ayucitra, 2012. The shape and appearance of starch granule were not destroyed after succinylation

process. These results also indicate that different concentration of succinic anhydride does not change the shape and appearance of starch granules.
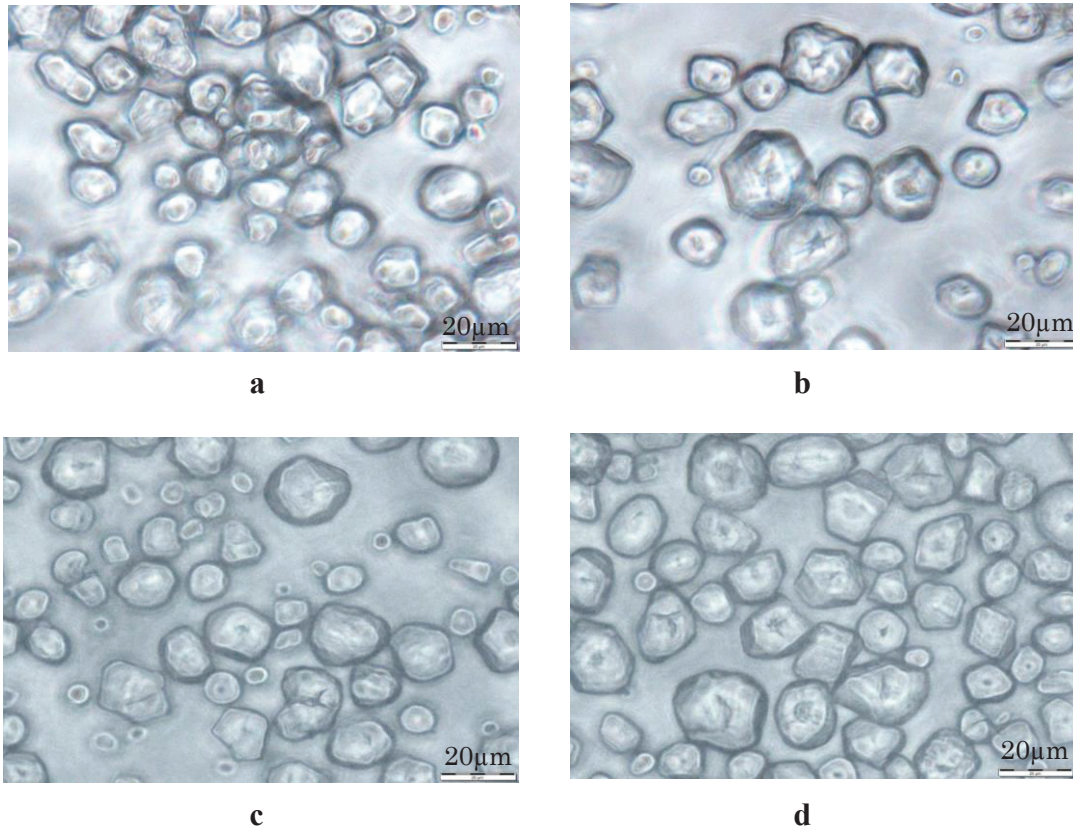
Figure 2.1. Micrographs of a) native, b) suc 2%, c) suc 4%, and d) suc 6%. Scale bar is 20 μm.

2.4 Conclusions

Our results exhibit that succinylation with over 2% can decrease solubility, swelling power, and paste clarity, while it has no effect on water binding capacity, stability ratio, PV and change of granule shape compared with the native starch. These results suggest that the low paste clarity properties in native can be overcome through succinylation process with 2% succinic anhydride. However, it could not overcome the low stability under heat and shear stress. Thus, it needs further investigation to find the suitable modification process to overcome the weaknesses of the native starch.

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CHAPTER 3

Effects of Dual Modification with Succinylation and Annealing on Physicochemical, Thermal and Morphological Properties of Corn Starch

3.1 Introduction

Starches are an abundant organic substance that is useful to the food industry (Chi et al., 2008 and Arocas et al., 2009). It has many applications, such as stabilizers, gelling agents, and thickeners (Liu et al., 2014). Native starches have a number of weaknesses, such as low stability on high temperature and shear stress, thermal decomposition, and high tendency of retrogradation (Jayakody and Hoover, 2008 and Xiao et al., 2012). Typically, modification processes are used to overcome these problems. Many modification methods have been used to improve starch properties. Starch modification processes are divided into physical, chemical and enzymatic processes (Zavarese and Dias, 2011).

Succinylation is a method of chemical modification of starch. It is an esterification reaction of a hydroxyl group in the starch molecule with succinic anhydride (Lawal, 2012). It results in higher viscosity, greater thickening power, and lower retrogradation rate (Olayinka et al., 2011). Previous studies on starch modification with succinylation have been conducted on yam starch (Lawal, 2012), on sorghum starch (Olayinka et al., 2011), on corn starch (Chi et al., 2007), on rice starch (Moin et al., 2016), and on corn and amaranth starch (Bhandari and Singhal, 2002). The succinylation process of starch modification confers many advantages such as high solubility in cold water, high viscosity, better thickening power, improved paste clarity, retarded retrogradation and

freeze-thaw stability (Moin et al., 2016). However, it has the shortcoming of instability during shearing at high temperatures (Ačkar et al., 2015). Therefore, another modification process is needed to overcome this weakness of the succinylation process. The annealing process was chosen because of its ability to increase the thermal and shear stability of starch (Hoover and Vasanthan, 1994b).

Unlike succinylation, annealing is a method of physically modifying starch. This process is widely used because it does not leave a residue as does the chemical process (Wang et al., 2014). This process modifies the starch without damaging the granules (Dias et al., 2010, Rocha et al., 2012; Singh et al., 2011). It is generally performed above glass transition temperature and below gelatinization temperature, with high (>60 %) or intermediate moisture content (40-55 %) (Jacobs and Delcour, 1998). Annealing has been used as a starch modification process with various starch sources. It has been shown to decrease swelling power in a number of sources of starch (Wang et al., 2014; Waduge et al., 2006; Hoover and Vasanthan, 1994a; Dias et al., 2010; Hormdok and Noomhorn, 2007; Adebowale et al., 2005a; Gomes et al., 2004 and Ali and Hasnain, 2016). Reduced breakdown viscosity (BD) after annealing has been reported by Dias et al., 2010; Lan et al., 2008; Ali and Hasnain, 2016 and Singh et al., 2011. Low BD and higher stability ratio indicate that high shear resistance and temperature of the starch (Corke et al., 1997; Shafie et al., 2016). Yadav et al. (2013) demonstrated that the stability ratio of native chestnut starch increased after annealing.

Recently, dual modification processes have been widely used in starch modification because they can improve the undesirable properties of the starch (Xiao et al., 2012). Various methods of dual modification have been done by many researchers, but there have been no reports about this dual modification (succinylation-annealing) process. Accordingly, there is no available information with regard to the effects of this

combination method on the physicochemical, morphological and thermal properties of starch. Thus, this study aimed to prepare starch by this dual modification (succinylation-annealing) method. The effects of annealing, succinylation and dual modification (succinylation-annealing) on physicochemical, morphological and thermal properties of these starches were investigated. The effect of the stability ratio on heat and shear stress of these starches was also evaluated.

3.2 Materials and methods

3.2.1 Materials

Corn starch (12.39% moisture content), succinic anhydride, and sodium carbonate were obtained from Nacalai Tesque, Inc. (Kyoto, Japan).

3.2.2 Succinylation process

Succinylated starch was prepared by reacting native starch with succinic anhydride with some modifications (Olayinka et al., 2011). Starch (50 g) was added to 125 mL of distilled water with 1 g of sodium carbonate and mixed with succinic anhydride (1 g). Succinylation was performed for 24 h at room temperature, with stirring using a magnetic stirrer. The mixture was filtered through filter paper (Whatman No. 1, GE Healthcare UK Ltd., Buckinghamshire, UK), and then washed with 20 mL of 95% ethanol. The precipitate was then washed with distilled water and dried in an incubator (Eyela LTI-100ISD, Tokyo Rikakikai Co. Ltd., Tokyo, Japan) at 25 °C for 24 h. The dry starch was ground and sieved (100 mesh) to obtain succinylated starch using a cyclone sample mill (model 3010-018, UDY Corporation, Fort Collins, CO, USA).

3.2.3 Annealing process

Annealing was performed according to the method described by Vamadevan et al. (2013), with slight modifications. Starch (62.5 g) was added to 125 mL of distilled water and heated in a glass beaker using a water bath at 55 °C for 24 h. The starch suspension was then centrifuged (KN-70, Kubota, Osaka, Japan) at 1665× g to separate the water from the wet starch. The wet starch was dried in an incubator (Eyela LTI-100ISD, Tokyo Rikakikai Co. Ltd., Japan) at 25 °C for 24 h, ground, and sieved (100 mesh) to obtain annealed starch using a cyclone sample mill (model 3010-018, UDY Corporation, Fort Collins, CO, USA).
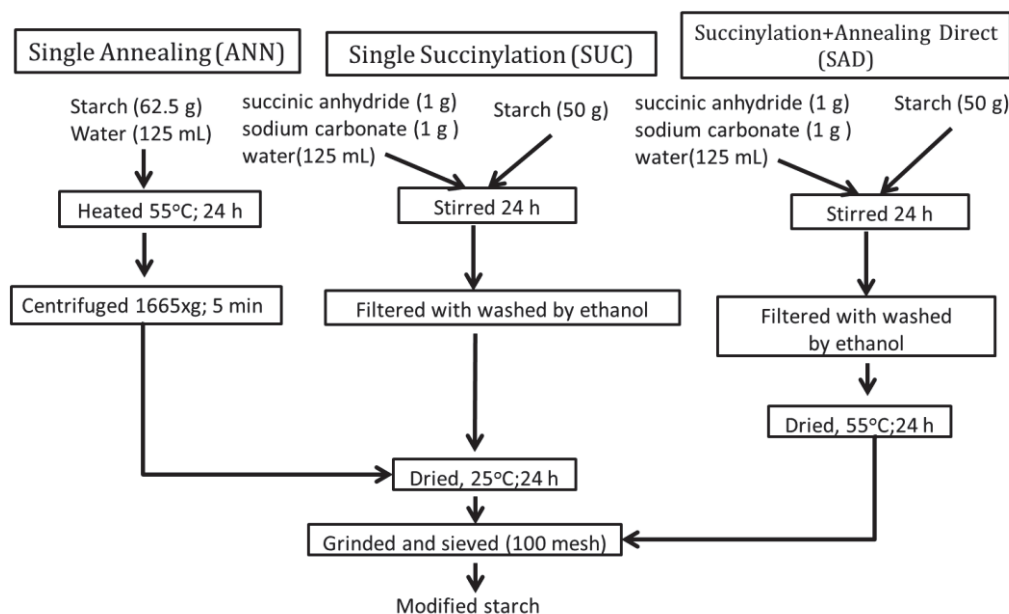
Figure 3.1. The process of starch modification

3.2.4 Dual modification process

Succinylated starch was obtained using the method described by Olayinka et al. (2011), with some of the above-mentioned modifications, except for the drying process. Wet starch with a moisture content of about 40% was annealed and dried in an oven at 55 °C for 24 h. The dry starch was ground and sieved (100 mesh) using a cyclone

sample mill (model 3010-018, UDY Corporation, Fort Collins, CO, USA) to obtain dual modified starch.

3.2.5 Fourier-transform infrared (FTIR) spectroscopy

FTIR spectra were recorded on an FTIR System (Spectrum 100 FTIR, Perkin Elmer, Waltham, MA, USA) using KBr pellets generated by way of the methodology described by Gbenga et al. (2014), with slight modifications. Starch (2 mg) was weighed, ground, and mixed uniformly with 200 mg of pure KBr powder. The starch was placed in an evacuable KBr die in a clear disk and then pressed using a hydraulic press. The sample was inserted into the FTIR system and scanned at a range of 400–4000 cm^{-1} to obtain a percentage of absorbance.

3.2.6 Degree of substitution (DS)

The DS was determined using the alkali saponification method. A weight of 0.5 g of starch was added to a 100 mL conical flask with 25 mL of 75% ethanol. Then, 20 mL of 0.5 M aqueous sodium hydroxide was added to the solution. The starch solution was stored at room temperature for 72 h, with occasional swirling of the flask. The excess alkali was back-titrated using 0.5 M hydrochloric acid (Jyothi et al., 2005). The following equation was used to calculate the percentage of succinyl and DS:

$$\% \text{ succinyl} = \frac{(\text{blank titre} - \text{sample titre}) \times 0.1 \times \text{molarity of acid} \times 100}{\text{weight of sample}}$$

$$\text{Degree of substitution (DS)} = \frac{162 \times \%\text{succinyl}}{10000 - (99 \times \%\text{succinyl})}$$

3.2.7 Water binding capacity

The procedure as like as described in Chapter 2, page 29.

3.2.8 Swelling power and solubility

The procedure as like as described in Chapter 2, page 30.

3.2.9 Paste clarity

The procedure as like as described in Chapter 2, page 30.

3.2.10 Pasting properties

The procedure as like as described in Chapter 2, page 31.

3.2.11 Differential scanning calorimetry

DSC measurements were conducted using a differential scanning calorimeter (Exstar SII-6200, Seiko Instruments Inc., Chiba, Japan) based on the method of Wang et al., (2017) with some modification. Starch (3 mg) was weighed and added to aluminum pans. Distilled water (9 µL) was added to the aluminum pans using a syringe to obtain a starch to water ratio of 1:3 (w/w). The pans were sealed and allowed to stand for 30 min at room temperature prior to analysis. The samples were heated from 20 °C to 130 °C at a heating rate of 10 °C/min. An empty aluminum pan was used as the reference. The gelatinization parameters were obtained using the Origin software (Version 2016, OriginLab Corp., Northampton, MA, USA).

3.2.12. X-Ray diffraction pattern

The X-Ray diffraction patterns of native and modified starches were observed using an X-Ray diffractometer (D8 Advance, Bruker AXS, Billerica, MA, USA) as previously described by Wang et al. (2014). The X-Ray diffractometer was equipped with a copper X-Ray tube operating at 40 kV and 40 mA. The starch samples were kept in a desiccator over a saturated solution of NaCl for 24 h prior to measurement. The diffraction intensity was measured from 10° to 30° as a function of 2ϴ, at a scanning speed of 1.67°/min and a step size of 0.04°.

3.2.13. Morphological properties

The procedure as like as described in Chapter 2, page 31.

3.2.14 Statistical analysis

Data were analyzed statistically using the Origin software (Version 2016, OriginLab Corp., Northampton, MA, USA). Analysis of variance and Tukey tests with a significance threshold of $p < 0.05$ were used to compare the differences among mean values.

3.3 Results and discussion

3.3.1 FT-IR Spectroscopy and Degree of substitution (DS)

FTIR spectra and DS of native and modified of starches are shown in Figure 3.1. FTIR measurements can clarify whether succinylation was performed on the modified starch. The results of FTIR spectroscopy showed a small new peak at 1572 cm^{-1} with the succinylated and dual modified starches. The DS of the succinylated and dual modified starches were both 0.15, reflecting the fact that succinylation was also performed in the

dual modified starch sample. The new peak at 1572 cm^{-1} suggested the existence of a carbonyl group (C–O) and that succinylation occurred in both the succinylated and dual modified samples. Consistent with previous studies, the asymmetric stretching vibration of carboxylate (RCOO^{-}) of octenyl succinic anhydride starch was located at 1572 cm^{-1} (Nagaoka et al., 2005; Zhang et al., 2004).
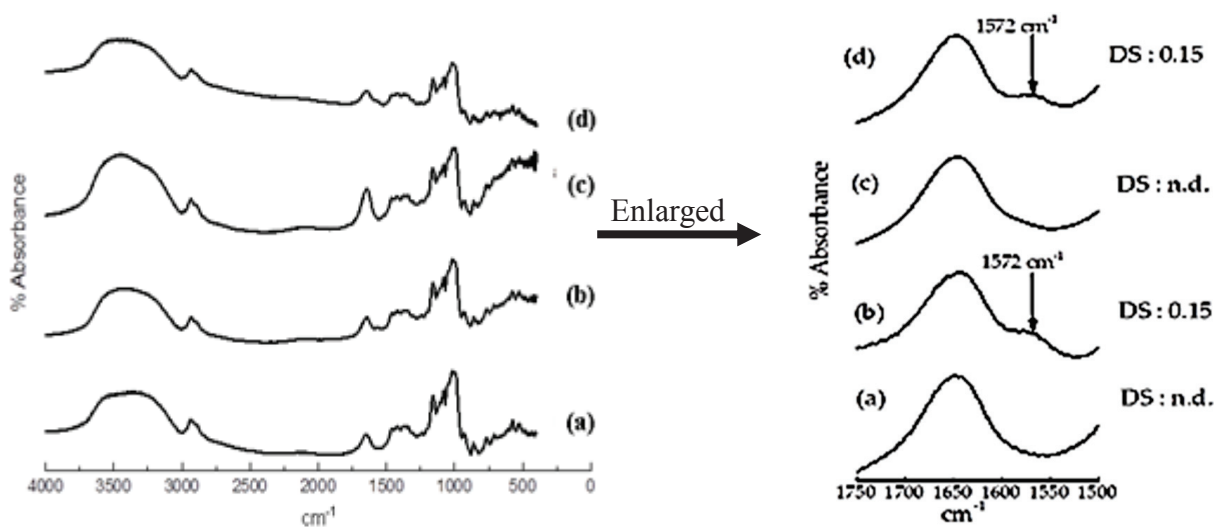
Figure 3.2. FTIR spectra for a) native, b) succinylated, c) annealed and d) dual modified corn starch

3.3.2 Water binding capacity (WBC)

The WBC of native, succinylated, annealed, and dual modified corn starches are summarized in Table 3.1. The hydrophilic tendency of starch can be measured by its WBC (Ali and Hasnain, 2016). The WBC of succinylated and dual modified starches were 0.84 and 0.83 g/g, respectively, both of which were higher than that of the native starch (0.69 g/g). Succinylation increased water binding compared with that in the native counterpart. Succinylation resulted in a more pronounced hydrophilic tendency and expansion of some amorphous regions (Arueya and Oyewaye, 2015). Moreover, Arueya and Oyewaye stated that succinylation introduced bulky functional groups, and

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their electrostatic repulsion led to percolation and absorption of water within the starch matrices. There was no significant difference in the WBC of dual modified starch compared with succinylated starch.

Table 3.1. Effect of annealing, succinylation and dual modification on physicochemical properties

Sample	WBC* (g/g)	Paste Clarity (%)	Swelling power (g/g)	Solubility (%)
Native	0.69 ± 0.03^b	65.4 ± 0.5^c	16.4 ± 0.2^b	11.2 ± 0.2^{ab}
Succinylated	0.84 ± 0.01^a	72.9 ± 1.3^a	17.3 ± 0.2^{ab}	12.7 ± 1.2^a
Annealed	0.66 ± 0.02^c	67.4 ± 0.7^{ab}	14.2 ± 0.4^c	9.7 ± 0.6^b
Dual Modified	0.83 ± 0.01^a	68.6 ± 0.5^b	17.7 ± 0.4^a	11.1 ± 0.8^{ab}

* : Water Binding Capacity

Values are mean of triplicate determinations $\pm$ SD. Means within columns with different letters are significantly different ($p < 0.05$).

Consistent with previous results in white sorghum starch Ali and Hasnain (2016), the WBC was decreased in annealed starch compared with that in the native starch. The lower WBC in annealed starch than in native starch is due to a decrease in the number of available water-binding sites after annealing, caused by amylose–amylose, and amylose–amylopectin interactions in the annealing process (Ali and Hasnain, 2016).

3.3.3 Swelling power and solubility

The swelling power and solubility values with different treatments are shown in Table 3.1. The swelling power increased slightly after the dual modification process, from 16.4 (native starch) to 17.7 g/g. There was no significant difference between the swelling power of succinylated starch, native starch, and dual modified starch. However, annealing decreased the swelling power of the native starch. This finding is in

agreement with previous reports on corn starch (Wang et al., 2014), barley starch (Waduge et al, 2006), wheat starch (Hoover and Vasanthan, 1994a), rice starch (Dias et al., 2010; Hormdok and Noomhorn, 2007), oat starch (Hoover and Vasanthan, 1994b), and sorghum starch (Adebowale et al., 2005; Singh et al., 2011; Ali and Hasnain, 2016). The annealing treatment of corn starch resulted in a lower swelling power value than that in the native starch. Waduge et al. (2006) reported that swelling power decreased in the post-annealing process of barley starches. This decrease in swelling power was due to the interaction of crystallite perfection and the interplay of amylose–amylose during the annealing process.

Several factors influence swelling power, such as the structure of amylopectin (Sasaki and Matsuki, 1998; Shi and Seib, 1992), complexes of V-amylose lipid (Tester and Morrison, 1990), amylose content (Morrison et al., 1993; Tester et al., 2000), enhancement of crystalline perfection (Waduge et al., 2006; Singh et al., 2011), increment in molecular organization (Gomes et al., 2004), and the level of interaction between amylose–amylose and/or amylose–amylopectin chains (Hoover and Manuel, 1996; Tester et al., 2000; Waduge et al., 2006; Hoover and Vasanthan, 1994 b; Gunaratne and Hoover, 2002; and Eerlingen et al., 1997). There was no significant difference in the solubility of native, succinylated, annealed and dual modified starches.

3.3.4 Paste Clarity

The paste clarity of succinylated, annealed and dual modified starches are presented in Table 3.1. The clarity of the starch pastes was increased following succinylation (72.9%), annealing (67.4%) and dual modification (68.6%) compared with the native starch (65.4%). Succinylation increased the paste clarity of corn starch compared with that of the native starch paste. A previous report by Bhandari and

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Singhal (2002) showed the same result: increased paste clarity of corn and amaranth starches after succinylation. The succinyl group is substituted for the hydroxyl groups on the starch molecules, inhibiting the formation of ordered structures after gelatinization and preventing retrogradation, resulting in a more transparent paste (Lawal, 2004).

The paste clarity value increased slightly after annealing compared with the native starch. This result is consistent with results reported by Ali and Hasnain (2016) in which annealing increased the paste clarity of white sorghum starch compared with that of native sorghum starch. Increased paste clarity of annealed sorghum starch was correlated with decreased solubility, and greater amylose leaching and amylopectin interaction led to higher turbidity of the starch paste (Perrera and Hoover, 1999). In the present study, there was no significant difference in paste clarity between dual modified starch and annealed starch.

3.3.5 Pasting properties

The pasting properties of the four starches are summarized in Table 3.2. The succinylated and dual modified starches had higher PV values than the native starch, consistent with previous findings of Olayinka et al. (2011), Hui et al. (2009) and Moin et al. (2016). In a study on potato starch, Hui et al. (2009) reported that the PV increased following octenyl succinic anhydride modification. The introduction of bulky hydrophilic succinate groups leads to increased starch chain expansion and PV (Moin et al., 2016 and Olayinka et al., 2011). In the present study, there was no significant difference between the PV values of annealed starch and the native starch.

The BD values of native, succinylated and annealed starches were 970, 1697, and 1481 mPas, respectively. Succinylated starch had a higher BD than the native starch.

Similar trends were observed in rice (Moin et al., 2016), yam (Lawal, 2012), cocoyam (Awokoya et al., 2011) and corn starch (Chen et al., 2014). Succinylation of the starch leads to partial degradation as the integrity of the starch granule cannot be maintained. The decrement of granule integrity led to an increase in BD when heat and shear stress were applied (Awokoya et al., 2011).

Table 3.2. Effect of annealing, succinylation and dual modification on pasting properties

Sample	Peak Viscosity (mPas)	Trough Viscosity (mPas)	Breakdown Viscosity (mPas)	Relative Breakdown	Stability Ratio
Native	2707 ± 43^c	1737 ± 37^c	970 ± 33^b	0.36 ± 0.01^c	0.64 ± 0.01^b
Succinylated	4111 ± 258^a	2414 ± 42^a	1697 ± 165^a	0.42 ± 0.02^b	0.58 ± 0.02^c
Annealed	2650 ± 49^c	1168 ± 104^d	1481 ± 31^a	0.56 ± 0.01^a	0.44 ± 0.01^d
Dual Modified	3171 ± 14^b	2188 ± 20^b	982 ± 13^b	0.31 ± 0.004^d	0.69 ± 0.004^a

Values are mean of triplicate determinations $\pm$ SD. Means within columns with different letters are significantly different ($p < 0.05$).

Annealing enhanced BD compared with the native starch. This was consistent with a study by Adebowale et al. (2005), which demonstrated that BD increased after annealing in sorghum starch. This indicates less stability at high temperature and under shear stress. Hormdok and Noomhorm (2007) also stated that annealing increased BD in rice starch. The relative BD is defined as the ratio of BD to PV (Arocas et al., 2009). The relative BD of succinylated and annealed starch increased significantly, from 0.36 (native starch) to 0.42 and 0.56, respectively. However, the BD of dual modified starch was significantly lower than that of the other three samples. Moreover, the dual process significantly decreased relative breakdown from 0.42 to 0.31 compared with

succinylation.

The stability ratio indicates the resilience of starch to heat and shear stress (Shafie et al., 2016), and is calculated as TV/PV. The stability ratio of starch was divided into: high stability ratio : > 0.95; medium stability ratio : 0.65-0.95; and low stability ratio : < 0.65 (Shafie et al., 2016). The stability ratio of succinylated and annealed starches was lower than that of the native starch. The stability ratio of dual modified starch increased from 0.58 (succinylated) to 0.69, suggesting that the process increased the stability ratio compared with succinylation. The interaction of succinylation and annealing resulted in good stability ratios. Succinylation led to an increase in PV, while annealing led to a decrease BD. Succinylation occurred in the amorphous regions because of amorphous regions as they were more accessible to chemical reactions than the crystalline regions (Lawal, 2012 and Van der Burgt et al., 2000). The introduction of the succinyl group increased the hydrophilic properties, leading to an increase in PV (Lawal, 2004, Arueya and Oyewaye, 2015, Moin et al., 2016 and Olayinka et al., 2011). This hydrophilic tendency led to an increase in the WBC of the starch. Succinylated starch has a higher WBC value than annealed starch; therefore, succinylated starch can absorb more water than annealed starch.

Annealing is correlated with the physical reorganization of the starch granule in the presence of water (Tester and Debon, 2000). The presence of more water leads to increased mobility of the amorphous regions to a crystalline state (Alcázar-Alay and Meireles, 2015), resulting in the starch being more resistant to heat and shear stress, thus reducing the BD.

3.3.6 Thermal Properties

The characteristics of endothermic transition were shown by the starches in DSC,

which appear between 60 °C and 80 °C. Table 3 shows the DSC gelatinization parameters of these four starches. DSC results suggested that the gelatinization temperature increased following annealing. These results are similar to those of previous reports on normal corn starch (Wang et al., 2014), barley starches (Waduge et al., 2006), wheat starch (Lan et al., 2008), and waxy barley starch (Qi et al., 2004). The gelatinization temperature increased as a result of the enhanced perfection of crystallites (Hoover and Vasanthan, 1994b; Jacobs and Delcour, 1998; Knutson, 1990; Tester et al., 1998, 2000; Waduge et al., 2006).

Table 3.3. Effect of annealing, succinylation and dual modification on thermal properties

Sample	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (mJ/g)
Native	66.8 ± 0.2[a]	73.5 ± 0.5[a]	81.7 ± 0.3[b]	7.9 ± 0.3[b]
Succinylated	67.2 ± 1.1[a]	74.0 ± 0.4[a]	82.0 ± 0.05[ab]	9.2 ± 0.9[ab]
Annealed	70.9 ± 0.1[b]	75.4 ± 0.1[b]	82.4 ± 0.1[ab]	8.2 ± 0.5[ab]
Dual Modified	67.6 ± 0.6[a]	74.1 ± 0.4[a]	82.6 ± 0.5[a]	9.7 ± 0.5[a]

T_o, T_p, and T_c are the temperatures of the onset, peak, and conclusion of the endothermic transition, respectively.

ΔH is the enthalpy gelatinization.

Values represent the mean of triplicate measurements ± SD. Means within columns with different letters are significantly different (p < 0.05).

There was no significant difference in enthalpy between native and annealed starch. No effect of annealing on enthalpy gelatinization (ΔH) has previously been reported in corn starch (Wang et al., 2014; Tester et al., 2000; Chung et al., 2009) on barley starch (Waduge et al., 2006), on rice starch (Hormdok and Noomhorn, 2007), or on wheat starch (Lan et al., 2008). The lack of change in enthalpy gelatinization *(ΔH)* in

barley starch after annealing indicated that annealing did not form a new double helix (Waduge et al., 2006), and there was no significant difference in gelatinization temperature and enthalpy gelatinization in corn starch after succinylation compared with the native starch. However, dual modification of corn starch increased the temperature of the conclusion of the endothermic transition (Tc) and ΔH compared with the native starch. There were no significant differences between annealing and succinylation with regard to gelatinization temperature and enthalpy.

3.3.7 X-Ray Diffraction Pattern

The X-Ray diffraction patterns of the native and modified starches are presented in Figure 3.2. The native and modified starches showed characteristic A-type diffraction patterns, with two peaks at 15.1 and 23.2, and a double peak at 17.1 and 18.0 (2Θ).
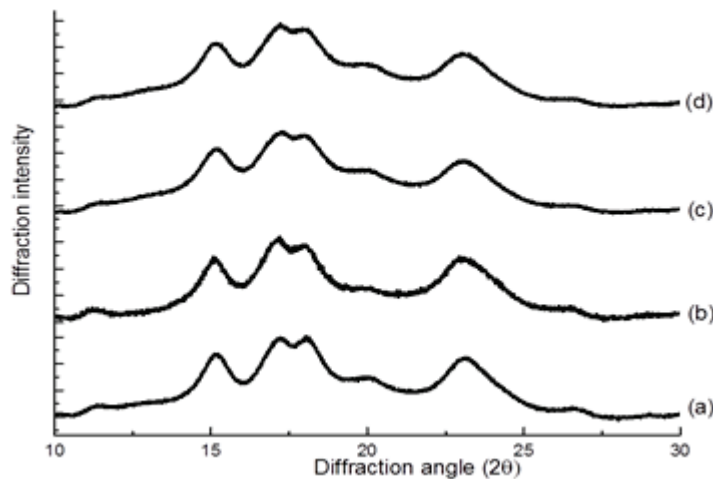
Figure 3.3. X-Ray diffraction pattern for (a) native, (b) succinylated, (c) annealed, and (d) dual modified corn starch.

These observations indicated that the annealing process did not alter the diffraction pattern of corn starch, consistent with previous findings (Wang et al., 2014; Dias et al., 2010; Rocha et al., 2012; Singh et al., 2011; Waduge et al., 2006; Wang et al., 2017; O'Brien and Wang, 2008 and Liu et al., 2009). Although annealing did not change the type of crystallinity pattern, it could increase the relative crystallinity (Liu et al., 2009).

Moreover, succinylation and dual modification did not change the diffraction patterns of starch.

3.3.8 Morphological properties

Corn starch granules were observed under a light microscope, shown in Figure 3.3. In agreement with the previous report from Wang et al., 2014 and Rocha et al., 2012, corn starch granules showed polyhedral and rounded shapes. Succinylation did not change the granule shape compared with the native starch. These observations are similar to those reported by Arueya and Oyewaye, 2015, Emeje et al., 2012 and Ayucitra, 2012. Furthermore, the shape, appearance and granule structure were not destroyed after succinylation. Likewise, the granule shape was unchanged after annealing compared with the native starch, consistent with previous findings of Wang et al., 2014; Rocha et al., 2012; Lan et al., 2008; Waduge et al., 2006; O'Brien and Wang, 2008, Kiseleva et al., 2005 and Ali and Hasnain, 2016.

Wang et al. (2014) suggested that the granule morphology of corn starch did not significantly change after annealing. The granules showed a variety of shapes, with small granules being spherical, and large granules being polyhedral. Rocha et al. (2012) also reported that there were no differences in granule shapes after annealing. The granule shape of corn starch remained unchanged after dual modification. Dual modified starch has also shown polyhedral and rounded shapes. This reveals that there is no difference between the shapes of dual modified starch and native starch, and suggests that dual modification does not affect the shape and appearance of starch granules.
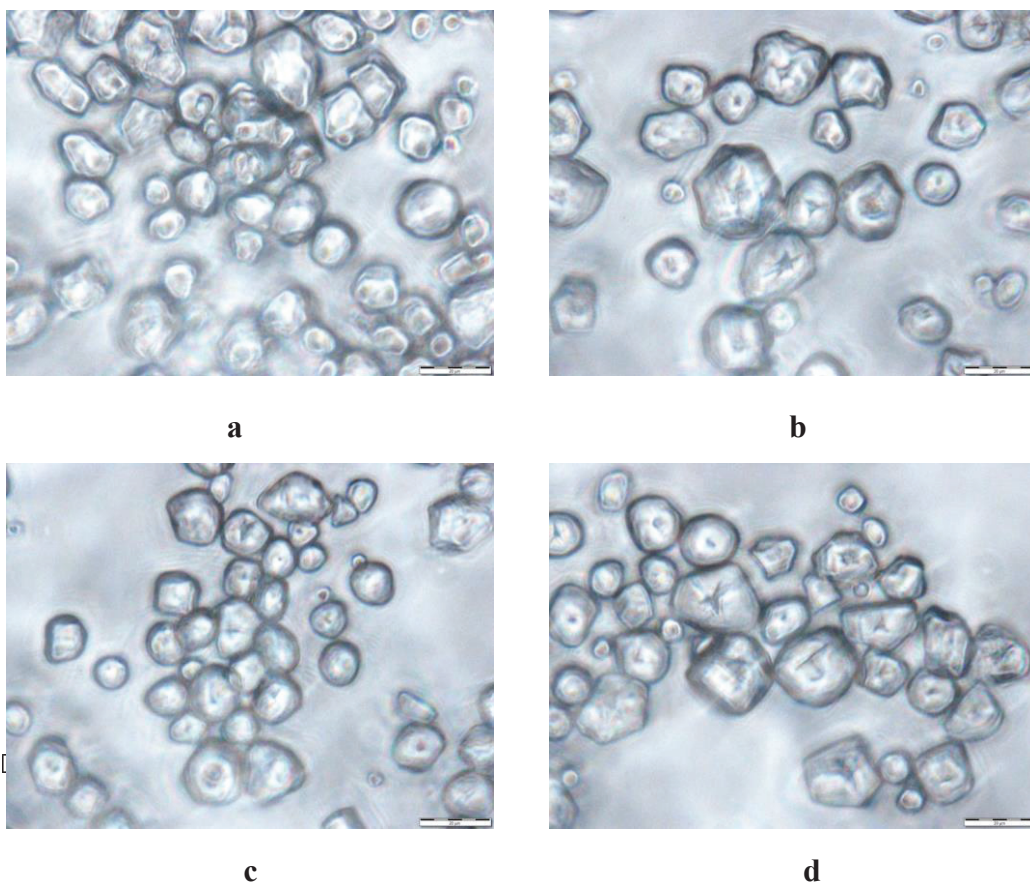
Figure 3.4. Micrographs of a) native, b) annealed, c) succinylated, and d) dual modified

corn starch. Scale bar is 20 μm.

3.3.9 Effect of different temperature of annealing with dual modification process on physicochemical and morphological properties

3.3.9.1. Water binding capacity

The water binding capacity (WBC) of native and dual modified starches was shown in Table 4.4. The WBC of all dual modified starches has higher than the native starch counterpart. The succinylation in the dual modification process leads to improving the WBC compare with native. The bulky functional groups were introduced

by succinylation process (Lawal, 2004). It is can increase the amount of water into the starch matrices. The WBC of SAD 30, 40, and 50 were 0.82, 0.85 and 0.88 g/g, respectively. There is no significant difference in the different temperature annealing on the WBC.

3.3.9.2. Paste clarity

Paste clarity of native and dual modified starches was summarized in Table 4.4. The dual modified starches have higher paste clarity compare with a native counterpart. Succinyl groups substitute the hydroxyl group of the starch. This is lead to inhibit retrogradation process and result in a more transparent paste (Lawal, 2004). The SAD 30 has no significant difference compared with SAD 40 and SAD 50. It is mean that different temperature of annealing has no effect on the paste clarity.

Table 3.4 **Effect of different temperature of annealing on the physicochemical properties**

Sample	WBC* (g/g)	Paste Clarity (%)	Swelling power (g/g)	Solubility (%)
Native	$0.69{\pm}0.04^b$	$65.4{\pm}0.5^b$	$16.4{\pm}0.2^a$	$11.2{\pm}0.2^a$
SAD 30	$0.82{\pm}0.02^a$	$68.6{\pm}0.9^a$	$13.6{\pm}0.4^c$	$11.1{\pm}0.2^a$
SAD 40	$0.85{\pm}0.03^a$	$68.2{\pm}0.8^a$	$14.9{\pm}0.1^b$	$11.8{\pm}0.8^a$
SAD 50	$0.88{\pm}0.01^a$	$67.1{\pm}0.6^{ab}$	$15.9{\pm}0.4^a$	$12.1{\pm}0.4^a$

* : Water binding capacity

Values are mean of triplicate determinations $\pm$ SD. Means within columns with different letters are significantly different ($p < 0.05$).

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3.3.9.3. Swelling power and solubility

The swelling power and solubility of the native and dual modified starches were presented in Table 4.4. Swelling power of SAD 30 and 40 has lower than native counterpart, but SAD 50 has no significance different with native. The native and all of the dual modified starch have no significant difference in the solubility. That is indicated that the different of annealing temperature has no effect on the solubility.

3.3.9.4. Morphological properties

The granule shape of the native and all dual modified sample was presented in Figure 4.3. The granules of corn starch have polyhedral and rounded shapes. The findings exhibited that dual modification process with different of annealing temperature had no effects on the changes of granule-shaped compare with native. Moreover, the different of annealing temperature does not change the shape and appearance of starch granules. Annealing process does not significantly change the granule morphology of corn starch (Wang et al., 2014).
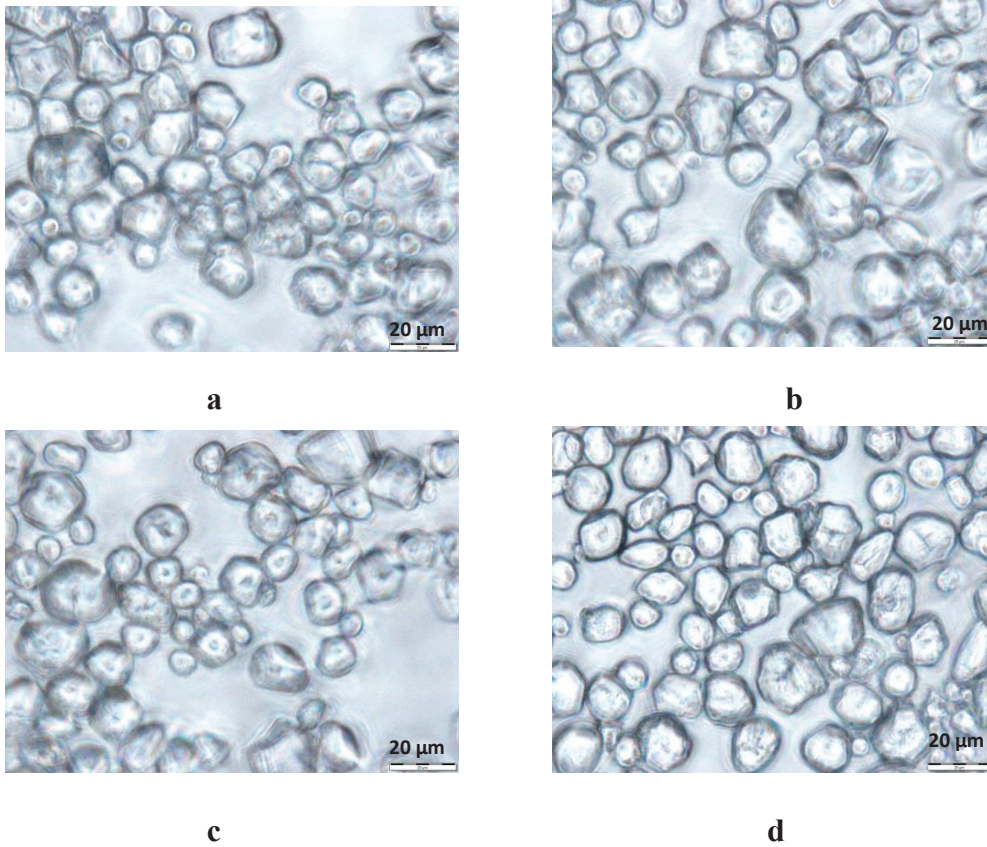
Figure 3.5. Micrographs of a) native, b) SAD 30, c) SAD 40, and d) SAD 50. Scale bar is 20 µm.

3.4. Conclusions

The preparation of dual modified corn starch was investigated. Our results show that dual modification increased the WBC, swelling power, PV, paste clarity, gelatinization temperature, and enthalpy gelatinization, while it had no effect on solubility, granule shape, and X-Ray diffraction pattern compared with the native starch. Dual modification was also shown to be more effective than succinylation at increasing the stability ratio. These results suggest that the undesirable properties of succinylated corn starch can be overcome by dual modification. The findings of this study are of potential use to the food industry in products, such as sauces that require starches resistant to heat and shear stress during processing (Mason, 2009). Further investigation

into the use of this dual modification process is required for other types of starch.

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CHAPTER 4

Conclusion

4.1 Conclusions from this works

Native starch has some weaknesses such as high opacity cooked paste and not stable viscosity in high temperature and shear stress. These weaknesses resulted in the limited use of native starch in the food industry. The starch modification was used to overcome the weaknesses and improve the properties of native starch.

In chapter 2, it is revealed that succinylation process was succeeded to improve the paste clarity of native starch. Succinylated starch with 2% succinic anhydride increased the paste clarity of the starch. However, use beyond 2% succinic anhydride could lower paste clarity. Succinylation with over 2% can decrease solubility, swelling power, and paste clarity, while it has no effect on water binding capacity, stability ratio, PV, and change of granule shape compared with the native starch. These results suggest that the low paste clarity properties in native can be overcome through succinylation process with 2% succinic anhydride. However, it could not overcome the low stability under heat and shear stress.

In chapter 3, the effects of dual modification with succinylation and annealing on the properties of corn starch were investigated. Physicochemical properties (water binding capacity, swelling power, solubility, and paste clarity) were increased after dual modification process. Dual modification process also increased gelatinization temperature and enthalpy gelatinization (ΔH), but it is no effect on morphological properties and X-Ray diffraction pattern. Dual modification process was succeeded to increase stability ratio compare to others samples. The dual modified starch is

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beneficiaries for the food industry, which requires starch with good stability at high temperature and shear stress, for example, sauces industry.

4.2 Future suggestions

Recently, dual modification process was used in the starch modification. Dual modification process has better properties than single modification. The results exhibited that dual modification with succinylation and annealing give a better result than single treatments. The different kind of starch has different physicochemical and functional properties. Thus, it needs further investigation to investigate the effect of this dual modification process with different sources of starch. Moreover, these results can be used in the starch modified industry, but it needs a further investigation.