Himjyoti Dutta
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Editors

Amylose

Properties, Structure and Functions

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PREFACE

Amylose is the linear polymeric fraction of starch which has its unique characteristics leading to its specific role in the application of starches and its own. Amylose forms a significant proportion of the macromolecular structure of starch. Written by a selected team of international authors, including academicians and researchers with special expertise on starch chemistry, technology and functions, the book *Amylose: Properties, Structure and Functions* is a unique approach to the multifaceted trends of amylose chemistry, properties, functionality and applications.

Under the collaborative editorial guidance of Dr. Himjyoti Dutta and Dr. Sanjib Kumar Paul, who are experienced in researches on starch, starch-based composite materials and other biomaterials, the book provides an overview of important scientific and technological approaches on amylose. Traditional and recent analytical methods for amylose purification and characterization have been thoroughly discussed in this book. The role of amylose in major starch sources suggesting specific usage in food and other complex edible and non-edible matrices have been covered. Recent findings on its unexpected properties, directing it to the ever growing world of functional biopolymers have been discussed. Amylose polymorphism and complex formation with non-starch components have been elaborated for optimum knowledge dissemination on its potential use as nano-scale material for food, drug, nutraceutical and pharmaceutical industries.

Looking into the unavailability of an exclusive book on amylose and its various modern aspects, the editorial team, with the collaboration of authors throughout the globe, executed the idea of bringing a reference book on amylose in the name of *Amylose: Properties, Structure and Functions*. The editors emphasized to include all the present day aspects of amylose to address the need of students, researchers and industry experts in a global perspective. Wide coverage of informations with recent findings along-with short and long term consequences and future prospects, a novel attempt was made to make the book as an ideal reference book on amylose for the readers.

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Chapter 6

AMYLOSE INCLUSION COMPLEXES

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ABSTRACT

Amylose is a naturally occurring linear polysaccharide, with a helical conformation of α-(1→4)-glycosidic linkages. Amylose is known as a host compound that can form inclusion complexes with a variety of low-molecular-weight compounds or small molecules, such as iodine, alcohol, the aroma compounds, fatty acids, and esters. Inclusion interactions occur in the hydrophobic part of the guest molecule with the cavity of amylose. Amylose inclusion complexes are generally called V-amylose or V-polymorph pattern. As a result of the conversion, the inside of the inclusion is hydrophobic, while the outside is hydrophilic. The ability of amylose to form inclusion complexes affects the quality attributes of almost all foodstuffs containing starch. Current developments show that inclusion complexes can be utilized in the food and pharmaceutical industries. Several factors affecting the amylose-inclusion complex include thermal treatment, lipid structure, and amylose chain length, in

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addition to pH, the ratio of starch and fatty acids, and temperature. The value of amylose-inclusion complexes can be determined using the complex index value. The molecular structure of amylose-inclusion complexes can be demonstrated by Fourier-transform infrared (FT-IR) spectroscopy, X-ray diffraction (XRD), differential scanning calorimetry (DSC) and ^{13}C solid-state CP/MAS NMR and electron paramagnetic resonance (EPR). The previous studies indicate that the application of amylose-inclusion complexes can be applied for various purposes. These applications include flavor and bioactive releases, composite films, retardation of starch retrograde, and emulsifying behavior. The results of research on amylose-inclusion complexes have shown that the material has extreme potential in food and pharmaceutical sector to play significant role in coming days.

Keywords: starch, amylose, inclusion complexes, V-amylose, molecular struture

1. INTRODUCTION

Starches are the mainly abundant biological source and high molecular weight carbohydrate in nature, as a result of photosynthesis. Starch can be found in all green plant tissues, including tubers, stems, roots, trunks, leaves, and others. Green plants produce starch for energy storage in a granular form. The utilization of starch is very comprehensive, either in food products (for example: bakery, ice cream, thicken sauces, soups, confectionery, syrups, snacks, soft drinks, beer, fat replacers, edible film) or in non-food applications (for example: pharmaceuticals applications, cosmetic, bioplastic and textile, paper, adhesives, packing material) (Amagliani, et al. 2016; Copeland, et al. 2009; Hay, et al. 2018; Konieczny and Loos 2018; Liu, et al. 2018; Mazzocchetti, et al. 2014). As a food ingredient, starch provides almost 70% of energy for a human diet and the glucose molecules given by the starch metabolism is fundamentally used as a substrate in the brain and red blood cells (Copeland, et al. 2009).

Starch is a polymeric carbohydrate of α-D-glucose units as a monomer. It is composed of two main components, namely amylose and amylopectin. Amylose is a linear polyglucan in which each glucose unit-linked via α-

(1→4)-glycosidic linkage, whereas, amylopectin is a branched polyglucan, composed of α-(1→4)-glycosidic linkage of glucose molecules with some additional α-(1→6) branch points (Ciric and Loos 2013; Hanashiro 2015; Lewandowski, 2015; van der Vlist, et al. 2008; van der Vlist, et al. 2012). The amylose consisting of 15-30% and amylopectin typically the major component of native starch granules (Copeland, et al. 2009; Hanashiro 2015; Manca, et al. 2015). The rest of the components cover a small number of lipids (Maphalla and Emmambux 2016), phosphate monoester (Polnaya, et al. 2012; Polnaya, et al. 2013), minerals, and protein/enzymes.

Amylose exhibits a unique ability to form inclusion complexes spontaneously with hydrophobic guest molecules (Condepetit, et al. 2006; Maphalla and Emmambux 2016). In the presence of suitable molecules, amylose would undergo a conformation transformation. Lu, et al. (2019) suggested that the presence of fatty acids would induce the formation of amylose helices and stretch fatty acids. Usually, the guest molecules embedded in the helices, and occasionally, the guest's molecules trapped between the helices (Rondeau-Mouro, Bail and Buleon 2004). The driving force for the helical conformation is the tendency of hydrogen bonds with water molecules and repulsive contact to the hydrophobic part of the starch. The complex formation produces a single, left-handed helix structure which is called V-type amylose complexes (Biais, et al. 2006; Eliasson 2004; Gelders, et al. 2004; Kawada and Marchessault 2010; Obiro, Ray and Emmambux 2012; Vamadevan, et al. 2014; Waduge, Xu and Seetharaman 2010; Wulff, Avgenaki and Guzmann 2005), that may crystallize in an antiparallel arrangement (Kong, et al. 2018). The use of the name V-type amylose as an equivalent to the amylose-inclusion complex has been explained by Putseys, Lamberts and Delcour (2010) in their article.

Colin and de Claubry first demonstrated the interaction of starch and iodine in 1814 (Saenger 1984). Starch can form inclusion complexes with many kinds of molecules or ligands, including aliphatic alcohols and ketones, fatty acids, aromatic aldehydes, hydrocarbons, iodine, dyes, pesticides, and emulsifier and lipid, dimethyl sulfoxide (DMSO), potassium bromide, potassium hydroxide, and aroma compounds

(Condepetit, Escher and Nuessli 2006; Gotanda, Yamamoto and Kadokawa 2016; Itthisoponkul, et al. 2007; Kim and Lim 2009; Kong, et al. 2018; Milani, et al. 2009; Wulff, Avgenaki and Guzmann 2005). The acting fraction is amylose, which can form a threaded building surrounding these other molecules (Lu, et al. 2019). Depending on the type of ligands, different types of V-amylose complexes are produced, which can be between V6 to V8 by their X-ray diffraction patterns, where the numbers represent the number of anhydro-glucose units per turn (Le Bail, Rondeau and Buleon 2005). In the case of the V6 type, which is mostly formed with fatty acids or glycerides, there are three subtypes of crystalline packing (V6-I, II and III) differing in the location of the guest ligands trapped within or between the single helices (Brisson, Chanzy and Winter 1991; Condepetit, Escher and Nuessli 2006) that is stabilized by hydrophobic interactions and hydrogen bonding (Condepetit, Escher and Nuessli 2006).

As a result of this transformation, central channel forms that pass through the axis of the helix, resulting in a hydrophobic helical cavity (Immel and Lichtenthaler 2000), while the outside surface produces a hydrophilic. The hydrophobicity caused by the axial O-1, H-3, H-5, and 6-CH_2 fragments, which engage the inner surface of the helix, while the hydroxyl groups be a factor in the hydrophilicity of the outer surface. The inner cavity is more hydrophobic, which is a guest molecule binding site with the same properties (Immel and Lichtenthaler 2000; Putseys, Lamberts and Delcour 2010). The alternating rotation of the inclusion complexes helix stabilized by various van der Waals forces and hydrogen bonds. The driving force for the formation of inclusion complexes is related to the hydrophobic interactions (Immel and Lichtenthaler 2000) that occur when guest molecules are transferred from an aqueous to a less polar environment (Wang, et al. 2017). An explanation of amylose-inclusion complexes about the formation, identity, and physicochemical properties have described by Putseys, Lamberts and Delcour (2010).

Several specific techniques such as homogenization (Lesmes, Barchechath and Shimoni 2008; Meng, et al. 2014a), steam jet-cooking (Fanta, et al. 2008), microwave heating (Felker, et al. 2013), and extrusion-cooking (Raphaelides, et al. 2010) have been applied to prepare V-amylose

complexes. These techniques aim to increase the solubility of amylose and ligands under the conditions of high-shearing and high-heating temperature, to increase complexation.

2. AMYLOSE-INCLUSIONS COMPLEXES WITH LIPIDS

In recent years, the increase in consumer preference for clean label starches is being observed (Arocas, Sanz and Fiszman 2009) compared to the synthetic chemicals. Lipids can be classified as food-friendly chemicals for clean label starches. The binding of lipids to starch molecules naturally or the addition of lipids to starch often changes their properties, for example, reducing swelling power in water (Ahmadi-Abhari, et al. 2013b; Vasiliadou, Raphaelides and Papastergiadis 2015), retarding retrogradation (Singh, et al. 2003; Tufvesson, et al. 2001), decreasing viscosity of gelatinized starch (Gelders, Goesaert and Delcour 2006), protection of oxygen sensitive molecules (Floros and Ziegler 2011; Lalush, et al. 2005; Lay Ma, Yang, Gu and Zhang 2009), carbon nanotubes (Yang, et al. 2008), suppress colon carcinogenesis (Zhao, et al. 2011), and increasing the content of resistant starch (Liu, et al. 2019; Putseys, et al. 2010;Wang, et al. 2016). Amylose can interact with lipids resulting in the formation of amylose-lipid complexes (Maphalla and Emmambux 2016) and it is schematically represented (Figure 1). The successive helical turns stabilized by many intra- and inter-helical van der Waals bonds and hydrogen bonds. In the amylose-lipid inclusion complex, the aliphatic (hydrocarbon chain) portion of the lipid located in the lipophilic core of the amylose helix, while the polar type located outside the helix. After amylose-inclusion complexed by palmitic acid, the gelatinization temperature leached amylose content, swelling power, and relative crystallinity were generally decreased (Kim, et al. 2017; Nakazawa and Wang 2004). The main factors leading to the formation of complexes are the solubility or dispersibility of the fatty acid in water. Tang and Copeland (2007) suggested that one of the disadvantages of palmitic acid to form complexes with amylose is that they are difficult to dissolve in water.

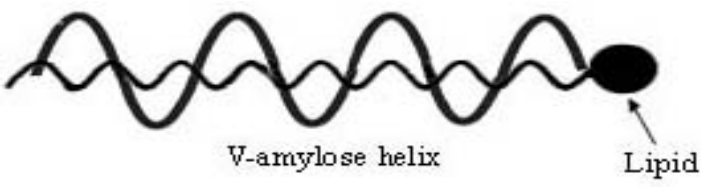

Figure 1. Schematic representation of the amylose-lipid inclusion complexes.

Lu, et al. (2019) suggested that the amylose in starch granules located as individual chains, which are between the chains of amylopectin in amorphous and semi-crystalline regions. Inclusion complexes cause the amylose chain or small amount of amylopectin helices to bind to fatty acids, while uncomplexed fatty acids dispersed between helices in the crystalline regions of the amorphous regions (Biais, et al. 2006; Lu, et al. 2019). Lu, et al. (2019) suggested that in the organization of lamellar structures, amylose-fatty acid complexes and amylopectin formed the crystalline phase, while the branching points of amylopectin, amylose, which does not form complex and free fatty acids will form the amorphous phase.

Previous studies showed that the amylose chain length (Garcia, et al. 2016; Kawai, et al. 2012; Zabar, et al. 2009; Zhou, et al. 2013), characteristics of lipids including lipid type, chain length and saturation (Kanicky and Shah 2002; Uri, Barchechath and Eyal 2008; Zhou, et al. 2013), and complexes temperature (Marinopoulou, et al. 2016) are very influential on the formation of the amylose-lipid complex. Amylose content is an essential factor affecting the formation of complexes. Garcia, et al. (2016) reported that the amylose content had a positive relationship with the number of amylose-lipid inclusion complexes, where exceptionally long amylose chains are useful for forming more stable V-type complexes. Whereas Exarhopoulos and Raphaelides (2012) suggested that the characteristics and level of inclusion complex crystallinity depend on the amylose content, chain length of fatty acids, and different heating methods.

2.1. Complexing Index

The complexing index value can be used to show the level of inclusion of amylose-lipids in complex formation. Li, et al. (2019), Meng, et al. (2014a) and Wang, et al. (2019) use complexing index values to indicate the level of inclusion complexes.

The complexing index was evaluated using the following equation (Li, et al. 2019; Meng, et al. 2014a; Wang, et al. 2019):

$$\text{Complexing index (\%)} = (A_{control} - A_{sample})/A_{control} \times 100\% \quad (1)$$

where, $A_{control}$ is the absorbance of the starch without guest molecule, and A_{sample} is the absorbance of amylose-inclusion complexes.

Li, et al. (2019) showed that the complexing index value increases with increasing concentration of palmitic acid, although at specific concentrations, the value decreases. Li, et al. (2019) revealed that the decline in value could be caused by increased cell-aggregation palmitic acid. Meanwhile, Lebail, et al. (2000) suggests that at high free fatty acids, lipids can get trapped in amylose chains without forming a complex. The results from the study of Wang, et al. (2019) showed the fact that the higher number of amylose molecules produced during the branch removal process encourages more formation of amylose-lipid inclusion (Jane, et al. 1999). Pullulanase debranching method is one of the best methods that can produce a high complexing index. The amylose polymer produced as a result of cutting by the enzyme provides more free polymers and can form inclusion complexes (Wang, et al. 2019). The complex index value is also positively correlated with DPPH radical scavenging activity (Li, et al. 2019).

Wang, et al. (2019) suggest that the different methods for producing amylose-inclusion complexes cause differences in the value of complexing indexes. The value of the complexing index of potato starch-lauric acid inclusion complexes was determined using the dimethyl sulfoxide heating method (26.92%), ultrasound treatment (33.24%), and the pullulanase debranching method (47.26%), and observed higher than the control

(22.12%). The dimethyl sulfoxide solvent used in the dimethyl sulfoxide heating method promotes amylose release from potato starch swollen. Therefore, dimethyl sulfoxide heating samples have higher complexing index value than control samples. The same stated by Singh, et al. (2006), that the formation of the amylose-lipid complex would inhibit the development of starch granules. In the ultrasound method, ultrasonic treatment destroys potato starch granules to release amylose molecules, which increases the chance of contact between amylose and lauric acids. Ultrasonic treatments increased the dispersion of lauric molecules in swollen potato starch paste (Liu, et al. 2018). Therefore, the value of the complexing index increases after the ultrasonic treatment of potato starch-lauric acid complexes. For the pullulanase debranching method, Zhang, et al. (2012) stated that the debranching pretreatment can facilitate the formation of V-type complexes. This result can be ascribed to the fact that higher the number of amylose molecules produced in the branch removal process encourages more formation of amylose-lipid inclusion complexes with the pullulanase debranching method (Jane, et al. 1999). The pullulanase debranching method shows a higher index value than the other methods. Wang, et al. (2019) suggested that there are relatively more free amylose molecules produced by the pullulanase debranching method involved in complexing reactions compared to other methods.

2.2. Amylose-Inclusion Complexes Structure

Structures and physicochemical properties of amylose-inclusion complexes need to be known to improve their use in future. Zabar, et al. (2010) through his research using an acidification method showed how to characterize the structure of amylose-inclusion complexes with fatty acids. The molecular structure of fatty acid molecular inclusion complexes was then verified using Fourier-transform infrared (FT-IR) spectroscopy, X-ray diffraction (XRD), differential scanning calorimetry (DSC) and ^{13}C solid-state CP/MAS NMR. The results of XRD analysis can be used to confirm the formation of amylose-inclusion complexes (Li, et al. 2019; Wang, et al.

2017; Zabar, et al. 2010). Lesmes, et al. (2009) suggested that the structure and physicochemical properties of V-amylose are essential to study because of their future use.

Li, et al. (2019) suggested that the use of FT-IR spectroscopy can monitor changes in starch structures that have experienced inclusion complexes. Starch complexes show structural changes compared to their native starch. There are two additional bands at 1705 cm^{-1} and 2846 cm^{-1} that show amylose-palmitic acid complexes.

Amylose inclusion complex with other molecules causes changes in molecular structure. This can be indicated by the emergence of a new peak based on XRD testing. The peaks formed are Bragg angels of 2θ = 7.40°, 13.10°, and 19.80°. The temperature of crystallization also affects the formation of new peaks, but not for all fatty acids. Zabar, et al. (2010) suggested that the amylose-stearic acid inclusion complexes do not produce new peaks, but complexes with linoleic acid and conjugated linoleic acid produce new peaks. The peak located in Bragg angels of 2θ = 14.90°, 17.10°, and 22.60°. These results indicate that the crystallization temperature induces the formation of type-A amylose crystals for inclusion complexes. Wang, et al. (2017) reported that the inclusion of new peaks formed in the high amylose maize starch (HAMS) complex-salicylic acid (SA), HAMS-1-naphthol (1-NPL) and HAMPS-2-naphthol (2-NPL). The three complexes also show differences in the new peaks formed (Table 1).

Complex X-ray diffraction patterns of all samples (except for linolenic acid) show two main peaks, corresponding to the Bragg (2θ) around 13.0° dan 20,0° and minor peaks at 7.0°, which present a typical V6-I type complex consists of six anhydro-glucose units per turn (Le Bail, Rondeau and Buleon 2005; Lesmes, et al. 2009). In the case of linolenic acid, especially at the reaction temperature at 90°C, it shows a double peak at 13.0° and a new peak at 18.0°. These results may indicate that the amylose-linolenic acid complex formed in V7 type crystals. Also, some researchers report that when V7-type complexes drained, the crystalline structure partially transformed into V6 form (Nuessli, et al. 2003; Rondeau-Mouro, Bail and Buleon 2004). It can be assumed that the linolenic acid is trapped inside and/or between a single amylose helix (V6-II and III). That the

peaks at around 20° degrees 2θ also found in low-amylose cereal starch and amylose-free potato starch (Vamadevan, et al. 2014; Varatharajan, et al. 2011; Varatharajan, et al. 2010), and therefore, can not be expressly demonstrated to be associated with amylose-lipid complexes (Bertoft 2017). This condition indicates that other peaks in XRD are needed to prove the formation of amylose inclusion complexes (Table 1). Based on the crystallization conditions (temperature and type of ligand), there are structural differences in the shape of the amylose-lipid complex in the solid-state, namely: type I (form I) and type II (form II). Type I demonstrates an amorphous X-ray pattern, while Type II shows a typical V-type pattern. The state of the complex represents an energy-favorable situation for both amylose and surfactant molecules, which is reflected by the fact that the complexes formed are difficult to separate. Type I polymorphs measured by DSC analysis is generally formed in a random arrangement with a single helix, which is not easily detectable by XRD analysis. However, the type I polymorphic complex, as observed in the DSC thermogram shows the type V6-I diffraction pattern, probably due to dehydration.

Five allomorphic families of compact-helix V-amylose have reported, in which three contain 6-fold helix, one contains 7-fold helix, and one contains 8-fold helix. Helbert (1994) proposed a nomenclature system for the compact-helix V-amylose based on the number of residues per turn and volume of inter-helical space. Consequently, the three 6-fold V-amylose families can be called as V6I, V6II and V6III as a function of the volume of inter-helical space. The7- and 8-fold V-amylose families can be called V7 and V8, respectively.

Probes such as salicylic acid (SA), 1-naphthol (1-NPL), and 2-naphthol (2-NPL), are used to show the amylose-inclusion complexes better. XRD is usually used to confirm the formation of inclusion complexes between high-amylose maize starch (HAMS) and probes. The results from the study (Wang, et al. 2017) indicated that the HAMS can be complexes with SA, 1-NPL, and 2-NPL, and produce HAMS-fluorescent probe inclusion complexes. The SA probe is observed to be the best fluorescent probe that is determined based on the degree of crystalline and the encapsulation rate.

Table 1. New peaks formed after the amylose-inclusion complexes with several guest molecules

Inclusion Complexes	Peak (2θ)													DC	References
	5°	6°	7°	11°	12°	13°	14°	16°	17°	18°	19°	21°	22°		
HAMS-SA		6.10			12.65			16.40						34.20	(Wang, et al. 2017)
HAMS-1-NPL	5.98				12.74			16.48						29.49	(Wang, et al. 2017)
HAMS-2-NPL		6.72		11.50						18.06				28.85	(Wang, et al. 2017)
Amylose-FA	5.0		7.4			13.1	14.9		17.1		19.80	21.7	22.6	28-43*	Zabar, et al., (2010); (Lu, et al. 2019)
Amylose-LA							14.9		17.1				22.6		Zabar, et al., (2010)
Amylose-CLA							14.9		17.1				22.6		Zabar, et al., (2010)
Amylose-palmitic acid			7.5		12.5							21.5	23.5		(Li, et al. 2019); (Wang, et al. 2016)
Amylose-x-DSA						13.5						20.5			(Kong, et al. 2018)
Amylose-fatty sodium salt					12.8						19.9				(Hay, et al. 2019)
Amylose-β-CD			7.3			13.0								14-17	(Tian, et al. 2010)
Amylose-FeA			7.5			13.0					19.5				(Kenar, et al. 2016)
dG50-LA-AP					12.88						19.80				(Liu, et al. 2019)

Description: HAMS = high-amylose Maize starch; 1-NPL = 1-napthol; 2-NPL = 2-napthol; FA = fatty acids; FeA = ferulic acid; dG50-LA-AP = balanced High amylose corn starch-lauric acid-atmospheric pressure; DC = degree of crystallinity; *DC samples produced by various methods.

The intensity of fluorescence probes can reflect the formation of inclusion complexes. The results from the study (Wang, et al. 2017) showed that the potential fluorescence probe method can be used to determine the capability inclusion complexes.

Based on thermal behavior analysis using DSC, it can be stated that the production temperature of amylose-inclusion complexes also affect the melting temperature (Zabar, et al. 2010). Other findings also showed almost similar results, like melting temperature of the complexes correlated with the melting temperature of the fatty acids (Tufvesson, Wahlgren and Eliasson 2003a). Lauric acid has a lower melting temperature than conjugated lauric acid or stearic acid. Besides melting temperature, melting enthalpy of stearic acid complexes is also higher than amylose-conjugated lauric acid and amylose-lauric acid complexes. Zabar, et al. (2010) suggested that the differences between stearic and lauric acids complexes might be due to differences in their chemistry. Some research (Tufvesson, Wahlgreen and Eliasson 2003a; Tufvesson, Wahlgreen and Eliasson 2003b) results showed that the thermal stability and melting enthalpy of the crystal complex depend on the chemical properties of the complexing ligands (chain length, unsaturation, nature of polar head groups), the degree of amylose polymerization and the conditions (temperature, time and solvent) used during complexation. Kong, et al. (2018) suggested that the based-on the measurements of DSC, fatty acid chain length increases the thermal stability of amylose-fatty acid inclusion complexes.

Analysis using ^{13}C and CP NMR shows that C1 and C4 carbon of amylose are susceptible to binding to a guest molecule, such as fatty acid. The peaks formed for carbon of fatty acids in the chemical shift range of 15-35 ppm. Differences in the type of fatty acids in the inclusion complexes can cause different molecular mobility. This difference is greatly influenced by fatty acid saturation. Molecular mobility of saturated stearic acid is higher than unsaturated linoleic acid, and hence amylose-linoleic acid inclusion complexes are more spatially defined structures than amylose-stearic acid complexes (Zabar, et al. 2010).

Electron paramagnetic resonance (EPR) spectroscopy can also be used to study the microenvironments of the biological system. One of them is

inclusion complexes (Bardelang, et al. 2006; Mezzina, et al. 2007). EPR provides essential information about molecular dynamics and local polarity of fatty acids and probes within these systems. Specific spin probes, especially those derived from fatty acids, may thus be used to interact with amylose and fatty acids in forming inclusion complexes. For example, 5- and 16-doxyl-stearic acids (5-DSA and 16-DSA) are derivatives of stearic acid and carry a doxyl ring moiety containing a nitroxide radical, which makes them EPR-active.

3. AMYLOSE-INCLUSIONS COMPLEXES WITH GLYCEROL MONOSTEARATE

Glycerol monostearate functions to bind materials, become lubricants during extrusion, prevent the development of extrudates, make extrudates not stick to each other, and reduce product cooking loss during the cooking process (Kaur, Singh and Singh 2005; Singh, Sharma and Singh 2000). Glycerol monostearate is known to form a helical inclusion complex with amylose (Gultom 2014). The complex can prevent starch granules from expanding, which can lead to reduced development strength and solubility. Fatty acids have hydrophobic and hydrophilic parts, such as glycerol monostearate. Therefore, it can be presumed that the amylose and glycerol monostearate can form the same structure like fatty acids.. The effect of adding glycerol monostearate in extrusion of corn grits reduces water solubility index, specific energy consumption, and expansion (product development) but increases water absorption index. This function is needed to make analog rice, which processed at high extrusion temperatures (Gultom 2014).

Glycerol monostearate form helical inclusion complexes with amylose by occupying the hydrophobic amylose helical core. The complex can prevent starch granules from expanding to reduce the strength of development and solubility. Glycerol monostearate includes an emulsifier that has a fat fraction with high melting temperature, has the role of

facilitating shear, uniformity of extrudate formation, and protecting the dough from stickiness so that the extrusion process becomes easier (Moscicki 2011).

4. AMYLOSE-FLAVORING INCLUSION COMPLEXES

The background of the hydrolysis study of inclusion complexes between amylose and flavoring compounds is far less comprehensive than lipids as ligands. However, monitoring of α-amylase catalysis during starch processing is attractive both for the production of resistant starch and for its effect on guest release, which modulates the perception of aroma and taste during consumption of certain products (Sajilata, Singhal and Kulkarni 2006). In this case, Heinemann, et al. (2005) studied hydrolysis of the inclusion complex between amylose and various flavoring compounds such as geraniol, γ-nonalactone and δ-dodecalactone when using porcine pancreatic α-amylase, even though hydrolysis was carried out directly on the dispersion system after the heating step (without drying steps). In subsequent research, Tietz, Buettner and Conde-Petit (2008) analyzed the interactions between menthone flavor and native tapioca flour in aqueous suspension, without performing special procedures intended to obtain inclusion complexes. Several studies of amylose-inclusion complexes with flavors such as linalool, citronellol, limonene, ß-pinene, geraniol, menthol (Ades, et al. 2012) and menthone (Ades, et al. 2012; Tietz, Buettner and Conde-Petit 2008), camphor, thymol (Heinemann, et al. 2005; Tapanapunnitikul, et al. 2007; Yeo, Thompson and Peterson 2016) etc. are reported.

Haryadi (1993) suggested that the amylose-inclusion complexes with flavor compounds are weak. The compound is released when amylose attacked by an enzyme. This behavior is used to trap or encapsulate flavors in the extrusion process and other processes. Many types of flavor have brewed in dried starch powder for various purposes. Furthermore, it was stated by Haryadi (1993) that complex inclusion also occurred in the storage of rice. As long as it is stored, some lipids on rice are broken into

monoglycerides and diglycerides. These fragments can form complexes with amylose. In new rice, there is a minimal amylose-lipid complex. A small portion of amylose released from rice grains during cooking. This contributes to the appearance of adhesiveness between rice grains. The waxiness of starch depends on the presence of oryzenin which dissolves and escapes from the granules, which interact with starch molecules. In rice that has been stored for a long time, some amylose forms a complex with lipids resulting from the breakdown of rice oil enzymes until it is insoluble and does not escape when cooking rice.

The formation of complex amylose inclusion will reduce the tendency of amylose to undergo retrogradation, thereby inhibiting the speed of increased viscosity during heating (Haryadi 1993; Suarni and Aqil 2013) and the further leaching of amylose (Putseys, et al. 2010).

5. INCLUSION COMPLEX WITH PUFA AND OMEGA 3

Zabar, et al. (2010) suggested that various encapsulation techniques had been carried out to improve control over the release of lipophilic nutraceuticals such as PUFAs and omega-three rich oils. Furthermore, the study of amylose inclusion-complexes showed that the complex could control the delivery system for PUFA. The results of the study show that there are two primary forms of polymorphic crystalline, namely types I and II. Type I consist of amorphous regions, while type II is semi-crystalline. Type II shows three peaks based on the XRD pattern, which is 7.4°, 13.1°, and 19.8° (Lesmes, et al. 2009). Based on transmission electron microscopy (TEM) testing, the form of inclusion of the amylose-fatty acid complex is uniaxial.

Zabar, et al. (2009) combine molecular-level investigations with nanostructure and microscopic characteristics. Research of Zabar, et al. (2010) shows that amylose inclusion complexes can be developed by different production methods, and are more suitable for food application. The amylose complex with 18:0 saturated stearic acid,18:2 linoleic acid, and 18:2 mixture of isomeric conjugated linoleic acid produced using the

acidification method (Zabar, et al. 2010). The XRD diffractogram shows the formation of amylose-fatty acid's inclusion. The three main peaks formed after the inclusion complex are as stated (Lesmes, et al. 2009). However, the inclusion of the amylose-linoleic acid or conjugated linoleic acid complex, which crystallized at 90°C, results in additional low-intensity peaks, namely 2θ = 14.9°, 17,1°, and 22.6°. These results indicate that the high crystallization temperature induces the formation of A-type amylose crystals. All complexes with stearic acid produce a new peak of 2θ = 21.7° which is V-amylose polymorphism.

DSC shows a complex thermal behavior of inclusion. Melting temperature increases with increasing production temperature. The type of fatty acids also determines a high melting temperature. Melting the linoleic acid temperature is lower than conjugated linoleic acid and stearic acid.

Zabar, et al. (2010) stated that the resolution of carbon C1 and C4 amylose is very sensitive to the inclusion complex of amylose-fatty acids, for linoleic acid or stearic acid, based on ^{13}C and CP NMR testing. There is a difference between linoleic acid and stearic acid, which may be due to molecular mobility in the sample. Their saturation largely determines increased molecular mobility of the inclusion complexes of amylose-fatty acids. The mobility of saturated fatty acids (stearic acid) is higher than that of unsaturated fatty acids (linoleic acid). Zabar, et al. (2010) suggested that the amylose complexes produced with linoleic acid demonstrate more spatial structures than the complexes produced with stearic acid.

The inclusion complex of amylose-fatty acids causes changes in the morphology of amylose particles. Zabar, et al. (2010) reported the changes between the titration becomes an acid condition (t = 0 h) and the completion of the crystallization process (t = 24 h). Surface roughness measurements also indicate this change. SEM images of amylose-fatty acids inclusion complexes are also affected by production temperatures, but this is not indicated by conjugated linoleic acid and stearic acid. Products produced at low temperatures (<60°C) shows smooth surface properties, and there is almost no difference at the micronic level. Production at high temperatures (~90°C) produces formation of amorphous structure. Amorphous structures are formed as a result of the presence of

bubbles as they approach the boiling point of water when precipitated. The interaction of amylose with fatty acids leads to the formation of molecular inclusion complexes, which arranged in the lamella packaged in aggregated spheroids. The presence of fatty acids induces segments in the amylose chain to form helices that bind fatty acids.

Thermogravimetric analysis was used to confirm the formation of amylose-inclusion complexes - the temperature range used for analysis is 50° to 600°C. Weight loss of the inclusion complexes starts at a temperature of 155° to 165°C and decomposed at more than 220° to 250°C. This shows that amylose-inclusion complexes cause thermal properties to be more stable (Wang, et al. 2017).

Lauric acid has been included in starch to prepare the amylose-lipid inclusion complexes (Chang, He and Huang 2013; Zhang, et al. 2012). Lauric acid is more strongly bound to amylose than other fatty acids with longer carbon chain lengths (Tang and Copeland 2007). Meng, et al. (2014b) found that the corn starch with long carbon chain fatty acids had index complexes value lower than corn starch with lauric acid because of low dispersivity in gelatin starch. According to Kawai, et al. (2012), melting enthalpy (the number of inclusion complexes) decreases with an increasing number of carbon atoms in saturated fatty acids containing lauric acid C12:0, myristic acid C14:0, palmitic acid C16:0, stearic acid C18:0.

6. The Crystallinity of Amylose-Fatty Acids Inclusion Complexes

A number of studies have been carried out on the formation of the amylose-lipid complex and its crystalline properties, reporting that these characteristics are strongly influenced by thermal treatment, lipid structure, and amylose chain length. Complex formation reactions at relatively low temperatures (90°C) forms complexes such as lamella (type II) because nucleation is slow, and propagation for crystallization is adequately

processed (Biliaderis and Galloway 1989; Karkalas, et al. 1995). Regarding the starch chain length and linearity appear to be the most important structural features for the formation and V-complex characteristics. It is reported that longer amylose chains preferred for the formation of stable complexes (Gelders, et al. 2004; Zhou, et al. 2013). Many researchers study the effects on the chemical structure of lipids in the formation of complex inclusions with starch. It reported that the longer the fatty acid chains in lipids form a more stable complex of heat, and the addition of double-bonds in fatty acids (unsaturated) interferes with the formation of the V-amylose complex (Eliasson and Krog 1985; Zabar, et al. 2009; Zabar, et al. 2010). Most studies of the formation of V-amylose complexes for fatty acids have been carried out during thermal analysis primarily using differential scanning calorimeters, and large-scale elaborate preparations.

7. EFFECT OF pH ON INCLUSION COMPLEXES

Seo, Kim and Lim (2015) reported that pH has a significant role on amylose-fatty acids inclusion complexes. The experiment uses stearic and oleic acids. Recovery of stearic acids are 74.72% at pH 7, while oleic acid is 72.68% at pH 6. The recovery value reaches a maximum when the pH is neutral. The recovery of starch also shows relatively similar results. Seo, Kim and Lim (2015) showed that the low pH conditions could cause partial hydrolysis when starch heated at high temperatures (121°C). Partial starch hydrolysis causes short amylose chain formation and causes the opportunity to form lower amylose-inclusion complexes. Also, alkaline conditions can increase the hydrogen bond between starch and water, which also affects the interaction between starch and fatty acids.

The formation of amylose-polyunsaturated fatty acids (linoleic and linolenic acids) is limited to inclusion complexes when reactions occur under acidic conditions (pH 5 and 6). Recovery of both polyunsaturated fatty acids and starch increases due to increased reaction pH. Yotsawimonwat, et al. (2008) suggested that an increase in PUFA recovery

with an increase in pH might be from an increase in solubility of PUFA by ionization. The pKa value also determines suitability for alkaline conditions. PUFA has a pKa value that is lower than stearic and oleic acids, causing PUFA to have higher suitability in alkaline conditions.

8. The Ratio of Starch and Fatty Acids

The weight ratio between starch and fatty acids in the inclusion complex can be plotted as a function of fatty acid recovery. The weight ratio of the two components within the complex is not constant but decreases as the recovery of fatty acids increases. This trend shows that the inclusion of multi-molecular fatty acids may be in the amylose unit chain, although the formation of the V-amylose complex is relatively unstable with unsaturated fatty acid glycerides (Eliasson and Krog 1985; Zabar, et al. 2010).

Stearic, oleic, and linolenic acid reached a maximum recovery value of about 75% by weight, but the maximum recovery for linoleic acid was much lower (less than 30%). These results indicate that the formation of complexes between amylose and fatty acids does not always have a positive correlation with the degree of saturation. In particular, the exceptionally high recovery rate of linolenic acid, even though it is prone to thermal oxidation, is very interesting (Seo, Kim and Lim 2015). Karkalas, et al. (1995) suggests that the free rotation of C-C bonds near unsaturated bonds allows unsaturated fatty acids to form quasi-linear conformations around double bonds. This structural transformation can produce behavior similar to saturated fatty acids. Oleic and linolenic acids produce the same results in complex formation, but linoleic acid shows lower results, although only several fatty acids tested. Based on these observations, it can be hypothesized that even unsaturated bonds numbered in fatty acids may not form quasi-linear or U-forms for suitable complex formations. Additional research must be followed to verify this hypothesis.

9. EFFECT OF TEMPERATURE

Ahmadi-Abhari, et al. (2013a) suggested that the temperature played an essential role in the susceptibility of starch enzymes. We observe that temperature at 60°C the action of amylase is much slower than at higher temperatures; therefore, a lower amount of reducing sugar is formed even after 240 minutes of digestion. The research of Ahmadi-Abhari, et al. (2013b) reported loss of starch crystalline at 60°C. The crystallinity loss was a prerequisite for the development of swelling and an increase in the suspension viscosity of starch, which does not occur below 60°C. Increasing the temperature of 5°C to 65°C increases the amount of reducing sugar as a function of digestive time. More than 60% of reducing sugars observed after 240 minutes of enzyme hydrolysis at 65°C. This sharp temperature effect was related to changes in the crystal structure of starch granules. By heating at a temperature that exceeds the temperature of gelatinization of starch, the rate of digestion increases because the crystalline phase melts, the entry of water and accessibility for the enzyme increases. That leads to a sharp increase in reducing sugar. When starch heated to 90-95°C, the amount of reducing sugar increases further to more than 70%. Interestingly, this increase is relatively small compared to the effect of losing crystallinity.

Seo, Kim and Lim (2015) s uggested that the crystallinity represented by peak intensity positively correlated with recovery of fatty acids: the lowest peak intensity as the reaction temperature of 50°C and the highest intensity at 90°C. All the fatty acids tested in this study show the ability of the type V crystal complex with amylose, especially when the reaction temperature is 90°C. The consistency between fatty acid recovery and crystallinity shows that the inclusion of fatty acids occurs primarily for the formation of long-range crystal structures detected by XRD. However, Seo, Kim and Lim (2015) suggested that the additional studies are still needed to understand the relationship between reaction parameters and structural characteristics of amylose fatty acids.

10. DIGESTIBILITY OF AMYLOSE-INCLUSION COMPLEXES

Starch digestibility determines the Glycemic Index; the level of glucose released into the blood. Unfortunately, rapid digestibility causes high GIs, resulting in overweight and obesity, ultimately contributing to several diseases, such as type II diabetes (Soong, Goh and Henry 2013). V-amylose would modulate the glycemic response due to an increased resistance to hydrolysis (Obiro, Ray and Emmambux 2012; Putseys, et al. 2010) and would help to improve glycemic regulation and may help in the prevention and management of insulin resistance and metabolic syndrome (Hasjim, et al. 2010; Lau, Zhou and Henry 2016).

Starch, based on digestibility can be classified in to three categories: RDS (starch which can be digested quickly, which is digested into glucose after 20 minutes), SDS (slow digestible starch, starch digested into glucose between 20 and 120 minutes) and RS (resistant starch, starch which cannot be digested but fermented in the large intestine) which is characterized by the level and duration of the glycemic response (Cummings 1992; Englyst, Kingman and Englyst, et al. 1999). In general, starch digestion is a complex process that is highly dependent upon the substrate, the enzyme adsorption by the substrate, and the presence of other components such as lipids and proteins (Lehmann and Robin 2007). It is possible to increase the resistance of starch components to enzyme hydrolysis. For example, endogenous lipids and phospholipids in cereal starch can complex with amylose (Kwasniewska-Karolak, Nebesny and Rosicka-Kaczmarek 2008) making amylose more susceptible to amylolytic enzymes (Zhang, Ao and Hamaker 2006; Zhang, Venkatachalam and Hamaker, 2006). *In vivo* and *in vitro* digestibility studies of the effects of these components have shown that they can slow enzymatic digestion (Singh, Dartois and Kaur 2010).

Recent studies have reported the low digestion of V-complexes (Putseys, Lamberts and Delcour 2010). The V-complex characterized by a specified X-ray diffraction pattern and formed between the aliphatic lipid chain and the amylose molecule. Lysophosphatidylcholine as a complexing agent who has demonstrated high complexing capabilities with amylose, as shown by DSC (Ahmadi-Abhari, et al. 2013b). Furthermore, the formation

of V-amylose has been shown to reduce the digestibility of starch and increase the resistance to enzymatic hydrolysis.

Another factor that affects starch hydrolysis is the formation of inclusion complexes between amylose and other natural components or added during starch processing. Native starch contains a proportion of natural lipids that form complex inclusions with amylose components when they gelatinise in the presence of these lipids. Two general results from various studies are that, first, the inclusion complex shows resistance to enzymatic hydrolysis compared to uncomplexed amylose and control starch and second, the rate of hydrolysis reaches a steady-state values several hours after the initiation of an enzymatic reaction (Ai, Hasjim and Jane 2013; Kawai, et al. 2012; Rodríguez and Bernik 2014). Putseys, et al. (2010) suggested that the amylose-lipid complex influenced the characteristics of starch gels and could reduce the rate of digestion. This is possible because of the release of the short amylose chain in a controlled manner, which then undergoes amylose crystallization. This amylose crystallization will form the RS.

On the other hand, Putseys, Lamberts and Delcour (2010) also proposed that with the inclusion complex, the hydrolysis process occurred in two stages. The first stage, characterized by high hydrolysis rate, is associated with enzymatic attacks to amorphous regions, such as amylose residues between helical structures; whereas the second stage achieved when hydrolysis involves the attack of the enzyme into the inclusion complex itself, which takes place at a slower rate. Li, et al. (2019) suggested that the amylose-palmitic acid had the potential to reduce cholesterol.

Ahmadi-Abhari, et al. (2013a) suggested that the complexation of amylose with lysophosphatidylcholine reduces the susceptibility of wheat starch granules to α-amylase, compared to native wheat starch, which rapidly degraded. Depending on the concentration of lysophosphatidylcholine, the amylose molecule develops an inclusion complex and becomes more easily degraded. Digestive differences between samples containing lysophosphatidylcholine and references, based on the amount of reducing sugars, illustrate the accessibility of lower

inclusion complexes to enzymes. The difference is increasing after digestion for 240 minutes. Conformation barriers to enzymatic attacks due to the new V-helix form, explain the decrease in α-amylolysis. Complex formation inhibits digestive enzymes to access glycosidic bonds throughout the helix. Depending on complex stability, this even leads to full resistance of the amylose-lysophosphatidylcholine complex to amylolysis. So, in principle (Ahmadi-Abhari, et al. 2013a) suggested that the formation of inclusion complexes causes the starch to be more difficult to digest. This result also proved through DSC analysis, which showed that there was a decrease in enzymes to access starch as a result of the formation of amylose-LPC complexes. Also, the inability of starch to swollen as a result of inclusion complexes causes low accessibility of amylase to starch molecules (Ahmadi-Abhari, et al. 2013a). The reduced digestive complexes increase the potential of amylose-fatty acid inclusion complexes to suppress colon carcinogenesis. Zhao, et al. (2011) suggests that rat fed cooked with inclusion complexes can reduce azoxymethane-induced preneoplastic lesions (colonic cancer precursors) in the colon of rat. Meanwhile, Liu, et al. (2019) expressed the same opinion, and there was an increase in slowly digestible starch and resistant starch contents for starch complexes.

CONCLUSION

Starch is part of the human diet, so that it gets much attention in the development of its use. Starch is composed of two main glucose polymers, amylose, and amylopectin. Amylose, a straight-chain polymer, has a special feature to form inclusion complexes with hydrophobic guest molecules, where the guests are embedded in the helical interior of starch. Hydrogen bonds and the repulsive contact to hydrophobic part of starch are two critical things to encourage inclusion complexes. Guest molecules or ligands generally form inclusion complexes with amylose such as aliphatic alcohols and ketones, fatty acids, aromatic aldehydes, hydrocarbons, iodine, dyes, pesticides, emulsifiers and lipids, DMSO, potassium bromide,

potassium hydroxide, and aroma compounds. Amylose-lipids inclusion complexes can cause changes in their physicochemical properties, such as reducing swelling power, retarding retrogradation, decreasing viscosity of gelatinized starch etc. The main factors that play a role in the formation of inclusion complexes are the solubility of fatty acids in water. The level of amylose-inclusion complexes can be shown using complexing index values. The higher value of the complexing index shows the high inclusion complexes that formed. The structure of amylose-inclusion complexes and physicochemical properties is essential to know to increase their utilization. The molecular structure of amylose-inclusion complexes can be demonstrated using FT-IR, XRD, DSC, NMR, and EPR. Analysis using XRD shows new peaks after the formation of inclusion complexes, but not always. The use of a probe scan further clarifies understanding using XRD about amylose-inclusion complexes. DSC can be used to study the effect of production temperature on inclusion complexes. The position of C1 and C4 carbon of amylose is susceptible to binding to a guest molecule. It can be indicated by the analysis using ^{13}C and CP NMR when there is a difference in molecular mobility. EPR spectroscopy can also be used to study microenvironments of inclusion complexes.

Amylose-inclusion complexes that have been studied to include complexes with fatty acids and flavorings. Changes in the crystallinity of amylose-inclusion complexes is influenced by various factors such as thermal treatment, lipid structure, and the length of the amylose chain. Amylose-inclusion complexes are strongly influenced by pH, the ratio of starch and fatty acids, and temperature. The recovery of starch and fatty acid's increases at pH is neutral. Unsaturated bonds, even in fatty acids, may not form suitable complex formations, although further research needed for verification. Temperature influences change in the starch crystal structure and positively correlated with the recovery of fatty acids. Amylose-inclusion complexes also influence the digestibility of starch. Inclusion complexes cause low starch digestibility due to resistance to enzymatic hydrolysis.

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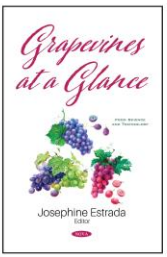

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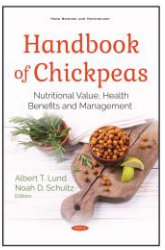

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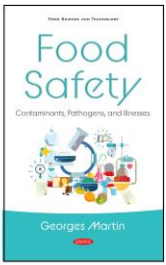

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