V-amylose Structural Characteristics, Methods of Preparation, Significance and Potential Applications

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Abstract

The amylose component of starch can form complexes known as V-amylose with amphiphilic or hydrophobic ligands. The V-amylose complexes are single, left-handed helices that are arranged as crystalline and amorphous lamellae, which may form distinct nano or micron scale structures. V-amylose has potential as a biomaterial for nanoencapsulation of sensitive bioactive and flavor ingredients, modification of rheological behavior of starch-containing products, reduction of starch retrogradation, and postprandial hyperglycaemia in diabetics. Various aspects of V-amylose structure, methods of preparation, factors that affect its formation, the significance and potential applications of the V-amylose complexes are reviewed.

Keywords V-amylose Complexes, Starch Nanoparticles, Starch Spherulites, Starch Digestibility, Starch Rheology, Nanoencapsulation

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Introduction

Starch is a major plant food material and ingredient that is made up of two glucose polymers; a linear amylose and branched amylopectin.[1] There has been increased interest in application of starch and its derivatives due to its biodegradability and availability, which make it a good biopolymer candidate for development of ingredients of food and pharmaceutical product components.

Amylose consists of mainly α-(1→4)-glucan linkages with a few occasional α-(1→6)-glucan linkages.[2] The amylose molecules are about 15 to 30 % of starch depending on the type of starch; starches having >40% amylose are referred to as high amylose starches.[2] Amylose can form single helical inclusion complexes generically known as V-amylose.[3-5] The V-amylose complexes have been shown to form with a diverse range of compounds such as alcohols,[6-8] fatty acids,[9] potassium hydroxide (KOH),[10] iodine,[11] flavor compounds,[12-15] and hydrophobic organic polymers.[16-22] V-amylose complexes have shown potential in various food-related applications such as nanoencapsulation of sensitive bioactive[23, 24] or flavor compounds,[12, 14] formation of amylose nanotubes,[25] and modification of starch rheological functionality.[26, 27] Although presently V-amylose in not yet commercially applied as an ingredient in food or pharmaceutical systems, its has been associated with starch functionality modulations that include modification of starch pasting properties,[27, 28] reduced retrogradation,[29] and increase of the resistant starch content in starch.[30] Since amylose is an inherent food component and natural ingredient, the application of V-amylose as an ingredient in foods products would raise limited legal or regulatory issues if the included ligands are food grade.

The present paper discusses aspects of the V-amylose structure, methods of preparation, factors that affect V-amylose formation, its significance and potential

applications. Areas that require focus for improved understanding and utilization of V-amylose complexes are considered.

Chemistry and Structure of V-amylose Complexes

V-amylose Helix Molecular Arrangement

The inner surface of the V-amylose complex helix is lined with methylene groups and glycosidic linkages, hence resulting in a hydrophobic helix cavity while the hydrophilic glycosyl hydroxyl groups are located on the surface of the helix.[11], Godet *et al*., [5, 31] using simulated molecular docking, pointed out that within the V-amylose helix, the H-5 atoms of the glycosyl residues in a six glycosyl residue ring could form van der Waals interactions with fatty acid methylene groups, hence also implying a hydrophobic internal environment. Rappenecker *et al*.[4] and Nimz *et al*.[32] described the intra and intermolecular contacts in V-amylose that are necessary for stability of polymeric and macrocycle 26-D-glucose cycloamylose V-amylose, respectively. They showed that the helical amylose structure and amylose-ligand interaction are stabilized by a series of molecular hydrogen bonds and van der Waals forces between the amylose glucose residues, water molecules and the ligand.[4, 32] Nimz et al.[32] demonstrated that the intrahelical amylose-ligand interaction is dominated by hydrogen-to-hydrogen van der Waals forces between the hydrogens bonded to the 3rd and 5th carbon of glucose molecules with the hydrogen from the aliphatic chain C-H for the cycloamylose V-amylose helix. The formation of the inclusion complexes therefore involves hydrophobic forces that enable transfer of a hydrophobic ligand component, such as the aliphatic fatty acid chain from a

hydrophilic polar phase, into the hydrophobic environment within the amylose helix cavity.[31, 33, 34]

Along glucose residues in the amylose chain, the V-amylose is stabilized by intramolecular hydrogen bonds (H-O….H-O) involving O2(1)…O3(2) [4, 32] with an extra O2 (1)…O6(7) in polymeric V-amylose[4] (the number after O indicates the carbon number in D-glucose to which the oxygen is covalently bonded while the number in parenthesis indicates the glycosyl residue number/position in the amylose chain). The intrahelical channel may contain water molecules that are hydrogen bonded to each other but not to the amylose.[4] Water molecules are mainly located in the interhelical spaces where they form a network of hydrogen bonds that maintain the V-amylose stability.[4] Water mediated interhelical hydrogen bonding occurs in a bidentate mode (glycosyl-O…H-O-H…O-glycosyl) to glucosyl residues at O5 and O6 or at O2 and O3 in cycloamylose helices.[32] The interhelical hydrogen bonds have been shown to occur between water molecules and O2, O3, O5, and O6 of the glucosyl residues in polymeric V-amylose.[4] Interhelical space water-water hydrogen bonding further occurs between the water molecules linked to the helix, such as the O-W… W-O (W = H-O-H) bonds reported by Rapnecker *et al*.[4] This leads to an extensive network of hydrogen bonds that can enable the build up of larger V-amylose structures or crystals.

Based on molecular modelling,[5, 31] Raman spectroscopy,[35] and nuclear magnetic resonance (NMR) data,[36-39] the carboxyl group of fatty acids (hydrophilic/polar component of the ligand) has been suggested to be located outside the amylose V-helix (Figure 1). Steric hindrance and electrostatic repulsions prevent the polar carboxyl group from entering the helix, hence leaving it at the helix entrance while the axes of the aliphatic group and the helix are superimposed.[31] NMR

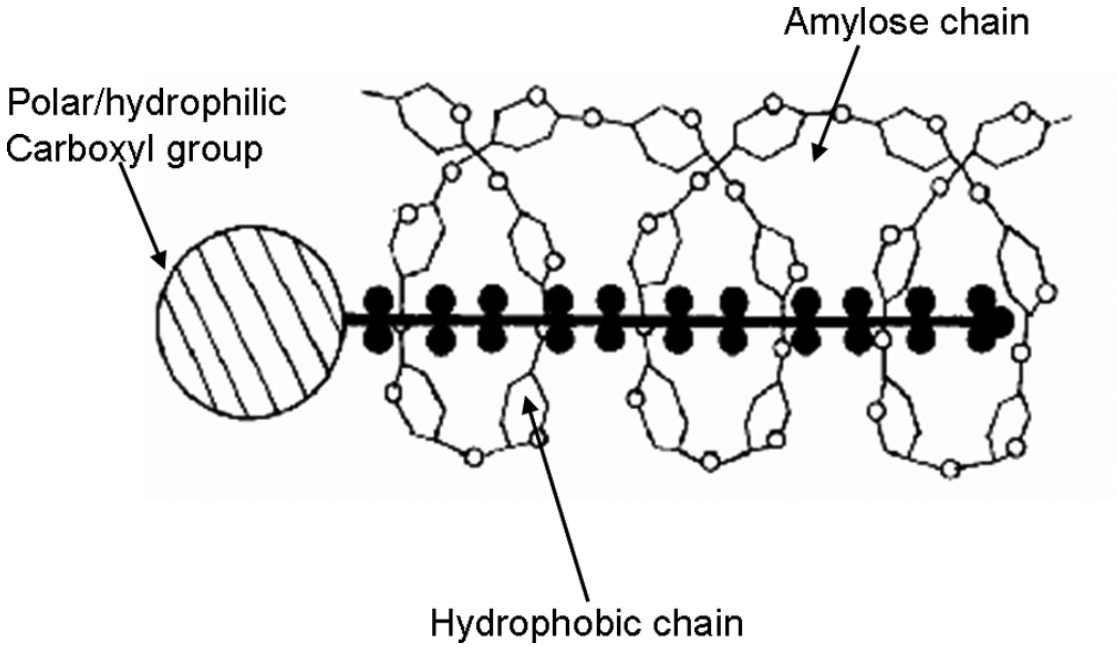

Figure 1. Schematic illustration of monostearin-amylose helical complex with the whole chain inside the helical space. (Adapted from[35])

relaxation also showed that the complexed fatty acid relaxes faster than a free one (non-complexed), suggesting that amylose is restrained in mobility after complex formation compared to non-complexed amylose.[38] The rigidity of the helix was shown to be insensitive to hydration effects.[36, 39] The complexed ligands are resistant to washing effects since they are retained in V-amylose upon washing (twice using a 50% v/v ethanol in water solution) compared to non-complexed ligands, which are easily removed on a single wash.[15] This implies that complexed ligands could resist washing and other similar processing effects that would lead to loss of the ligand. Non-complexed ligands (lipid), rather than forming V-amylose complexes, may self associate to form micellar bodies.[40]

Based on variations in NMR shifts and X-ray *d*-spacings, Kawada and Marchessault[37] suggested that the ligand hydrophobic component (e.g., fatty acid aliphatic chain) is flexible and could be located either entirely within the helix or

partly out. Shogren *et al*.[41] suggested that V-amylose helix ligands occur as pairs within the helix in a side-by-side arrangement or as two end-to-end ligands in a linear arrangement depending on the amount of water in the helix environment. Alternation between the two arrangements was postulated to be accompanied by a change in helix diameter and the ligands were considered to take up the different conformations in order to obtain energetic stability in the different water content environments.[41] These arrangements were supported by molecular modelling studies that showed that two low energy solutions were possible for the carboxyl group; one with a single fatty acid molecule per helix and the other with two.[5, 31] These hence did not rule out the existence of both the single ligand and the two per ligand per helix arrangements. The arrangements could probably be explained to result from the different ligand aliphatic chain stereo-conformations and head group sizes used since it was suggested that aliphatic chains in an all-*trans* conformation and alcohols with slim head groups can smoothly fit in the V-amylose channel.[32]

The V-amylose helix diameter is controlled by the size of the complexing agent, leading to helices with 6, 7 or 8 glucosyl residues-per-turn.[4, 13, 42-44] Six glycosyl residues per turn are for lipids or linear alcohols, 7 for branched chain alkyl compounds, while 8 residues are for more bulky compounds.[4, 13, 42-44] These are referred to as V6, V7 and V8-amylose, respectively.[43, 45] The V6 helix, which is the most widely studied, has a helix pitch of about 0.8 nm with a 1.32-1.36 nm rise per monomer.[4, 5] Depending on the crystal unit cell interhelical space distance, the V-6 type was suggested to be divided into V6I, V6II, and V6III[43]. The symbols I, II and III indicate crystal unit cells of slightly increased volume and different location of the ligand molecules[7, 8, 46]. The ligand molecules are located entirely within the helix for V6I, while some are found in the interhelical spaces for V6II and V6III[15]. Other

researchers, however, suggest that V-amylose mainly falls in the V6 and V8 categories and V7 is a form of V6III[12, 45].

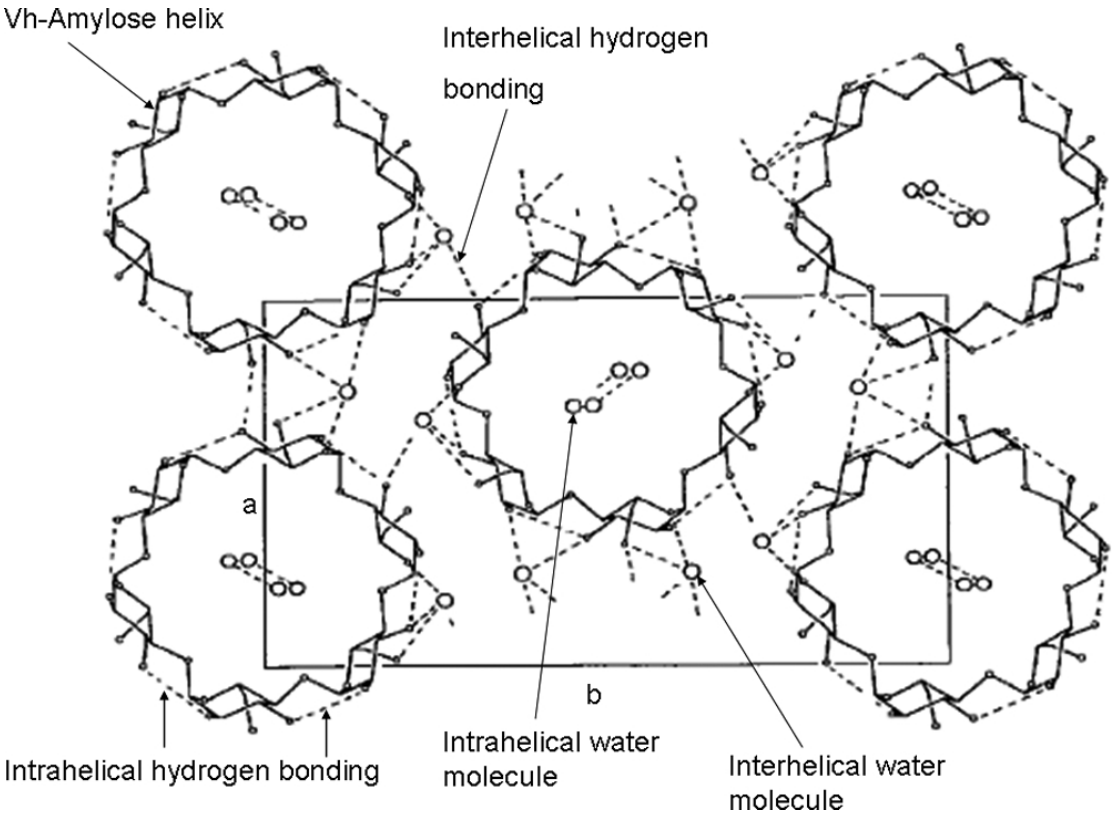

Figure 2. V_h-amylose Crystal unit cell in viewed in the *a,b* plane showing helix spatial arrangement and associated hydrogen bonding . (Adapted from [4])

The molecular association of amylose with the ligands, under suitable conditions, can produce crystalline structures.[33, 47] The constituent V-amylose crystals of the structures may occur in anhydrous (V_a) or hydrated form (V_h).[4] The crystals formed are characterized by X-ray diffraction peaks with *d*-spacing of 0.12, 0.68, and 0.44 nm for a hydrated V-amylose (V_h) complex and 1.13, 0.65, and 0.43 nm for an anhydrous V-type (V_a).[4, 46, 48] The V-amylose crystals are orthogonal structures with dimensions ranging from a = 1.36-1.37 nm, b = 2.37-2.58 nm, and c = 0.78 - 0.81 nm.[4, 6, 46, 48] In space, the single helices are organized into the standard orthogonal space group

$P2_12_12_1$ arrangement and may have up to 4 or 16 water molecules in the interhelical space within each crystal unit cell for V_a and V_h, respectively (Figure 2).[4, 6, 10] The 8-fold helices (V8) on the other hand, may occur as tetragonal *P*41212 or *P*43212 space group with unit cell parameters *a* = b = 2.2844 nm and c = 0.7806 nm[49]. The helical structure of the V-amylose was recently discussed by Putseys *et al*.[50] and Putaux *et al*.[51] from a historical perspective.

Types of V-amylose Complexes

V-amylose is also divided into type I and type II depending on the melting temperature of the crystalline components.[52, 53] Type I generally has melting temperatures below 100 °C, while Type II has melting temperatures above 100 °C. [52, 53] Type I complexes consist of a partially-ordered structure with no distinct crystalline regions, while type II complexes are made up of distinct crystalline and amorphous regions.[54] Type II can be subdivided into Types IIa and IIb.[55] The two differ slightly in the degree of crystallinity or perfection of the ordered domains.[55] Both Types IIa and IIb have melting temperatures above 100 °C although that of Type IIb is higher (> 121 °C).[55] Generally, three DSC endothermic peaks may be observed during melting of V-amylose complexes.[56, 57] There is a peak at about 62 ºC , a middle peak in the range 90-104 ºC, and the a peak at >104 ºC.[55-60] These were considered to correspond to uncomplexed lipids,[61] type I [58-60] and type II amylose-lipid complexes, respectively.[58-60] If type I complexes are annealed at high temperature (90 °C), they are transformed into type II complexes.[60] Tufvesson and Eliasson[57] suggested a mechanism for the ordering of amorphous and crystalline regions with heating. In the mechanism, heating and cooling induces transformations between types I and II amylose-lipid complexes, free amylose and a liquid crystalline

phase (Figure 3). When Type I complexes are heated, they transform to a liquid crystalline phase and then to type II complexes, which also break down to form a mixture of amylose and a liquid crystalline phase with further heating. These changes may be facilitated by a higher moisture content in the system.[62]

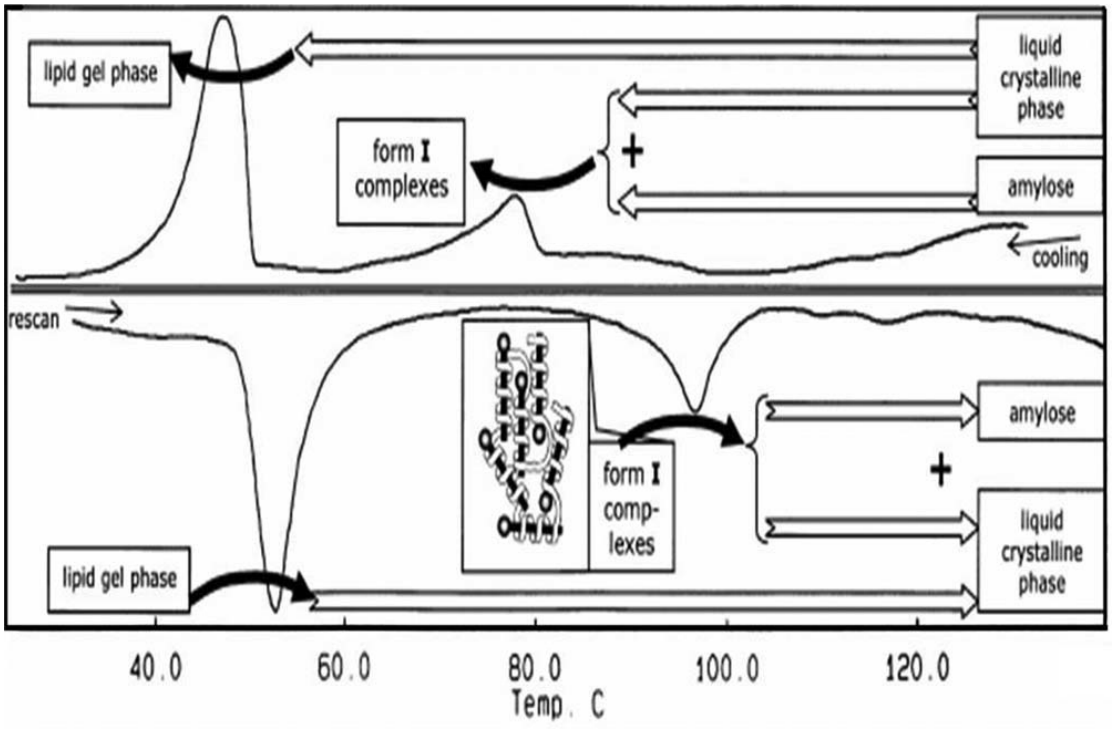

Figure 3. Transformation between free amylose, liquid gel phase, type I complexes, and liquid crystalline phase on heating and cooling of an amylose-lipid complex system. Bold arrows indicate heating induced transformation while reverse arrows would indicate cooling transformation. (Adapted from [57])

V-amylose Structures: From Nano to Micron Level

The V-amylose suprastructure is made up of crystalline regions of helices interspaced by amorphous regions.[9, 15, 24, 33, 63, 64] The ligand may be positioned in three locations; within the helices, between the helices of the crystalline regions or dispersed in the amorphous regions.[15] A lamellar-like structural organization of amylose V-complexes was suggested based on electron X-ray scattering, X-ray diffraction (XRD)

data, and V-amylose hydrolysis with alpha-amylase followed by gel permeation chromatography.[52, 65] In the lamellar structural organization, the crystalline regions consisting of the V-amylose crystals make up one lamellar region while the amorphous uncomplexed components make up another region with the two

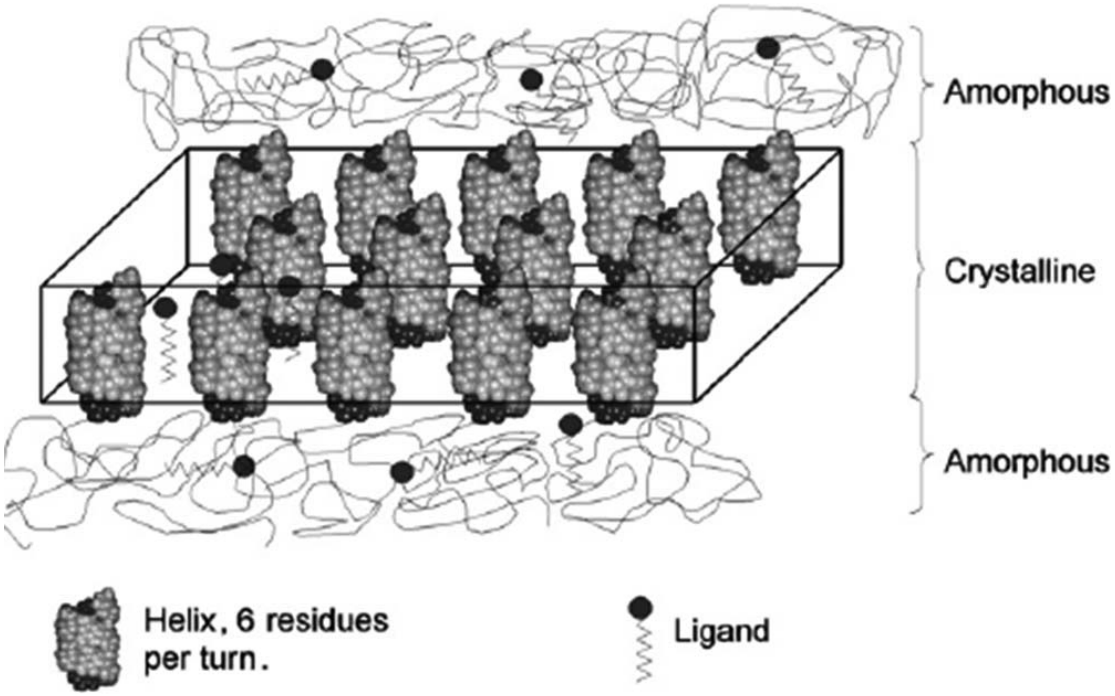

Figure 4. Schematic representation of the lamellae-like structure of amylose-ligand complexes and possible ligand of molecules trapped. (Adapted from [15])

alternating (Figure 4).[15] It was postulated that the polysaccharide chains were arranged with their chain axes perpendicular to the surface of the lamellae.[52, 65] Several studies have suggested that the lamellae folding length of V-amylose is about 10 nm.[15, 23, 52, 63, 65, 66] The crystalline lamellar thickness strongly depends on amylose chain length and to a lesser extent on fatty acid chain length.[67] The degree of amylose polymerization hence may be a major factor in determining the structural organization of V-amylose at nanoscale. A quantitative verification of the lamellar structure of the V-crystals superstructures was recently provided by Zabar *et al*.[68]

using SAXS (small-angle X-ray scattering system). They found that the dimensional scattering intensity fitted a 'modified lamellar model' and a Guinier approximation of the radius of gyration.[68] The 'modified lamellar model' describes randomly arranged well-defined domains, which are made up of alternating layers of crystalline and amorphous material. V-amylose has been postulated to consist of aggregates or supramolecular structures of nanosize (at least 100nm on one dimension).[23, 63] The size and shapes of nano-scale supramolecular structures formed however may depend on the preparation method. Lalush *et al*.[23] obtained distinct nanoscale spherical shapes with a diameter of 150 nm and elongated structures with a width of 43-160 nm using two different methods involving water/DMSO/ligand heating and KOH/ligand heating with pH reduction (HCl), respectively.

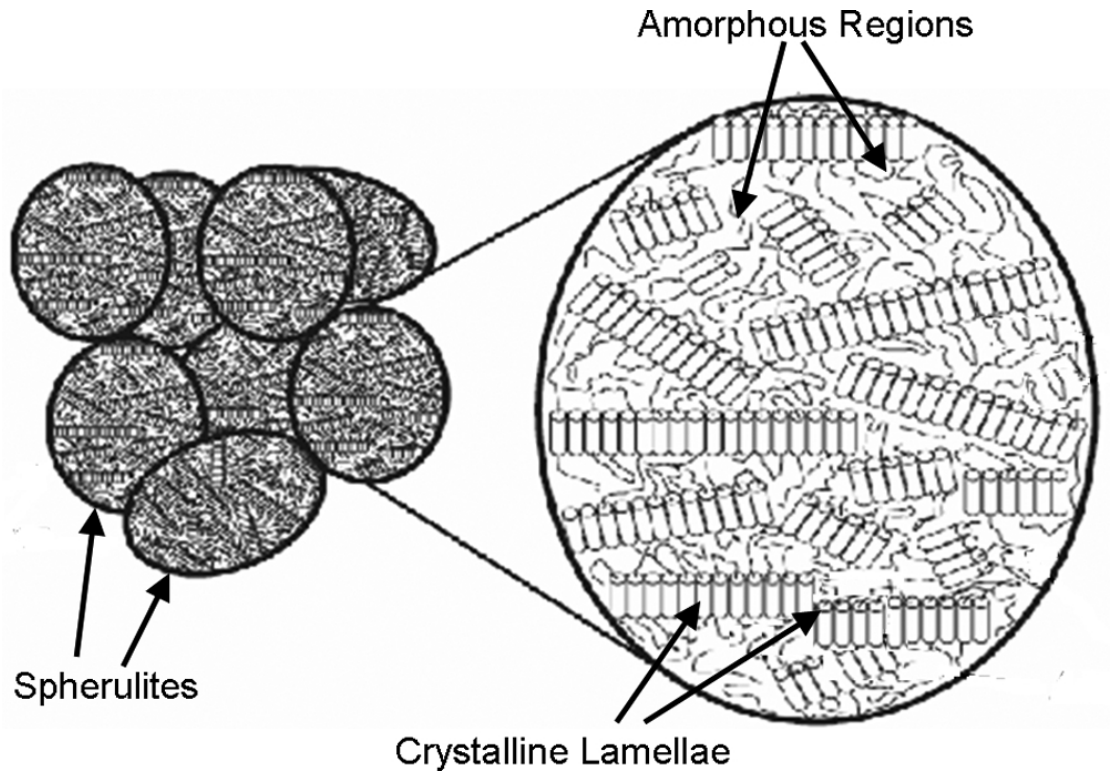

Figure 5. Suggested model for the organization of amylose molecular inclusion complexes into helical segments organized in lamellae interspersed in amorphous regions to form spheroids which tend to aggregate. (Adapted from [63])

V-amylose lamellae have been suggested to pack into nanoscale structures that tend to aggregate to form micron-sized structures for V-6III complexes (Figure 5).[63] Three different types of micron-sized spherulite morphologies have been reported: spherical, sintered (‘‘snowball”) and torus/disc shaped.[69] Spherulite morphology is governed by a complex interaction between reactant and experimental variables such as the presence of added oil, starch concentration,[70] cooling rate, ligand concentration, stirring during cooling,[47] final heating temperature,[69] structure of included ligand (fatty acid),[71] and starch type[72] among others. It was postulated that the crystalline lamellae in spheroids are radially oriented since banded maltese cross patterns of birefringence could be observed in spherical spherulites.[47, 72]

Given the different V-amylose suprastructural arrangements that have been observed and postulated at nano or micron scale under different preparation conditions, there is need for further research to enhance understanding of the formation of V-amylose structures. This could facilitate the production and application of tailor made V-amylose structures in different food and pharmaceutical products.

V-amylose Complex Preparation Methods

Methods for preparing amylose-lipid complexes can be loosely grouped into classical methods, enzymatic methods, and thermo-mechanical methods. Some of these methods utilize top-down and bottom-up approaches at various degrees to produce V-amylose structures of different sizes at nano and micron scale.

Classical V-amylose Preparation Methods

These mainly involve mixing an amylose-containing starch fraction with the ligand substance under shear-less heating/moisture conditions. Generally, this procedure involves dissolving the starch or amylose in a diluent such as dimethylsulfoxide (DMSO), potassium hydroxide (KOH) solution and/or water, adding the ligand and then incubating at elevated temperatures to allow for complex formation to occur.[69] This is usually followed by a pH reduction and/or cooling stage to enable crystallization, and then centrifugation may be used to isolate V-amylose[53, 55, 73, 74]. Incubation at 60 ºC yields a relatively amorphous type I V-amylose, while at ≥ 90 ºC, semi-crystalline type II V-amylose complexes are formed.[33, 55, 73] Technical variations in the classical method include parboiling,[75, 76] shear-less heating of an amylose/ligand/diluent mixture at elevated temperature (up to 140 ºC),[77] and autoclaving.[78] Tufvesson *et al*. [56, 57] and Rajesh *et al*.[69] demonstrated that heating starch/ligand/water mixtures in a DSC can also be used for V-amylose preparation. Classical methods may yield complexes with crystal lamellae thickness within the range 5-100 nm depending on the preparation conditions.[69] Lalush *et al*.[23] demonstrated that distinct nanocrystalline structures could be formed using the classical methods, while Gelders *et al*.[33] suggested that these methods generally give polydisperse V-amylose populations, indicating that the complexes can form different structures. This would present problems in controlling the size of the resultant crystalline structures and hence challenges for applications in which V-amylose structures of particular dimensions are required.

Enzymatic Methods

Enzymatic methods for V-amylose preparation can be classified into two main forms; entirely enzymatic and partially enzymatic methods. Entirely enzymatic V-amylose synthesis utilizes a bottom-up approach to catalyse preparation of the V-amylose complex from glucosyl residues, while the partially enzymatic preparation uses a top-bottom approach involving enzymatic starch hydrolysis in conjunction with classical complex preparation procedures. Both of these methods have been used to produce V-amylose complexes whose structure was shown to consist of nano-scale dimensions.

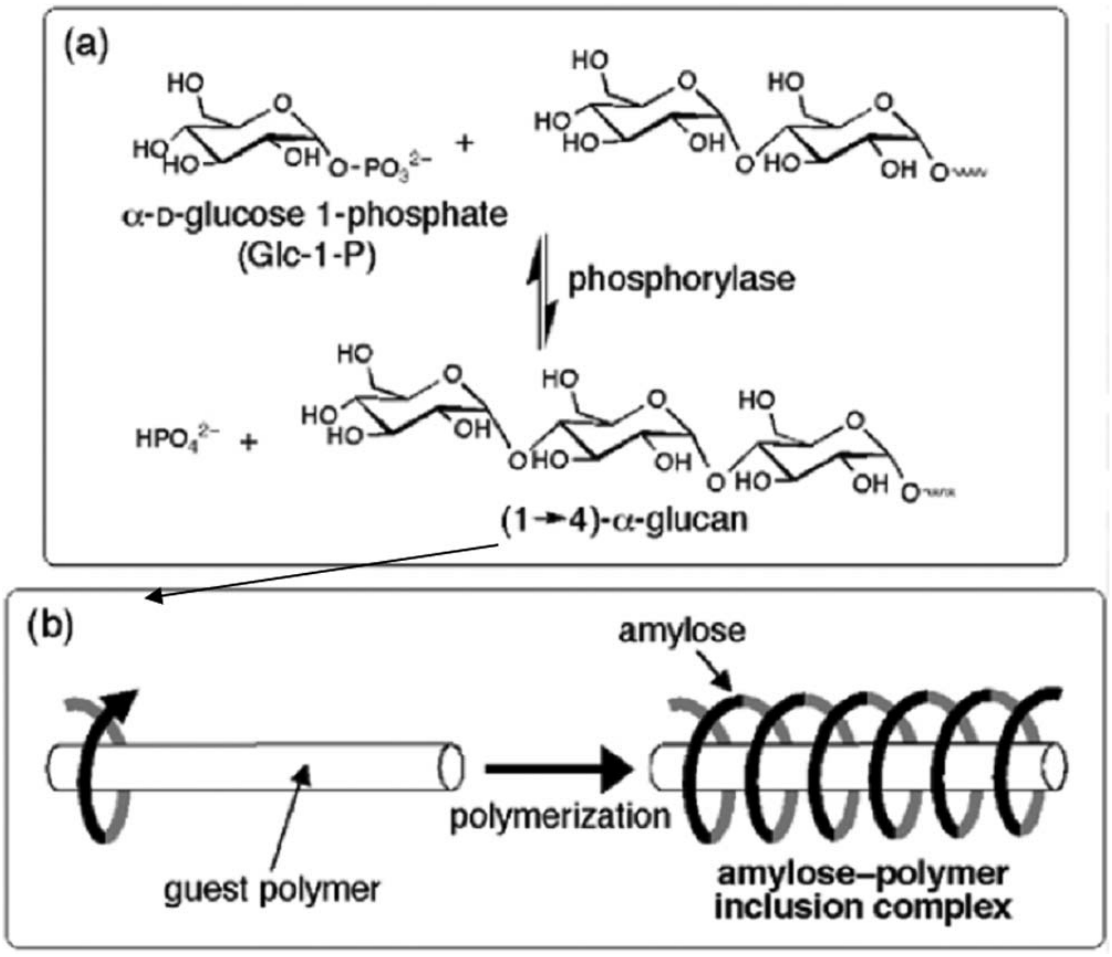

Figure 6. (a) Formation of (1-4)-α-glucan (amylose). (b) Concept of "vine-twinning" polymerization. (Adapted from[17])

Entirely Enzymatic Methods: These methods utilize glucose phosphorylase to catalyse synthesis of V-amylose from glucose phosphate in the presence of appropriate hydrophobic ligands. Such a procedure for preparing V-amylose complexes, referred to as 'vine twining,' recently was introduced.[16-18, 20] In this method, amylose is synthesized from glucose 1-phosphate around hydrophobic long chained carbon polymers to yield V-amylose in form of single-walled amylose nanotubes (Figure 6).[16-18, 20] The amylose is formed by adding one glucose molecule at a time to give single-walled, left-hand helices along the lengths of the hydrophobic hydrocarbons (Figure 6).[16-18, 20] The V-amylose may be isolated as single-walled V-amylose nanotubes by dispersion of the reaction products in an appropriate solvent such as DMSO or water.[25] Examples of different hydrocarbon types that have been utilized as ligands in the synthesis of the V-amylose include telechelic poly(ε-caprolactone)s (PCLs),[20, 22] polyesters,[21] polyethers,[18] and poly(ester-ether)s.[22] This method could be applied for preparation of potential food ingredients by use of food-grade, long carbon chain ingredients.

Application of glucose 1-phosphorylase for the production of V-amylose complexes was also achieved by Gelders *et al*.[64] They used potato phosphorylase to prepare V-amylose with monoglyceride as the ligand. The V-amylose chains were synthesized from a reaction of a primer (a short chain of α-1,4- glucose units) and glucose-1- phosphate, which was catalysed by potato phosphorylase (EC 2.4.1.1). They were able to attain two separate monodisperse V-amylose crystal populations. Putseys *et al*.[79] later demonstrated that by controlling the reaction conditions, such as enzyme dosage, lipid saturation level, glucose phosphate to primer ratio and lipid chain length, it was possible to attain V-amylose starch of predetermined dimensions. They proposed a synthesis mechanism in which the primer is initially elongated until

an amylose chain of sufficient length to complex the first lipid/ligand molecule is obtained, and then chain extension occurs together with subsequent complexation until the complex becomes insoluble and precipitates.[79] The V-amylose complex crystals (grouped helices) were suggested to grow from a fringed micellar organization (rod shapes) or by folding of amylose helices in U-tube shape, continuously until they were able to form extensive crystal lamellae (Figure 7).[64]

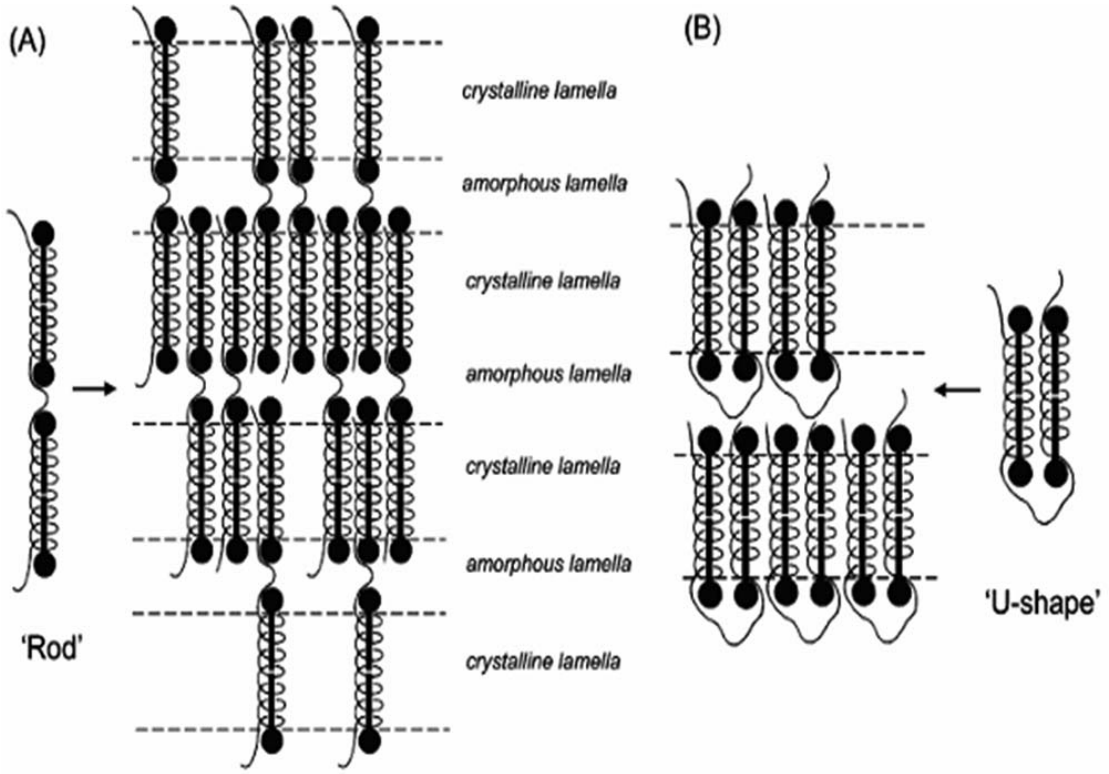

Figure 7. Schematic drawing of the postulated semi-enzymatically prepared V-amylose complex morphology: (A) a fringed micellar organization, represented by rods or (B) folding of the amylose helices into U-shapes, lining up in crystalline lamellae. (Adapted from [64])

Generally, these entirely enzymatic methods, which use a bottom-up approach, show relatively more potential for commercial application since studies have demonstrated large scale production[80] and control of the sizes of resultant V-amylose.[79]

Partially Enzymatic Methods**:** Partial application of enzymes for production of V-amylose complexes mainly involves modification of classical V-amylose synthesis by addition of an enzyme-catalyzed starch/amylose depolymerization step before or after complex formation. Gelders *et al.*[33] produced V-amylose complexes by depolymerising starch using β-amylase before complexing the resultant dextrins mixture with an appropriate ligand. It was suggested that V-amylose complexes made up of amylose with predetermined DP (Degree of polymerization) could be produced by controlling the conditions in the procedure.[33] They later demonstrated that complexes made up of two or more dextrin subpopulations can be obtained when complexes formed by classical methods are further hydrolyzed using pancreatic alpha-amylase.[67] Kim *et al.* [81, 82] produced nanocrystals of V-amylose complexes by combining classical preparation concept, membrane filtration with V-amylose α-amylolysis. Initially, starch with or without depolymerization was dissolved in DMSO and then allowed to filter gravimetrically across a membrane (10 μm) to complex with butanol in a chamber at elevated temperature (70 °C). The complexes formed were then treated with α-amylase for removal of uncomplexed amylose or amorphous starch components in order to obtain V-amylose nanocrystals. The V-amylose nanocrystals obtained were 23-72 nm in length with precomplexing starch depolymerization and 10-20 nm[81] without prior depolymerization. This method, however, gave low yield (>10%) of nano-complexes, which was attributed to loss by hydrolysis (85–90%) of the starch initially complexed.[81] This presents a major limitation of utilization of this top-bottom approach for production of V-amylose of particular desired dimensions; hence, the need for more research on utilizing similar

approaches for production of V-amylose complexes with specific required dimensions.

Thermo-Mechanical Methods

Thermo-mechanical methods involve simultaneous use of heating and shearing effects to produce V-amylose complexes. Several methods have been tried including steam jet cooking, extrusion cooking, dual feed homogenization, and RVA (rapid visco-analyzer) pasting. Thermo-mechanical methods accelerate gelatinization/pasting and starch granule degradation, which enhances formation of complexes.

Steam Jet Cooking: Several studies have been reported on the utilization of steam jet cooking for preparation of V-amylose starch.[47, 70-72, 83] It is thought to enhance ligand (fatty acid) solubility under high-temperature and high-shear conditions, hence facilitating complex formation.[83] Steam jet cooking results in formation of a mixture of micron range spherulites that are spherical or disc shaped and/or submicron spherical particles.[47] The proportion of the species depends on the cooling rate and stirring conditions during cooling. Submicron particles are obtained with rapid cooling while mixture of disc-shaped and spherical particles are obtained after slow cooling with stirring.[47] The morphology of formed particles is also influenced by the type of starch used; for example, wheat starch was shown to form only spherical spherulite particles with slow cooling while maize starch formed both torus and spherical forms.[72] Different proportions of spherulite morphologies, yields, and sizes are formed with different fatty acids, pH conditions, and fatty acid concentrations.[71] The diverse variation in spherulite morphology with experimental conditions and reactants results from the interactive influence of the variables on the rates of crystalline lamellae formation, spherulite nucleation and spherulite growth.[71]

Homogenization: A continuous, dual-feed homogenization process for the formation of V-complexes using three starches of different amylose/amylopectin ratio was recently developed by Lesmes and Shimoni.[84] The main aspect of the technique was use of homogenizer shearing and heating for amylose complexation based on the classical methods. The method gave micron-sized V-amylose complex particles.[84] They showed that pre-dissolving high amylose corn starch or normal corn starch in a hot alkali solution leads to a bi-modal population or a mixed population of V-type particles, respectively. Based on easy release of complexed stearic acid by these microparticles on digestion with pancreatin, it was suggested that they could serve as a delivery system for bioactive compounds in the GIT (gastrointestinal tract).[84, 85]

Extrusion: Studies have demonstrated formation of amylose-lipid complexes due to native or added fatty acids during extrusion of different starches.[86-90] These studies, using both twin and single screw extruders, have mainly focused on providing evidence of V-complex formation and effects of the presence of fatty acids on functionality of resultant extrudates. During extrusion, V-amylose complexes started forming at about the same point as gelatinization, with the maximum amounts occuring at the end of the screw.[91] The complexes are formed earlier in a twin screw extruder, indicating that a twin screw extruder is a better option for amylose-yield complexation than a single screw extruder.[91]

The influence of extrusion conditions, such as screw speed, moisture content, barrel temperatures, and shear rates, on V-amylose formation during extrusion has also been assessed. Using response surface methodology (RSM), Teresa de Pilli *et al*.[88] showed that the feed moisture is a major variable that significantly influences the formation of starch–lipid complexes. The highest values of V-amylose melting

enthalpy and complexing index (3.67 J/g and 94.34%, respectively) were obtained with the smallest level of feed moisture (21.17%). Barrel temperature and screw speed also modulate the formation of V-complexes.[92] Maximum complexation occurs at relatively low barrel temperature (<120 ºC) and screw speeds (20 and 91 rpm).[92] Such low shear rates would allow for sufficient contact time for complex formation. Bhatnagar and Hanna (1994),[90] using RSM on a twin screw extruder, demonstrated the effects of temperature, moisture, and shear rate. They found that the optimum conditions for complex formation were 100-140 ºC, 140 rpm, and feed moisture content of 19% w/w. Application of α-amylolysis on V-amylose prepared by extrusion of starch was demonstrated to increase the crystallinity of the extrudate by Lopez *et al*.[93] This provides a possible avenue for improving V-amylose produced by extrusion. The studies done so far on V-amylose preparation by extrusion, however, have not been able to clearly identify the underlying structural and molecular changes that occur during V-amylose preparation by extrusion. Understanding these changes would enable optimization of amylose-lipid complex production of through extrusion.

RVA and Amylograph Pasting: Some studies have demonstrated formation and preparation of V-amylose using the laboratory scale RVA and Amylograph instruments. Tang and Copeland,[40] using X-ray diffraction and iodine complexing index, suggested that amylose-lipid complexes are formed during an analytical RVA starch pasting that involves holding at 95 °C for 2 min. Preparation of V-amylose using a Brabender amylograph by heating a rice starch/palmitic, stearic or myristic acid slurry at 95 °C with holding times of 30-90 min was demonstrated by Kaur *et al*.[94] The presence of increased amounts of added ligands lead to decreased maxima and ratios of absorbances at 630 and 520 nm,indicating formation of V-amylose.[94]

Using a prolonged RVA holding time (32-45 min holding) at 82-95 °C with different shear rates, Nelles *et al*.[95] showed a biphasic commercial maize starch pasting that they suggested to result from V-amylose formation. A high viscosity second pasting peak was formed with the prolonged holding, which disappeared when the maize starch was defatted. This indicated that the peak was probably due to interaction between starch and native lipids through V-amylose formation.[95] V-amylose formation during the second pasting peak was latter supported by XRD data,[96, 97] DSC assessment,[97] decreased solution amylose content,[98] and a positive stearic acid dose-viscosity response.[96, 99] RVA and Brabender viscoamylograph pasting for V-amylose production provides a direct control on heating and cooling rates and gives a simple method of studying and production of relatively large quantities of V-amylose complexes at laboratory scale compared to a DSC scan. However, the structure of the V-amylose complexes formed using these methods needs to be assessed in more detail.

The future of V-amylose preparation methods

The different V-amylose preparation methods give products with varied yields, structures, and within different preparation times. Most of the preparation methods have focused on providing evidence of V-amylose presence or formation and less on the structure of the resultant V-amylose, which could affect its functionality and potential applications. Although structures with different distinct regular structures, such as spherulites, have been produced, there is a high variability in the yield, structure and morphology. The formation of the structures appears to be governed by nanoscale interactions between ingredients and the experimental conditions. Work at the nanoscale, however, is still inconclusive. Studies that have tried to assess the

structures beyond the helix and the crystal unit level have hypothesised an ordered arrangement at the nano and micron scale. Controlling this order and structure could allow for preparation of structurally tailor-made V-amylose for particular applications.

There is need for more research at the nano level using techniques such as SAXS, AFM (atomic force microscopy), scanning transmission electron microscopy (STEM), transmission electron microscopy (TEM), small angle laser scattering (SALS), and dynamic scattering particle size distribution among others. These methods could be used in conjunction with high-performance liquid chromatography (HPLC) or field flow fractionation (FFF) combined with online photon correlation spectroscopy (PCS) and mass spectrometry (MS) to increase convenience.[100] In order to utilize the relatively large quantities of data that may be obtained through the various methods, application of increasingly powerful computational methods to enable faster calculations and simulations for model and theory development may be required.[101]

Factors that Affect V-amylose Complex Formation

V-amylose formation is affected by several factors, which can be grouped into reactant factors and experimental factors. Reactant factors include starch type, water content of the starch, starch degree of polymerization, starch/ligand concentration ratio, and the structure of the included molecule. Experimental factors that affect the V-amylose complex formation include the complexation temperature, complexation time, and medium pH.

Effect of Starch Type on V-amylose Complex Formation

The starch source, amylose content, and degree of polymerization affect V-amylose formation. The effect of crop variety on the formation of V-amylose complexes has been studied by several researchers. During rice parboiling for complex formation, the rice variety has significant impact on the complex yield.[76] V-amylose formed by starches isolated from different wheat varieties show different degrees of crystallinity, thermal melting temperatures, and enthalpies of complex dissociation.[102, 103] The influence of the variety or starch type on the yield of amylose-lipid complexes most likely results from the different rates of starch granule penetration by the ligands (lipids) due to differences in granule microstructure, including the presence or absence of surface pores/channels on the granule.[27] The amylose content, which varies between varieties, may also be a major influence on yield and crystallinity of amylose-lipid complexes. Starches with higher amylopectin contents tend to form fewer or no complexes than those with less amylopectin.[72, 96, 104] High amylose corn starches, such as Hylon®, Amylo-maize 70® and Gelose©, would therefore be appropriate for higher V-amylose yield, as demonstrated in some studies.

Effect of Degree of Starch Polymerization on V-amylose Complex Formation

The amylose DP has been shown to modulate V-amylose complex formation by several researchers. V-amylose yield is more sensitive to amylose molecular weight than the number of carbons of the fatty acids.[105] The complex yield and crystallinity increases with the degree of amylose polymerization below DP 400, but decreases at higher DP of 950.[33] The increase in crystallization yields with amylose chain length could be explained by a combination of increased amylose solubility and the ability to form complexes that can precipitate in the crystallization medium.[105] The decrease in

crystallization yield at higher DP probably results from the presence of more amylose chain than that required for complexing the ligand (fatty acid). The excess amylose chain length then contributes to the amorphous V-amylose component,[5, 74] which would lead to an increase in the proportion of amorphous lamellae in the V-amylose suprastructure.[9, 105] The optimum DP for V-amylose formation is still not clear given the 400-950 range was not investigated. However, the optimum DP probably depends on the structure of the included ligand since Kubik and Wulff [34] showed that the enthalpy of complex formation reaches a maximum at DP 400 and DP 250 for 4-tert-butylphenol and sodium dodecyl-sulphate, respectively. Partial depolymerization of amylose to some extent is therefore necessary in order to obtain V-amylose of higher crystallinity for top-bottom approaches of V-amylose preparation.

The minimum DP for complexing ligands apparently depends on the ligand included. The minimum amylose size for complexing with fatty acids is around 30-40 glucosyl residues to complex palmitic acid and 20-30 glucosyl residues for lauric and caprylic acids, which is about the chain length to accommodate two fatty acids per chain.[105] It was also illustrated that, irrespective of the complexation temperature, the critical minimum DP for complex formation and precipitation was 35 and 40 for complexes with glycerol monostearate (GMS) and docosanoic acid (C22), respectively, which also corresponded to the length needed to accommodate two of each of the ligand molecules.[33] The minimum required DP for complexation and precipitation therefore apparently depends on the acyl chain length.[79] It was postulated that the general minimum DP is 30, suggesting that amylopectin, which has short side chains (DP = 20), cannot form inclusion compounds.[105]

Effect of Starch Moisture Content on V-amylose Complex Formation

Moisture content influences the type of crystalline structure that results. It was found that moisture content impacts whether and to what extent type II amylose V-complexes are formed. V-amylose complexes are readily formed at low (25%) and intermediate (40%) moisture conditions, while high moisture contents (66% w/w) seem to inhibit complex formation and crystallization.[76] At lower moisture contents (36-64 %, wet basis), water is suggested to be necessary as plasticizer during V-amylose formation.[106] Increased water content probably hinders the system from attaining the activation energy required for complex formation.[107] At high moisture (65%) content, the effect however may depend on the starch type since other studies have shown maize starch readily forms V-amylose complexes.[108] The heating conditions (time, temperature and presence of shearing effects) may also play a role at higher moisture contents as an increase in the second peak paste viscosity was observed with decreasing moisture content (92-87% w/w) during RVA biphasic starch pasting (90 °C, 160 rpm, 120 min).[95] The second peak viscosity has been attributed to V-amylose formation. [27, 95]

Effect of Ligand Structure and Concentration on V-amylose Complex Formation

The acyl chain (hydrophobic component) length, degree of unsaturation, concentration, type of polar head of the ligand, and the presence of native starch lipids have been shown to significantly affect the type, yield and structure of the resultant V-amylose complexes.[55, 57, 60, 109] Increasing fatty acid unsaturation leads to the formation of less crystalline complexes compared to fully saturated fatty acids.[55, 68] It was observed that *cis*-unsaturated fatty acids complex poorly with amylose, giving low yields and enthalpies of dissociation.[73] This was attributed to inefficient

complexing by the fatty acid, which was depicted as nonlinear or kinked due to the *cis*-double bond. [14, 6] Karkalas and Ma[55] suggested that the *cis-trans* effect influences the crystal structure more than the yield. They postulated that the amylose helix needed to be expanded from six glucosyl residues per turn to seven in order to accommodate the unsaturated portion of the acyl chain, as happens with other bulky ligand molecules. Decreasing the molecular flexibility through increasing saturation results in increased order within the lamella, i.e. higher crystalline fraction proportion, larger average crystalline lamellar thickness, and larger particle radius of gyration.[24, 68]

There is a decrease in V-amylose yield with increased chain length.[110] This has been attributed to the activation energy required for complex formation, which increases with increasing acyl chain length.[110] Increased activation energy is explained to result from the extra energy required to induce more hydrophobic interactions between the ligand and the amylose helix.[58, 111] Godet *et al.*,[61] however, explained the reduced ability to form complexes at higher acyl chain length to result from reduced solubility. They stated that the more soluble the included ligand, under given conditions, the more V-amylose that is formed[61, 112].

V-amylose complex formation increases with the amount of the fatty acids (ligands) used.[40] There is an optimum concentration range for fatty acids to form complexes with starch.[106, 107] A ratio of 10:1 (amylose : fatty acid) by weight was found optimal.[106, 107] It has, however, been demonstrated that maximal complex formation occurs at different concentrations for different types of fatty acid.[40] The optimal concentration for each fatty acid is related to the water solubility and critical micellar concentration of the lipid and, above a certain concentration, the lipids tend to self-assemble in preference to forming V-amylose complexes.[40] This may explain

the low complex formation with stearic acid in some studies, while other studies reported high complexation.[40, 73, 83] The critical micelle concentration for fatty acids and water solubility decrease as carbon chain length increases,[40] so smaller amount of longer-chained lipids are required for complex formation compared to the shorter-chained fatty acids under the same conditions.

Effect of Heat on V-amylose Complex Formation

The complexation temperature, duration of heating, and cooling rate affect the type of V-amylose crystal structures formed during complex formation. The particular temperature regimes used during V-amylose preparation through a given technology are important in determining the yield and V-complex structures formed. During steam jet preparation of amylose-lipid complexes, the specific conditions, especially the endpoint heating temperature, the cooling rate and the final quench temperature, determine the formation and morphology of resulting spherulites.[47, 69, 72] For extrusion cooking, the heating temperature/barrel temperature was also shown to play a significant role during the formation of amylose-lipid complexes.[90]

V-amylose Type I is obtained at lower heating temperature (60 ºC) through rapid nucleation, which results in a random distribution of helices without forming distinct crystallites[33]. Precipitation/crystallization temperature appears optimal at >90 ºC where Type II complexes are formed[33, 52, 62]. At these temperatures, both crystallinities and enthalpies are maximized.[7] Type II is considered a separate thermodynamic state from Type I with a high energy-barrier between the two types; hence, the higher temperatures required for its formation.[56, 107]

Longer heat treatment favors formation of complex type II V-amylose at the expense of type I.[56, 57] A shorter heat cycle mainly favors formation of Type I

complexes even though a certain amount of Type II complex may also form.[56] A longer heating cycle favors formation of complex type II V-amylose with the effect being larger for longer fatty acids (longer hydrophobic component) than for shorter ones.[56] Ligands with shorter acyl chains however require relatively shorter heat cycles to form type II V-amylose complexes compared to those with longer acyl chains.[56, 57]

Annealing has been illustrated to affect complex formation.[62, 113] Complexing agents induce metastable, less perfected crystalline structures that are inclined to reorganization upon heating, presumably via lamella thickening[62] or reorganization of amorphous and crystalline lamellae.[113]

Effect of pH on V-amylose Complex Formation

Complex formation is affected by the pH of the complexation medium. Complexes with neutral lipids (e.g., monoglycerides) are readily formed as insoluble precipitates in neutral aqueous media while in contrast, insoluble complexes with ionizable fatty acids are formed only at pH below 7 and in the presence of electrolytes [55]. The ionizable carboxyl group in fatty acid ligands makes the initial aggregation of complexes more sensitive to pH and salt concentration.[55] The hydrocarbon chain length of the fatty acid has an important impact on the effect of pH on complex formation. When pH< pK_a, short chain fatty acids (e.g., 8:0) form V-amylose complexes more easily than longer chain fatty acids (> 10:0).[114] Longer chain fatty acids (> 10:0) form V-amylose complexes more easily when pH > pK_a.[114] It was suggested that the ionized form of the short-chain fatty acid at pH > pK_a causes less transfer of the fatty acid into the hydrophobic cavity of the helix. The increased complex formation for longer chain fatty acids at pH > pK_a could be attributed to

increased solubility of these longer-chain fatty acid when present in a dissociated and ionized form.[114]

Effect of other Additives and Starch Modifications

The presence of other potential amylose complexing agents and starch acetylation has been demonstrated to affect the formation of V-amylose complexes. Starch acetylation in particular reduces the complex formation ability of the amylose polymer.[115] Cyclodextrin (β), which also forms inclusion complexes, apparently competes with amylose for ligands and potentially forms a three-in-one complex involving amylose and the ligand.[116] A similar three-way amylose-protein-ligand complex was suggested to form when V-amylose complex formation was induced in the presence of whey protein isolate.[117-119] The presence of whey protein decreased the amount of starch–FFA complex and DSC melting enthalpy although surprisingly a more crystalline XRD order was observed.[120] An increase in relative crystallinity and crystal size of V-amylose was also noted to occur with increasing amounts of added sorbitol.[121] Given these limited observations, the effects of other food ingredients on the formation of V-amylose needs to be researched further in order to understand their interactions in practical food systems.

Significance and Potential Application of V-amylose Complexes

The functional properties of food products are affected when starch forms complexes with monoglycerides, free fatty acids and lysophospholipids.[55] V-amylose complex formation has been demonstrated to affect the hydrolytic stability or digestibility, pasting, gel formation, flow properties, and retrogradation of starch.

Effect of V-amylose Complexes on Starch Digestibility

The formation of V-amylose complexes reduces the digestibility of starch and is suggested to modulate the glycaemic response.[9, 15, 67, 73, 111, 122] Formation of the single helices of V-amylose is suspected to induce resistance to enzymatic hydrolysis.[123] Resistant starch type III was shown to partly consist of V-amylose complexes.[124] Some studies however show that resistant starch and V-amylose are separate entities with V-amylose complexes being more prone to enzymatic hydrolysis than resistant starch.[125] Its is reported, though, that hydrolysis of complexed starch occurs at slower rate than noncomplexed starch, although both forms are finally digested *in-vivo*.[126]

Several studies have attempted to assess the mechanism involved in the increased resistance of starch to enzymatic hydrolysis with V-amylose formation. The more saturated and longer the hydrophobic chain length of included ligand, the higher the resistance to enzymatic digestion.[24] Resistance to enzymatic hydrolysis also depends on the type of V-amylose complexes present. The rate of degradation is highest in Type I, followed by Type IIa, and least in Type IIb V-amylose complexes.[59] This indicates an increase in resistance with increased helical order/crystallinity. During hydrolysis the amorphous regions are degraded first, followed by the more crystalline regions.[15] There is an increase in crystallinity with hydrolysis to an optimum, beyond which it decreases due to degradation of crystalline regions.[9, 15] The enzymatic hydrolysis therefore initially increases complex stability by degrading less stable or amorphous regions and leaving the more stable ones.[44] Increased stability of remaining V-amylose complexes after partial enzymatic hydrolysis is also explained as a result of an annealing effect.[109] The effect is suggested to involve induced growth of more stable crystallites due to hydrophobic

aggregation and rearrangement of undigested crystal stacks and lamellae.[109] The enzymes were postulated to initially hydrolyze the interconnecting amylose chains between the helices (which make up the amorphous component), thereby leaving only the more crystalline regions.[67] Complexes formed from lower DP amylose (DP60) hence show more resistant to hydrolysis because they have less interconnecting amylose chains.[67] This indicates that if amylose of appropriate DP was used, the resultant complexes would have higher crystallinity, stability and probably be of predetermined dimensions.[65, 67]

Addition of already prepared V-amylose to unmodified starch was also shown to increase the resistant starch content. This was postulated to result from controlled release of short chains of amylose that crystallized to form resistant starch[30]. Therefore, when starch with induced or added V-amylose is consumed by diabetics, it may therefore reduce postprandial hyperglycemia due to the increased resistance to hydrolysis that would lead to slowed digestion. Animal studies are needed, however, in order to verify the potential resultant reduction of postprandial blood insulin and C-peptide levels.

Effect of V-amylose on Starch Pasting and Viscous Behaviour

The pasting behavior of starch is affected by the presence of lipids (fatty acids), which leads to formation of amylose-lipid complexes. There is reduced granule swelling, leaching of amylose,[127-130] a decreased or absent initial pasting viscosity peak,[27, 131] good viscosity stability in conditions of high temperature or shear, stable cold paste viscosity,[132] and no or reduced starch gel formation.[27, 133, 134] This pasting behavior is similar to that of cross-linked starch[132] and provides a possible means of improving the mouthfeel of starch-containing foods.[27]

Some theories have been put forward to explain the reduction in paste viscosity when starches are pasted with amylose complexing agents. It was suggested by Eliasson *et al*.[104] that complex formation was accompanied by formation of a layer of amylose-lipid complexes on the surfaces of granules that prevents leaching of uncomplexed amylose out of the granule and entrance of water into the granule. This layer is also responsible for the formation of intergranule linkages through hydrogen bonding between amylose-lipid complexes that are found on the surfaces of the granules. This mechanism was questioned because partially destroyed granules exhibited viscosity changes similar those of whole granules when pasted with added fatty acids.[77, 135] It was thereby suggested that the reduction in paste viscosity resulted from a rigid network of structures in the granules due to amylose-lipid complexation.[77, 129, 135] The structures prevented granule swelling and leaching of amylose. Richardson *et al*.[134] however suggested the reduction in granule swelling and amylose leaching to result from increased hydrophobicity of the starch granules, which prevented uptake of water, reduced granule swelling and ultimately reduced the paste viscosity.

After a prolonged pasting time (30-60 min) beyond the initial peak viscosity, which involves granule swelling and amylose leaching, the paste viscosity increases again for starches pasted with added or native fatty acid.[27, 95, 98] The second increase in pasting viscosity may be up to 3 times the first one and coincides with the formation of more V-amylose complexes.[96-98] It was shown that during this second increase in paste viscosity, the starch granules are completely disintegrated[98] and no micron scale structures could be observed.[97] Although the underlying molecular and structural changes that occur during this second peak are not clear, it may also provide a novel method of improving starch functionality in food systems.

Although studies have shown decreased or nongelling with V-amylose formation with fatty acid at high concentration (up to 10% solids),[27, 133, 134] other studies involving used of flavor compounds, such as menthone, decanal and fenchone, have instead demonstrated an induced gelation at low starch concentrations.[26, 136, 137] The influence of V-amylose complex formation on starch gelation could therefore be dependent on the starch-ligand concentration ratio. Other factors cannot be ruled out though since the effect on the rigidity of starch gels depends on the botanical origin of the starch, lipid acyl chain length, and the molecular conformation of the lipid.[133] On the other hand, the aqueous dispersions of dried V-amylose complexes show high spreadability and have rheological properties similar to commercial shortening at high concentration.[138] Starch pasting for a short or prolonged duration also shows a similar effect with a reduced power law flow index.[27] These studies show a possible route for utilization of V-amylose as a fat replacer.

Effect of V-amylose on Starch Retrogradation

Formation of V-amylose complexes slows down retrogradation of starch. It is suggested that formation of V-amylose complexes competes with the retrogradation process for amylose in the starch system.[125, 139] It was demonstrated using DSC that the formation of amylose-monopalmitin complexes occurs preferably to the recrystallization that accompanies retrogradation.[123, 140] The retrogradation endotherm of starch is indeed decreased on formation of amylose-lipid complexes.[44] It has been also suggested that the reduction in retrogradation may also result from amylopectin-lipid complexes.[139] The decreased retrogradation mechanism is therefore probably due to an interaction between the two phenomena. The reduction in retrogradation was suggested by D'Silva *et al.*[27] to be the underlying cause of a

nongelling phenomenon of teff and maize starch, which were pasted for a prolonged time with added stearic acid. Formation of amylose-lipid complexes also reduces the extent of synerisis in starches.[131] Staling in starch-containing foods may therefore be decreased by inducing complex formation or addition of the complexes.[111] Indeed, Krog and Jansen[29] demonstrated retardation of retrogradation in bread as a result of complex formation.

Encapsulation of Flavor and Bioactive Substances

Starch has been shown to form inclusion complexes with a wide range of compounds that serve as flavor components, nutraceutical, pharmaceutical or bioactive substances. The complex formation is not only limited to linear compounds but has also been demonstrated to occur for more bulky and very small inclusion compounds.[12, 141, 142] However, in case of impossible or limited inclusion due to an unsuitable bioactive ligand structures, complex formation could be initiated by converting them to fatty acid esters.[143] Use of amylose through V-amylose has advantages of ready availability, relatively low cost, nontoxicity, biodegradability, and capacity for targeted delivery at particular GIT locations. [144] This targeted delivery allows for application of reduced active ingredient dosages because the included ligand is released only where its needed or absorbed.[144]

Formation of V-amylose complexes has been demonstrated to lead to increased oxidative stability,[145] decreased volatility,[145] increased thermal stability, and to modulate the release properties of the flavor compounds. Inclusion levels of up to 10.4 % w/w have been reported.[78] The increased stability would enable application in foods that use relatively mild heat processing, such as pasteurization, and also confer protection during storage and processing. For example, Gokmen et

al.[146] recently showed a reduction of flax seed oil oxidation, as well as reduced acrylamide and hydroxymethyl furfural (HMF) levels during bread baking as a result of added V-amylose.

Flavor compounds demonstrated to form the inclusion complexes have included fenchone, menthone, and geraniol;[13] decanal;[26] hexanol, hexanal and hexanon, 1-heptanol-1-decanol, ketones;[147] and lactones[148, 149] among others. The structure and formation of amylose-lipid inclusion complexes with flavor compounds was extensively reviewed by Conde-Petit *et al.*[12] Coinclusion of complexes also been suggested,[12, 78] indicating the possibility of using the complexes in food systems that utilize a mixture of flavors. Coinclusion however leads to a reduction in the overall inclusion content compared to single flavor compounds.[78] The inclusion of complexes is also limited by the water solubility of the included compounds[112] and leads to gel-like changes in the starches,[26] which maybe desirable in some products. On the other hand, the potential application of the amylose-flavor compounds for increased flavor retention has been demonstrated in a model sponge cake food system.[150]

Biodegradable biopolymers such as starch are recommended for delivery of bioactive pharmaceutical and nutraceutical substances.[84] The delivery of sensitive bioactive pharmaceutical or nutraceutical ligands to the lower parts the GIT has been shown to be possible through protection by encapsulation with amylose.[23, 24, 151]Amylose complexes with lipids are not available to mouth and gastric digestion but become available under pancreatic conditions to release the included molecule.[85, 152] This potential application has been demonstrated *in vitro* for stearic acid and polyunsaturated fatty acids (PUFA)[24]. Nanoencapsulation of CLA was demonstrated by Lalush *et al.*[23] and patent application made based on complexes produced by the

continuous homogenization method.[85] A recent study by Cohen *et al.*[153] has shown the *in vivo* V-amylose nanoencapsulation capacity for improved bioavailability bioactive ingredients. They reported that the plasma concentration of the ligand (genistein) can be twice as high as the controls with V-amylose nanoencapsulation.[153] However, there is still a need for more information on this practical application of V-amylose complexes, which may be obtained through use of other food grade ligands with both acute and chronic administration studies.

Concluding comments

V-amylose presents a potential for application of starch in novel ways. Research conducted so far on the structure and functionality of V-amylose provides a significant platform for application and utilization of V-amylose. The research shows that V-amylose complexes are single left-handed helices that are arranged into crystalline and amorphous lamellae, which may form distinct nano or micron scale structures. They can affect the rheological properties of starch, starch digestability, starch retrogradation, and can be used for nanoencapsulation of bioactive or sensitive substances. Further utilization of V-amylose could involve application as a fat replacer, protection of sensitive bioactive or flavor food or pharmaceutical ingredients, increasing the resistance of starch to hydrolysis for diabetics, and reduction of bread staling.

Several aspects, however, need to be addressed in order to enable better understanding and utilization of V-amylose in food and pharmaceutical systems. The V-amylose amylose structural organization at micron and nano-level is still mostly hypothetical and the factors that affect structural changes, especially at nanoscale, are still to be elucidated. With proper understanding of the nano and micron level structural

organization, it will be possible to apply tailor-made structural and functional forms of V-amylose to applications such as delivery of bioactive substances, encapsulation of sensitive flavor compounds, and modification of rheological properties of foods. Focus should be placed on the formation and rearrangements of V-amylose crystalline and amorphous lamellae as affected by preparation and processing factors since they have been shown to be the main structural entities at nano and micron level. This will allow for optimization of yield and more efficient preparation and will pave the way for commercialized production. This will require imaging and structure probing techniques utilized in nanotechnology, such as SAXS, AFM, TEM, STEM, SALS, and dynamic scattering particle size distribution. These techniques generally used in the area of nanotechnology can be used in conjunction with molecular level assessment techniques, such as Fourier transform infra-red spectroscopy (FTIR), XRD, Raman spectroscopy, and high performance size exclusion chromatography (HPSEC).

As regards the modification of food rheology, although the complexes have been shown to modify starch pasting, gelling and flow properties, there are very few reported direct applications in food systems; hence, the need for more applied level research. Along with this, there is need for more studies on the interactions of V-amylose complexes with other food ingredients that can affect V-amylose functionality since it has been shown that the presence of other potential ligands may affect the functionality of the resultant V-amylose[78].

Further studies also need to be conducted in animals to assess the observed *in vitro* slow digestion or hydrolytic resistance property of the V-amylose *in vivo* with both acute and chronic testing of various (bioactive) ligands. The effects of V-amylose injection on postprandial plasma glucose concentration, insulin and C-peptide should

be assessed in order to establish the potential application in diabetics for a reduced postprandial hyperglycaemia.

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