

CHAPTER II

LITERATURE REVIEW

2.1. Bambara Groundnut

Vigna subterranean is a member of the family Fabaceae. It has the common name as Bambara Groundnut (Bambara nut, Bambara bean, earth pea, or ground-bean). Bambara Groundnut is the most legume indigenous of Africa after peanut and cowpea. It is a hardy crop and has been recognized as an important nutritious food source. It has seven varieties of BG based on their seed coat color. In addition, they are difference in shape and size. Bambara Groundnut normally found in Nigeria and also found in Indonesia, Malaysia, Philippines, and the South of Thailand. This legume has low fat, high essential amino acids and high protein: so it is often referred to a complete food. Normally, Bambara groundnut is used in many kinds of product, for example snack (kangu), vegetable milk, low fat yoghurt, and puddings (Gwala et al., 2019, Minka & Bruneteau, 2000, Oyeyinka & Oyeyinka, 2018, Brough, Azam-Ali, & Taylor, 1993, Falade, Ogundele, Ogunshe, Fayemi, & Ocloo, 2015).

2.1.1 Nutrition

Bambara groundnut often refers to high nutrition food. Yao et al (2015) studied amino acids in Bambara groundnut Ci12 landrace (*Vigna subterranea* (L.) Verdc.) seeds produced in Côte d'Ivoire. It was found that this legume seeds contained the protein of 18.8% and suggested that it was rich in essential amino acids, for example, isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine. Glutamic (209.5 mg/g crude protein) and aspartic acids (146.1 mg/g crude protein) are the major non-essential amino acids, while leucine (102.1 mg/g crude protein) and lysine (80.2 mg/g crude protein) are the principal essential amino acids (table 1). A similar result was reported in by Adeyeye & Olaleye, (2012) who studied Bambara groundnut in Nigeria. Leucine and lysine content are also high, especially in dehulled and whole seeds of Bambara groundnut (Adeyeye & Olaleye, 2012).

Aremu, Mamman, & Adebisi (2013) studied fatty acids of six varieties of Bambara groundnut seed in Nigeria. The six varieties of Bambara groundnut that were chosen in this research are different in seed coat color which were cream with black eye, cream with black speck, cream, cream with violet spotted, light brown and dark brown. They reported that saturated fatty acids were palmitic (C16:0), stearic acid (C18:0), arachidic acid (C20:0) and behenic acid (C22:0) (Table 2.2). The monounsaturated fatty acids were palmitoleic (C16:1), oleic (C18:1) while the polyunsaturated fatty acid were linoleic (C18:2) and linolenic (C18:3). Linoleic acid was found to be the highest content among all fatty acids, followed by palmitic acid, oleic acid, and stearic acid.

Bambara groundnut also contains high phenolic compounds. Vivien, (2018) reported that sixty-two bioactive compounds were present in the raw Bambara groundnut. The majority of the phenolic compounds identified in Bambara groundnuts were flavonoids (Nyau, Prakash, Rodrigues, & Farrant, 2015), for instance, quercetin (which have anti-inflammatory properties), catechins (which is an antioxidant demonstrating a significant risk reduction for cardiovascular diseases), ferulic acid (which is used in anti-aging skin care products).

Table 2.1 Amino acid contents and amino acid score (AAS) of protein in Bambara groundnut

Amino Acid	Crude Protein (mg/g)	FAO Report Protein ^a (mg/g)	AAS
Histidine	38.6 ± 2.3	16	2.41
Isoleucine	54.5 ± 0.0	30	1.82
Leucine	102.1 ± 0.1	61	1.67
Lysine	80.2 ± 5.2	48	1.67
Threonine	44.3 ± 3.2	25	1.77
Tryptophan	6.0 ± 0.0	6.6	0.91
valine	62.4 ± 2.3	40	1.56

Table 2.1 Amino acid contents and amino acid score (AAS) of protein in Bambara groundnut (Cont.)

Amino Acid	Crude Protein (mg/g)	FAO Report Protein ^a (mg/g)	AAS
Methionine	6.4 ± 0.1	-	-
Cysteine	24.1 ± 0.0	-	-
SAA	30.5	23	1.33
Tyrosine	31.3 ± 1.3	-	-
Phenylalanine	76.9 ± 2.0	-	-
AAA	108.2	41	1.88
Aspartic acid	146.1 ± 5.1	-	-
Serine	68.5 ± 3.5	-	-
Glutamic acid	209.5 ± 4.3	-	-
Proline	53.6 ± 1.7	-	-
Glycine	46.5 ± 3.4	-	-
Alanine	51.4 ± 1.4	-	-
Arginine	74.8 ± 6.0	-	-
AAS	0.91	-	-
Limiting amino acid	Tryptophan	-	-

^a Recommended amino acid scoring patterns for older child, adolescence, adult group, SAA: sum of sulphur amino acids (methionine + cysteine), AAA: sum of aromatic amino acids (phenylalanine + tyrosine), AAS: mg of essential amino acid in test protein/mg of essential amino acid in recommended amino acid scoring patterns in FAO report. (Yao et al., 2015)

Bambara groundnut also contains high phenolic compounds. Vivien, (2018) reported that sixty-two bioactive compounds were present in the raw Bambara groundnut. The majority of the phenolic compounds identified in Bambara groundnuts were flavonoids (Nyau et al., 2015), for instance, quercetin (which have anti-inflammatory properties), catechins (which is an antioxidant demonstrating a significant risk reduction for cardiovascular diseases), ferulic acid (which is used in anti-

aging skin care products). In addition, flavonoids also showed a free radical scavenging property. This study suggested that bambara groundnuts may be useful as nutraceuticals due to that it contains various phenolic compounds from the flavonoids class.

Table 2.2 Fatty acid composition (%) of six varieties of Bambara groundnut seed oils

Fatty acid	Cream with Black Eye	Cream with Black Speck	Cream	Cream with Violet Spotted	Light Brown	Dark Brown	Mean	SD	RSD
Palmitic acid (C16:0)	22.38	22.24	21.79	23.27	22.38	21.79	22.31	0.50	2.22
Stearic acid (C18:0)	11.20	10.94	11.69	10.98	9.89	10.41	10.85	0.57	5.25
Arachidic acid (C20:0)	0.63	0.70	0.43	0.68	0.63	0.43	0.58	0.11	19.96
Behenic acid (C22:0)	0.47	0.44	0.82	0.20	0.47	0.82	0.54	0.22	40.74
Palmitoleic acid (C16:1)	3.44	4.52	3.97	3.52	4.72	3.67	3.97	0.52	12.67
Oleic acid (C18:1 ω 9)	18.07	17.54	18.49	17.62	18.08	18.49	18.05	0.37	2.05
Linoleic acid (C18:2 ω 6)	35.43	35.05	34.04	35.19	35.44	35.62	35.13	0.52	1.48
Linolenic acid (C18:3 ω 3)	8.38	8.57	8.77	8.53	8.39	8.77	8.57	0.17	1.87

Source: (Aremu, Mamman, & Adebisi, 2013)

2.1.2 Physical and Functional properties

Sirivongpaisal (2008) studied about the functional properties of Bambara groundnut (BG) flour and starch. They obtained the BG seed from local market in Pattalung province. Scanning electron microscopy was used to study the morphology of starch granules. The shape of Bambara groundnut starch was oval and round, and it has smooth surface as shown in Figure 2.1

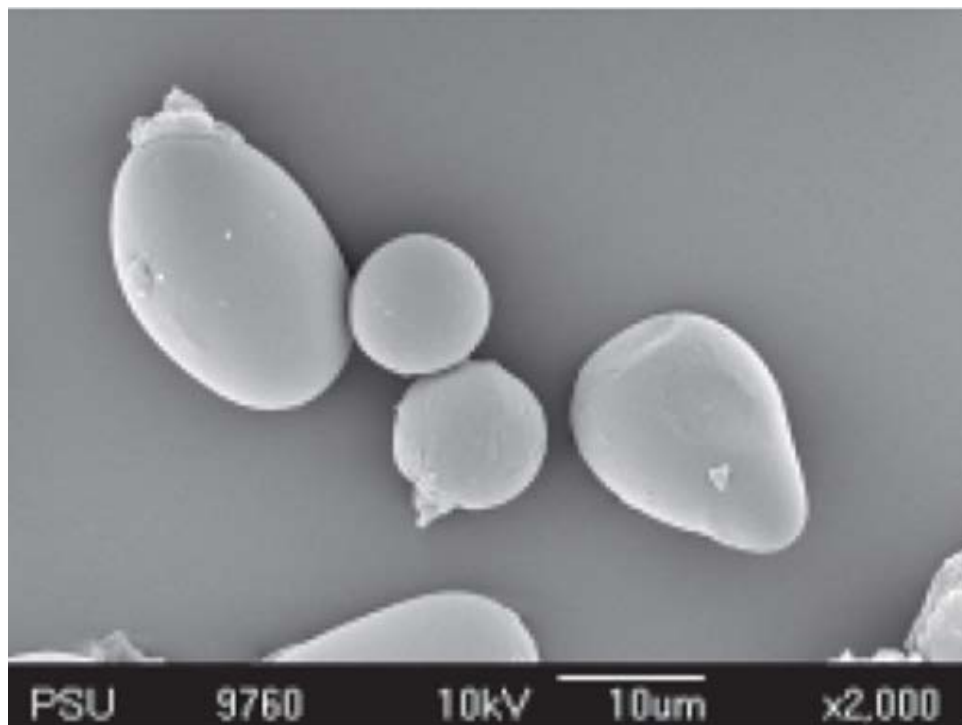


Figure 2.1 Scanning electron micrograph of starch granules from Bambara groundnut at magnification 2000X (Piyarat, 2008)

A crystalline structure of starch generally is divided into 3 types which are A-, B-, and C-type. From X-ray diffractogram, the Bambara groundnut starch showed an A-type diffraction pattern because strong peaks were found at 2theta of 5.78, 5.17, 4.86 and 3.78 °A (Kaptso et al., 2015; Piyarat, 2008). In contrast, Afolabi et al., (2018) and Oyeyinka et al., (2017) reported a C-type diffraction pattern that is a mixture of A- and B-types. It is possible to observe differences in crystallinity patterns of starch from the same species.

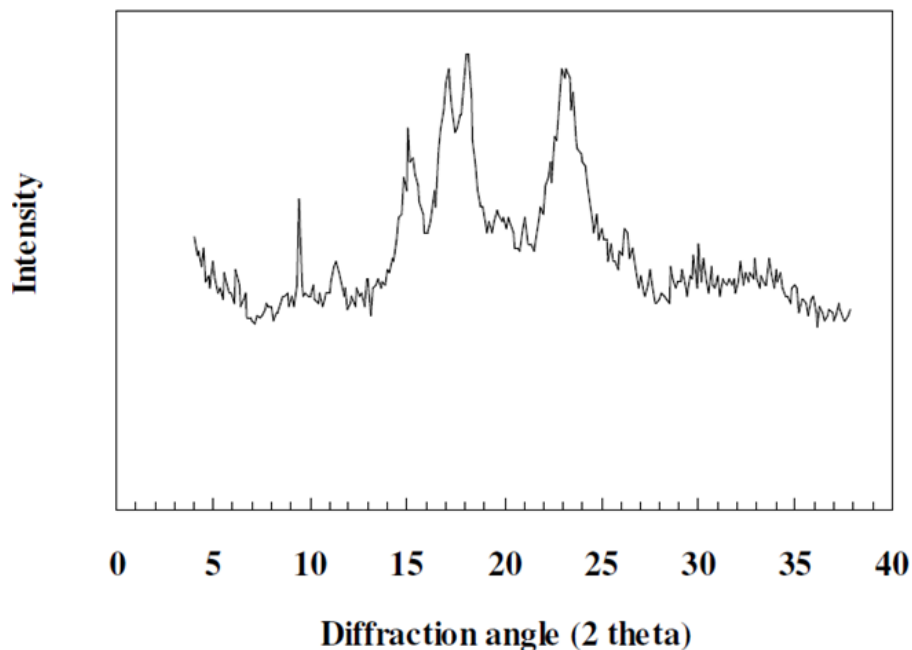


Figure 2.2 X-ray diffraction pattern of Bambara groundnut starch (Piyarat, 2008)

Piyarat, (2008) studied about swelling power and solubility of Bambara groundnut flour and starch at the temperature range of 50 to 95°C. The results showed higher of swelling power, when temperature is increased (Adebowale, Adeniyi Afolabi, & Lawal, 2002; T. A. Afolabi, Opara, Kareem, & Oladoyinbo, 2018; Piyarat, 2008). However, the swelling values of Bambara groundnut flour was reported lower than starch because lipid and protein are present in its composition.

Gelatinization properties of Bambara groundnut was studied using differential scanning calorimeter (DSC). Piyarat (2008) reported the gelatinization of starch was 71.69, 75.33, and 79.17°C for the onset (T_o), midpoint (T_p), and end (T_c) of gelatinisation respectively, and the enthalpy (ΔH) of gelatinization was 11.73 (J/g), while T_o , T_p , T_c , and ΔH of Bambara groundnut flour was 76.84, 80.75, 85.82°C and 6.00 (J/g) respectively. They suggests that gelatinization properties was related with a degree of homogeneity of crystallites within granules of Bambara groundnut starch This results showed that $T_c - T_o$ of Bambara groundnut flour was higher, while its enthalpy of gelatinization was lower as compared to enthalpy of starch. It was

explained that the other composition of flour such as proteins, lipid, or fiber could interact with starch (Czuchajowska, Klamczynski, Paszczynska, & Baik, 1998).

The Brabender viscograph technique was used to study the pasting properties of Bambara groundnut flour and starch in Piyaat's study (Piyaat, 2008). The pasting characteristic of flour showed a lower peak viscosity during holding period at 95°C. The viscosity profile of Bambara groundnut flour was slightly lower and more stable when compared to Bambara groundnut starch. It suggested that the flour is more resistant to heat than the starch. This could be explained by the interaction of starch and other components, lipid and proteins, during heating.

For *in vitro* digestibility, the different varieties of Bambara groundnut, which were maroon, brown and cream was studied and they had 11.5-12.3% RDS, 15.9-16.9% SDS and 69.7%-72.6% RS contents. (Oyeyinka, Singh, Venter, & Amonsou, 2017). In addition, Oyeyinka et al., (2019) studied the starch fractions of raw Bambara groundnut starch compared to those of cooked Bambara groundnut starch. It was found reported that raw red Bambara groundnut starch has 22% of RDS, 48% of SDS, and 30% of RS. After cooking, the SDS and RS fractions were lower to be, 9.7% and 17% respectively, and the RDS fraction was higher to be, 73%. The difference of these results may be related to many factors including amylose to amylopectin ratio, granule size, degree of crystallinity, type of crystalline polymorphic form, molecular structure of amylopectin.

2.2 Starch

Starch is an end-product from photosynthesis and used as energy source in plant. It can found in leaf of green plant, root, seed, fruits, or stems. Starch is white, tasteless, and odorless powder. It was used for different purposes such as textile, paper, or food products.

Starch is the polysaccharides consisting of glucose units, which link with glycosidic bonds. The components of starch are amylose and amylopectin. Amylose is a linear chain that is linked with α -(1-4) glycosidic bonds. For amylopectin, glucose

units is linked with α -(1–4) glycosidic bonds for a linear structure and linked with α -(1–6) glycosidic bonds for the branching points. The relative weight percentage of amylose is in range of 18 to 33% and 72–82% for amylopectin (Bul  on, Colonna, Planchot, & Ball, 1998). The structure of starch was described as the “cluster” model, as suggested by a semicrystalline structure. Short branch chains of amylopectin are in crystalline lamella regions, while the branch point is in amorphous region (Bello Perez & Agama-Acevedo, 2017; Robyt, 2008). P  rez & Bertoft, (2010) suggested that amylose is intermixed with amylopectin at the branching points.

The crystalline structure of starch is classified as two types (A and B). (P  rez & Bertoft, 2010) The structure of A and B type is different in crystal packing and water content. From the calorimetric studies, A-type starch has higher temperature than B-type starch. This indicated that an A-type is more tightly pack arrangement of double helices and more stable compared with a B-type starch (Gidley, 1987). I

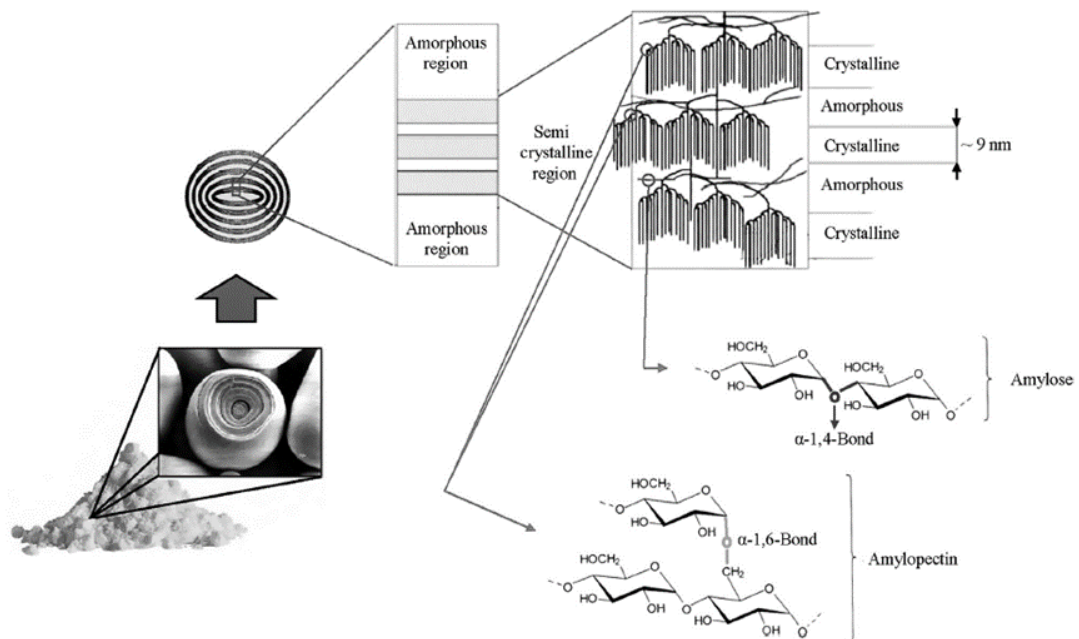


Figure 2.3 Projection of starch granule (Bello Perez & Agama-Acevedo, 2017)

2.3 The nutritional classification of starch

Based on its *in vitro* enzymatic hydrolysis and the rate of glucose release, starch is divided into 3 types, which are rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) (Englyst, Kingman, & Cummings, 1992).

2.3.1 Rapidly digestible starch (RDS) and Slowly digestible starch (SDS)

RDS is starch which is converted to glucose molecules in 20 min of enzyme digestion, while SDS is the fraction which is digested in 100 minutes. Both RDS and SDS are completely digested in the small intestine.

2.3.2 Resistant starch (RS)

Resistant starch (RS) is the starch that is resistant to normal digestion in humans and animals, and fermented by the colonic microflora in the large bowel. Many studies suggest that RS has an important role in energy metabolism and weight management. Normally, RS can be divided into four types base on the mechanism by which the starch resists digestion (Murphy, Douglass, & Birkett, 2008).

2.4 Definition and classification of resistant starch

Resistant starch type 1 (RS1): RS1 is the starch that trapped within the food matrix. Thus, it can protect starch granules from the digestion of enzymes. Starch can release when the foods are milled or grinded foods, which make it more accessible to digestive enzymes.

Resistant starch type 2 (RS2): This type of RS starch is resistant to enzyme because of the nature of the starch granule, composition and conformation of the granule. The starch that is eaten as raw starch or has a high gelatinization temperature in which granules do not gelatinize during cooking is a source of RS2.

Resistant starch type 3 (RS3): RS3 or retrograded starch is the starch that cooked and cooled. This process makes the structure of starch granules having more crystalline regions that are very resistant to digestive enzymes.

Resistant starch type 4 (RS4): This starch is undergone the chemical modification, including dextrinization, etherification, esterification and oxidation, and cross-linking with difunctional reagents. These make the structure of the starch to be interference with the digestive enzymes.

2.5 Amylose-lipid complex as resistant starch type 5

Amylose lipid complex is defined as the inclusion of lipid molecules in amylose (figure 3). Single-helix amyloses that are co-crystallized with compounds (e.g., fatty acids or alcohols) are given the generic name of V-amylose.

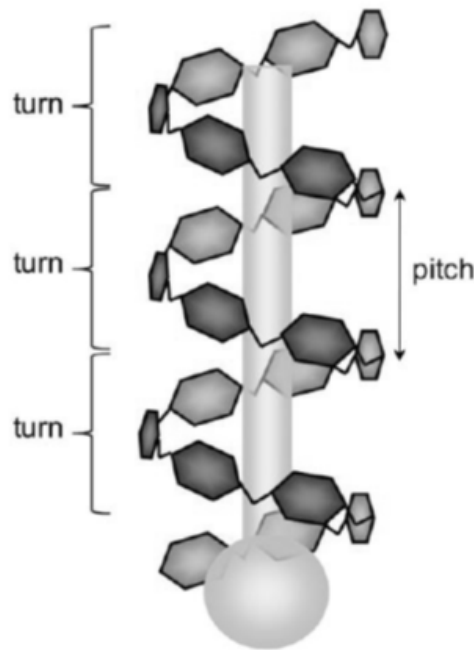


Figure 2.4 Amylose lipid complex structure (Panyoo & Emmambux, 2017)

V-amylose complexes have been used to enhance starch pasting properties, prepare novel starches with slowly digestible property and protect volatile and sensitive ligands such as polyunsaturated fatty acids. It has been proposed as a new source of resistant starch (RS type 5) in addition to the four earlier proposed sources. Amylose-lipid complex has more advantages comparing with other types of RS in that it is more heat stable than RS2 and requires less extensive physical or chemical processing than that of retrograded amylose (RS3) and chemically modified starch (RS4) (Hasjim, Ai, & Jane). In starch granules, amylose-lipid complex has been shown to retard granule swelling during heating in excess water and reduces the enzyme accessibility to hydrolyze the starch granules. Ai et al, (2013) studied the cooking of tapioca, normal maize and high-amylose maize starches in the presence of corn oil, soy lecithin, palmitic acid (PA), stearic acid (SA), oleic acid (OA) or linoleic acid (LA).

The results showed the reduction of the susceptibility of the starch to enzyme hydrolysis, indicating that the amylose-lipid complex was formed during the cooking of starch and lipids that decreased the enzyme digestibility of starch.

In addition, lipid structure also influences on the complexing ability of amylose-lipid complex. A saturated long-chain fatty acid produces a more enzyme-resistant amylose complex than a shorter or an unsaturated fatty acid (Kitahara, Suganuma, & Nagahama, 1996). The kink structure of cis-double bond disrupts the alignment of the fatty acid in the cavity of amylose single helix and the dissociation temperature of amylose complexed with an unsaturated fatty acid is lower than that of amylose complexed with a saturated fatty acid of the same hydrocarbon chain-length (Tufvesson, Wahlgren, & Eliasson, 2003).

2.5.1 Types of V-amylose complexes

Based on the melting temperature, V-amylose is divided into type I and type II. Generally, type I has melting temperatures below 100°C, while type II has melting temperatures above 100 °C. Type I complexes consist of a partially ordered structure with no distinct crystalline regions, whereas type II complexes are made up of distinct crystalline and amorphous regions. (Obiro, Sinha Ray, & Emmambux, 2012)

2.5.2 V-amylose complex preparation methods

For classical V-amylose preparation methods, an amylose-containing starch fraction was mixed 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.

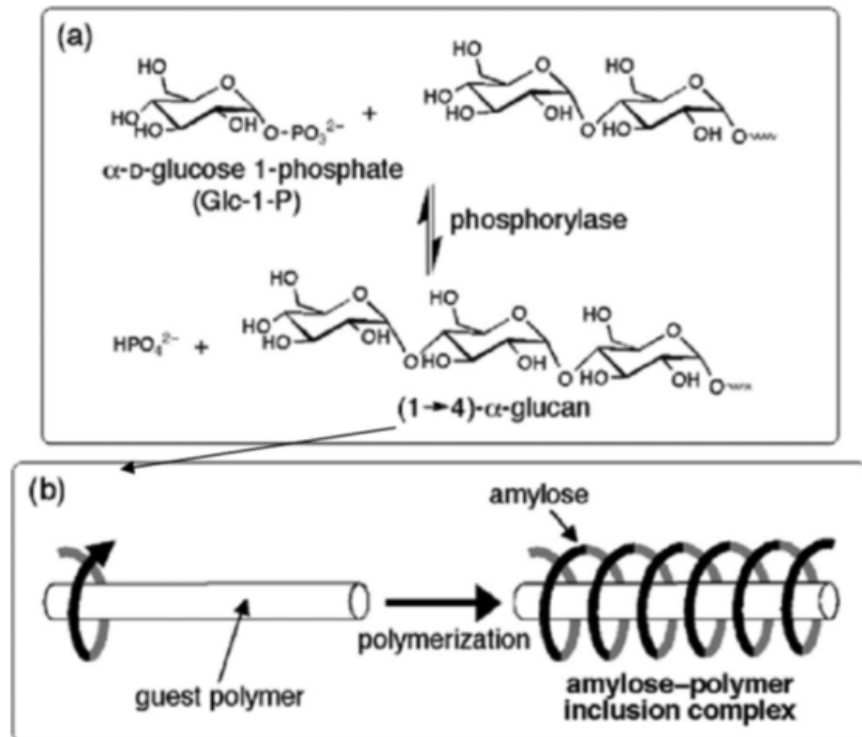


Figure 2.5 Concept of “vine-twinning” polymerization.

2.6 Factors affecting starch digestibility

Several factors affect starch digestibility, both extrinsic and intrinsic properties of starch granules. Extrinsic factor is starch granule surface characteristics such as porosity of granule and intrinsic properties such as packing of amorphous and crystalline regions (Gallant, Bouchet, Buléon, & Perez, 1992) or interaction of amylose with other components such as lipids and proteins.

2.7 Rheology of starch

Dynamic rheological property is one of the functional properties of starch to determine its applications. Starch thickener is often utilized in food industry and viscosity is one of the important parameter in the characterization of rheological property. Dynamic rheological property is defined as the deformation and flow behaviors responding to a stress. For starch paste and gel, it depends on many factors, including the chemical structure of starch, starch concentration, pasting conditions (e.g., temperature, shear rate, and heating rate), and storage conditions (temperature and time). Oyeyinka, (2017), studied the rheology properties of Bambara groundnut starch. The results showed a decrease in viscosity with increasing shear rates suggesting a breakdown in the starch structure by the applied shear stress. This is the behavior of pseudoplastic macromolecules.

2.8 Modified Bambara groundnut starch

Physical modification is popular method that use to improve the quality of starch. Annealing and heat-moisture treatment were used for improve digestibility of Bambara groundnut starch (T. A. Afolabi et al., 2018). The native starch was treated by annealing at 50°C, for 48 h, while heat-moisture treatment was conducted at 100°C for 16 h at 25% moisture content. After treated with heat-moisture treatment, the resistant starch content was higher than the native starch. In contrast, the RS of the annealed starch was lower as compared with the native starch. It was explained that some components of starch interacted with each other during heat moisture treatment therefore restricting accessibility of starch chains to the hydrolyzing enzymes.

Oxidation, acetylation and carboxymethylation were the chemical modification that was studied to prepare the modified Bambara groundnut starch. (Adebowale et al., 2002; T. Adeniyi Afolabi, 2012) The effect of chemical modification on physicochemical properties of Bambara groundnut starch was investigated. For swelling power, the acetylated and carboxymethylated starch had a higher swelling power as a result of the presence of functional groups which led to the loss of crystallinity and the increase of amorphous region, while that of oxidized starch was

lower because of the weakness of the internal structure of starch granules (Adebowale et al., 2002; T. Adeniyi Afolabi, 2012). However, all of these modified starch showed lower peak temperature than the native starch. Carboxymethylated starch showed the increased peak viscosity, while acetylated and oxidized starch showed the decreased peak viscosity.

Not only physical and chemical modification, but also lipid complex formation was used for improving the quality of Bambara groundnut starch. Stearic acid, linoleic acid, and lysophosphatidylcholine were added in the native BG starch (Oyeyinka et al., 2017). The lipids interacted with starch which showed a V-amylose single helical structure, then restricting an enzyme accessibility into the starch granule. This indicates that the starch-lipid complex can decrease the starch hydrolysis rate. In addition, the melting temperature of starch-lipid complex was 97.5 to 100.9°C that indicated the Type-I V-amylose complexes. The higher melting temperature of starch-lipid complex showed their thermal stability, which was higher than the native starch.

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