Chapter 18

The Structure and Characteristics of Sago Starch

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Abstract The crystalline structure of sago starch related to the gelatinization characteristics is reported in this chapter, although the sago starch synthesis at the molecular biological level is still under discussion. Sago starch granules are oval with a temple bell-like shape, a mean diameter of 37.59 μm , and exhibit a Maltese cross, indicating the presence of some common internal ordering. Sago starch from its X-ray diffraction pattern shows a peak at around 5.6, 17, 18, and 23° (2 θ for Cu K α), which corresponds to 1.58, 0.521, 0.492, and 0.386 nm, respectively. Sago starch is classified as a C type (a mixture of A type and B type as an accessory), containing slightly higher content of B type in the basal portion of sago palm trunk. Low viscosity of sago starch amylopectin is explained by the presence of smaller molecule with a slightly higher number of long chains than the high-viscosity amylopectin. The gelatinization temperature and enthalpy of sago starch determined by a differential scanning calorimeter (DSC) are 69.4–70.1 °C and 15.1–16.6 J g⁻¹, depending on the moisture content, degree of a crystallinity within the granule, granule size, and the amylose to amylopectin ratio. The observation of granular birefringence (Maltese cross) under polarized light is one of the useful tools to determine the gelatinization behavior of sago starch.

18.1 Starch Synthesis

Starch production is the most important agricultural activity for peace in the world. Plants can produce glucose and its dehydrated and condensed substances as starch by photosynthesis from carbon dioxide, temporarily accumulating starch in leaves, twigs, and buds, which serve as energy stores (Fig. 18.1). Toward the end of the growing season, starch is transformed into sucrose prior to translocation. This means that sucrose is the starting substance of storage starch synthesis. From sucrose in the cell, glucose-1-phosphate, glucose-6-phosphate, and adenosine

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Fig. 18.1 Starch production by photosynthesis (Akazawa 1965, partly modified)

diphosphate glucose (ADPG) are synthesized, and, finally, ADPG is produced from glucose-1-phosphate and glucose-6-phosphate. The glucose residue of ADPG is dehydrated and condensed by granule-bound starch synthase (GBSS) and soluble starch synthase (SSS) to make amylose and amylopectin chains. GBSS relates to the production of amylose which exhibits a low amount of branching. Meanwhile, the long chains of α -1,4 glucans are partly cut by branching enzymes, and thereafter α -1,6 glucans are produced by the dehydration reaction of OH at C1 and OH at C6. Large amounts of non-reductive edges in the chains of starch are able to become elongated edges by SSS, and new side chains of non-reductive are formed by branching enzymes. Over-numbers of binding part of α -1,6 binding are cut down by cutting enzymes, resulting in side chains being reduced to form amylopectin. When GBSS is lost, only the waxy type of starch containing no amylose will be formed. On the other hand, when the activity of SSS becomes lower, high amylose starch will be formed. At the same time and/or after the elongation of amylose and amylopectin chains occur, polymorphs are formed during high dimension of structure formation. Although this phenomenon in starch science is one of the important issues, scientific light has not completely elucidated it until now (Fig. 18.2).

No information on sago starch synthesis in relation to starch synthase is available at the present time.

Fig. 18.2 Starch production in storage organs (*F-6-P* fructose-6-phosphate, *G-1-P* glucose-6-phosphate, *ADPG* adenosine diphosphate glucose, *GBSS* granule-bound starch synthase, *SSS* soluble starch synthase)

18.2 Starch Conformation

Starch contains polymer chains of glucose units with a high degree of regularity, crystalline clusters of double helices (Kainuma and French 1971, 1972), which consist of two glucans: amylopectin and amylose. Amylopectin is the major component in most starches. It is the extensively branched structure and is composed of short chains of α -(1,4)-linked D-glucosyl units that are interconnected through α -(1,6)-linkages (Vamadevan and Bertoft 2015). Amylose is a minor component and is essentially longer linear chains than amylopectin.

The X-ray diffraction technique is popular for the analysis of the crystalline structure of starch and has developed rapidly. Until the 1970s, it was not known that amylose has a crystalline structure and exhibited a monoclinic and hexagonal structure with a double helix (Wu and Sarko 1978a, b; Imberty and Perez 1988, Imberty et al. 1988). However, it is still unclear if the helical structure is right- or left-handed. One unit (a total of 12 glucose residues consisting of 6 glucose residues making a double helix forming 1 unit) of A-type starch, monoclinic structure, holds eight water molecules (Fig. 18.3). Meanwhile, one unit of B-type starch, hexagonal structure, holds 36 water molecules in the cavities of the structure. In addition, C-type starch also was proposed to be a mixture of A and B type (Hizukuri and Nikuni 1957). However, C type always consists of A and B type.

The synthetic processes and mechanisms of starch by synthetic enzymes in amyloplast have been elucidated. However, the working processes of A-, B-, and C-type starch are not yet clear. Synthesized ADPG forms α -1,4 glucoside bonding by dehydration shrinkage with OH group of non-reductive edges of glucose, and the

Fig. 18.3 Crystalline structure of A-type and B-type amylose (Imberty et al. 1991, partly modified)

elongation of glucose gradually occurs to make maltose and/or maltotriose. The basic unit of six glucoses, consisting of A-type starch, is maltose. Three units of maltose form a basic unit of amylose and amylopectin, in the case of A type. On the other hand, two units of maltotriose form a basic unit of amylose and amylopectin for B type. Two basic units are closely hydrated to each other, allowing hydrogen bonding to produce a double helix. Double-helical units can precipitate from the soluble state of starch to high molecular starch in amyloplast.

The fragments of low molecular starch form the double helix structure, associated with the formation of stable three-dimensional structures at a low energy level. The conformation analysis has been developed in protein science and elucidated the structure of the domains. When amyloplast is present at a high temperature and a low moisture content, the restriction of free movement in the solution of amyloplast and the limitation of water molecules result in the formation of A-type starch, monoclinic crystalline structure, which is found in aboveground starch accumulation organs. Meanwhile when amyloplast is at a low temperature and high moisture content, the double helix can easily move and hold water molecules resulting in the formation of hexagonal crystalline structure in belowground starch accumulation organs.

The C-type starch structure derived from both A type and B type consists of exsolution-like materials formed by GBSS and SSS under a given temperature and concentration. Most sago starch is C-type starch, so-called C_A type, including B-type starch as accessory.

Fig. 18.4 Scanning electron microscope image of sago starch granules from Leyte, Philippines

Fig. 18.5 Particle size distribution of sago starch from Leyte, Philippines (Nishiyama et al. 2015)

18.3 Sago Starch Structure

18.3.1 Crystalline Structure of Sago Starch

Sago starch granules are oval with a temple bell-like oval shape (Fig. 18.4). Scanning electron microscopy provided a clear shape and size of native sago starch granules from Leyte, Philippines, which varied from 8 to 240 μm in diameter with the mean value of 37.59 μm (Fig. 18.5), which is relatively larger than that found by Kobayashi (1993), suggesting that the stroma developed the septum-like structure in an amyloplast and finally produced the temple bell-like shape.

Fig. 18.6 Light and polarized light microscope images of sago starch (Nishiyama et al. 2015)

Fig. 18.7 XRD pattern of sago starch from Leyte, Philippines (Nishiyama et al. 2015) (*Corn*, Kosakai Pharmaceutical Co.; *potato*, Miyazawa Pharmaceutical Co.; *sago*, collected from Leyte, Philippines)

The observation of sago starch granules under a polarized light microscope is effective to see their crystalline properties (Nishiyama et al. 2015) (Fig. 18.6). Sago starch granules exhibit a distinctive Maltese cross (Fig. 18.6 B, C), which indicates the presence of some common internal ordering.

The X-ray diffraction (XRD) pattern of starch granules of corn, potato, and sago at the nanoscopic level shows that starch consists of thin lamellar domains (Nishiyama et al. 2015) (Fig. 18.7). Takahashi et al. (1981), Kawabata et al. (1984), and Yatsugi (1986) used XRD to analyze the structure of sago starch and to compare the starch of corn, potato, mung bean, cassava, and arrowroot. Yatsugi (1986) reported four sago starches did not show differences in their XRD patterns, although it clearly indicated the difference in the first and fourth ring among them.

The XRD pattern of sago starch collected from various portions of the trunk at different growth stages (9–14.5 years after transplanting) and the relative crystallinity of sago starch, corrected by the internal standard of CaF_2 , ranging from 22 to 40°, were reported by Hamanishi et al. (1999, 2000). Ahmad et al. (1999a), Okazaki et al. (2008) (Fig. 18.8), and Yaacob et al. (2011) also found that sago starch showed

Fig. 18.8 XRD patterns of sago starch from Leyte, Philippines (Okazaki et al. 2008). Sago starches were extracted from the lower trunk (0–80 cm) to the apical (600–660 cm) portion. The numbers 1, 2a, 2b, 3, 4a, 4b, 5, 6, and 7 show the peaks

a peak at around 5° of X-ray diffraction. The X-ray diffraction pattern of sago starch indicates the peaks at around 5.6 , 17 , 18 , and 23° , which corresponds to 1.58 , 0.521 , 0.492 , and 0.386 nm, which are classified as a C_A type of starch (Cai et al. 2014), a mixture of A type and B type of starch. Sago starch is classified as C type, containing A type mainly and B type as an accessory. Srichuwong et al. (2005) found that sago starch extracted from 14 local varieties of sago palm in Vanuatu was of the C or C_A type.

XRD is a useful tool to determine the crystalline degree of sago starch and to evaluate its thermodynamics. The crystalline index of sago starch was 609, calculated by the equation (1), which shows the similar crystalline index to the result obtained by Katsumi et al. (2014) (Fig. 18.9).

Recently, Polnaya et al. (2013) also revealed that native sago starch from Indonesia was characterized by a weak diffraction peak at $2\theta = 5.67^\circ$ and broad peaks at $2\theta = 15.30^\circ$, 17.12° , 18.08° , and 23.46° , which indicated the C type. In addition, Uthumporn et al. (2014) showed that the XRD patterns of sago starch at different growth stages from Malaysia were C type. However, the XRD analysis of sago starch was not sufficient to provide an understanding of the flexibility of sago starch structure during wet and dry processes.

Fig. 18.9 X-ray diffraction after waveform separation (Katsumi et al. 2014) (*Amylopectin*, MP Biomedicals Inc.; *amylose*, Sigma-Aldrich Inc.; *corn*, Kosakai Pharmaceutical Co.; *potato*, Miyazawa Pharmaceutical Co.; *sago*, collected from Leyte, Philippines. Crystalline index = $\text{peak height}_{3b} / \text{FWHM}_{3b} + \text{peak height}_{4a} / \text{FWHM}_{4a}$ (1) *FWHM*: full width at half maximum)

Okazaki et al. (2014) found that the scarcity of change in sago starch structure (from non-spiny and spiny palms) was due to heating up to 60 °C in the case of low moisture content according to X-ray diffraction. However, over 60 °C the dehydration caused structural changes in sago starch.

18.3.2 Amylopectin and Amylose in Sago Starch

Amylopectin is made up of chains of α -(1,4)-linked D-glucosyl units that are interconnected through α -(1,6)-linkages, containing 10^7 – 10^8 of molecular weight (degree of polymerization: 12,000–40,000) (Hizukuri 2003). On the other hand, amylose is mainly composed of a liner structure of glucose units linked by α -(1,4) bonds with 10^5 – 10^6 of molecular weight (degree of polymerization: 600–36,000) (Hizukuri 2003), entangled with amylopectin. These components represent approximately 98–99% of the dry weight of starch (Tester et al. 2004). Amylose itself comprises 15–35% of the starch by weight. High percentages of amylose give starch less stickiness and a low gelatinization temperature.

Takeda et al. (1989) reported that the low viscosity of sago starch amylopectin is explained by the presence of a smaller molecule with a slightly higher number of long chains than the high-viscosity amylopectin. The amylopectin chain distribution of sago starch is composed of four fractions with the different chain lengths of Fraction I ($22.9 \pm 0.8\%$), Intermediate Fraction ($3.9 \pm 0.4\%$), Fraction II ($17.2 \pm 0.4\%$), and Fraction III ($56.0 \pm 1.4\%$) (Ishii et al. 1990). Takahashi and Hirao (1994) reported that Fraction III of sago amylopectin was 52.3%, intermediate in value between sweet potato and mung bean amylopectin. Furthermore, Srichuwong et al. (2005) reported that the mean chain length of amylopectin taken from 14 varieties of sago starch from Fiji, Samoa, and Vanuatu was 41.0% for the degree of polymerization (DP) of 6–12, 53.8% for DP 13–24, and 5.2% for DP 25–30.

18.3.3 *Gelatinization Characteristics of Sago Starch*

Starch gelatinization is the disruption of molecular order within the starch granule manifested in irreversible changes in properties such as granular swelling, native crystallite melting, loss of birefringence, and starch solubilization (Maaruf et al. 2001). Starch undergoes a glass transition in the amorphous components prior to the gelatinization when the starch granule is heated in the presence of water (Vamadevan and Bertoft 2015).

The behavior of sago starch gelatinization was observed by a light microscope, a polarized light microscope equipped with a micro-heater, X-ray diffraction, and Brabender amylograph. The highest viscosity of 8% sago starch suspension was determined with a Brabender amylograph to be 830 B.U. around 92.5 °C (Takahashi et al. 1981). Arai et al. (1981) revealed that the viscosity of 8% sago starch suspension was lower than that of 8% sweet potato, corn, cassava, wheat, and 4% potato suspension. In addition Kawabata et al. (1984) reported that 6% sago starch suspension showed the lowest viscosity at 135 B. U. with a small breakdown, compared to cassava, arrowroot, and potato. Yasugi (1986) compared the amylograms of four kinds of 5% sago starch suspension to corn and cassava starch suspension and concluded that good quality sago starch had low viscosity. The viscosity of six sago starches from Papua New Guinea was determined at 30–70 °C with a rotational viscometer and was inversely related to the handling temperature and showed different temperature sensitivity (Sopade and Kiaka 2001). Takeda et al. (1989) found that amylose and amylopectin from sago starches with low and high viscosity on amylography differed in molecular structure. The maximum viscosity of sago starch with high and low viscosity was 735 and 280 B. U., respectively. They concluded that amylose controlled the pasting by the interaction with other materials such as lipid and/or intermolecular hydrogen bonds between other amylose and amylopectin.

Fig. 18.10 Micrographs of sago starch from Papua New Guinea under normal and polarized light in conjunction with a λ -plate during gelatinization (Okazaki et al. 2016) (Bar is 50 μm . The numbers in parentheses represent the temperature during gelatinization)

The gelatinization temperature and enthalpy of starch determined by a differential scanning calorimeter (DSC) depends on the moisture content and degree of crystallinity within the granule and also on granule size and the amylose to amylopectin ratio (Ahmad et al. 1999b). Ahmad et al. (1999b) showed that the gelatinization peak temperature and enthalpy varied from 69.4–70.1 $^{\circ}\text{C}$ and from 15.1–16.7 J g^{-1} , respectively, among 11 sago starches. The gelatinization peak temperature of sago starch was higher than those of corn, cassava, potato, and pea. Furthermore, Ahmad and Williams (1999a, b) Ahmad et al. (1999a, b) and Maaruf et al. (1999, 2001) reported that the presence of salts and sugars affected the gelatinization properties of sago starch. Intrinsic viscosity decreased with increasing temperature, but the critical concentration remained fairly constant over the range of temperatures (Nurul et al. 2001). Siau et al. (2004) attempted to make cationic sago starch using 3-chloro-2-hydroxypropyltrimethylammonium chloride (0.01–0.10 mol L^{-1}) and sodium hydroxide (0.03–0.86 mol L^{-1}) and found that the gelatinization enthalpy of cationic sago starch was lower, compared with native sago starch. The enthalpy of a transition is explained as corresponding to the amount of crystal order (or double helical structure) in the starch suspension (Mohamed et al. 2008). The structural transformation of sago starch modified by osmotic pressure and heat moisture was observed by DSC (Pukkahuta and Varavinit 2007). The gelatinization peak temperature of sago starch increased linearly with increasing osmotic pressure and heat moisture.

The behavior of sago starch granules accompanied by gelatinization was shown by observing granular birefringence (Maltese cross) under polarized light (Okazaki et al. 2016) (Fig. 18.10). The blue and yellow color areas, indicating radial alignments, represent the regions of crystallinity of sago starch. The loss of color on heating indicated the disappearance of radial alignment of crystallinity. The disruption

of crystallinity occurred on the proximate surface of the eccentric hilum and was propagated from the proximate surface to the distal surface of the eccentric hilum. The initial, middle, and end gelatinization temperatures determined at a hot stage were 78.0, 80.0, and 83.0 °C for sago starch from Papua New Guinea.

References

- Ahmad FA, Williams PA (1999a) Effect of salts on the gelatinization and rheological properties of sago starch. *J Agric Food Chem* 47:3359–3366
- Ahmad FA, Williams PA (1999b) Effect of sugars on the thermal and rheological properties of sago starch. *Biopolymers* 50:401–412
- Ahmad FA, Che Man YB, Asbi BA et al (1999a) Differential scanning calorimetry: gelatinisation of sago starch in the presence of sucrose and sodium chloride. *J Sci Food Agric* 79:2001–2009
- Ahmad FA, Williams PA, Doublier J-L et al (1999b) Physico-chemical characterisation of sago starch. *Carbohydr Polym* 38:361–370
- Akazawa T (1965) Biosynthesis of starch. *Kagaku to seibutsu (Chem Biol)* 3:282–291. (in Japanese)
- Arai Y, Kaneko T, Ito T (1981) Rheological and chemical properties of sago starch. *Japan J Trop Agric* 25:41–46. (in Japanese)
- Cai CH, Cai JW, Man JM et al (2014) Allomorph distribution and granule structure of lotus rhizome C-type starch during gelatinization. *Food Chem* 142:408–415
- Hamanishi T, Hatta T, Jong FS et al (1999) Physicochemical properties of starches obtained from various parts of sago palm trunks at different growth stages. *J Appl Glycosci* 46:39–48. (in Japanese)
- Hamanishi T, Hatta T, Jong FS et al (2000) The relative crystallinity, structure and gelatinization properties of sago starch at different growth stages. *J Appl Glycosci* 47:335–341
- Hizukuri S (2003) Molecular structure of starch. In: Fuwa E, Komaki T, Hizukuri S, Kainuma K (eds) *Encyclopedia of starch science*. Asakura Shoten, Tokyo, pp 11–38. (in Japanese)
- Hizukuri S, Nikuni J (1957) X-ray diffractometric studies on starches. Part II. Structure of “C”-crystallite. *J Agric Chem* 31:525–527. (in Japanese)
- Imberty A, Perez S (1988) A revisit to the three-dimensional structure of B-type starch. *Biopolymers* 27:1205–1221
- Imberty A, Buleon A, Tran V et al (1991) Recent advances in knowledge of starch structure. *Starch/Stärke* 43:375–384
- Imberty A, Chanzy H, Perez SA et al (1988) The double-helical nature of the crystalline part of A-starch. *J Mol Biol* 201:365–378
- Ishii Y, Nagashima N, Kawabata A et al (1990) A study of chain length distribution in amylopectins from tropical starches. *Starch Sci (Denpun Kagaku)* 37:251–257. (in Japanese)
- Kainuma K, French D (1971) Nageli amylopectin and its relationship to starch granule structure. I. Preparation and properties of amylopectins from various starch types. *Biopolymers* 10:1673–1680
- Kainuma K, French D (1972) Nageli amylopectin and its relationship to starch granule structure. II. Role of water in crystallization of B-starch. *Biopolymers* 11:2241–2250
- Katsumi N, Okazaki M, Yonebayashi K (2014) New proposal for “crystalline index” of starch. *Sago Palm* 22:25–30
- Kawabata A, Sawayama S, Nagashima N (1984) Some physico-chemical properties of starches from cassava, arrowroot and sago. *J Japan Soc Starch Sci* 31:224–232

- Kobayashi S (1993) Utilizing characteristics of sago palm. *Sago Palm* 1:25–32. (in Japanese)
- Maaruf BA, Ghani YB, Che Man YB et al (1999) A differential scanning calorimetric (DSC) study on the effects of annealing and sugar-emulsifiers on gelatinization of sago starch (*Metroxylon sagu*). *J Food Process Preserv* 23:443–462
- Maaruf AG, Che Man YB, Asbi BA et al (2001) Effect of water content on the gelatinization temperature of sago starch. *Carbohydr Polym* 46:331–337
- Mohamed A, Jamilah B, Abbas KA et al (2008) A review on physicochemical and thermorheological properties of sago starch. *Amer J Agric Biolog Sci* 3:639–646
- Nishiyama S, Okazaki M, Katsumi N et al (2015) Surface charge on sago starch granules. *Sago Palm* 23:77–83
- Nurul IMD, Abdul MDM, Azemi BMN (2001) Effect of temperature and starch concentration on the intrinsic viscosity and critical concentration of sago starch (*Metroxylon sagu*). *Starch/Stärke* 53:90–94
- Okazaki M, Igura M, Kimura SD et al (2008) Development of the structure of sago starch. In: Toyoda Y, Okazaki M, Quevedo M, Bacusmo J (eds) *Sago: its potential in food and industry*. TUAT Press, Tokyo, pp 127–135
- Okazaki M, Katsumi N, Nishiyama S et al (2014) Thermal denaturation properties of non-spiny and spiny sago palm starches under low moisture content. *Sago Palm* 22:31–37
- Okazaki M, Katsumi N, Nishiyama S et al (2016) Polymorph and crystallinity disruption of sago starch during gelatinization. *Sago Palm* 24:20–25
- Polnaya FJ, Haryadi MDW et al (2013) Effects of phosphorylation and cross-linking on the pasting properties and molecular structure of sago starch. *Int Food Res J* 20:1609–1615
- Pukkahuta C, Varavinit S (2007) Structure transformation of sago starch by heat-moisture and osmotic-pressure treatment. *Starch/Stärke* 59:624–631
- Siau CL, Karim AA, Norziah MH et al (2004) Effects of cationization on DSC thermal profiles, pasting and emulsifying properties of sago starch. *J Sci Food Agric* 84:1722–1730
- Sopade PA, Kiaka K (2001) Rheology and microstructure of sago starch from Papua New Guinea. *J Food Eng* 50:47–57
- Srichuwong S, Sunarti TC, Mishima T et al (2005) Starches from different botanical sources I: contribution of amylopectin fine structure to thermal properties and enzyme digestibility. *Carbohydr Polym* 60:529–538
- Takahashi S, Hirao K (1994) Studies on the physical and the chemical properties of sago starch, and its performance when used in the preparation of Japanese sweets. *Bul Fac Home Econ, Kyoritsu Women's Univ* 40:59–64. (in Japanese)
- Takahashi S, Kitahara H, Kainuma K (1981) Properties and cooking quality of starches. Part I, chemical and physical properties of starches from mung bean and sago. *J Japan Soc Starch Sci* 28:151–159. (in Japanese)
- Takeda Y, Takeda C, Suzuki A (1989) Structures and properties of sago starches with low and high viscosities on amylography. *J Food Sci* 54:177–182
- Tester RF, Karkalas J, Qi X (2004) Starch - composition, fine structure and architecture. *J Cereal Sci* 39:151–165
- Uthumporn U, Wahidah N, Karim AA (2014) Physicochemical properties of starch from sago (*Metroxylon sagu*) palm grown in mineral soil at different growth stages. *Global Conf on Polymer Compos Mater (PCM 2014)*:1–11. doi:[10.1088/1757-899X/62/1/012026](https://doi.org/10.1088/1757-899X/62/1/012026)
- Vamadevan V, Bertoft E (2015) Structure-function relationships of starch components. *Starch/Stärke* 67:55–68
- Wu H-CH, Sarko A (1978a) The double-helical molecular structure of crystalline B-amylose. *Carbohydr Res* 61:7–25
- Wu H-CH, Sarko A (1978b) The double-helical molecular structure of crystalline A-amylose. *Carbohydr Res* 61:27–40

- Yaacob B, Mohd CIMA, Kamaruddin H et al (2011) Optimization of reaction conditions for carboxymethylated sago starch. *Iran Polym J* 20:1–14
- Yatsugi T (1986) Problems of sago starch manufacturing. In: Yamada N, Kainuma K (eds) *Proceedings of 3rd international sago symposium*. Sago Palm Research Fund, Tokyo, pp 201–207

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