Thermal Denaturation Properties of Non-Spiny and Spiny Sago Palm Starches under Low Moisture Content

Masanori Okazaki[1,*], Naoya Katsumi[1], Shun Nishiyama[1]
Ladie Anne Palermo[2] and Masato Igura[2]

[1]Ishikawa Prefectural University, 1-308, Suematsu, Nonoichi, Ishikawa 921-8836, Japan

[2]Tokyo University of Agriculture and Technology, 2-24-16, Nakacho, Koganei 184-8588, Japan

*Corresponding author

Abstract: The sago palm (*Metroxylon sagu*) can accumulate 200 kg or more of starch per palm in its trunk. The characteristics of sago starch are different in the growing stages, portions of trunk, and cultivars. The differences between starch grains of non-spiny and spiny sago cultivars have not yet been fully characterized. In this study, an optical and scanning electron microscope, X-ray diffraction (XRD), and ^{13}C cross-polarization/magic angle solid-state nuclear magnetic resonance (^{13}C CP/MAS NMR) were used to analyze the structure of sago starch. The grain size of non-spiny sago starch was slightly smaller than that of the spiny one. The heated non-spiny sago starch samples showed that heating from 40 to 80°C resulted in the combining of 4a and 4b X-ray diffraction patterns, changing the reflecting face of the starch with a distance of 0.52 nm. The difference between non-spiny and spiny sago starches was not remarkable in ^{13}C CP/MAS NMR spectra. The NMR signal peak of C_1 at 102 ppm increased with increasing heat temperature up to 80°C and decreased with increasing heating temperature after 80°C. It is concluded that the crystalline structures of non-spiny and spiny sago starches are affected by temperatures above 60°C based on the X-ray diffraction pattern and the fact that the conformation flexibility of the sago starch structure by heating was limited to the surface portion from the X-ray diffraction patterns and NMR spectra.

Keywords: [13]C cross-polarization/magic angle solid-state nuclear magnetic resonance, heating, non-spiny sago starch, spiny sago starch, X-ray diffraction

Introduction

Non-spiny and spiny sago palms (*Metroxylon sagu*) have been known to grow in the lowlands of Southeast Asia (Celiz, 2000). There is no genetic diversity among them (Barahima et al., 2008; Kondo, 2010). The basic physicochemical characteristics of sago starch, moisture content, crude fat, fiber, crude protein, amylose content, viscosity, molecular weight of amylose and amylopectin, gelatinization temperature, and crystalline structure, have been reported by Ahmad et al. (1999), Kainuma and Hatta (2003), Leong et al. (2007), Okazaki et al. (2008), and Tie et al. (2008).

Hamanishi (2002) found similarities of amylose content, thermal properties, and gelatinization in non-spiny and spiny sago starches and the discrepancy of hardness and adhesion properties measured by a tensipresser (Taketomo Electric Co.). However, little data on the comparison of physicochemical properties of non-spiny and spiny sago starches is available at the present time. Okazaki et al. (2008) performed the characterization of different sago cultivar starches using an X-ray diffraction technique, ^{13}C cross-polarization/magic angle solid-state nuclear magnetic resonance (^{13}C CP/MAS NMR), and differential scanning calorimetry (DSC) and concluded that both non-spiny and spiny sago starches were similar in structure and thermal properties under the saturated moisture condition in an aluminum DSC capsule. Few NMR results on starch were available to expect to structural organization at the molecular level (Gidley and Bociek, 1985; Veregin et al., 1986; Horii et al.,

1987; Gidley and Bociek, 1988; Bogracheva et al., 2001; Tang and Hills, 2003). Still more limited information on the NMR of sago starch was obtainable (Okazaki et al., 2008; Tie et al., 2008).

However, there were no data on the thermal behavior of different sago cultivar starches in a low moisture condition, though the hydrolysis of cassava (*Manihot esculenta*) starch (Atichokudomchai et al., 2004) and Chinese yam (*Dioscorea batatas*) starch (Wang et al., 2009) caused a change in the resonance intensity of amorphous and semi-crystalline fractions.

In this study, the thermal denaturation of sago starch from non-spiny and spiny palms under low moisture content was elucidated by X-ray diffraction and ^{13}C CP/MAS NMR.

Materials and Methods

1. Sago starch samples

Two different sago (non-spiny and spiny) starch samples were used in this study. In Leyte, Philippines, the names of folk varieties were unknown. Sago starches were collected in 2006. The sago starch from the middle portion of sago palms was extracted by macerating the pith in water in a blender. The starch was separated from the pulp by squeezing the pulp in a cotton cloth and then air-dried. The moisture content of the samples from non-spiny and spiny sago palms was 12.1 and 12.2% (w/w), respectively.

2. Optical and electron microscopy observation

Light microscopy was performed to characterize starch granular morphology. Starch samples were mounted on a glass slide. Samples were viewed under normal and polarized light at a magnification of 100 in an optical microscope (Meijitechno MT 9000). Visual observation of starch granular morphology and the presence of characteristic Maltese cross were examined. The scanning electron microscope images were obtained using a Hitachi Miniscope TM-1000.

3. X-ray diffraction pattern of sago starch

Starch samples were mounted on a glass plate for X-

ray diffraction. This was carried out by an X-ray diffractometer (MiniFlex, Rigaku and Max-Labo Automatic Diffraction) equipped with Cu Kα radiation operating at 30 kV, 15 mA or 40kV, 30 mA and scanning (2θ) between 2 and 30° at a rate of 2° per minute and a sampling interval of 0.01°. The changes in starch structures by heating were examined through X-ray diffraction analysis when the temperature reached 40, 50, 60, 70, and 80ºC using a Rigaku RINT-2100 VPC/N X-ray Diffractometer (Rigaku). The heating temperature (40–80°C) condition used was decided, owing to the DSC results on sago starch (Wattanachant et al., 2002), indicating that the gelatinization temperature of sago starch ranged from 60 to 74°C.

4. Solid-state cross-polarization/magic angle spinning ^{13}C NMR spectra of sago starch

The starch samples were dried in a Taitec VA-810 freeze dryer, placed in a 69.0 µL container, and analyzed by a Jeol Detum ECA/ECX solid-state CP/MAS ^{13}C NMR spectrometer operating at a ^{13}C resonance frequency of 500 MHz, spinning rate 10 kHz, point number 2048, using scanning numbers 128 and 256, at room temperature. The starch samples were heated from 60 to 300°C for 24 hours in the probe to investigate the thermal changes in sago starch with temperature, since Oya and Takahashi (1987a, b) baked muffins and breads at 190 and 200°C, respectively, to show the application of sago starch to baked foods.

Results and Discussion

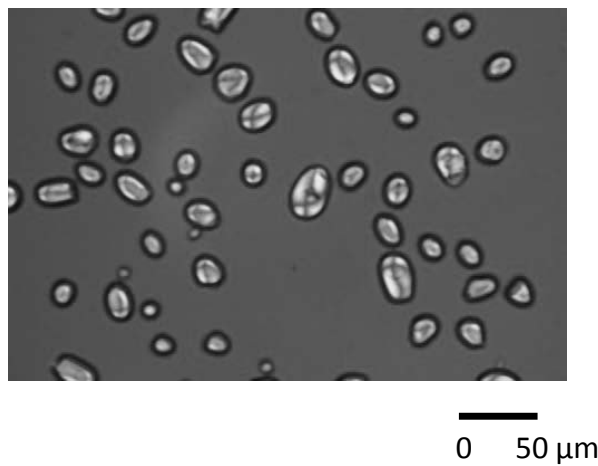**1. Sago starch granules under microscope**

The light microscopic images viewed under normal light and polarized light of the non-spiny and spiny sago starch granules are shown in Figs. 1 and 2. Sago starch grains were oval and temple-bell shaped and 30–35 µm in mean diameter (Ahamad et al., 1999; Takahashi, 2010). There was an increasing tendency in the mean long and short diameters of starch grains extracted from the apical to basal portion of the non-spiny and spiny sago palms. The mean long and short
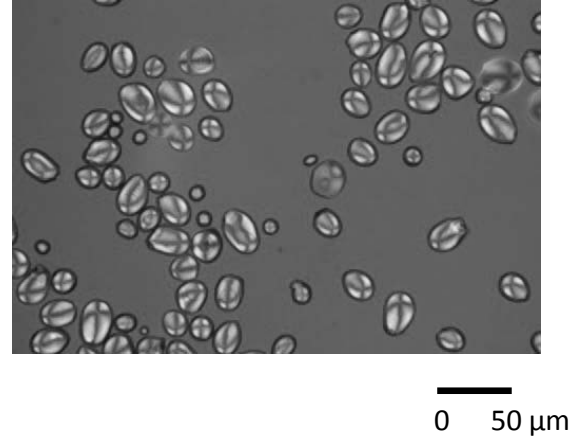
0   50 μm
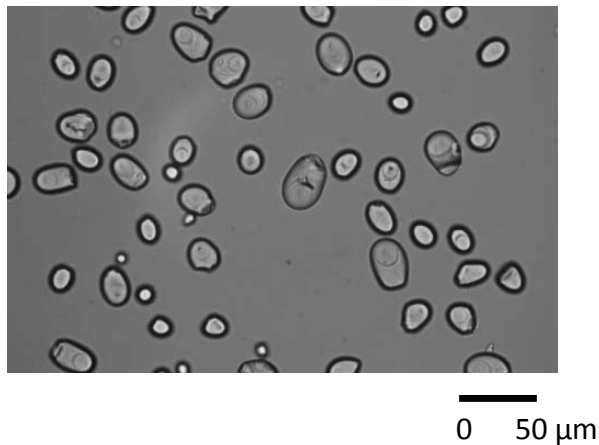
Open nicol
Non-spiny sago starch

0   50 μm
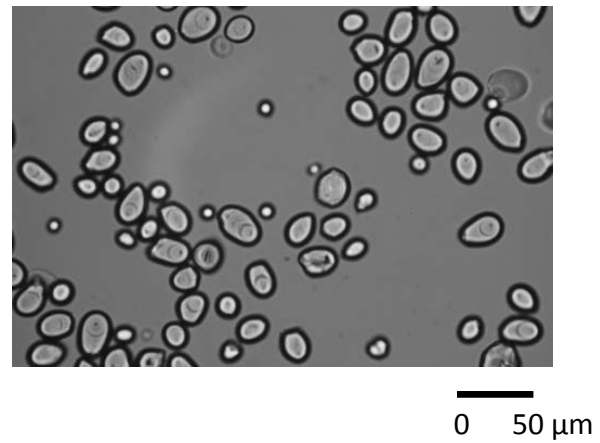
Open nicol
Spiny sago starch

0   50 μm

Crossed nicol
Non-spiny sago starch


0   50 μm

Crossed nicol
Spiny sago starch

Fig. 1. Sago starch granules under optical microscope (x 100)

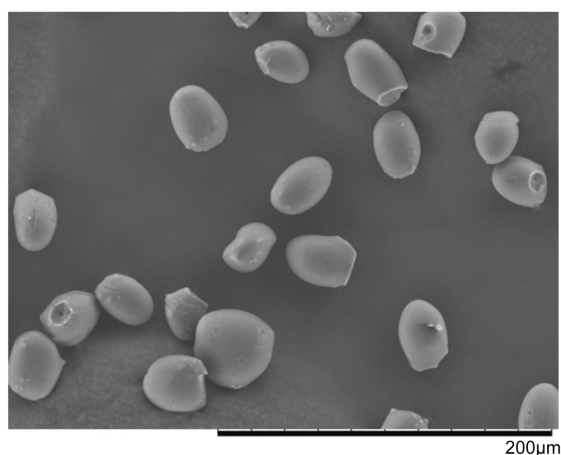**Fig. 2.** Sago starch granules under optical microscope

diameters of the non-spiny sago starch grains were smaller than those of the spiny sago starch grains (Tsujimoto et al., 2008). Under polarized light (using gypsum plate: 530 nm), starch grains showed a Maltese cross (Figs. 1 and 2). The appearance of the Maltese cross was evident in all samples, which connotes a high degree of internal organization. Sago starch grains contained a crystalline structure and exhibited birefringence. Electron microscope images with a magnification of 500 are shown in Fig. 3. The difference between the mean values of long and short grain diameters of non-spiny and spiny sago palms was found under a scanning electron microscope (Fig. 3) rather than a microscope (Figs. 1 and 2).

2. X-ray diffraction of sago starch

The X-ray diffraction patterns of sago starch are shown in Fig. 4. The crystallinity of starch can be often described as three types of X-ray diffraction patterns, A, B, and C, which are mostly observed in native starches. The drier and warmer conditions, similar to those in cereals, made starch A. Cool and wet conditions were thought to be analogous to the in vivo condition of tubers, which made starch B (Imberty et al., 1991). The type C pattern, sago starch, is thought to be the combination of both types A and B (Takahashi, 1986; Hamanishi, 2002; Celiz, 2000; Hamanishi et al., 1999; Okazaki et al., 2008). The type A pattern exhibited a doublet at 17 and 18° and a
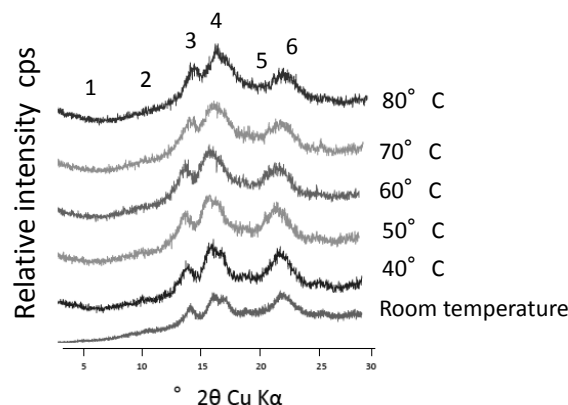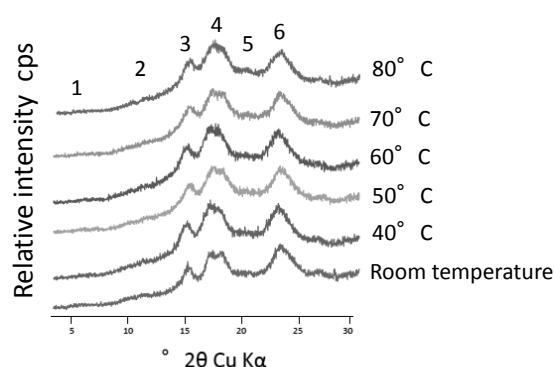
Non-spiny sago starch
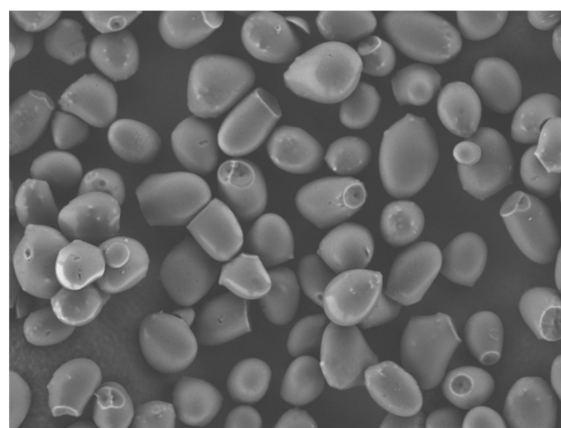
Non-spiny sago starch

Spiny sago starch

Fig. 3. Sago starch granules under electron microscope

Spiny sago starch

Fig. 4. X-ray diffraction pattern of sago starch
The number shows the peak of the crystalline structure.
The temperature ranged from room temperature to 80 °C.

single peak at 23°, which was clear in corn starch. While the type B pattern is characterized by a peak at 5–6°, a single peak at 17°, and a doublet at 22 and 24°, which was clear in potato starch. However, the type C crystalline structure has combination peaks at 5–6, 10, 15, 17, 18, and 22–24°, with characteristics of both types A and B, which means a solid solution. Sago starch exhibited the type B crystalline structure, dominated by a type A structure, which exhibited a distinct doublet at 17 and 18°, a single peak at 23°, and an indefinite peak at 5–6° (Fig. 4). The X-ray diffraction pattern of non-spiny sago palm starch (room temperature) was similar to that of spiny sago palm starch (room temperature).

3. Thermal changes in the X-ray diffraction pattern of sago starch

X-ray diffraction has been used for preliminary investigation of the changes in crystalline structure during processing and conditioning (Smits et al., 1998). In this study, the diffractograms of sago starch revealed a noticeable change in structure by heating (Fig. 4). The non-spiny sago starch had a breaking 4b peak starting at 60 °C to form a singlet peak. The doublet 4th peak was breaking to make the singlet peak, starting from 60 °C, although the appearance of starch grains was not changed by heating (data not shown). The spiny sago starch, however, still had a doublet peak at 17 and 18° with the heating treatment of 70 and 80 °C.

This shows that, at higher temperatures than room temperature, type A and C starches have a tendency to change their 4th peak doublet to type B's singlet. The difference between non-spiny and spiny sago starches with heating treatment in the range of 70–80°C was distinct in X-ray diffraction patterns.

In a model of a solid-state transition from type B to type A, the double helices were preserved while the water was removed, and the helices rearranged to fill the void left by the removal of water at conditions of low humidity and high temperature (Imberty et al., 1991). This is in agreement with the X-ray diffractograms at heated conditions, wherein the 4th doublet peak of types C and A altered to form a 4th singlet peak, which is a characteristic peak of the type B starch. Transition was at the solid state, and crystallinity was kept, as confirmed by the appearance of Maltese cross under polarized light of the heated starch samples.

Okazaki et al. (2008) reported that the non-spiny sago palm starch was classified into three groups, due to gelatinization properties, by DSC. They suggested that the gelatinization behavior of sago starch taken from different portions of the trunk, based on the ratios of type A to type B, which holds the cavity in the hexagonal system structure of the type B starch (Imberty et al., 1991).

4. Changes in the ^{13}C NMR spectra of sago starch by heating

The resonance signal peaks at around 70, 80-78, 85 and 102 ppm of the CP/MAS ^{13}C NMR are shown in Fig. 5. About 102 ppm was assigned to the C_1 site of individual glucose units in starch (Gidley and Bociek, 1985). They also found that type A starch showed three peaks at 98.2, 99.2, and 100.4 ppm, and type B starch exhibited 2 peaks at 99.2 and 100.0 ppm. Tang and Hills (2003) also reported that the peaks at 61.9
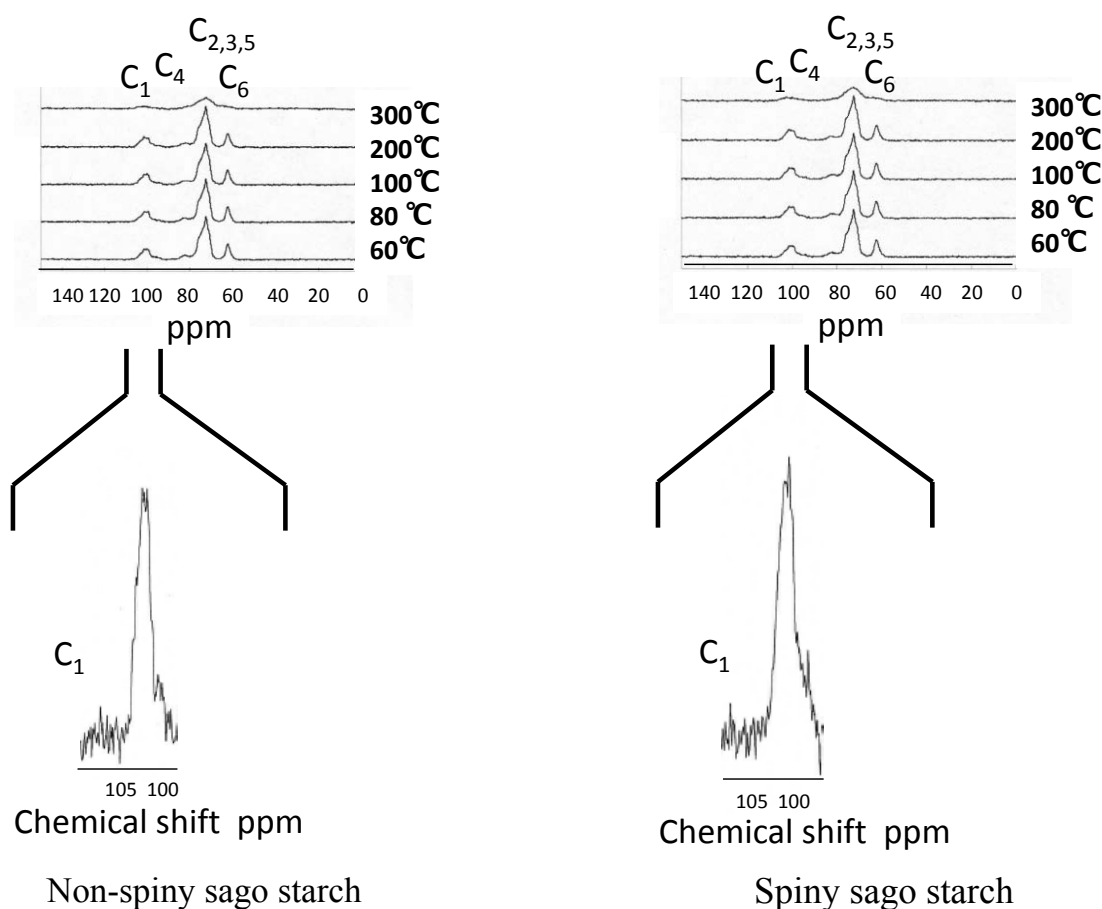

Fig. 5. NMR spectra of sago starch heated at 60–300 °C

ppm, at 98.9, 99.8 and 101 ppm and at 78–84 ppm and 92–104 ppm of type A starch could be assigned to the single peak for C_6, the three-peak pattern for C_1, and the wide range peak pattern for the amorphous starch signals, respectively. The amorphous starch signals were often much broader than the crystalline starch ones due to broader conformational distributions (Gidley and Bociek, 1988). In the case of type B starch, the distinct two-peak pattern of C_1 at 99.5 and 100.3 ppm and a peak for C_6 at 61.6 ppm were observable (Tang and Hills, 2003). For type C starch, the spectral patterns of C_1 at 98–104 ppm and C_6 at 60–62 ppm provided the similarity to those of type A starch (Bogracheva et al., 2001).

The C_1 signal peaks of non-spiny and spiny sago starches increased with increasing the heating temperature up to 80°C and decreased with increasing temperature after 80°C (Fig. 5). Dehydration by heating led to remarkable changes of the molecular mobility of starch.

Conclusion

Sago, non-spiny and spiny, has type C starch, which contains mainly type A starch, with type B as an accessory. The mean long and short diameters of sago starch grains extracted from the non-spiny sago starch were slightly shorter than those of the spiny one. The heated non-spiny and spiny sago starch samples showed that heating at 40–80°C provided the combining of 0.52 nm and 0.49 nm of the reflecting surface based on the results of X-ray diffraction patterns. The difference between non-spiny and spiny sago starches was not remarkable in ^{13}C CP/MAS NMR spectra. It is concluded from the X-ray diffraction patterns and ^{13}C CP/MAS NMR spectra that the crystalline structure of non-spiny and spiny sago starches is affected by temperatures above 60°C and the fact that the conformation flexibility of the sago starch structure was limited to the surface portion.

Acknowledgment

References

Ahmad, F. B., P. A. Williams, J. L. Doublier, S. Durand and A. Buleon 1999 Physico-chemical characterization of sago starch. Carbohydrate Polymers 38:361-370.

Atichokudomchai, N., S. Varavinit and P. Chinachoti 2004 A study of ordered structure in acid-modified tapioca starch by ^{13}C CP/MAS solid-state NMR. Carbohydrate Polymers 58: 383-389

Barahima, A., H. Ehara, M. H. Bintro, H. Sudarsono and Surahman, M. 2008 Genetic diversity of the sago palm (*Meteroxylon sagu*) in Indonesia, based on the genes encoding the biosynthesis of waxy starch. *In*: Sago: Its potential in Food and Industry. (Toyoda, Y., M. Okazaki, M. Quevedo and J. Bacusmo eds.) TUAT Press (Tokyo) 35-44.

Bogracheva, T. Y., Y. L. Wang and C. L. Hedley 2001 The effect of water content on the ordered/disorders structure in starches. Biopolymers 58: 247-259.

Celiz, L. L. 2000 Sago palm (*Metroxylon sagu*) cultivation in the Philippines and its genetic background. Ph D thesis. Tokyo University of Agriculture and Technology, 165 pp.

Gidley, M. J. and S. M. Bociek 1985 Molecular organization in starches: A C CP/MAS NMR study. Journal of the American Chemical Society 107: 7040-7044.

Gidley, M. J. and S. M. Bociek 1988 ^{13}C CP/MAS NMR studies of amylose inclusion complexes, cyclodextrins, and the amorphous phase of starch granules: Relationships between glycoside linkage conformation and solid-state ^{13}C chemical shifts. Journal of the American Chemical Society 110: 3820-3829.

Hamanishi, T. 2002 Studies on physicochemical properties of sago starch obtained at different growth stages of the trunk and cooking characteristics of sago starch. p.1-146, Ph. D thesis,

Kyoritsu Women's University.

Hamanishi, T., T. Hatta, F. S. Jong, S. Takahashi and K. Kainuma 1999 Physicochemical properties of starches from various parts of sago palm trunks at different growth stages. Journal of Applied Glycosicence 46: 39-48.

Horii, F., H. Yamamoto, A. Hirai, R. Kitamura 1987 Structural study of amylose polymerphs by cross-polarization-magic-angle spinning, ^{13}C-N. M. R. spectrometry. Carbohydrate Research 160: 29-40.

Imberty, A., A. Buleon, V. Tran and S. P. Nantes 1991 Recent advances in knowledge of starch structure. Starch 43: 375-384.

Kainuma, K. and T. Hatta 2003 Starch grain and its solid structure. *In*: Encyclopedia of Starch Science. (Fuwa, E., T. Komaki, S. Hizukuri and K. Kainuma eds.) Asakura Shoten (Tokyo) 39-49. (in Japanese)

Kondo, T. 2010 Utilization of sago starch. *In*: Sago Palm. (The Society of Sago Palm Studies ed.) Kyoto University Press (Kyoto) 263-264. (in Japanese)

Leong, Y. H., A. A. Karim and M. H. Norziah 2007 Effect of pullulanase debranching of sago (*Metroxylon sagu*) starch at subgelatinization temperature on the yield of resistant starch. Starch/Särke 59: 21-32.

Okazaki, M., M. Igura, S. D. Kimura, S. B. Lina, S. Matsumiura, T. Nakato, K. Takahashi, M. A. Quevedo and A. B. Loreto 2008 Development of the structure of sago starch. *In*: Sago: Its potential in Food and Industry. (Toyoda, Y., M. Okazaki, M. Quevedo and J. Bacusmo eds.) TUAT Press (Tokyo) 127-135.

Oya, C. and S. Takahashi 1987a Application of sago starch to baked foods (Part 1). Journal of Cookery Science of Japan 20: 362-370.

Oya, C. and S. Takahashi 1987b Application of sago starch to baked foods (Part 2). Degree of gelatinization and texture of sago starch bread. Journal of Cookery Science of Japan 20: 371-377.

Smits, A. L. M., P. H. Kruiskamp, J. J. G. van Soest and J. F. G. Vliegenthart 1998 The influence of various small plasticisers and malto-oligosaccharaides on the retrogradation of (partly) gelatinized starch. Carbohydrate polymers 51: 417-424.

Takahashi, S. 2010 Traditional extraction method of sago starch. *In*: Sago Palm. (Yamamoto, Y. ed.) Kyoto University Press (Kyoto) 237-242. (in Japanese).

Tang, H. R. and B. P. Hills 2003 Use of ^{13}C MAS NMR to study domain structure and dynamics of polysaccharides in the native starch granules. Biomacromolecules 4: 1269-1276.

Tie, A., A. A. Karim and D. M. A. Manan 2008 Physicochemical properties of starch in sago palms (*Meteroxylon sagu*) at different growth stages. Starch/Starke 60: 408-416.

Tsujimoto, M., N. Akiyama, M. Okazaki and M. Nakata 2008 Physicochemical properties and medicinal quality of sago starch. Abstracts of the 17th of The Society of Sago Palm Studies, p. 56-59. (in Japanese)

Veregin R. P., C. A. Fyfe, R. H. Marchessault and M. G. Tayler 1986 Characterization of the crystalline A and B starch polymerphs and investigation of starch crystallization by high-resolution ^{13}C CP/MAS NMR. Macromolecules 19: 1030-1034.

Wang, S., J., J. L. Yu, Q. H. Zhu, J. G. Yu and F. M. Jin 2009 Granular structure and amorph position in C-type Chinese yam starch granule revealed by SEM, ^{13}C CP/MAS MNR and XRD. Food Hydrocolloids 23: 426-433.

Wattanachant, S., S. K. S. Muhammad, D. Mat Hashim and R. A. Rahman 2002 Suitability of sago starch as a base for dual-modification. Songklanakarin Journal of Science and Technology 24: 431-438.