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Difference in the Amylopectin Chain Distribution of Sago (*Metroxylon sagu*) Starch from Papua New Guinea and the Philippines

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Abstract: The amylopectin chain distribution of sago starch collected from Papua New Guinea (PNG) and the Philippines (PLP) was studied using a high-performance ion-exchange chromatograph (HPIC) equipped with a pulsed amperometric detector (PAD). The branch chain lengths with the degree of polymerization (DP) 6–12 (Gr1), DP 13–24 (Gr2), DP 25–36 (Gr3), and DP≧37 (Gr4) designated as short, intermediate1, intermediate2, and long amylopectin chains were 25% (Gr1), 58% (Gr2), 12% (Gr3), and 4% (Gr4) (peak area percentage) for PNG and 25% (Gr1), 60% (Gr2), 12% (Gr3), and 4% (Gr4) for PLP, respectively. Both PNG and PLP sago starches possessed a greater abundance of short amylopectin chains (Gr1) than those of corn and potato starches.
Keywords: amylopectin, chain distribution, Papua New Guinea, Philippines, sago starch

Introduction

Sago (*Metroxylon sagu*) starch has been a staple food in Southeast Asia and the South Pacific area. Japan imported about 20 thousand tons per year from Malaysia and Indonesia (Agriculture & Livestock Industries Cooperation, 2015) for dusting powder. Sago starch powder is easily extracted from the stem of the sago palm grown in a harsh, swampy environment. The traditional extraction methods for sago starch can be classified into two levels: the domestic level and the small-scale processing plant level (Karim et al., 2008). The extracted sago starch exhibits a C-type (Ishii et al., 1990; Wang et al., 1988) diffraction pattern (a mixture of A and B types). Wide-angle X-ray diffraction can be used to determine A-, B-, and C-type crystallinity (Wang et al., 1988). The precise peak positions have been realized by computational peak fitting (Cairns et al., 1997; Katsumi et al., 2015).

Newly accumulated starch in the apical portion of the sago palm revealed a C-type structure that is dominantly affected by an A-type structure. Developed sago starch granules accumulated in the basal portion of the sago palm showed the C type, but the relative percentage of the B-type structure gradually increased, compared to that of the A-type structure (Hamanishi et al., 1999; Okazaki et al., 2008). A starch granule, including that of sago starch, consists of two major components: amylose built from long linear chains and amylopectin containing much shorter linear chains than amylose. These components represent approximately 98–99% of the dry weight (Tester et al., 2004). The amylose (20–30% of total sago starch) is composed of the chains with the apparent glucose units of 600 to 36000, connected through (1→4) α-*D*-glucosidic linkages (Takeda et al., 1989). Meanwhile, the amylopectin (70–80% of total sago starch) shows the branched macromolecule forms with respect to the glucose units of 12000 to 40000, with the interconnection by (1→6) α-*D*-glucosidic linkages (Takeda et al., 1989). The amylopectin, therefore, is on average 10^6 to 10^7 molecular weight, which relate to the structure of starch and viscosities.

Takeda et al. (1989) also revealed that the low-viscosity amylopectin of sago starch was a smaller molecule with a slightly higher amount of long chains than the high-viscosity amylopectin. The amylopectin chain distribution of sago starch determined by Ishii et

al. (1990) revealed four fractions, with the different chain lengths of Fraction I (22.9 ± 0.8%), Intermediate Fraction (3.9 ± 0.4%), Fraction II (17.2 ± 0.4%), and Fraction III (56.0 ± 1.4%). Takahashi and Hirao (1994) reported that Fraction III of sago amylopectin was 52.3%, an intermediate value between that of sweet potato and mung bean amylopectin. Furthermore, Srichuwong et al. (2005) showed that the maximum chain length of amylopectin for the A and C types (including sago) was present at DP 11–12 and for the B type at DP 13. No report on the amylopectin chain distribution of sago starch produced in different areas under similar climate conditions has yet been published.

The objective in this study is to show the amylopectin chain distribution of sago starch collected from Papua New Guinea (PNG) and the Philippines (PLP).

Materials and Methods

1. Sago starch samples

Sago starch samples were collected from Port Moresby (latitude 9° 26' south, longitude 147° 13' east) of Papua New Guinea (PNG) and Baybay, Leyte, of the Philippines (PLP) (Okazaki et al., 2016). The PNG sample was extracted from different portions of a palm and mixed in Port Moresby on June 30, 2001 (hearing investigation). The air-dried sample was sent to Japan and washed with distilled water three times on July 14, 2001. After air-drying, the PNG sample was sieved using a 125 μm screen. The PLP sample was taken from the middle portion of a palm log on September 2, 2011, in Baybay and mixed. The air-dried sample was sent to Japan and washed with distilled water three times on October 5, 2011. After air-drying, the PLP sample was sieved using a 125 μm screen.

The annual mean temperature in Port Moresby and Cebu (latitude 10° 17' north, longitude 123° 54' east) on Mactan Island, 150 km west of Baybay, is 27.3 and 28.0°C, respectively. The annual precipitation is 1012 mm in Port Moresby and 1260 mm in Cebu. The climate conditions of Port Moresby and Baybay are quite similar.

The moisture content of sago starch was 12% for PNG and 13% for PLP. Corn (Kosakai Pharmaceutical Co., Tokyo, Japan) and potato (Miyazawa Pharmaceutical Co., Tokyo, Japan) starch samples were used as references. Isoamylase from *Pseudomonas amyloderamosa* was purchased from Hayashibara Co., Ltd. (Okayama, Japan). Other reagents were of analytical grade and were obtained commercially.

2. X-ray diffraction

X-ray diffraction was performed by an X-ray diffractometer (Mini Flex, Rigaku, Tokyo, Japan) operating at 30 kV and 15 mA. Copper Kα radiation was utilized with a nickel filter. The operation angle was 3 to 45° for 2θ. The scanning speed was recorded at 2° min^{-1}.

3. Analysis of branch chain distribution of sago amylopectin

Ten milligrams of sago starch was used for hydrolysis (debranching) of α-1,6 bonding by isoamylase to get α-glucans at 37°C for 24 h. The starch hydrolysates were analyzed by a high-performance ion-exchange chromatograph (HPIC) using a CarboPac PA 100 column (4 x 250 mm, Dionex, Sunnyvale, CA, USA) equipped with a pulsed amperometric detector (PAD) (ICS-3000, Dionex). The flow rate was 1.0 mL min^{-1}, and the injection volume was 25 μL. The eluent phase consisted of solutions A (0.125 mol L^{-1} NaOH) and B (1.0 mol L^{-1} CH_3COONa) using the following gradient: 0–9 min, 7.5% eluent B; 9–18 min, 7.5–18.0% B; 18–40 min, 18.0–22.5% B; 40–60 min, 22.5–50% B. The column was equilibrated with 7.5% eluent B for 30 min between runs.

Results

1. X-ray diffraction pattern of sago starch

Sago starch samples show diffraction peaks at 5.6, 17, 18, and 23° (2θ) for Cu Kα, which corresponded

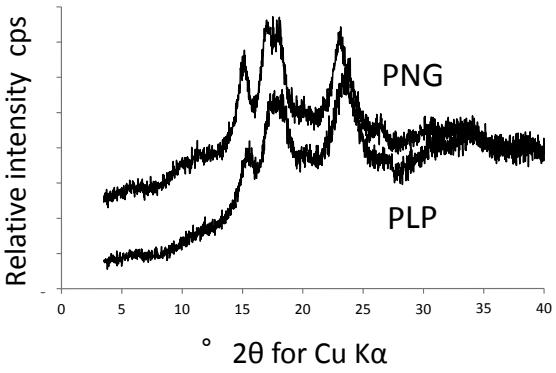

Fig. 1. X-ray diffraction pattern of the PNG and PLP sago starch samples

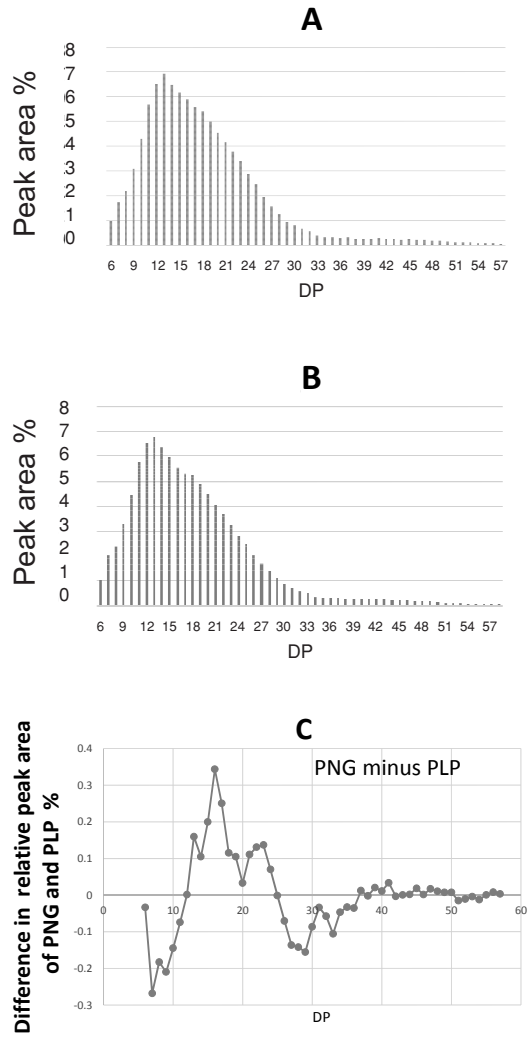

Fig. 2. Branch chain distribution of amylopectin in sago starch and differential values between PNG and PLP
A: PNG (Papua New Guinea)
B: PLP (Philippines)
C: Difference in relative peak areas of PNG (A) and PLP (B)

to 1.6, 0.52, 0.49, and 0.39 nm, respectively, for the reflection surface distance (Fig. 1). The separation of the peaks at 17 and 18° of two samples was not clear. For the PLP sago starch sample, the diffraction peak at 5.6° was smaller than that of the PNG sago starch sample. Two sago starch samples were identified to be of the C type (almost A-type accompanied by B-type as accessary).

2. Amylopectin branch chain distribution of sago starch

The peak area percentages of the amylopectin branch chain distribution in PNG and PLP sago starches are shown in Fig. 2A and B. The chains of up to DP 57 were detected with the peak separation. These amylopectin branch chain distributions had the peak at DP 13 and the small shoulder at DP 17–19 for both the PNG and PLP sago starches. The sago amylopectin branch chain was characterized by the large amount of short chains with a DP up to 12. The PNG sago amylopectin branch chain length resembled the PLP one. The difference in the amylopectin chain distribution of two sago starches was observed in the peak area percentage (Fig. 2C); DP 6–12, DP 13–24, DP 25–36, and DP $\geqq$ 37 designated as short, intermediate1, intermediate2, and long amylopectin chains were 25, 58, 12, and 4% of the peak area percentage for PNG and 25, 60, 12, and 4% for PLP, respectively (Table 1). These results suggested the similarity of the amylopectin chain length distribution for PNG and PLP. We summarize the percentages of the amylopectin branch chains of starches in Table 1. The DP $\leqq$ 12 branch chain percentages for PNG and PLP were relatively larger than those of corn and potato starch. On the other hand, the DP 13–36 branch chain percentages for PNG and PLP were smaller than those of corn and potato starch.

Discussion

The amylopectin molecular weight of sago starch was approximately 10^6 (Potter and Hassid, 1948; Takeda et al., 1989). Several papers on sago

Table 1. Relative area percentages of the amylopectin branch chains of starch

	Gr1 DP 6-12	Gr2 DP 13-24	Gr3 DP 25-36	Gr4 DP ≧37	Gr1/Gr2	Gr1/(Gr2+Gr3)	Gr1/(Gr2+Gr3+Gr4)
	Relative area %						
PNG	25	58	12	4	0.43	0.36	0.34
PLP	25	60	12	4	0.42	0.35	0.33
Corn	21	60	15	4	0.35	0.28	0.27
Potato	19	61	15	5	0.31	0.25	0.23

DP: Degree of polymerization
Gr1: Short branch chain length (DP 6–12)
Gr2: Intermediate1 branch chain length (DP 13–24)
Gr3: Intermediate2 branch chain length (DP 25–36)
Gr4: Long branch chain length (DP≧37)

Table 2. Reports on the amylopectin branch chain of sago starch

	Amylopectin branch chain of sago starch	Remarks
Potter and Hassid (1948)	Molecular weight of amylopectin 1 x 10^6	Calculated molecular weight of deacetylated product Osmotic pressure measurement
Zainon et al. (2010)	Molecular weight of debranched sago starch $1.87 \pm 0.4 \times 10^5$ g/mol	Gel permeation chromatography multi-angle laser light scattering
Takahashi et al. (1981)	Peaks at DP 14-15 and DP 45	Debranched with isoamylase, Gel-filtration
Takeda et al. (1989)	Weight average chain length Low-viscosity A B1 B2 B3 B4 11.4 18.7 43.2 75.5 145 High-viscosity A B1 B2 B3 B4 11.3 18.3 42.8 61.0 133	Debranched with isoamylase, Gel-filtration
Ishii et al. (1990)	Average chain length Fr. I Intermediate Fr. Fr. II Fr. III 22.9 3.9 17.2 56.0 (%)	Debranched with isoamylase, Gel-filtration
Hizukuri (2003)	Average chain length Total A B1 B2 B3 B4 Chain length 11 18 43 61 133 Weight (%) 100 29.0 46.2 20.3 3.5 0.5 Molar (%) 100 45.7 44.8 8.4 1.0 0.1	
Srichuwong et al. (2005)	Avarage chain length DP 6-8 DP 9-12 DP 13-24 DP 25-30 9.0 28. 1 56.2 6.7 (%)	Debranched with isoamylase labeled with 8-amino-1,3,6-pyrenetrisulfonic acid, Fluorophore-assisted capillary electrophoresis
This study	Relative area percentage Gr1 Gr2 Gr3 Gr4 DP 6-12 DP 13-24 DP 25-36 DP≧37 PNG 25 58 12 4 (%) PLP 25 60 12 4 (%)	Debranched with isoamylase, High-performance ion-exchange chromatograph equipped with a pulsed amperometric detector

amylopectin branch chain lengths have been published (Table 2). There are the differences in sago amylopectin chain length distribution among the studies.

Takahashi et al. (1981) reported the peaks at DP 45 and DP 14 to 15 of the debranching sago amylopectin performed with the gel filtration of Sephadex G-50 (1.9 x 100 cm) after the hydrolysis using isoamylase from *Pseudomonas amyloderamosa*. The amylopectin branch chains of sago starch with different viscosities were investigated by Takeda et al. (1989), who indicated that the low-viscosity amylopectin (average chain length: 22) was a smaller molecule (A chain) with slightly higher amount of long chains than the high-viscosity amylopectin.

Ishii et al. (1990) reported that using debranching with the isoamylase amylopectin of sago starch provided 56.0 ± 1.4% for the peak chain length of 13 to 15 (Fr. III). However, Hizukuri (2003) revealed that amylopectin of sago starch provided 29.0% (weight) at a chain length of 11 and 46.2% (weight) at a chain length of 18. A recent study by Srichuwong et al. (2005) suggested that sago amylopectin indicated 37.1% for DP 6–12, 56.2% for DP 13–24, and 6.7% for DP≧25 and provided that the amylopectin of sago starch had a higher percentage of DP < 13 than that of the A-type (corn) and B-type (potato) starch. The percentage of the shorter chain length (DP 6–12)

obtained in our study was lower than that in Srichuwong et al. (2005). The reason for the large difference in the shorter chain length percentage of sago amylopectin between this study and that of Srichuwong et al. (2005) is not yet clear.

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