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**Note**

# Amylose and Amylopectin Unit-chain Distributions of Ginseng, Sanchi Ginseng, Pinellia Tuber, Alisma Rhizome and Coix Seed Starches

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# Amylose and Amylopectin Unit-chain Distributions of Ginseng, Sanchi Ginseng, Pinellia Tuber, Alisma Rhizome and Coix Seed Starches

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**Abstract: Amylose and amylopectin unit-chain distributions of starches from the roots of *Panax ginseng* C.A. Meyer (PG) and *Panax notoginseng* (Burk.) F.H. Chen (PN), tuber of *Pinellia ternata* (Thunb.) Breitenbach (PT), rhizome of *Alisma orientale* Juzepczuk (AO) and seed of *Coix lacryma-jobi* Linné var. *ma-yuen* Stapf (CL) were investigated by means of a gel filtration method. The amylose contents of the PG, PN, PT, AO and CL starches were 15.4–28.2, 25.9–35.7, 20.1–34.9, 26.6–35.2 and 17.2–26.4%, respectively. The ratios of subfractions (Fr. III/Fr. II) of amylopectin in PG, PN, PT and AO starches were 0.82±0.19, 0.93±0.13, 1.22±0.25 and 1.23±0.05, respectively. Those of CL starches were 0.97 and 3.49. The weight-average chain-lengths ($\overline{CL}_w$) of amylopectin in PG, PN, PT, AO and CL starches were 33–47, 28–35, 22–32, 25–28 and 23–38, respectively. It was confirmed that the ratio of subfractions (Fr. III/Fr. II) was negatively correlated with $\overline{CL}_w$. The weight-average degrees of polymerization ($DP_w$) of amylose from PG, PN, PT and CL starches were 2720–3590, 3240–3730, 2390–2710 and 3090–3880, respectively.**

**Key words: amylose, amylopectin, chain-length, crude drug starch**

The physicochemical properties of starches from crude drugs have been examined to make profitable the use of starch after extraction of the active ingredients.[1,2] The crude drugs used were the roots of *Panax ginseng* C.A. Meyer (PG) and *Panax notoginseng* (Burk.) F.H. Chen (PN), tubers of *Pinellia ternata* (Thunb.) Breitenbach (PT), rhizomes of *Alisma orientale* Juzepczuk (AO) and seeds of *Coix lacryma-jobi* Linné var. *ma-yuen* Stapf (CL).[1,2] Properties such as gelatinization and digestibility of starch granules are considered to be related to the molecular structure of amylose and amylopectin. The molecular structures of amylose and amylopectin from PG, PN, PT, AO and CL starches have not been reported. This paper reveals amylose and amylopectin unit-chain distributions of the crude drug starches.

## MATERIALS AND METHODS

***Materials.*** The starches of PG, PN, PT, AO and CL were the same as those used previously.[1,2] The country of cultivation and year of harvest for the crude drugs are described in Table 1. The starch was deproteinized by the method of Suzuki *et al.*[3] and defatted by the method of Schoch.[4] The starch was screened with 400 mesh or less and kept in a tight container. Isoamylase from *Pseudomonas amyloderamosa* (crystal, ultracentrifugally and electrophoretically pure, approx. 59,000 U/mg protein) was purchased from Hayashibara Biochemical Laboratories Inc., Okayama, Japan. The enzyme was used without further purification. Shodex standard samples for gel permeation chromatography, plullans (P-800, Mw: 788,000; P-400, 404,000; P-200, 212,000; P-100, 112,000; P-50, 47,300, P-20, 22,800; P-10, 11,800; P-5, 5,900) were purchased from Shoukou Tsuushou Co., Inc., Tokyo, Japan. The columns Toyopearl HW-50S and HW-65S were purchased from Tosoh Co., Inc., Tokyo, Japan. All other reagents and solvents were of the highest grade commercially available.

***Measurements of amylose content and amylose and amylopectin unit-chain distributions by gel permeation chromatography.***[5,6] Isoamylase solution (215 IU/mL) was prepared by adding isoamylase (20 μL) to 50 mM citrate buffer (pH 3.5, 5.49 mL). After a mixture of starch (30 mg) and distilled water (0.5 mL) was heated for 10 min in a boiling water bath, the paste was fully gelatinized with 5 M sodium hydroxide (0.5 mL). After adding 50 mM citrate buffer (2 mL) and 6 M hydrochloric acid (0.4 mL) to the gelatinized solution and then adjusting the pH to 3.5 with 1 M hydrochloric acid, the mixture with isoamylase solution (30 μL) was incubated at 40°C for 20 h, then cooled to room temperature, lyophilized and kept at −20°C.

After the column of Toyopearl HW-50S or HW-65S was washed with distilled water and 20 mM sodium carbonate buffer (pH 9.5) and then degassed, it was charged

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Abbreviations: PG, root of *Panax ginseng* C.A. Meyer; PN, root of *Pnax notoginseng* (Burk.) F.H. Chen; PT, tuber of *Pinellia ternata* (Thunb.) Breitenbach; AO, rhizome of *Alisma orientale* Juzepczuk; CL, seed of *Coix lacryma-jobi* Linné var. *ma-yuen* Stapf.

**Table 1.** Amylose content and amylopectin unit-chain distribution of ginseng, sanchi ginseng, pinellia tuber, alisma rhizome and coix seed starches on Toyopearl HW-50 S.

| Crude drug starch sample | Amylose | Amylopectin | | Ratio of Fr. III/Fr. II |
|---|---|---|---|---|
| | Fr. I (%) | Fr. II (%) | Fr. III (%) | |
| PG-1 | 28.2 | 39.4 | 32.3 | 0.82 |
| PG-2 | 19.5 | 46.4 | 34.1 | 0.73 |
| PG-3 | 15.4 | 50.4 | 34.3 | 0.68 |
| PG-4 | 20.8 | 46.1 | 33.1 | 0.72 |
| PG-5 | 22.2 | 36.4 | 41.4 | 1.14 |
| PN-1 | 28.4 | 35.2 | 36.4 | 1.03 |
| PN-3 | 35.7 | 34.4 | 30.0 | 0.87 |
| PN-4 | 25.9 | 36.5 | 37.8 | 1.04 |
| PN-6 | 32.9 | 37.6 | 29.4 | 0.78 |
| PT-1 | 20.6 | 34.9 | 42.7 | 1.22 |
| PT-2 | 34.9 | 34.8 | 30.3 | 0.87 |
| PT-3 | 25.1 | 31.6 | 43.3 | 1.37 |
| PT-4 | 20.1 | 32.8 | 47.1 | 1.44 |
| AO-1 | 32.0 | 31.2 | 36.8 | 1.18 |
| AO-2 | 26.6 | 32.0 | 41.4 | 1.29 |
| AO-3 | 31.4 | 31.1 | 37.5 | 1.21 |
| AO-4 | 35.2 | 28.6 | 36.3 | 1.27 |
| AO-5 | 26.7 | 33.2 | 40.1 | 1.21 |
| CL-2 | 17.2 | 43.5 | 39.3 | 0.97 |
| CL-3 | 26.4 | 16.4 | 57.2 | 3.49 |

PG, root of *Panax ginseng* C.A. Meyer; PG-1, China Jilin, red ginseng, two-year-old root harvested in 1999; PG-2, China Jilin, two-year-old root harvested in 1999; PG-3, China Heilongjiang, two-year-old root harvested in 1999; PG-4, Korea, four-year-old root harvested in 1997; PG-5, Korea, six-year-old root harvested in 1997; PN, root of *Panax notoginseng* (Burk.) F.H. Chen; China Yunnan, Wenshan, three-year-old root; PN-1, head number (HN)= 20, harvested in 1999; PN-3, HN=80, harvested in 1998; PN-4, HN=120, harvested in 1998; PN-6, HN=200, harvested in 1999. PT, tuber of *Pinellia ternata* (Thunb.) Breitenbach; PT-1, number of spherical tubers per unit dried weight, 1400–1600 grains/kg, Sichuan, China, harvested in Oct. 1998; PT-2, 2700–3000 grains/kg, Guizhou, China, harvested in Oct. 1999; PT-3, 3000 or more grains/kg, Guizhou, China, harvested in Feb. 1999; PT-4, 700–800 grains/kg, Sichuan, China, harvested in Oct. 1998; AO, rhizome of *Alisma orientale* Juzepczuk AO-1, Sichuan, China, harvested in Dec. 2000; AO-2, Sichuan, China, harvested in Apr. in 1998; AO-3, Sichuan, China, harvested in Aug. 1998; AO-4, Sichuan, China, harvested in Mar. 2001; AO-5, Sichuan, China, harvested in Feb. 1998; CL, seed of *Coix lacryma-jobi* Linné var. *ma-yuen* Stapf; CL-2, Thailand, harvested in Apr. 1998; CL-3, Thailand, coated with astringent skin, harvested in Apr. 1998. Fr. I, amylose content; Fr. II, amount of longer chains of amylopectin; Fr. III, amount of shorter chains of amylopectin.

in a glass column (2.2×90 cm) and successively equilibrated by elution with 20 mM sodium carbonate buffer at a flow rate of 12 mL/h for 2 days.

The suspension of starch debranched by isoamylase in distilled water (0.5 mL) was fully dissolved with 5 M sodium hydroxide (0.5 mL) and diluted to 4.0 mL with distilled water. The sample solution (3 mL) was added to the column described above, which was eluted with 20 mM sodium carbonate buffer and fractions of 3 mL were collected using an Advantec SF-2120 (Advantec Toyo Kaisha, Ltd., Tokyo, Japan).

The total amount of carbohydrate in each fraction was determined using a calibration curve for maltose by the phenol-sulfuric acid method.[7] The total carbohydrate content was obtained by subtracting absorbance of sodium carbonate buffer from that of the diluted fraction solution. The percent recovery of passage through the Toyopearl HW-50S column of starches debranched by isoamylase was measured. The average recovery (89.8–94.5%) for 20 starch samples was 91.0±1.1%. Although the real fraction solution was mixed at various ratios with both part of the starch sample solution and sodium carbonate buffer, total carbohydrate was measured using sodium carbonate buffer as a blank. Thus, it seems that a result close to 100% recovery has really been obtained.

The average unit-chain distributions of amylopectin and amylose were obtained by the method of Hizukuri.[6] Standard plullan (1.0 mg) was added to a bottle containing 20 mM sodium carbonate buffer (4 mL, pH 9.5) and kept refrigerated for 24 h. After the agglomerate particles had swelled to the fullest extent, they were stirred gently until they had dissolved homogeneously with distilled water. The standard solution of plullan was filtered with a 0.45 μm mesh filter. The universal calibration curves for calculating the average molecular weight of amylopectin and amylose were obtained using plullans (P-50, P-20, P-10 and P-5) on Toyopearl HW-50S and plullans (P-800, P-400, P-200 and P-100) on Toyopearl HW-65S, respectively.

## RESULTS AND DISCUSSION

### *Amylose content.*

Amylose contents (corresponding to the total amount of Fr. I[5]) of five crude drug starches on gel filtration are shown in Table 1. The amylose content of PG starches was 15.4–28.2% (AV±SD=21.2±4.7%). It was reported that the amylose content of Korean ginseng starch decreased with the age of the root, from 70.5% (one-year-old) to 53.6% (five-year-old),[8] and that the starch of Korean ginseng was composed only of amylose.[9] The amylose content of PG-4 and PG-5 starches from Korean ginseng was 20.8–22.2%, being fairly low compared with values reported in the literature.[8,9] The amylose content of PG starches approximated that (24.6%[10] by gel filtration and 23%[11] by amperometric titration) of potato starch. The amylose content, 25.9–35.7% (30.7±4.4%) of PN starches was larger than that in PG starches. The amylose content of PT starches was 20.1–34.9% (25.2±6.9%). The amylose content of AO starches was 26.6–35.2% (30.4 ±3.7%), approximating that (26%)[12] of AO starch cultivated in Japan. The amylose content of CL starches, seed starch, was 17.2-26.4%, being similar to that (Fr. I, 14.2–16.7%)[13] of endosperm starches of a Japonica type "hybrid rice", to that (Fr. I, 6.4–27.2%)[14] of rice starch, and to that (26.8%)[15] of maize starch. No relationship[16] between the amylose content of starches in Table 1 and the average diameter of their starches[1,2] was observed.

### *Amylopectin unit-chain distribution.*

The amounts of longer chains (Fr. II) and shorter chains (Fr. III) of amylopectin and the relative amount of these subfractions (Fr. III/Fr. II) are also listed in Table 1. The ratios of subfractions (Fr. III/Fr. II), which determined tentatively the chain-length distribution of amylopectin,[17] were 0.68–1.37 except that (3.49) of CL-3

**Table 2.** Average chain-length of amylopectin of ginseng, sanchi ginseng, pinellia tuber, alisma rhizome and coix seed starches.

| Crude drug starch sample | $\overline{CL}_w$ |
|---|---|
| PG-1 | 34 |
| PG-2 | 37 |
| PG-3 | 47 |
| PG-4 | 39 |
| PG-5 | 33 |
| PN-1 | 28 |
| PN-3 | 35 |
| PN-4 | 29 |
| PN-6 | 31 |
| PT-1 | 23 |
| PT-2 | 22 |
| PT-3 | 31 |
| PT-4 | 32 |
| AO-1 | 25 |
| AO-2 | 28 |
| AO-3 | 27 |
| AO-4 | 26 |
| AO-5 | 25 |
| CL-2 | 38 |
| CL-3 | 23 |

For abbreviations see Table 1. $\overline{CL}_w$, weight-average chain-length.

starch. The ratio of subfractions has been reported to be 2.44–2.73[13] and 2.7–3.2[14] in rice starches, and 1.3[5] and 1.7[10] in potato starches. The subfraction ratios of PG, PN, PT and AO starches were 0.82±0.19, 0.93±0.13, 1.22 ±0.25 and 1.23±0.05, respectively. The results indicate that the amounts of shorter chains of amylopectin were approximately equal to or less than those of longer chains in PG and PN starches and a little greater than those of longer chains in PT and AO starches. Extensive scatterings of subfraction ratios were observed between CL-2 and CL-3 starches. The scatterings of ratios (0.9–6.9)[15] of subfractions of starches from maize and related plants starches had been reported.

The weight-average chain-lengths ($\overline{CL}_w$) of amylopectin are shown in Table 2. The $\overline{CL}_w$ of PG and PN starches were 33–47 (AV±SD=38±6) and 28–35 (31±3), respectively, slightly larger than that (35)[18] of potato starch. The $\overline{CL}_w$ of PT and AO starches were 22–32 (27±5) and 25–28 (26±1), respectively. It was reported that the $\overline{CL}_w$ of amylopectins of A-, B- and C-type starches were in the range 23–29 (average, 26), 30–44 (36) and 26–29 (28), respectively,[17] and that A-type starches have shorter chains than B-type starches.[17] PG starch was classified as $C_B$-type[1] being very close to B-type and PN, PT, AO and CL starches were all classified as $C_A$-type.[1,2] Since the ratios of B-type from X-ray diffractograms[1,2] ranked in the order PG>PN>PT÷AO>CL, our results agreed with the relation between $\overline{CL}_w$ and crystalline structure reported by Hizukuri.[17]

The relationship between average chain-length of amylopectin and the relative amount of subfractions Fr. III/Fr. II is shown in Fig. 1. The correlation coefficient was −0.587 ($p<0.01$). The result indicates that amylopectins with large average chain-lengths such as PG starches have

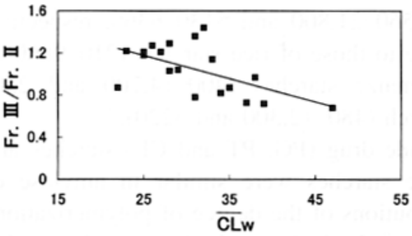


**Fig. 1.** Relation between average chain-length of amylopectin and relative amount of subfractions Fr. III/ Fr. II.

Fr. II, amount of longer chains of amylopectin; Fr. III, amount of shorter chains of amylopectin; $\overline{CL}_w$, weight-average chain-length of amylopectin; correlation coefficient, $r=-0.587$ ($n=19$, $p<0.01$). The plot of CL-3 was eliminated because of its remarkably large ratio of subfractions in Table 1.

**Table 3.** Amylose unit-chain distribution of ginseng, sanchi ginseng, pinellia tuber and coix seed starches on Toyopearl HW-65S.

| Crude drug starch sample | Distribution of degree of polymerization | $\overline{DP}_w$ |
|---|---|---|
| PG-1 | 490–7170 | 2830 |
| PG-2 | 590–9910 | 2720 |
| PG-3 | 650–8370 | 3590 |
| PG-5 | 870–9910 | 3540 |
| PN-1 | 270–7170 | 3240 |
| PN-4 | 500–7170 | 3580 |
| PN-6 | 400–7170 | 3730 |
| PT-1 | 950–6660 | 2670 |
| PT-2 | 1440–8370 | 2390 |
| PT-3 | 730–6210 | 2700 |
| PT-4 | 340–4300 | 2710 |
| CL-2 | 3090 | 3090 |
| CL-3 | 3880 | 3880 |

For abbreviations see Table 1. $\overline{DP}_w$: weight-average degree of polymerization. Data of AO starches could not be obtained because of their deficiencies.

smaller ratios of Fr. III/Fr. II, that is, smaller proportions of the shorter chain fraction, Fr. III, as suggested by Hizukuri.[17]

The smaller the ratios of Fr. III/Fr. II, the larger the enthalpy of crude drug starches[1,2] was, as reported by Noda, *et al.*[19] The negative correlation between the ratio of Fr. III/Fr. II and digestibility of crude drug starches by α-amylase[1,2] was observed ($r=0.986$, $p<0.01$). It was, therefore, confirmed that gelatinization and digestibility properties of starch are closely related to the molecular structure of amylopectin.

***Amylose unit-chain distribution.***

Amylose unit-chain distributions of crude drug starches on gel filtration are shown in Table 3. The distributions of the degree of polymerization of amyloses from PG, PN, PT and CL starches were 490–9910, 270–7170, 340–8370 and 3090–3880, respectively. The weight-average degrees of polymerization ($\overline{DP}_w$) of amyloses from PG, PN, PT and CL starches were 2720–3590, 3240–3730, 2390–2710 and 3090–3880, respectively. These values were close to those of amyloses from lily (2300), tapioca (2660) and sweet potato (3280) starches.[20] The distributions of the degree of polymerization and $\overline{DP}_w$ of amyloses from the crude drug starches were smaller than those of potato starches (560–21,800 and 5130–6360, respectively)[21] and were close to those of rice starches (210–9860 and 3090–3230),[21] maize starches (400–14,700 and 2500),[21] and kudzu starch (480–12,300 and 3220).[18]

The crude drug (PG, PT and CL) starches and the rice and maize starches were similar in amylose content[13-15] and distributions of the degree of polymerization and $\overline{\mathrm{DP}}_w$ of amylose.[21] Both the crude drug starches and the potato starch were similar in the ratio of subfractions (Fr. III/Fr. II) of amylopectin[5,10] but they differed in enthalpy.[1,2,22] The crude drug starches are expected to be available for starch gelatinized at low energy as compared with potato, rice and maize starches.[22]

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## ニンジン，サンシチニンジン，ハンゲ，タクシャ，ヨクイニン澱粉のアミロースとアミロペクチンの単位鎖長分布


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ニンジン *Panax ginseng* C.A. Meyer (PT) とサンシチニンジン *Panax notoginseng* (Burk.) F.H. Chen (PN) の根，ハンゲ *Pinellia ternata* (Thunb.) Breitenbach (PT) の根茎，タクシャ *Alisma orientale* Juzepczuk (AO) の根茎，ヨクイニン *Coix lacryma-jobi* Linné var. *ma-yuen* Stapf (CL) の種子から調製された澱粉のアミロースとアミロペクチンの単位鎖長分布をゲルろ過法により調べた．澱粉のアミロース含量は，それぞれ PG，15.4–28.2%；PN，25.9–35.7%；PT，20.1–34.9%；AO，26.6–35.2%；CL，17.2–26.4% であった (Table 1)．澱粉のアミロペクチンの短鎖区分/長鎖区分 (Fr. III/Fr. II) の比は，それぞれ PG，0.82±0.19；PN，0.93±0.13；PT，1.22±0.25；AO，1.23±0.05 であり，CL は 0.97 と 3.49 であった (Table 1)．アミロペクチンの重量平均単位鎖長は，それぞれ PG，34–47；PN，28–35；PT，22–32；AO，25–28；CL，23–38 であった (Table 2)．短鎖区分/長鎖区分 (Fr. III/Fr. II) の比と重量平均単位鎖長 ($\overline{\mathrm{CL}}_w$) の間には負の相関性が認められた (Fig. 1)．アミロースの重量平均重合度は，それぞれ PG，2720–3590；PN，3240–3730；PT，2390–2710；CL，3090–3880 であった (Table 3)．