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A comparative study of edible canna (*Canna edulis*) starch from different cultivars. Part II. Molecular structure of amylose and amylopectin

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Abstract

Molecular structures of starches isolated from Japanese-green, Thai-green and Thai-purple cultivars of edible canna (*Canna edulis* Ker) were investigated. The absolute amylose content ranged from 19 to 25%. Degrees of polymerization (DPn) values of amylose determined by fluorescence-labeling method were 1590 for Thai-purple, 1620 for Japanese-green and 1650 for Thai-green cultivars. Mole% of branched fraction of amyloses from edible canna starches examined by a HPLC system after β-amylolysis of labeled amyloses was 13–16%. Branch chain-length distributions of amylopectin analyzed by HPSEC after debranching with isoamylase, followed by fluorescence-labeling of unit chain, showed bimodal distribution with the DPn range of 25–28. The amylopectin of edible canna starches contained high amounts of organic phosphorus (391–420 ppm). The distribution profile of phosphorylated chains, separated from non-phosphorylated chains by DEAE-Sephadex A-50 chromatography, indicated that the phosphate groups were located mostly in long B-chains of amylopectin molecules.

Keywords: Edible canna; Cultivar; Amylose; Amylopectin

1. Introduction

Starch is the major carbohydrate reserved in plants and an important part of our nutrition. Starch consists of two polysaccharides, amylose and amylopectin. Amylose is essentially a linear molecule containing glucose unit linked by α-1,4 linkages with a few branches (Hizukuri, Takeda, Yasuda, & Suzuki, 1981). Amylopectin has large molecular weight and highly branched structure constructed from hundreds of short α-1,4 glucan chains, which are interlinked by α-1,6 linkages. The botanical source of starch is of great importance for the characteristics and ratio of amylose and amylopectin. In addition, variations in the fine structure of both polysaccharides, including molecular size, average degree of polymerization, average-number of chains, average chain length (CL), and the chain length distribution profile can lead to a difference in physicochemical properties of starch granules.

Jane and Chen (1992) studied on reconstituted starches made from amyloses and amylopectins from different botanical sources of starch. The studies showed that amylopectin branch chain length strongly affected paste properties, i.e. viscosity, gel strength and light transmittance. The long branch chain amylopectin and intermediate size amylose contributed the greatest synergistic effect on viscosity. Suzuki, Hizukuri, and Takeda (1981) and Takeda, Takeda, and Hizukuri (1983) reported that the chain length or molecular weight of amylose is related to the tendency of retrogradation. In addition, Reddy, Subramanian, Ali, and Bhattacharya (1994) found that the amylose content had a close correlation with the paste viscosity of rice starch. The starch granules with higher amylose contents were relatively rigid, elastic and strong, while those with lower amylose contents were soft, elastic and broken down more easily.

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The molecular structures of starches from many botanical sources have been reported (Hizukuri, 1985; Hizukuri, Kaneko, & Takeda, 1983; Hizukuri et al., 1981; Takeda, Hizukuri, & Juliano, 1987a; Takeda, Takeda, & Hizukuri, 1989). However, edible canna starch has not been subjected to detailed study. Although there have been some reports on the molecular structures of this starch and these revealed that starch from edible canna was a B-type starch with average degrees of polymerization (DPn) of amylopectin branch chain length ranged from 22.5 to 28.9 (Hanashiro, Abe, & Hizukuri, 1996; Jane et al., 1999; Santacruz, Koch, Svensson, Ruales, & Eliasson, 2002). The amylose contents of edible canna starch were 22.7–23.8% (Jane et al., 1999; Santacruz et al., 2002). However, information on the fine structure of amylose, and some properties of amylopectin, e.g. blue value, β-amylolysis limit, phosphorus contents and phosphorus linked to C-6 of the glucosyl residue, is unavailable. Furthermore, all the reports mentioned above reported on single cultivar of edible canna starch.

In the previous study (Thitipraphunkul, Uttapap, Piyachomkwan, & Takeda 2003) we reported the physicochemical properties of edible canna starch from three cultivars and suggested that the gelatinization and retrogradation properties are influenced by their molecular structures. Therefore, efforts were made here to characterize the fine structure of amylose and amylopectin and correlate between the molecular structures and some physicochemical properties of edible canna starches.

2. Materials and methods

2.1. Materials

Three cultivars of edible canna (Thai-purple, Thai-green and Japanese-green) were grown on experimental plots under identical environmental condition at the Corn and Sorghum Research Center, Kasetsart University, Bangkok, Thailand. Nine-month rhizomes were harvested for starch extraction. Cassava tuber (Kasetsart 50 variety) was provided by Rayong Filed Crops Research Institute, Rayong, Thailand. Mung bean was purchased from local supplier in Bangkok.

Crystalline *Pseudomonas* isoamylase was the product of Hayashibara Biochemical Lab. Inc., (Okayama, Japan). Sweet potato β-amylase was further purified from a commercial product (Sigma Chemical Co., St Louis, MO) by the method of Marshall and Whelan (1973). Other chemicals were of analytical grade and used without other treatments.

2.2. Starch isolation

The slurry of canna rhizomes was prepared by adding some water into approximately 1-cm cubes of cleaned rhizome, and then ground in a blender. The pulp in slurry was removed by screening through a bolting cloth and the suspension obtained was filtered through a 88 μm sieve. The filtrate was allowed to settle until a dense, firm starch layer was deposited. The supernatant was decanted and starch cake was rewashed at least three times. The starch cake was then dried in an oven at 50 °C for 15 h. Cassava starch was isolated in the same manner whereas mung bean was soaked overnight in water before grinding.

2.3. Starch fractionation

Starch was defatted by three replication of dissolution in dimethyl sulfoxide and precipitation with ethanol (Takeda, Hizukuri, & Juliano, 1986) before fractionation.

Preparation of amylose fraction. Amylose fraction was prepared by dispersion and selective precipitation method according to a procedure described by Takeda et al. (19876. The purity of amylose specimens was confirmed by gel-permeation chromatography on Toyopearl HW-75 F (Tosoh, Tokyo, Japan) (Takeda, Shirasaka, & Hizukuri, 1984).

Preparation of amylopectin fraction. Amylopectin fraction was prepared by precipitation with ethanol according to a procedure described by Takeda et al. (1986).

2.4. Analytical methods

Iodine affinity. The iodine affinities (IA, g/100 g) of amylose and amylopectin fractions were determined by the amperometric titration method (Larson, Gilles, & Jenness, 1953) with some modifications (Takeda et al., 1987a; Takeda, Hizukuri, Takeda, & Suzuki, 1987b).

Blue value and λ_{max}. Blue value and λ_{max} was determined according to the method described by Suzuki et al. (1981). The absorbance of the amylose (or amylopectin)–iodine complex was measured in a solution (8 mM acetate buffer, pH 5.0, 100 ml) containing 4 mg of amylose/amylopectin, 8 mg of iodine, and 80 mg of potassium iodide, using a spectrophotometer. Blue value is defined as the absorbance at 680 nm measured under the above conditions. The wave-lengths for absorbance measurement were scanned for determination of λ_{max}.

β-Amylolysis limit and isoamylolysis. β-Amylolysis limit was conducted and calculated as described by Suzuki et al. (1981). Isoamylolysis was performed according to the method of Suzuki et al. (1981).

2.5. Molecular structure of amylose

The number-average degree of polymerization (DPn). Two methods, colorimetric and fluorescence-labeling methods, were used for determination of DPn values. For colorimetric method, DPn was calculated by dividing total carbohydrate by its reducing value. Total carbohydrate and reducing value were measured by phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956)

and the modified Park–Johnson method (Hizukuri et al., 1981), respectively.

Determination of DPn by fluorescence-labeling method was carried out according to the procedure described by Hanashiro and Takeda (1998). 2-Aminopyridine was used as the fluorescent reagent. The chromatograms of labeled amylose were derived from a HPLC system, using three columns (7.5×75 mm^2), TSKgel G6000PW, G4000PW and G3000PW (Tosoh Co., Tokyo, Japan) connected in series. The fluorescence response and refractive index response were detected by a fluorescence detector (FS-8010, Tosoh) and a refractive index detector (ERC-7512, Erma Inc., Tokyo, Japan), respectively. DPn of amylose was calculated from the ratio of RI response to fluorescence response (RI/F) using a calibration line, which was obtained from the RI/F values of standard amyloses.

Average chain length (CL). The CL of amylose was determined by dividing total carbohydrate by its non-reducing value. Non-reducing value was measured by the modified rapid Smith degradation method ((Hizukuri et al., 1981).

Mole fraction of linear and branched amylose. Amylose was labeled with 2-aminopyridine and hydrolyzed by β-amylase in the same manner as described for β-amylolysis limit. The β-amylolysate was then analyzed by the HPLC system as mentioned above. Mole fraction (%) of branched amylose was calculated as (peak area of β-limit dextrin from amylose fraction/peak area of whole amylose) × 100. Mole fraction (%) of linear amylose was obtained from deduction of branched fraction from whole amylose.

2.6. Molecular structure of amylopectin

Average chain length (CL) and molar-based distribution of unit chains. CL was determined using three methods, colorimetric method with the rapid Smith degradation (Hizukuri & Osaki, 1978), colorimetric method with isoamylolysis (Suzuki et al., 1981) and fluorescence-labeling method with isoamylolysis (Hanashiro, Takawa, Shibahara, Iwata, & Takeda, 2002). The molar-based distribution of unit chain of amylopectin was determined by gel-permeation HPLC with columns of Shodex OHpak SB-803HQ and SB-802.5HQ × 2 (Hanashiro et al., 2002).

Individual chain-length distribution. The individual chain-length distribution of amylopectin debranched with isoamylase was examined by high performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) (Hanashiro et al., 1996).

Phosphorus contents. Phosphorus was determined as inorganic phosphate (Fiske & Subbarow, 1925) after treatment of the amylopectin samples with hot perchloric acid (Allen, 1940). Phosphorus linked to C-6 of the glucosyl residue was assayed as glucose-6-phosphate using glucose-6-phosphate dehydrogenase after acid hydrolysis of amylopectin (Hizukuri, Tabata, & Nikuni, 1970).

3. Results and discussion

3.1. Structure of amyloses

Table 1 summarizes the properties of amylose from canna, mung bean and cassava starches. All amylose specimens were similar in iodine affinity, blue value, and λ_{max}, but their molecular sizes were different. The amylose of Thai-purple (DPn 1590), Japanese-green (DPn 1620) and Thai-green (DPn 1650) canna starches were comparable in size among each others, but smaller than those from mung bean (DPn 2200) and cassava (DPn 3220) starches. The DPn values obtained were in the same order as those reported as 2000 by Oates (1990) and 1900 by Biliaderis, Grant, and Vose (1981) for mung bean and as 3390 by Hizukuri et al. (1981) for cassava. The DPn values determined by

Table 1
Properties of amyloses

Property	Japanese-green canna	Thai-green canna	Thai-purple canna	Mung bean	Cassava
Iodine affinity (g/100 g)	20.6	20.2	19.7	19.6	20
Absolute amylose contents (%)	25	22	19	28	18
Blue value	1.29	1.29	1.24	1.26	1.3
λ_{max} (nm)	629	631	631	632	629
Number-average DP (DPn)					
Colorimetric method	1620	1650	1590	2200	3220
Labeling method	1630	1550	1440	2110	2930
Average chain length (CL)	450	471	420	350	410
Average number of chain (NC[a])	3.6	3.5	3.8	6.3	7.9
β-Amylolysis limit (%)	85	84	84	76	75
Linear amylose (mole%)	87	84	84	84	66
Branched amylose (mole%)	13	16	16	16	34
NC of branched amylose[b]	21	17	17	34	21

[a] NC = DPn/CL (colorimetric method).
[b] NC of branched amylose = [(NC of whole amylose) − (1 − mole fraction of branched amylose)]/(mole fraction of branched amylose).

the colorimetric method were close to those determined by the fluorescence-labeling method. The amyloses from canna starches could be categorized into the small-to-medium size, when compared to those from other starches such as potato, 4370; sweet potato, 3230; barley, 1580; wheat, 1220; maize, 1030 (Hanashiro & Takeda, 1998).

The CL of all amyloses was not significantly different (350–471). However, the average-number of chain per molecule of amylose (NC) from canna starches (3.5–3.8) was considerably lower than those from mung bean (6.3) and cassava (7.9) starches. The β-amylolysis limit of canna amylose was 84–85%, which was higher than those of mung bean (76%) and cassava (75%) amyloses. These values correlated with NC, as NC increased, the β-amylolysis limit decreased. It suggests that higher degree of branching, lower β-amylolysis limit would be obtained.

NC was calculated using the whole amylose basis, i.e. it was assumed that the branching of amylose was uniformly distributed in all amylose molecules. However, it is possible that amylose might exist both in linear (without branching) and branched forms as reported previously (Takeda et al., 1987b). To verify this, the distribution profiles of β-amylolysate of labeled amyloses were investigated. The results obtained are shown in Fig. 1. As shown in Fig. 1, the β-amylolysate was clearly separated into two fractions: G2/G3 fraction and β-limit dextrin. Since the linear chain has no branching, therefore β-amylase can hydrolyze the chain from non-reducing end until maltotriose or maltose with labeling reducing end was obtained. That is, the peak of G2/G3 represents a mole fraction of linear chain amylose. In contrast, the enzyme hydrolyzed the branched amylose from all non-reducing ends and ended up near branching points, leaving the backbone of branched amylose as β-limit dextrin. The retention time of β-limit dextrin was similar to that of whole amylose (Fig. 2), suggesting that branched amylose was larger than linear chain amylose. In addition, broad distribution of β-limit dextrins indicated that the branch linkages located randomly on molecule of branched amylose.

The values of mole fraction of linear and branched amylose are presented in Table 1. The mole% of branched fraction of canna and mung bean starches were comparable (13–16 and 16%, respectively), whereas that of cassava starch (34%) was considerably higher. The higher extent of branched chain fraction of cassava could be somewhat one factor responsible for lowering the β-amylolysis limit. NC of branched amylose was

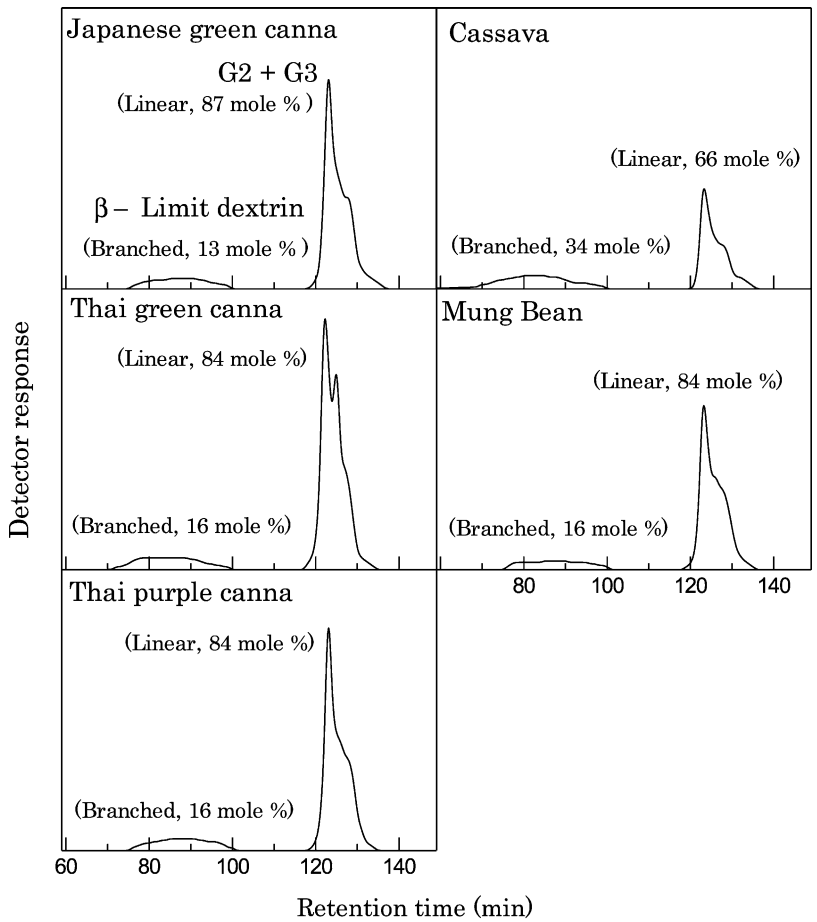

Fig. 1. Gel-permeation HPLC chromatograms of β-amylolysate of fluorescence-labeled amyloses.

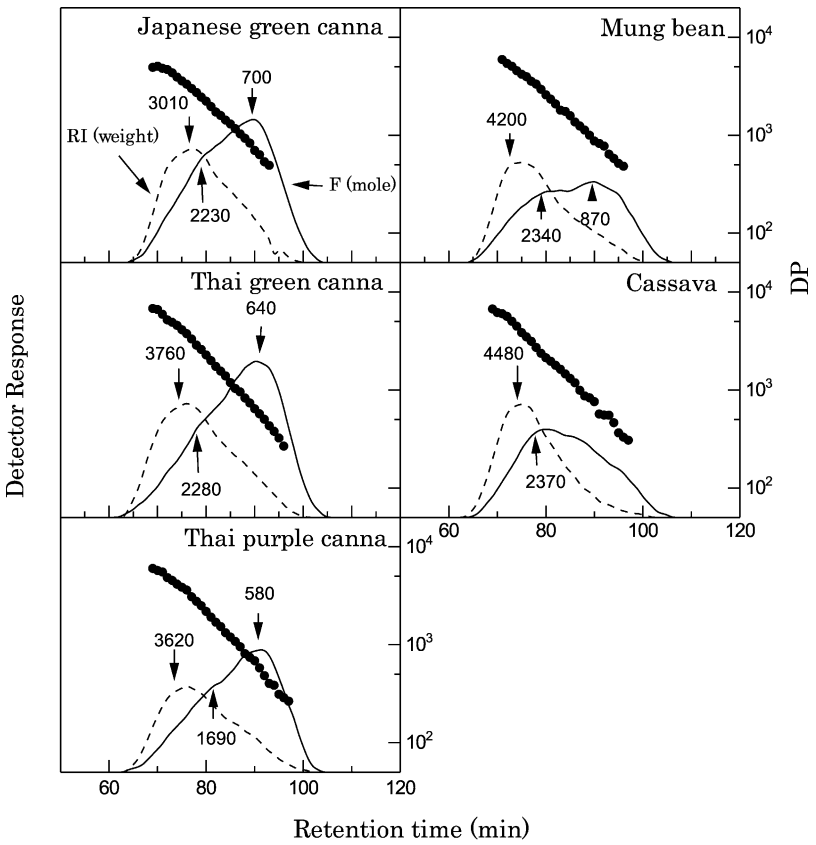

Fig. 2. Gel-permeation HPLC chromatograms of fluorescence-labeled amyloses. (—) Fluorescence response, F; (– – –) differential refractometry, RI; (●) DP. Arrows indicate DP values.

calculated to be 17–21, 34 and 21 for canna, mung bean and cassava starches, respectively.

Fig. 2 shows the distributions of fluorescent-labeled amyloses determined by gel-permeation HPLC with RI and fluorescence detectors. The RI and fluorescent profiles represent weight- and molar-based distributions, respectively. The chromatograms indicated that canna amylose had a peak and a shoulder similar to amyloses from barley starch (Yoshimoto, Takenouchi, & Takeda, 2002), normal rice, wheat and maize amyloses (Hanashiro & Takeda, 1998). The molar-based distribution showed that all the amylose of canna starches and mung bean starch contained two molecular species differing in molecular size, and the small species was predominant. The amylose from cassava starch, by contrast, the large species was abundant.

3.2. Structure of amylopectins

The properties of canna, mung bean and cassava amylopectins are summarized in Table 2. Canna

Table 2
Properties of amylopectins

Property	Japanese-green canna	Thai-green canna	Thai-purple canna	Mung bean	Cassava
Iodine affinity (g/100 g)	0.54	0.63	0.62	0.52	0.13
Blue value	0.16	0.17	0.17	0.11	0.06
λ_{max} (nm)	555	555	556	552	536
Average chain length (CL)					
Isoamylolysis	25	26	28	24	21
Smith degradation	25	26	28	24	21
Labeling method	25	25	27	23	20
β-Amylolysis limit (%)	60	60	62	59	57
Phosphorus					
Organic (ppm)[a]	391	397	420	100	67
Link to C-6 (ppm)	269	275	299	15	33
C-6/organic (%)	69	69	71	15	49

[a] Linked to glucosyl residues.

amylopectins have slightly higher IA (0.54–0.63 g/100 g) than mung bean (0.52 g/100 g) and obviously higher IA than cassava (0.13 g/100 g) amylopectins. A similar tendency was observed in the blue value and λ_{max}, suggesting that canna amylopectins had more long chain forming a complex with iodine than mung bean and cassava amylopectins. The amylopectins from all cultivars of canna had higher CL (25–28) than mung bean (24) and cassava (21) amylopectins. This CL of canna amylopectins are comparable to the value (28.9) previously reported by Jane et al. (1999), but significantly higher than that (21.9) reported by Santacruz et al. (2002). Among the canna amylopectins, Thai-purple had the highest CL of 28 followed by Thai-green (26) and Japanese-green (25). The canna amylopectins had similar β-amylolysis limit of 60–62%, which was higher than mung bean (59%) and cassava (57%) amylopectins.

The canna amylopectins contained a large amount of organic phosphorus (391–420 ppm), and among them, Thai-purple amylopectin was the highest one. These values are relatively high when compared to other root and tuber starches. Among the starches, potato was found to contain the largest quantity of organic phosphorus (890 ppm dsb), followed by taro (210 ppm dsb) (Hoover, 2001). The organic phosphorus determined was supposed to be in phosphate monoester form, since phospholipids were removed at the step of preparing amylopectin samples. The proportion of the phosphorus at C-6 of glucosyl residue was 69–71%, which was similar to the sweet potato (56–69%) amylopectin (Hizukuri et al., 1970). These values appeared to be characteristics of root and tuber amylopectins. The remainders were supposed to be bound to C-3 (Hizukuri et al., 1970).

The chain-length distributions of canna, mung bean and cassava amylopectins were examined by gel-permeation HPLC after debranching with isoamylase followed by fluorescence-labeling of unit chains (Hanashiro et al., 2002). Chromatograms obtained are shown in Fig. 3. All three canna amylopectins displayed a bimodal distribution profiles on molar basis, as well as weight basis, with the first peak at DP 40–41 and second peak at DP 12–14, while mung bean and cassava showed two distinct peaks at DP 36–39 and DP 10–11 with a shoulder at DP 16–17. The profiles of canna amylopectins resemble that reported by Jane et al. (1999), but Santacruz et al. (2002) found a slight shoulder at DP 22–24. According to the designated unit chain of amylopectin (A, B_1, B_2, B_3,...) as proposed by Hizukuri (1986), the second peak are likely to be A + B_1 and the first peak seem to be equivalent to B_2 + B_3. CL of each unit chain is shown in Table 3. As expected, CL of all canna amylopectins is longer than those of mung bean and cassava amylopectins.

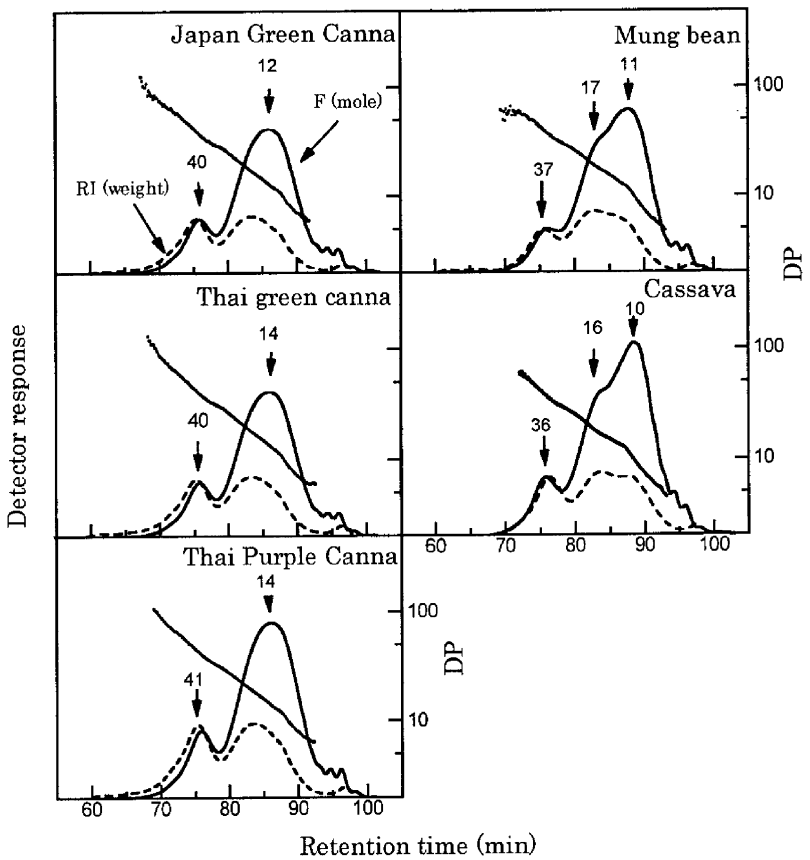

Fig. 3. Gel-permeation HPLC chromatograms of fluorescence-labeled unit chain of amylopectin. (—) Fluorescence response, F; (– – –) differential refractometry, RI; (●) DP. Arrows indicate DP values.

Table 3
Average chain-length and amount (mole%) of the fractions of amylopectin unit chain

Source	Average chain-length			Unit chain (mole%)			$A + B_1$
	A	B_1	$B_2 + B_3$	A	B_1	$B_2 + B_3$	$B_2 + B_3$
Japanese-green canna	14		50	84.2		15.5	5
Thai-green canna	14		52	84.3		15.7	5
Thai-purple canna	15		53	84.0		16.0	5
Mung bean	10	21	48	59.1	31.4	9.5	10
Cassava	9	24	42	62.2	26.0	11.8	8

The mole ratios of $A + B_1$ to $B_2 + B_3$ are 5 for all canna, 10 for mung bean and 8 for cassava amylopectins. These results indicate that canna amylopectins contain relatively high amount of the connecting chains (across clusters), comparing with mung bean and cassava amylopectins. The relative abundance of connecting chains probably is a characteristics of B-type starches as suggested by Hanashiro et al. (2002) and Hizukuri (1986). The results obtained from this experiment support this suggestion as well, since in our former report (Thitipraphunkul et al. 2003) canna starches have been shown to be B-type starches.

Due to the results that phosphorus are existing in a fairly large amount in canna amylopectins, it is interesting to investigate the location of phosphorus in various unit chains. The phosphorylated chains of amylopectin were separated from non-phosphorylated chains by using DEAE-Sephadex A-50 chromatography, according to the method described by Takeda and Hizukuri (1981). The distribution profiles of phosphorylated unit chains are shown in Fig. 4. Comparing with the distribution profiles of total unit chains shown in Fig. 3, it is clearly seen that the peak of short chain at retention around 85 min (DP below 14) almost totally disappeared. It implies that the short chain units are non-phosphorylated chains. In addition, the reversal of relative detector responses of short chains to long chains in case of total unit chains and phosphorylated unit chains of canna amylopectins was obviously observed. Mung bean and cassava amylopectins, even though it is not plainly seen, also displayed the same trend. These results lead to a conclusion that the phosphate groups are located mostly in long B-chain, which is in agreement with previous report by Takeda and Hizukuri (1982).

To demonstrate the individual unit chain distributions of amylopectins, the non-phosphorylated chains fraction of amylopectins were then analyzed by HPAEC-PAD. The distribution profiles are shown in Fig. 5. The results

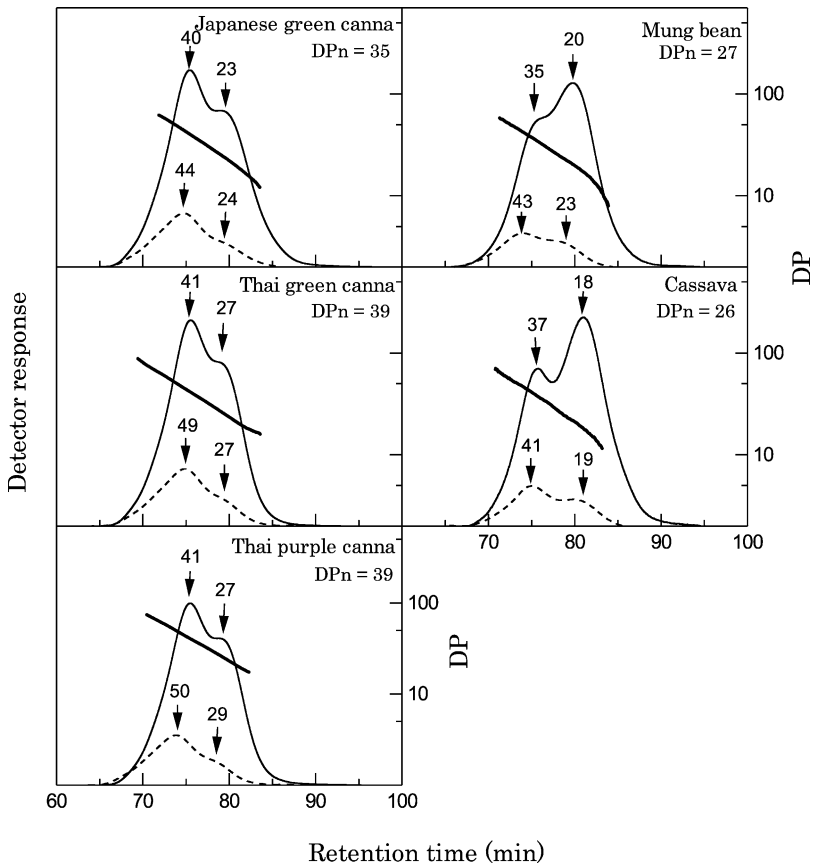

Fig. 4. Gel-permeation HPLC chromatograms of fluorescence-labeled phosphorylated unit chain of amylopectin. (—) Fluorescence response, F; (– – –) differential refractometry, RI; (●) DP. Arrows indicate DP values.

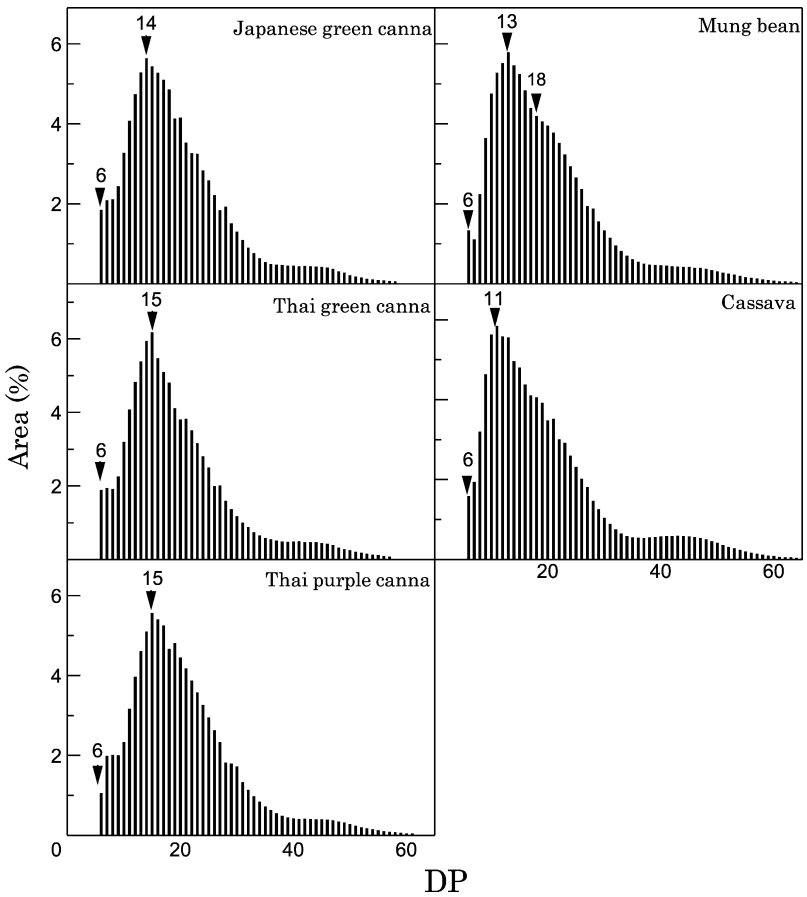

Fig. 5. Chain-length distributions of non-phosphorylated unit chain of amylopectins analyzed by using an HPAEC-PAD.

obtained by using HPAEC-PAD are generally consistent with those obtained by using gel-permeation HPLC. However, shoulders can be seen when the unit chains of canna amylopectins were analyzed by HPAEC-PAD. Although the phosphorylated chains were taken out, the profiles of all amylopectins are still similar to the distribution profiles of total unit chains in Fig. 3. These results confirm the former conclusion that the short chain units are non-phosphorylated chains and the long B-chain are existing as both phosphorylated and non-phosphorylated chains.

3.3. Relationship of molecular structure and functional properties

In our previous report (Thitipraphunkul et al. 2003), canna starches showed higher viscosity than mung bean and cassava starches, especially Thai-purple starch cultivar gave substantially higher viscosity. The difference in pasting properties of starch could be attributed to amylose and lipid contents and branch chain-length distribution of amylopectin. It was proved in this experiment that canna starches had a larger proportion of long amylopectin branch chains than the cassava and mung bean amylopectins. It is plausible that the long branch chains of the amylopectin interact to a greater extent (entangling) with amylose than do the short amylopectins to give higher viscosity of starch (Jane & Chen, 1992). High viscosity of canna starch could be also attributed to high phosphorus contents in amylopectin of canna starches. Among canna starches, Thai-purple cultivar had a different pasting profile from the other two cultivars. This suggested that not only the structure of amylopectin but also the low amylose content and smaller size of amylose of Thai-purple canna, affected the intermolecular forces within the starch granules, resulting in a higher breakdown than Japanese-green and Thai-green canna starches.

For the thermal properties of starches, higher amount of long outer chains in amylopectin molecules (Table 3) of canna starch could increase the efficiency of packing within the crystalline region of starch granules, resulting in higher T_0, T_p and ΔH of gelatinization determined by DSC. These results agreed with the studies of Noda et al. (1998).

The rate of retrogradation depends on a number of variables, including the structures of amylose and amylopectin, ratio of amylose to amylopectin, temperature, starch concentration, botanical source of the starch, and presence and concentration of other ingredients. In general, cereal starches retrograde more rapidly than the tuber and root starches. The percentages of retrogradation (%R) of starches determined by DSC were 67%

for Thai-green canna, 71% for Thai-purple canna, 83% for Japanese-green canna, 76% for mung bean and 34% for cassava starches (part I). It is interesting that canna starch, one of root and tuber starches, has a tendency to retrograde in the same level as the cereal mung bean starch. The difference in retrogradation of canna and mung bean starches could be attributed primarily to molecular size, structure of amylose and amylose content. There have been reported that small size, low value of average-number of chains and high content of amylose could enhance the retrogradation of starch (Lansky, Kooi, & Schoch, 1949; Suzuki et al., 1981; Takeda et al., 1983). Canna starches had smaller size of amylose and lower value of average-number of chains of branched molecule of amylose than those of mung bean starch (Table 1), but the amylose content was lower. Thus, the same level of retrogradation of canna and mung bean starches could be due to the integrated effects of these three factors. The results of light transmittance of gelatinized starch at different time storage at 25 °C (Thitipraphunkul et al. 2003) also confirmed that canna starches had high retrogradation.

The present investigation has demonstrated that there were similarities in molecular structure of both amylose and amylopectin of all three canna cultivars. However, Thai-purple canna starch showed slightly lower number-average DP and CL of amylose.

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