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EFFECTS OF AMYLOPECTIN UNIT CHAINS ON THE STARCH PASTING CHARACTERISTICS

Abstract

Structures and properties of starches isolated from Japanese rice and different botanical sources were investigated. Amylopectin unit chain-length distributions were analyzed using HPLC and HPAEC-PAD for comparing the distributions of unit chains of isoamylase-debranched amylopectins. Thermal properties were measured by DSC. It was found that there were at least five groups of Japanese rice amylopectins having different unit-chain distributions. The four kinds of starches (Akenohoshi, Haiminori, Tashukei 431, and Kenkei 2064) had the amylopectins of long chain-length compared with the other rice starches. It was observed that the gelatinization temperature of their four native starches were higher and the enthalpy change (•H) of gelatinization of their four retrograded starches showed larger than the other rice starches. In the starches of different botanical sources, the ratios of unit chains of DP6-12 (Fr.A) of amylopectins of quinoa, barley, buckwheat, Japanese radish, and tulip were higher, and the gelatinization temperatures were lower than the values of other starches. The ratios of Fr.A of amylomaize V and VII were lower, and the gelatinization temperatures were higher than the respective values of other starches. There were highly negative relationships between the ratio of short unit chain-length in amylopectin and gelatinization temperature.

Introduction

The crystalline regions in starch granules are mainly composed of amylopectin. The X-ray diffraction patterns of starch granules change by the unit-chain length distribution of amylopectin [1]. Amylose has a close relation to starch retrogradation. Amylopectin also had the great influence with the phenomena of gelatinization and retrogradation of starch [2]. We found that the gelatinization temperature has a close relationship with the chain-length distribution of amylopectin of maize starches isolated from mature kernels of mutants with starch-modifying endosperm genes, and that the enthalpy change of gelatinization has a highly relation to the amylopectin content

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of them [3, 4]. This objective of this paper is to know the relationship between the chain-length distributions of amylopectins and the properties of gelatinization of starch by investigating various kinds of Japanese rice starches and starches of different botanical sources.

Materials and methods

Materials

The rice endosperm starches were prepared from milled rice obtained from the Ministry of Agriculture, Forestry and Fisheries of Japan by the cold and dilute alkali method of *Yamamoto* et al. [5, 6] in the laboratory. Normal and waxy maize and wheat starches were gifts of Sanwa Denpun Kogyo Co., Ltd. Amylomaize V and VII were gifts of Oji Corn Starch Co., Ltd. Potato starch was a gift of Tokachi Farmers' Cooperative. Sweet potato starch was a gift of Kagoshima Keizairengokai. The other starches of different botanical sources were prepared in the laboratory by a modification of *Schoch's* method [7]. The purification of amylopectin from rice starch were performed by the method of *Lansky* et al. [8].

Methods

Absorption curves of starch-iodine complexes were recorded with a Hitachi 3210-type recording spectrophotometer with mixtures containing 1 mg of starch, 2 mg of iodine and 20 mg of potassium iodide per 25 ml [9]. Measurement of gel filtration of isoamylase-debranched starch materials by Toyopearl HW55S-HW50S were described previously [10, 11]. Gel-permeation HPLC of isoamylase-debranched starch was performed by using the method of *Hizukuri* [12]. A high-performance anion-exchange chromatography with a pulsed amperometric detector (HPAEC-PAD) of isoamylase-debranched materials of starch was performed by using a Dionex model DX-300 system (Dionex Corp., Sunnyvale, CA, U.S.A.) according to the method described by *Koizumi* et al. [13, 14] with a minor modification. The thermograms of native and retrograded starches were recorded on a differential scanning calorimeter (DSC) (Setaram Micro DSC III, Caluire, France) by a modification of method of *Inouchi* et al.[3, 4]. X-ray diffractometry was performed by the method of *Hizukuri* et al. [15].

Results and discussion

The Tab. 1 shows that the λ max and blue value of iodine-starch absorption complex, and the amylose contents of starch determined by measurements of gel filtration, and the ratios of fractions of amylopectin determined by HPAEC-PAD. There are low amylose, middle content of amylose, high amylose, and waxy rice starches from the results of the λ max, blue value, and amylose contents. Fig. 1 demonstrates that

Tabela 1

Some structural characteristics of starches of Japanese rice and waxy maize.

Name of cultivar	Iodine complex spectra		GPC	HPAEC-PAD			
	λ max. (nm)	Blue value (at 680nm)	A.C. (9b)	Fr.A (96)	Fr.Bl (~)	Fr.B2 (9'0)	Group
Rice starch							
Saikai236	546	0.13	9.8	30.1	65.5	4.4	E
Saikai215	548	0.12	9.6	30.1	65.5	4.3	E
Milky queen	550	0.13	7.4	29.6	65.5	4.8	D
Kanto194	551	0.14	8.1	32.1	63.3	4.6	F
Hokuriku161	551	0.14	9.5	31.6	63.1	5.3	E
Ou344	555	0.15	10.8	31.6	64.0	4.4	E
Hokuriku180	561	0.16	11.0	33.8	63.0	3.2	F
Soft158	562	0.17	12.6	31.3	65.1	3.6	E
Haiminori	567	0.21	15.7	25.0	69.5	5.5	C
Hokkai280	568	0.17	14.0	33.0	62.2	4.8	F
Kanto195	571	0.23	20.5	31.1	64.6	4.3	E
Akenohoshi	572	0.21	15.7	24.9	70.4	4.7	C
Hinohikari	572	0.18	18.5	31.1	64.4	4.5	E
Koshihikari	572	0.22	18.5	29.2	65.8	5.0	D
Kanto186	572	0.23	17.8	31.2	64.6	4.2	E
Kanto188	572	0.23	17.5	31.0	64.1	4.9	E
Kanto198	573	0.23	19.6	30.9	64.8	4.3	E
Saikai231	574	0.23	18.1	29.7	65.6	4.7	D
Hokuriku184	574	0.26	20.3	31.1	63.9	5.0	E
Kenkei 2064	574	0.37	21.0	16.9	76.0	7.1	A
Hokkai287	576	0.22	18.9	32.2	63.9	3.9	F
Sasanishiki	578	0.22	21.2	31.2	66.0	2.8	E
Kirara397	578	0.22	21.2	31.1	65.2	3.7	E
Ou368	578	0.23	20.7	30.1	65.9	4.0	D
Hokuriku183	578	0.29	21.8	30.8	64.5	4.7	E
Nihonbare	582	0.27	23.0	30.8	65.0	4.2	E
Chugoku134	591	0.28	25.7	31.6	64.1	4.6	E
Hoshiyutaka	592	0.31	28.0	31.3	64.1	4.6	E
Kanto181	592	0.35	26.5	31.3	63.3	5.5	E
Yumetoiro	592	0.33	30.8	29.3	65.7	5.0	D
Waxy rice starch							
Hakuchomochi	523	0.05	0.0	30.6	64.9	4.5	E
Hakutomochi	532	0.06	0.0	30.7	64.3	5.0	E
Hiyokumochi	532	0.07	0.0	30.6	64.8	4.6	E
Hokkaimochi286	531	0.06	0.0	32.4	63.1	4.5	F
Asamurasaki	528	0.07	0.0	30.9	64.4	4.7	E
Hokurikumochi181	532	0.07	0.0	30.5	66.1	3.4	D
Tashukei431	535	0.09	0.0	24.7	70.8	4.5	C
Chugokumochi167	533	0.07	0.0	29.9	65.0	5.1	D

Saikaimochi223	532	0.07	0.0	29.4	64.2	6.4	D
Saikaimochi225	532	0.07	0.0	32.1	63.3	4.6	F
Saikaimochi227	530	0.07	0.0	30.7	65.2	4.1	E
Saikaimochi235	523	0.08	0.0	30.6	66.4	3.0	E
Waxy maize starch	535	0.09	0.0	23.9	72.2	3.9	B

A,C. : Amylose contents

Fr.A(96) : Ratio of tot al peak area of DP 6~12
Fr.Bl(96) : Ratio of total peak a rea of DP 13~36
Fr.B2(9ó) : Ratio of total peak a rea of DP >37

Group A : Fr.A 23.0%
Group B : 23.OsFr.A<24.5%
Group C : 24.5SFr.A<29.0%
Group D :29.OSFr.A<30.5%
Group E : 30.5SFr.A<32.1%
Group F : 32.1sFr.A

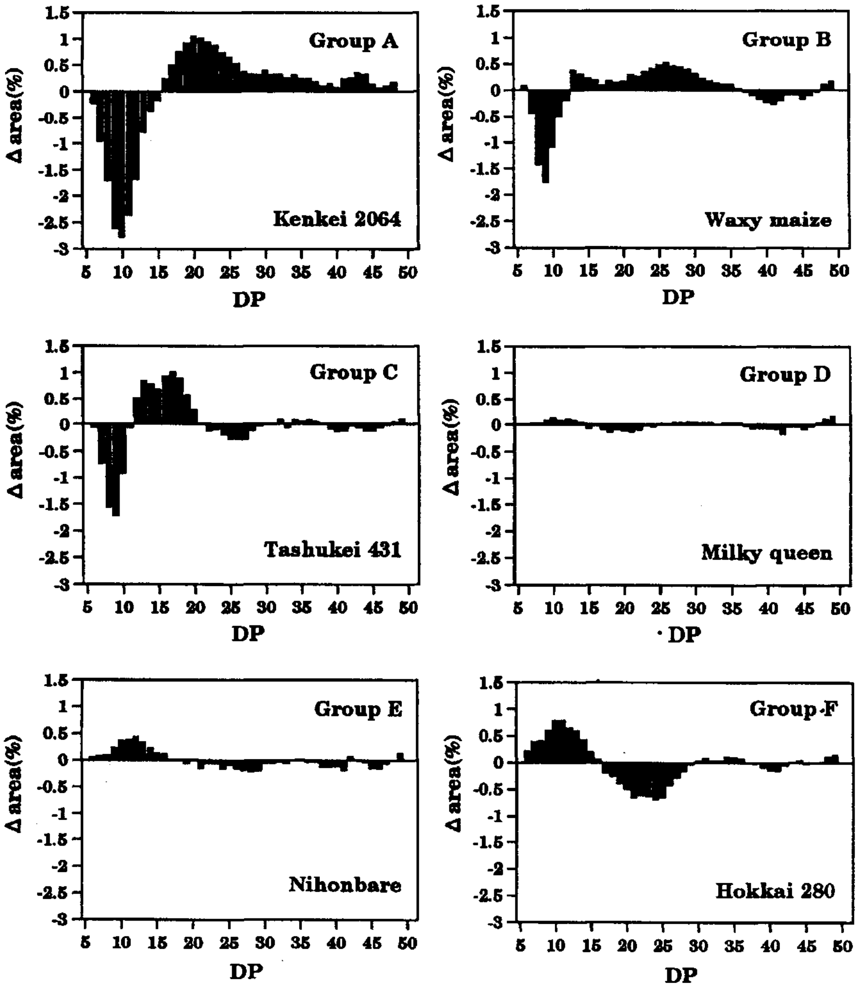

Fig. 1. Differences in chain-length distributions of debranched amylopectins of several rice varieties and waxy maize with comparison to Koshihikari rice amylopectin.

the differences in chain-length distributions of debranched amylopectins of several rice varieties and waxy maize with comparison to rice amylopectin of Koshihikari, the varieties accepted as the most delicious rice by Japanese people and had the best crop of rice in all varieties of Japanese rice, shown by the ratios of peak area of a PAD. It emerged that the chain-length distribution of rice amylopectin belonged to at least five groups (Group A, C, D, E, and F) determined by Fr.A contents (the ratio of total peak areas of DP 6-12). Most of Japanese rice starches belong to groups D, E, and F. The starches of Akenohoshi, Haiminori, Tashukei431 (Group C), and Kenkei2064 (Group A) had the amylopectins of longer chain-length compared with the other rice starches. Kenkei 2064 is an amylose-extender (*ae*) mutant rice.

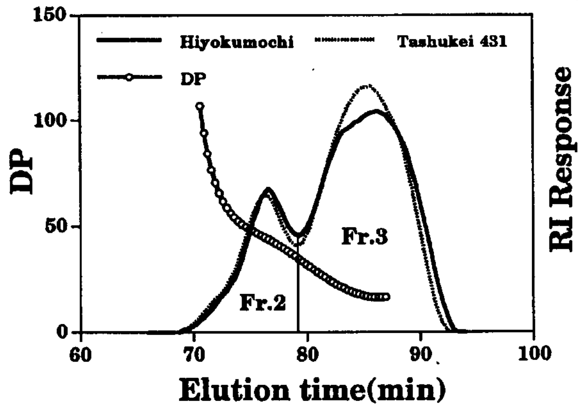

Fig. 2. Elution profiles of debranched waxy rice starches by HPLC-RI-LALLS.

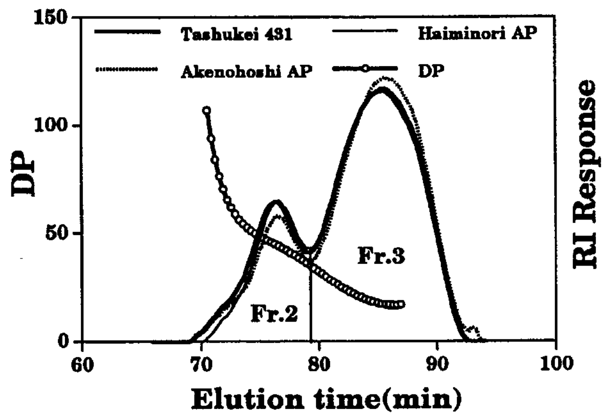

Fig. 3. Elution profiles of amylopectin (AP) of starches by HPLC-RI-LALLS.

Fig. 2 shows the elution profiles of debranched waxy rice starches of the most popular Japonica waxy rice (Hiyokumochi; Group E) and Tashukei431(Group C) by Gel-permeation HPLC. The average chain-length at vertex of Fr.3 of Hiyokumochi starch was about 15, and that of Tashukei431 was about 16-17. The elution profiles of debranced amylopectins purified from Haiminori and Akenohoshi starches which belong to the same group (Group C) were similar to that of Tashukei431. It could make sure that the unit chain lengths of amylopectins of these three rice starches (belong to Group C) were longer than the amylopectins of other starches except for Kenkei2064 from the results of Fig. 2 and Fig. 3.

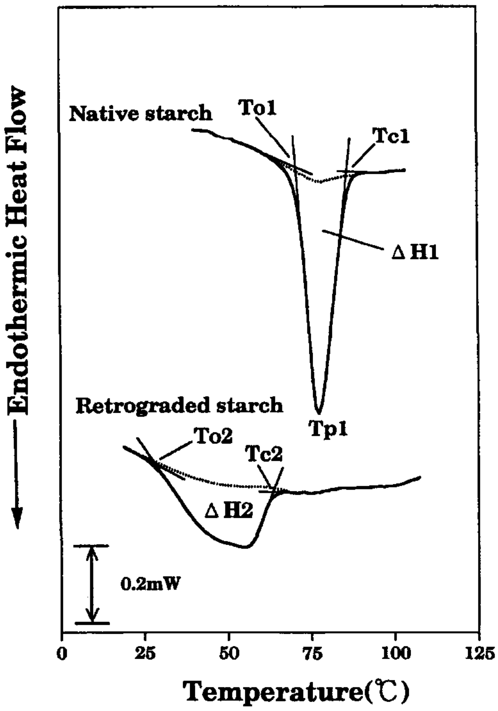

Fig. 4. Thermograms of native and retrograded rice starches of Tashukei 431 measured by micro DSC III (Retrograded starch was prepared by keeping the gelatinized starch in DSC pan in 5°C for 7 days).

Fig. 4 shows thermograms of native and retrograded starches of Tashukei431. The gelatinization temperatures and enthalpy changes of gelatinization of native and retrograded starches of rice and waxy maize are shown in Tab. 2. It was observed that the To1, Tp1, and Tc1 of native starches and Tc2 of retrograded starches of Akenohoshi, Haiminori, Tashukei431, and Kenkei2064 were higher and the •H2 of their retrograded starches were larger than the other rice starches. The To1, Tp1, and Tc1 of native starch of waxy maize were higher than common rice starches (belong to Group D, E, and F), and •H1 of native starch of waxy maize was larger than common rice starches, and the Tc2 of retrograded starch of waxy maize was higher and •H2 of retrograded starch of waxy maize was larger than common rice starches.

Tabela 2

Some thermal characteristics of native starches of Japanese rice and waxy maize and retrograded ones.

Name of cultivar	Native starch				Retrograded starch		
	To1 (°C)	Tp1 (°C)	Tc1 (°C)	ΔH1 (J/g)	To2 (°C)	Tc2 (°C)	ΔH2 (J/g)
Rice starch							
Saikai236	58.2	65.6	73.9	12.7	20.4	57.2	1.8
Saikai215	58.5	66.4	73.4	12.0	28.8	57.5	1.7
Milky queen	58.1	65.7	73.3	11.5	23.0	57.7	1.3
Kanto194	58.7	65.3	75.6	11.8	24.8	57.9	2.6
Hokuriku161	57.3	64.9	73.2	11.6	26.8	61.3	2.4
Ou344	54.5	62.1	70.9	10.9	26.9	56.3	1.5
Hokuriku180	54.2	60.7	69.0	10.4	21.6	57.1	1.2
Soft158	54.9	62.2	70.9	10.9	31.3	56.5	2.1
Haiminori	70.6	74.9	80.3	14.1	26.8	61.9	8.8
Hokkai280	54.2	60.7	69.0	10.4	21.6	57.1	1.2
Kanto195	56.4	62.1	70.0	11.1	22.4	59.1	3.2
Akenohoshi	71.6	75.3	80.0	12.9	27.4	62.5	9.7
Hinohikari	57.0	64.2	73.4	10.0	27.7	58.6	3.9
Koshihikari	58.8	64.3	72.2	11.9	32.0	56.6	2.9
Kanto186	56.6	62.8	70.5	11.4	33.4	57.5	2.5
Kanto188	58.8	63.5	69.9	10.4	31.6	57.5	1.8
Kanto198	58.0	63.6	70.9	10.9	27.6	57.1	1.8
Saikai231	58.7	64.1	71.3	11.1	31.6	56.8	2.3
Hokuriku184	58.6	64.0	71.4	10.7	29.7	57.2	2.1
Kenkei 2064	72.2	77.1	82.2	13.5	25.9	75.0	8.7
Hokkai287	53.8	59.8	67.0	9.7	21.9	57.5	4.4
Sasanishiki	56.4	62.7	71.1	10.0	28.1	57.1	2.8
Kirara397	54.6	60.1	67.3	10.1	27.0	57.1	1.8
Ou368	56.1	62.2	68.8	9.5	28.1	56.3	3.6
Hokuriku183	56.9	63.5	71.1	11.9	24.7	57.5	3.4
Nihonbare	56.8	63.5	69.2	9.5	25.1	55.9	2.9
Chugoku134	54.5	62.6	69.8	10.2	29.1	56.8	2.6
Hoshiyutaka	55.6	62.3	69.7	10.2	29.8	57.7	3.3
Kanto181	54.7	60.6	67.6	10.6	24.4	57.6	4.9
Yumetoiro	55.6	61.0	67.0	9.7	27.0	58.7	4.0
Waxy rice starch							
Hakuchomochi	50.8	59.7	75.6	11.5	26.0	55.9	1.1
Hakutomochi	56.4	65.4	79.3	10.2	29.9	57.8	0.8
Hiyokumochi	55.4	64.6	80.8	11.8	27.6	57.9	1.2

Hokkaimochi286	50.5	60.2	75.9	10.0	22.3	58.5	2,0
Asamurasaki	51.6	61.8	76.8	8.3	25.7	58.2	0.8
Hokurikumochi181	56.7	64.5	79.1	14.4	22.1	58.2	1,8
Tashukei431	71.0	77.1	86.0	15.9	27.9	63.3	10.9
Chugokumochi167	55.6	63.7	76.4	11.5	25.5	58.8	2,2
Saikaimochi223	57.5	65.0	79.6	11.5	-	-	-
Saikaimochi225	57.2	65.0	75.4	11.5	-	-	-
Saikaimochi227	56.9	64.6	75.8	12.3	30.1	58.1	0.9
Saikaimochi235	56.7	65.3	75.3	9.4	33.3	58.4	0.8
Waxy maize starch	62.5	68.2	78.6	14.3	26.0	62.6	6,4

To1 : Onset temperature (native starch)
Tp1 : Peak temperature (native starch)
Tc1 : Conclusion temperature (native starch)
ΔH1 : Enthalpy change (native starch)

To2 : Onset temperature (retrograded starch)
Tc2 : Con clusion temperature (retrograded starch)
ΔH2 : Enthalpy change (retrograded starch)

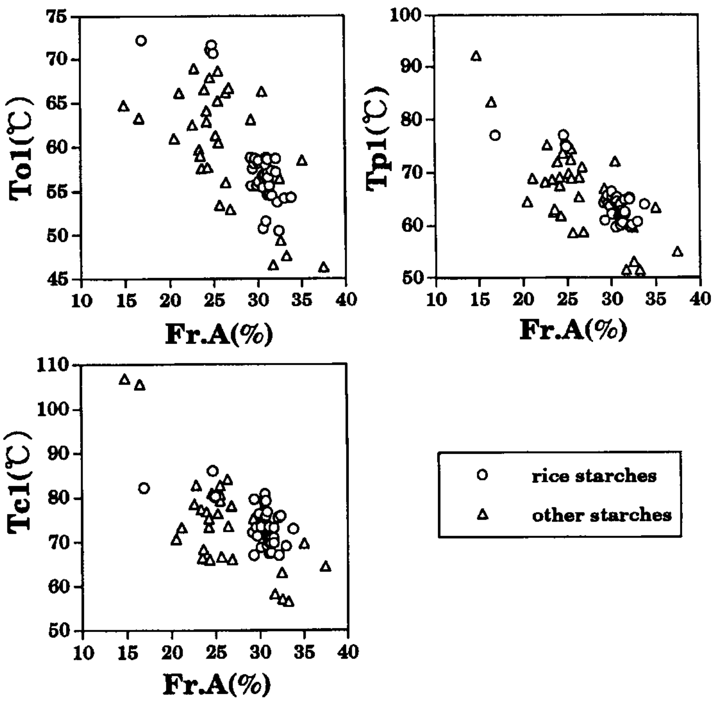

Fig. 5. Relationship between Fr.A and gelatinization temperatures.

Tab. 3 shows the λ max and blue value of iodine-starch absorption curves, the ratios of Fr.A, Fr.B1, and Fr.B2, the groups determined by Fr.A contents, and X-ray diffraction patterns of starches of different botanical sources. There were no relationship between X-ray diffraction patterns and the groups determined by Fr.A contents, however, the chains with chain lengths longer than DP12 were mainly supposed to determine the X-ray diffraction patterns of starch granules.

Tabela 3

Some structural characteristics of starches of different botanical sources.

Sample	Iodine complex spectra		HPAEC-PAD				X-ray diffraction pattern
	λ max. (nm)	Blue value (at 680 nm)	Fr.A (%)	Fr.Bl (%)	Fr.B2 (%)	Group	
Job's tears	528	0.06	23.73	70.92	5.35	B	A
Proso millet	531	0.07	25.25	68.72	6.03	C	A
Waxy maize	535	0.09	23.90	72.20	3.90	B	A
Sorghum	536	0.08	22.83	72.53	4.64.	A	A
Amaranth	538	0.09	30.54	63.32	6.14	E	A
Kiwifruit	580	0.44	26.42	66.24	7.34	C	B
Taro	584	0.20	25.56	69.46	4.98	C	A
Potato	586	0.45	20.52	73.87	5.61	A	B
Quinoa	587	0.32	37.45	57.37	5.18	F	A
Sweet potato	589	0.40	24.61	70.79	4.60	C	C
Amylomaize V	590	0.67	16.55	77.69	5.76	A	B
Pumpkin	591	0.43	21.13	74.72	4.15	A	B
Normal maize	592	0.38	24.23	71.02	4.75	B	A
Wheat	593	0.41	26.87	66.76	6.37	C	A
Job's tears (nonwaxy)	593	0.38	24.00	71.16	4.84	B	A
Horse radish	593	0.46	25.66	65.70	8.64	C	B
Barley	594	0.39	32.51	65.06	2.43	F	A
Amylomaize VII	595	0.80	14.85	75.10	10.05	A	B
Indian lotus	596	0.42	23.50	70.45	6.05	B	B
Buckwheat	597	0.43	35.07	60.60	4.33	F	A
Dogtooth violet	597	0.49	31.69	61.82	6.49	F	B
Japanese radish	597	0.42	33.24	62.61	4.15	F	B
Foxtail millet	598	0.42	24.25	69.52	6.23	B	A
Lily bulb	601	0.58	24.32	67.44	8.24	B	B
Tulip	602	0.48	32.57	59.71	7.72	F	B
Tapioka	602	0.40	29.27	65.41	5.32	D	C
Konjak	603	0.42	25.52	70.28	4.20	C	A
Bamboo shoot	604	0.43	25.55	70.26	4.19	C	C
Kudzu arrowroot	604	0.41	26.38	68.73	4.89	C	C
Loquat (Seed)	613	0.50	23.63	71.15	5.22	B	C
Sago	616	0.47	26.79	67.80	5.41	C	C

Fr.A(96) : Ratio of total peak area of DP 6~12
Fr.Bl(96) : Ratio of to tal peak area of DP13~36
Fr.B2(96) : Ratio of total peak area of DP >37

Group A : Fr.A<23.0%
Group B : 23.OSFr.A<24.5%
Group C : 24.5sFr.A<29.0%
Group D : 29.OsFr.A<30.5%
Group E : 30.5sFr.A<32.1%
Group F : 32.1sFr.A

Tabela 4

Some thermal characteristics of starches of different botanical sources.

Sample	Native starch				Retrograded starch		
	Tol (°C)	Tpl (°C)	Tcl (°C)	ΔH1 (J/g)	To2 (°C)	Tc2 (°C)	ΔH2 (J/g)
Job's tears	59.7	68.7	77.3	12.9	27.1	60.9	4.9
Proso millet	61.3	69.8	76.4	14.6	28.0	59.5	5.6
Waxy maize	62.5	68.2	78.6	14.3	26.0	62.6	6.4
Sorghum	68.9	75.2	82.8	16.3	29.4	62.3	8.8
Amaranth	66.3	72.1	78.2	10.6	27.7	59.5	4.7
Kiwifruit	66.1	69.1	73.5	16.1	29.8	67.8	4.2
Taro	68.6	74.5	80.7	12.5	27.7	62.6	7.7
Potato	61.0	64.5	70.6	17.4	27.4	72.1	7.5
Quinoa	46.3	55.0	64.5	9.9	26.0	54.2	1.4
Sweet potato	67.8	73.7	81.0	13.3	29.2	66.8	7.1
Amylomaize V	63.3	83.3	105.5	15.3	-	-	-
Pumpkin	66.1	68.9	73.3	16.0	28.8	71.5	7.6
Normal maize	62.9	67.5	73.3	12.9	29.7	61.4	6.3
Wheat	52.9	58.8	66.1	9.2	28.0	59.6	3.8
Job's tears (nonwaxy)	66.5	72.0	76.8	13.6	28.1	62.4	7.3
Horse radish	53.4	58.6	66.7	14.7	28.5	67.2	4.9
Barley	56.4	59.6	63.1	11.2	28.7	57.5	2.7
Amylomaize VII	64.7	92.1	106.8	10.1	-	-	-
Indian lotus	59.0	62.6	66.4	13.7	28.2	65.2	7.3
Buckwheat	58.5	63.4	69.7	12.0	27.5	57.0	4.0
Dogtooth violet	46.6	51.5	58.3	13.7	29.5	64.6	4.1
Japanese radish	47.6	51.5	56.6	11.3	29.6	62.5	3.3
Foxtail millet	64.1	69.1	75.2	10.2	29.0	62.4	4.4
Lily bulb	57.7	61.8	66.0	13.9	28.3	68.4	5.6
Tulip	49.4	53.1	57.1	13.3	28.1	62.8	3.9
Tapioka	63.1	67.0	75.2	13.9	27.9	61.5	4.1
Konjak	65.2	72.4	82.7	14.2	28.3	65.0	6.9
Bamboo shoot	60.5	68.9	79.4	12.3	28.5	63.3	6.3
Kudzu arrowroot	56.0	65.4	84.1	14.6	29.5	65.8	7.6
Loquat (Seed)	57.6	63.1	68.3	11.6	30.1	64.9	7.2
Sago	66.6	71.0	78.2	16.1	28.6	63.8	7.2

Tol : Onset temperature (native starch)
Tpl : Peak temperature (native starch)
Tcl : Conclusion temperature (native starch)
ΔH1 : Enthalpy change (native starch)

To2 : Onset temperature (retrograded starch)
Tc2 : Conclusion temperature (retrograded starch)
ΔH2 : Enthalpy change (retrograded starch)

Tab. 4 shows the gelatinization temperatures and the enthalpy change of gelatinization of native and retrograded starches of different botanical sources. The amylomaize V and VII starches with low content of Fr.A had higher temperature for gelatinization. The ratios of Fr.A of amylopectins of quinoa, barley, buckwheat, Japanese radish, dogtooth violet and tulip were higher, and the gelatinization temperatures were lower than the other starches. There were highly negative relationships between the Fr.A contents in amylopectins and the To1, Tp1, and Tc1 of starches of rice and the other botanical sources. This result shows that amylopectins in starch granules with shorter chain-length distributions have lower temperature of gelatinization.

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WPŁYW ŁAŃCUCHÓW AMYLOPEKTYNOWYCH NA CHARAKTERYSTYKĘ KLEIKOWANIA SKROBI

Streszczenie

Zbadano strukturę i właściwości skrobi wydzielonych z ryżu japońskiego i innych odmian botanicznych roślin. Pozorna zawartość amylozy w nich została określona przez pomiar intensywności widm absorpcyjnych kompleksów z jodem skrobi, przed i po amylolizie, pozbawiającej amylopektynę odgałęzień (izoamylaza).

Za pomocą HPLC ustalono rozkład długości łańcuchów bocznych amylopektyny a następnie, posługując się wysokorozdzielczą chromatografią anionowymienną z pulsacyjną detekcją amperometryczną (HPAEC-PAD), porównano ten rozkład z rozkładem w innych odmianach botanicznych skrobi przed i po amylolizie. Właściwości termiczne określono za pomocą skanningowej kalorymetrii różnicowej (DSC). Stwierdzono, że ze względu na rozkład długości łańcuchów amylopektynowych japońską skrobię ryżową można podzielić na 5 grup. Skrobie Akenohoshi, I-taiminori, Tashukei 431 i Kenkei 2064, miały dłuższe łańcuchy boczne w amylopektynie niż inne skrobie. Te cztery skrobie kleikowały przy wyższych temperaturach. Zmiany entalpii kleikowania tych czterech skrobi po zretrogradowaniu były wyższe niż dla

innych skrobi. Stosunek długości łańcuchów bocznych zawierających 6 i 12 jednostek glukozowych w odgałęzieniach w skrobiach, z chinoi, jęczmienia, gryki, rzodkwi japońskiej i tulipana był wyższy, a temperatury kleikowania niższe niż w przypadku innych skrobi. Stosunek liczby takich łańcuchów dla wysokoamylozowych skrobi kukurydzianych był niższy, a temperatury ich kleikowania, były wyższe niż te dla poprzednio wymienionej grupy skrobi.