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Development of Endosperm and Synthesis of Starch in Rice Grain

III. Starch property as affected by the temperature during grain development*

Guang-Cen HE**, Kiyoshi KOGURE and Hiroshi SUZUKI
(*Faculty of Agriculture, Kagawa University, Miki-cho, Kagawa-ken 761-07, Japan*)
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Abstract: *Japonica* rice plants (cultivar, Koshihikari) were treated at high and low temperatures in the early and late ripening stages. Starches prepared from the outer layer and inner portions of these rice grains were tested for their physicochemical properties. Generally, starch in the inner portion of rice grains was affected by temperature in the early ripening stage; whereas in the outer layer it was more affected in the late ripening stage. Amylose content was determined by iodine colorimetry and iodine binding capacity method. The amylose content of the outer layer and inner portion starches increased at low temperatures during grain development. Miniviscographic parameters of both starches were affected mainly by temperature in the early ripening stage and modified by that in the late ripening stage. The differential scanning calorimetric (DSC) transition temperature and gelatinization heat increased under high temperatures for the inner portion starch in the early ripening stage and for the outer layer starch in the late ripening stage. While the lipid content of inner portion starch increased at high temperatures in the early ripening stage, the lipid content of the outer layer starch decreased under high temperature in the late ripening stage. The fatty acid composition of lipids of the outer layer starch responded to temperature in a manner different from that of the inner portion starch.
Key words: Amylographic property, Amylose content, Differential scanning calorimetry, Fatty acid, Rice grain, Ripening period, Starch.

米粒の生長並びに米デンプン合成に関する研究 第3報 登熟期間における温度が米粒内外部デンプンの性質に及ぼす影響：何 光存・木暮 秩・鈴木 裕（香川大学農学部）

要 旨：稲の登熟期の前期と後期に温度を変えて，米粒の外部と内部デンプンの理化学性質に如何に影響するかを検討した．米粒の内部デンプンは登熟前期，外部デンプンは登熟後期の温度に影響されることが分かった．ヨウ素呈色法とヨウ素親和力で測定したデンプンのアミロース含量は登熟期の温度により変わり，低温で登熟した米デンプンのアミロース含量が高かったが，高温で登熟した米デンプンは低い値を示した．外部デンプンのアミロース含量は内部より低く，登熟後期の温度の影響が大きかった．ミニビスコグラフィで得られたアミログラフィ特性値には，高温で登熟したデンプンは低温で登熟したデンプンより粘度上昇温度が低く，粘度値が高く，ブレークダウンが大きかった．外部デンプンは内部デンプンより低い粘度上昇温度と高い最高粘度を示した．DSC（示差走査熱量測定）によるデンプンの糊化吸熱特性は，高温で登熟したデンプンの転換温度点と糊化吸熱量が低温で登熟したデンプンより全般的に高かった．また DSC 特性は内部デンプンで登熟前期の温度，外部デンプンで後期の温度に影響された．脂質含量については，内部デンプンの方が登熟前期の高温により増加，外部デンプンの方が後期の高温により減少した．脂肪酸の組成も登熟温度により変化した．
キーワード：アミログラフィ特性，アミロース含量，示差走査熱量測定，脂肪酸，デンプン，登熟期，米粒.

The physicochemical properties of rice starch have been proved to change with the environmental conditions during grain ripening period[2]. The effect of temperature on starch property has been documented by many researchers in an attempt to understand the mechanism of the change and to improve the eating quality of rice cultured under improper temperature conditions[1,13,15]. Amylose content, X-ray diffraction pattern, amylographic properties and the molecular structure of starch and the eating quality of rice are known to be affected by temperature during ripening[1,15,16]. Nevertheless, our knowledge on this subject is based on the data of whole rice grain.

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** Present address: Hubei Academy of Agricultural Sciences, Wuhan, China.

In previous reports, it was found that rice grains developed in a stepwise manner from the inner portion to the outer layer. The inner portion starch was synthesized mainly at the early ripening stage, and the outer layer starch at the late ripening stage. Furthermore, there was a tendency that amylose was synthesized more actively at the late ripening stage. The portion of starch that was synthesized at the early ripening stage was more resistant to the degradation by glucoamylase and hydrochloric acid[4,5].

Since the starches in the different portions of rice grain accumulated in different ripening stages, their property might change with environmental conditions at that time. In this experiment, rice plants were subjected to temperature treatments at different stages of grain development, and the properties of starches from the outer layer and inner portions of the grains were investigated.

Materials and Methods

1. Rice plants

Japonica variety, cv. Koshihikari was used for this experiment. Seeds were sown in seedling plates on June 19, 1987. On July 8, three seedlings were each transplanted to 1/2,000 are pots with 10 hills in the outer cycle and one hill in the center. Plants were grown in a glasshouse under natural conditions. To get a uniform heading the tillers were removed as soon as they emerged.

2. Temperature treatment

At heading day (August 28), the plants were transferred to phytotrons for temperature treatments as shown in Table 1. The temperature was exchanged on 20th day for L-H treatment and the 13th day for H-L treatment. The rice panicles were harvested at maturity and stored in a low temperature room (5°C) at once.

3. Rice milling and starch preparation

About 100g perfect brown rice grains were soaked in water at 5°C for 24 hours, then wet-milled to milling degree of 75% by the method described in previous papers[5,6]. And starch granules were prepared by 1.2% sodium dodecylbenzen sulfonate (SDBS) washing method.

4. Properties of starch granules

Amylose contents of rice starch were determined by the simplified method[8]. The testing mixture contained 5 mg rice starch, 2 mg iodine and 20 mg potassium iodine per 100 mL. Iodine binding capacity was determined with the Automatic Recording Titrator ART-3 (Hirama Laboratories, Japan) at conditions: temperature, 10°C; titration speed, 2 mL min^{-1}. A differential scanning calorimetry analysis was conducted on the differential scanning calorimeter (DSC) equipped with the 990 Thermal Analyzer (Du Pont Ltd.). The DSC cells were previously boiled in water. About 3 mg of starch granules (dry weight, read to 0.01 mg) were added to enough water to give a final starch concetration of 30% were sealed in. The samples were kept at room temperature overnight. The endotherm of starch gelatinization was scanned from 30°C to 110°C, under the following conditions: temperature raise rate, 10°C/min; atmosphere, air; chart speed, 5°C/cm; and DSC sensitivity, 1 mV/cm. The reference was 80 mg of aluminum oxide. The gelatinization heat (Δ H) was obtained from the peak area.

An amylographic measurements were conducted on the miniviscograph (Toyo Seisakusho Ltd., Japan), using 0.9 g of starch (dry weight) placed in pot added with water to make a total of 12 g. The temperature program was the same as the standard Brabender amylography[2,3].

Starch lipids were extracted with 6 volumes of water-saturated-butanol (WSB) at 95°C for five hours[11]. Methyl esters of fatty acids were prepared by heating the extracted lipids with 15% boron trifluoride-methanol solution. A gas chromatograph (GC-4CM, Shimadzu Inc., Japan) equipped with column packed with DEGS (80/100 mesh, Gasukuro kogyo Inc., Japan) was used to analyze the methyl esters. The fatty acids of rice starch lipids were identified by the retention time method. The amount of each fatty acid was determined on the peak area recorded by the Chromatopac against the internal standard of margaric acid.

Results and Discussion

1. Effect of temperature on the ripening duration

As shown in Table 1, the total ripening duration was long for rice plants grown under low temperature and short for those grown under high temperature. The difference between the L-L treatment and H-H treatment was 12 days. The yield of brown rice was

Table 1. Temperature conditions during the ripening stage and yield of rice*.

Treatment	Temperature (day/night°C) early stage	late stage	Ripening duration (days)	Brown rice yield (g/pot)
L-L	22/17	22/17	42(20+22)	50
L-H	22/17	32/27	37(20+17)	46
H-L	32/27	22/17	35(13+22)	45
H-H	32/27	32/27	30(13+17)	36

* Data in ( ) represent the days of the early repening stage and the late ripening stage. The day and night temperature treatment hours was 12.

Table 2. Amylose content and iodine binding capacity of rice starches developed under different tempratures.

Treatment	Amylose content (%)		Iodine binding capacity (mg/100mg starch)	
	* inner	outer	inner	outer
L-L	18.7	15.1	2.36	2.47
L-H	15.8	11.3	1.91	1.67
H-L	15.4	14.4	1.75	2.01
H-H	13.4	10.6	1.37	1.16

* Starch obtained from outer layer and inner portion of rice endosperm.

also affected by ripening temperature and showed a positive correlation with the ripening duration. There are many reports that an optimum temperature is, in general, 21°C to 25°C for *Japonica* type in the average temperature of day and night[10], that a reduction in the duration of grain growth by high temperature occurs below 30°C[19], and that furthermore a high night temperature accelerates the grain development[12].

In this experiment, the reduction ratio of the duration of the low temperature plant for the high temperature one was 65% in the early ripening stage and 77% in late one. Consequently, the brown rice grains of H-H treatment were found to be smaller in size[14] and have more imperfect grains than those of L-L treatment. Though the grain qualities, especially 1000-grain weight did not determined, the discoloured[9] and roughened leaves seemed to show lowering the leaf activity.

2. Amylose content

Amylose content of starch was higher in rice grains matured at low temperatures and *vice versa* (Table 2). The difference in amylose content between the L-L and H-H treatments was about five percentage points for both the outer layer and inner portion starches. Amylose content of starch from the inner portion of rice grain was mainly affected by the temperature in the early ripening stage and that of starch from the outer layer mainly by temperature in the late ripening stage. The difference between starches from the outer layer and inner portions was three percentage points in both the L-L and H-H treatment. It became larger in rice grain of L-H treatment and smaller of H-L treatment. The fact that amylose content of starch from the different layers in rice grain was affected by temperature in different ripening stages supported the concept that the physiological gradient existed within the rice grain[7]. The values of iodine binding capacity showed the similar tendency as amylose content. However, the iodine binding capacity of starch from the outer layer of grain was higher than that from the inner portion when the treating temperature in the late ripening stage was low, and *vice versa*. This deviation between the iodine colorimetric value and the iodine binding capacity value perhaps resulted from the molecular structure of starch, for not only the amylose but also the long chain of amylopectin complexed with iodine.

3. DSC parameters

The transition temperatures and gelatinization heat of starch detected on DSC from the treated rices are shown in Table 3. These parameters varied with the temperature dur-

Table 3. DSC characteristics of rice starches developed under different temperatures.

Treatment	portion	Phase transition temperature*				ΔH
		Ts°C	To°C	Tp°C	Tc°C	(J/g)
L-L	outer	54.5	58.2	64.2	74.0	14.0
	inner	53.7	55.6	63.0	73.1	14.5
L-H	outer	57.6	59.3	67.8	83.1	16.0
	inner	54.6	56.6	64.0	81.3	16.8
H-L	outer	58.1	61.4	67.2	79.3	16.0
	inner	59.1	62.0	68.3	80.8	16.2
H-H	outer	62.2	65.3	72.0	83.5	16.2
	inner	62.1	65.3	71.0	83.0	17.8

* Phase transition temperature : Ts, endotherm start temperature ; To, onset temperature ; Tp, peak temperature ; Tc, conclusion temperature.

ing grain development. The general tendency of change is that the higher temperature during ripening caused the higher transition temperatures and gelatinization heat of starch in DSC.

The difference of transition temperatures between starches from the outer layer and inner portions varied with the treatments. The transition temperatures were essentially the same in the outer layer and inner portion starches in the H-H treatment, and higher in the inner portion starch than in the outer layer starch in the H-L treatment. It turned higher in the outer layer starch than in the inner portion starch in the L-L and L-H treatments. The gelatinization heats seem to be higher in the inner portion starch in all the four treatments.

4. *Miniviscographic properties*

The whole operating procedure of miniviscography followed that of standard Brabender amylography with a small scale of sample in a small cup. The results obtained with this instrument are considered to reflect the pasting property by the standard amylography. All the parameters were affected mainly by the temperature in the early ripening stage and modified by that in the late ripening stage (Table 4). The pasting temperature fell and the viscosity values rose for starch as the temperature during ripening got higher. The change of pasting temperature was just inverse to the transition temperature in DSC endotherm curves in this experiment. The outer layer starches showed a lower pasting temperature, higher peak viscosity, and bigger breakdown than the inner portion starches in all the four treatments, although the difference between the two portion starches became smaller in the H-L treatment that had high temperature in the early ripening stage and low temperature in the late ripening stage.

5. *Fatty acid composition of starch lipids*

The nonstarch lipids in rice grains have been studied in detail[17,18]. However, information about the starch lipids as affected by temperature during the ripening period is scarce. In the present experiment, the lipid content and fatty acid composition in starch were found to be affected by the temperature during grain development (Table 5). The lipid content in starch from the inner portion of grain increased under high temperatures in the early ripening stage ; whereas the lipid content in the outer layer starch showed an inverse response to the temperature, and it decreased under high temperature in the later ripening stage. For the fatty acid composition, the myristic acid and oleic acid contents increased, and the palmitic acid and linoleic acid contents decreased in the inner portion starch lipids as affected by high temperatures in the early ripening stage. The change in fatty acid composition of the outer layer starch lipids appeared to be more complicated. The oleic acid content in the outer layer starch lipids showed similar tendency of changes as in the inner portion starch lipids, increased by the higher temperature in both the early and late ripening stages. The myristic acid and palmitic

Table 4. Miniviscographic properties of rice starches developed under different temperatures.

Treat-ment	Portion	Past. temp. °C	Viscosity (U)*			Breakdown (U)*	Setback (U)*
			peak	minimum	final		
L-L	outer	79.3	330	206	426	124	220
	inner	86.0	228	200	350	28	150
L-H	outer	74.8	378	214	355	164	141
	inner	82.4	268	220	364	48	144
H-L	outer	74.8	510	255	464	255	209
	inner	77.0	500	295	546	205	251
H-H	outer	——	——	270	385	——	115
	inner	74.8	515	325	520	190	195

* U is miniviscographic unit.

Table 5. Fatty acid composition of internal lipid of rice starches developed under different temperatures.

	Percentage of fatty acid*							% of
	14:0	16:0	18:0	18:1	18:2	18:3	20:1	starch
L-L								
outer	1.60	46.2	3.56	15.3	29.8	1.93	1.60	0.579
inner	2.01	47.7	4.34	12.6	31.3	1.51	0.54	0.531
L-H								
outer	2.23	49.9	3.24	17.0	26.3	1.09	0.28	0.429
inner	2.08	46.7	5.79	15.8	26.3	1.87	1.46	0.491
H-L								
outer	2.10	45.7	3.54	18.0	28.2	1.48	0.89	0.549
inner	2.26	43.9	6.10	16.1	27.0	1.89	2.71	0.578
H-H								
outer	2.25	45.9	3.41	25.3	21.4	1.10	0.62	0.389
inner	2.39	43.9	5.56	21.6	24.2	1.24	1.09	0.541

* Fatty acids: 14:0, myristic acid, 16:0, palmitic acid, 18:0, stearic acid, 18:1, oleic acid, 18:2, linoleic acid, 18:3, linolenic acid, 20:1, eicosenoic acid.

acid contents decreased, but the linoleic acid content increased under low temperatures in the late ripening stage. The results above indicate that the lipid content and fatty acid composition in the outer layer and inner portion starches were affected by temperatures during the different ripening periods and responded in a different manner.

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* In Japanese with English summary.