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Effect of Annealing Temperature on Gelatinization of Rice Starch Suspension As Studied by Rheological and Thermal Measurements

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The effect of annealing temperature (T_a) on the rheological behavior of 10 wt % rice starch suspension was investigated by the dynamic viscoelasticity, the differential scanning calorimetry (DSC), and the amount of leached out amylose and the swelling ratio of starch suspension. The rheological behaviors of the annealed samples are classified into three types in terms of T_a: T_{a1}, 48 and 55 °C, which are much lower than the gelatinization temperature, T_{gel} (=62 °C); T_{a2}, 58, 60, and 62 °C, which are almost the same as T_{gel}; and T_{a3}, 65, 68, 70, and 73 °C, which are much higher than T_{gel}. For the samples annealed at T_{a2}, the onset temperature of the storage and the loss moduli, G' and G'', increased with increasing T_a, and G' and G'' in the temperature range from 65 to 90 °C gradually increased though smaller than those for the nonannealed sample, the control. This can be understood by the partial gelatinization; i.e., the leached out amylose prevents further amylose from leaching out. The rheological property of the samples annealed at T_{a1} is not so different from that of the control, and the samples annealed at T_{a3} are almost gelatinized. The rheological behavior of starch suspension can be controlled by T_a.

KEYWORDS: Annealing; rice starch; gelatinization; rheological behavior; dynamic viscoelasticity; differential scanning calorimetry (DSC); leached out amylose

INTRODUCTION

To modify the thermal and rheological properties of starch, two hydrothermal treatments, annealing, and heat-moisture treatment that are based on the combination of the moisture with the heating, are commonly used (*1*−*3*). In the annealing, starch is incubated in excess water (>60%) or at intermediate water content (40−55%) at a temperature over the glass transition temperature and below the gelatinization temperature, T_{gel}, of the starch granule (*2*−*4*). In the heat-moisture treatment, starch is kept at low moisture content (18−27%), usually at a higher temperature like 100 °C (*5*, *6*). Annealing, which mainly increases T_{gel} and the degree of the starch crystallization (*2*, *3*), is well-known as an easy, cheap, and safe method to improve physicochemical properties of starch compared with chemical methods, cross-linking, etherification, and esterification. In addition, annealing is industrially helpful to improve the processing and the storage of starch foods which dominate the texture because annealing unconsciously may occur during a variety of food processing. For example, when the crop soaked in excess water is milled to isolate starch, the annealing of starch occurs (*7*, *8*). Further, the heating of choux paste before baking is an important process to make the specific shape of the choux puff (*9*), and this thermal treatment is similar to annealing (*10*).

However, the annealing effect on the viscosities of various starch suspensions has been the matter of debate. It has not been clarified whether the onset temperature at which the viscosity of the annealed starches starts to increase on heating, T_o, shifts to higher temperatures or to lower temperatures compared with that of the nonannealed sample, and whether the viscosity of starch suspension after annealing increases or decreases. There are some contradictions among the data as for the annealing effect as shown in **Table 1** referred from Jacobs and Delcour's review (*2*). For example, contradictory results were reported for the annealing effect on T_o of the viscosity for wheat starch obtained with the Brabender Visco-Amylograph (BVA) measurements; Hoover and Vasanthan (1994) reported that T_o after annealing increased by 0.5 °C on BVA (eighth row in **Table 1**; *15*), but Jacobs et al. (1995, 1996) reported that T_o shifted to lower temperatures by annealing, from 83 to 80 °C on BVA (ninth row; *16*, *17*). On the other hand, the annealing effect on T_o of rice starch depends on the measurement method. Although the value of T_o for the annealed rice starch shifted to lower temperatures from 77 to 74 °C (14th row; *16*, *17*) on BVA, T_o

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Table 1. Classification of the Effect of Annealing on the Rheological Behavior for the Starch Suspensions in Terms of the Origin of Starch and the Rheometers Used for Measurements Such as Brabender Visco-Amylograph (BVA), Rapid Visco-Analyzer (RVA), and Dynamic Viscoelastic Meter

	annealing			viscosity			literature
rheometer	starch	T_a[a] (°C)	time (h)	starch concn (%)	T_o[b,f]	V_p[c,f]	cited
Ostwald Viscometer	potato	52	0.5–10	1	+	−	11[d]
Corn Industries Viscometer	potato	49	18	5	+	−	12[d]
Brabender Visco-Amylograph (BVA)	potato	52	92	5	=	−	1[d]
		50, 52.5, 55	1.0–20	4	+	−	13
		50	72	5	+	−	14[d]
		50	72	6	+	−	15[d]
		50	24	6.6	+	−	16[d], 17[d]
	wheat	50	72	6	+	+	15[d]
		45	24	6.6	−	+	16[d], 17[d]
	lentil	50	72	6	+	−	15[d]
	oat	50	72	6	+	+	15[d]
	rye	50	1	6.3	+	−	18[d]
	pea	50	24	6.6	=	+	16[d], 17[d]
	rice	55	24	6.6	−	+	16[d], 17[d]
Rapid Visco-Analyzer (RVA)	potato	50	24	6.6	+	−	16[d], 17[d]
		52	16	6.6	+	−	19[d]
	wheat	45	24	6.6	−	+	16[d], 17[d]
	pea	50	24	6.6	+	+	16[d], 17[d]
	rice	55	24	6.6	=	+	16[d], 17[d]
	cassava	50	24–192	15.3	+	−	20
	finger millet	50	48		=	−	21
Dynamic Viscoelastic Meter	potato	50	20	15	+	−	22
	potato (Danshaku)	50	20	15	=	=	22
	wheat	50	20	15	+	−	22
	corn	50	20	15	+	−	22
	tulip	50	20	15	+	=	22
	katakuri[e]	50	20	15	+	=	22
	rice	T_{a1}: 48, 55	0.25	10	+	=	present study
		T_{a2}: 58, 60, 62	0.25	10	+	=	present study
		T_{a3}: 65, 68, 70, 73	0.25	10	−	=	present study

[a] Annealing temperature. [b] Onset temperature at which the viscosity of starch suspension starts to increase. [c] Peak viscosity of the sample during heating. [d] From Jacobs and Delcour's review (*2*). [e] *Erythronium japonicum*. [f] +, increase; −, decrease; and =, unchanged.

~ 85 °C determined by a Rapid Visco-Analyzer (RVA) was not changed by annealing (19th row; *16*, *17*). In the present study, the dynamic viscoelastic measurements were performed, and it is found that T_o of the annealed rice starch shifted to higher temperatures from 63 to 64 °C, when rice starch suspension was annealed in almost the same temperature range used in BVA and RVA (48 and 55 °C, 28th row). Furthermore, although the annealing effect on the peak viscosity (V_p) obtained by BVA and RVA depends on the origin of starch, the annealing effect on V_p of wheat (24th row; *22*) and rice starches (28th row; the present study) obtained by the dynamic viscoelastic measurement is different from that obtained by BVA (8th, 9th, and 14th row; *15*−*17*) and RVA (17th and 19th row; *16*, *17*). Therefore, origins of this inconsistency are caused possibly by (a) the measurement method, i.e., rheometer, heating rate, and the sample volume used for the measurements and/or by (b) the origin of starch, i.e., the starch composition such as amylose−lipid complex, and the size and shape of starch granules. For the former, BVA and RVA, which are commonly used to investigate the behavior of the viscosity for starch suspension, apply a strain or stress which is large enough to destroy a weak starch gel being formed on heating. The measurements of the dynamic shear viscoelasticity and the storage and the loss moduli, G' and G'', have an advantage in understanding the true annealing effect on the viscosity in comparison to these two rheometers, BVA and RVA, because a strain or stress which is low enough not to break the starch gel structure is required for the measurements. In addition, the heating rate used for the RVA measurements is much higher than that used for the BVA measurements and the dynamic viscoelastic measurements, i.e., BVA, 1.5 °C/min; RVA, 6.43 or 13 °C/min; and the dynamic viscoelastic measurements, 1 °C/min. The sample volume used for the measurements differs among the measurement methods, i.e., BVA, 500 mL; RVA, 25 mL; and the dynamic viscoelastic measurements, 2−3 mL.

Although rice starch foods are frequently eaten in Asia, there has been no paper on rice starch that has reported the annealing effect on the rheological behavior in the dynamic viscoelastic measurements, as far as we know. For the annealed potato, potato (Danshaku), wheat, corn, tulip, and katakuri (*Erythronium japonicum*) starches, the annealing effects on the viscosity obtained with the dynamic viscoelastic measurements were reported by Sekine et al. (*22*), as follows. The onset temperature (T_o) of G', a temperature at which G' starts to increase, for those annealed samples increased or unchanged, and the peak of G' on heating decreased or unchanged compared with that of each nonannealed sample, but the origin of the increase in T_o of G' and that of the decrease in G' on heating had been hardly discussed (*22*). In addition, a study for the effect of annealing temperature (T_a) on the viscosity of starch suspension has never been performed.

The aims of the present paper are to obtain reliable data for the annealing effect on the rheological behavior of rice starch on the dynamic viscoelastic measurements and to understand the origin of annealing effect in combination with the results obtained by the differential scanning calorimetry (DSC) measurements, and the estimation of the amount of leached out amylose and the swelling ratio of starch suspension. Further, the effect of the T_a on the viscosity of starch suspension is investigated, because the obtained information is helpful to understand the change of physicochemical properties of the

Table 2. Composition of Dry Rice Starch

moisture	13.2%
protein	0.3%
lipid[a]	0.5%
ash	0.1%
amylose content[b]	9.7%
mean granular diameter	5.77 ± 0.04 μm

[a] Estimated by acid hydrolysis. [b] Method of McCready and Hassid (*28*).

starch foods and to control the texture of rice starch foods which are frequently eaten in Japan.

Moreover, the study of rice starch will be important to understand the effects of the size of starch granule on annealing because the granule size of rice starch is smaller than that of other starches such as corn and potato starches (*23*−*25*) commonly used in industry. Since rice starch used in the present study contains the amylose−lipid complex which prevents starch granules from swelling (*26*, *27*), information for the annealing effect on the viscosity of starch suspension including amylose−lipid complex will be obtained. It will be useful to understand the effect of starch composition on annealing because the investigation of rice starch will complement an accumulation of the study such as **Table 1** to consider the annealing effect in terms of the starch composition.

MATERIALS AND METHODS

Materials. Nonwaxy rice starch was kindly supplied by Shimada Kagaku Kogyo. Co. Ltd. (Niigata, Japan). The composition of dry rice starch is shown in **Table 2**. The content of lipid was estimated by acid hydrolysis. Amylose content was determined from the standard curve made by using amylose (AS-1000, weight-average molecular weight = 1000000, Ajinoki Co., Aichi, Japan) and amylopectin (from potato starch, Nacalai Tesque Inc., Kyoto, Japan). This quantitative analysis was carried out by the method of McCready and Hassid (*28*). The mean granular diameter of native rice starch was estimated by using laser diffraction particle size analyzer (SALD-200V ER, Shimadzu Co., Kyoto, Japan).

Preparation of Rice Starch Suspension with and without Annealing for the Dynamic Viscoelastic Measurements. To make 10 wt % rice starch suspension based on the moisture content (13.2%) in the dry starch, distilled water (88.5 g) and starch (11.5 g) were put into a 300 mL Erlenmeyer flask, and starch was dispersed at 25 °C for 90 min with degassing under vacuum to remove the air. The moisture content in the dry starch was taken into account during the preparation of all the samples in all the measurements. A part of the soaked sample was taken out with stirring and was quickly used for the dynamic shear viscoelastic measurements. There was not any decantation of the sample. It is written as "control" or "25 °C" in the present study. Further, to make the annealed samples, the other soaked suspensions were heated from 25 °C to the annealing temperature (T_a), while maintaining stirring. After the temperature of those samples had reached each T_a, the samples were held at the same T_a with continued stirring for 15 min. The prepared annealed samples were immediately used for the dynamic shear viscoelastic measurements, after they were taken out in the same way as the control.

Dynamic Viscoelastic Measurements. The dynamic viscoelastic measurements were carried out by a strain controlled rheometer (Rheosol G-3000, UBM Co., Kyoto, Japan). The prepared suspension or the annealed sample was placed in the gap (51 μm) between the cone (angle, 3.964°; diameter, 39.95 mm) and the plate. The sample between the cone and the plate was covered by a semi-hermetic case, and a small amount of 100 cSt silicone oil was put around the sample to prevent the evaporation of water in the sample on heating. The sinusoidal oscillation of 0.039° (strain, 0.01) at 1.28 Hz was applied, and temperature was raised at 1 °C/min. The temperature at which the measurement of the samples annealed at each T_a started was slightly lower than each T_a because the temperature of the annealed samples is lowered during placing of the sample in the plates. The actual starting temperature of the dynamic viscoelastic measurements for 10 wt % rice starch suspension with and without annealing at different temperatures for 15 min is shown in **Table 3**. The way T_a is selected in the present study is mentioned later. The onset temperature of the storage and the loss moduli, G' and G'', is defined as a temperature at which G' and G'' start to increase on heating, and is written as "T_o" in the present study.

Table 3. Actual Starting Temperature of the Dynamic Viscoelastic Measurements for 10 wt % Rice Starch Suspension with and without Annealing at Different Temperatures for 15 min

T_a[a] (°C)	actual starting temp (°C)
25 (control)	25
48 (T_{o1})	40
55	45
58	45
60	48
62 (T_{p1}, T_{gel})	50
65	50
68	50
70	50
73 (T_{c1})	50

[a] Annealing temperature.

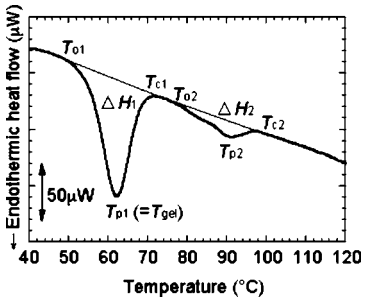

Figure 1. Typical differential scanning calorimetry (DSC) heating curve for 10 wt % rice starch suspension without annealing. Heating rate is 1 °C/min. T_{gel} is the gelatinization temperature. For the definition of the thermal characteristics, T_{o1}, T_{p1}, T_{c1}, ΔH_1, T_{o2}, T_{p2}, T_{c2}, and ΔH_2, see text.

Differential Scanning Calorimetry (DSC) Measurements. Figure 1 shows a typical differential scanning calorimetry (DSC) heating curve of 10 wt % rice starch suspension without annealing. The DSC measurements were performed by DSC6100 (Seiko Instruments Inc., Chiba, Japan). Two endothermic peaks are observed, when 10 wt % nonwaxy rice starch suspension is heated. It is well-known that the lower temperature DSC peak is caused by melting of amylopectin and the higher one by melting of amylose−lipid complex (*29*). The temperature at which amylopectin starts melting is called the onset temperature of the lower temperature DSC peak (T_{o1}), and the temperature at which it finishes melting is called the conclusion temperature of the peak (T_{c1}). The peak temperature is designated as T_{p1} and is called "the gelatinization temperature", T_{gel}, in the present study. Characteristic temperatures, T_{o2}, T_{c2}, and T_{p2} of the higher temperature DSC peak obtained by the melting process of amylose−lipid complex are also defined in the same manner. The gelatinization enthalpies, ΔH_1 and ΔH_2, are evaluated by integrating each peak.

The starch (4.6 mg) and distilled water (35.4 mg) were put into a 70 μL Ag pan, and the total weight of the sample was 40.0 mg. Then, the sample pan was sealed and kept at 25 °C for 90 min. A reference pan was filled with distilled water of the same weight of the sample (40.0 mg). The thermal treatment of all the sample preparations mentioned later was carried out in a calorimeter. The sample and the reference pans were placed in a calorimeter, heated from 25 to 120 °C, and this sample pan is called "control" or "25 °C" in the present study. To make the annealed samples, the other soaked samples without annealing were heated from 25 °C to each T_a with the reference pan and held at the same T_a for 15 min. The annealed samples were cooled

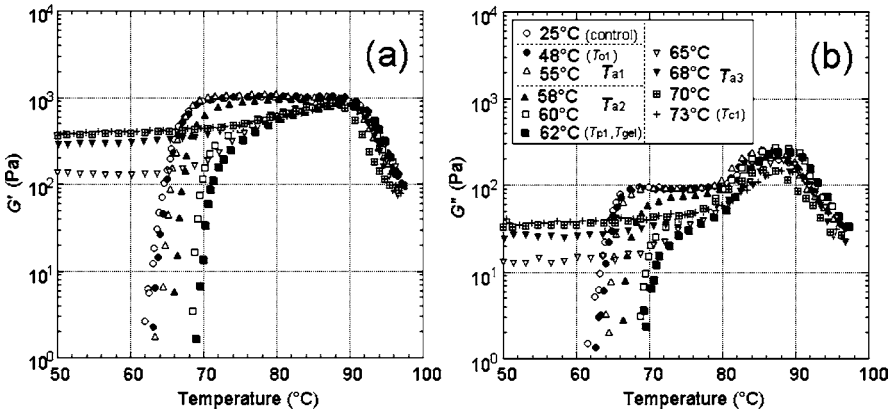

Figure 2. Temperature dependence of (**a**) the storage modulus, G', and (**b**) the loss modulus, G'', for 10 wt % rice starch suspension with and without annealing at different temperatures for 15 min. The condition of dynamic shear viscoelastic measurements is as follows: strain, 0.01; frequency, 1.28 Hz; geometry, cone and plate (angle, 3.964°; diameter, 39.95 mm); and heating rate, 1 °C/min. T_a and T_{gel} are annealing temperature and the gelatinization temperature, respectively.

to 25 °C, held at 25 °C for 5 min, and reheated to 120 °C. All the heating and cooling rates were 1 °C/min. To make the baseline stable, the selected cooling temperature of the annealed samples used for the DSC measurements was lower than that used for the dynamic viscoelastic measurements.

Estimation of the Amount of Leached Out Amylose and the Swelling Ratio for the Starch Suspension. The estimation of the amount of leached out amylose and the swelling ratio for the starch suspension give a good indication of the degree of the starch gelatinization because they increase on the starch gelatinization (*15*, *26*, *30*).

The dry rice starch (0.345 g) and distilled water (9.655 g) were put in a 20 mL screw-cap tube (diameter, 25 mm; height, 45 mm), the total sample weight was 10.00 g, and the concentration of the prepared sample was 3 wt %, based on the reported papers (*26*, *30*). All the samples were soaked with stirring at 25 °C for 90 min. The thermal treatment of all the sample preparations was carried out in a glass bath (diameter, 100 mm; height, 80 mm) with a circulator, Digital Temperature Controller (PolyScience, Pennsylvania). A soaked sample was heated from 25 to 80 °C at 1 °C/min, and this is called "control" or "25 °C" in the present study. The other soaked samples were heated from 25 °C to various T_a's (58, 62, 65, and 70 °C) while maintaining stirring. After the temperature of these samples had reached each T_a, the samples were held at the same T_a with continued stirring for 15 min. When these annealed samples were cooled to 50 °C, they were heated from 50 to 80 °C at 1 °C/min. All the samples including the control were taken out at 50, 60, 70, and 80 °C on heating and were stirred at 25 °C. The cooled samples were centrifuged at 1500*g* for 20 min at 25 °C (CENTRIFUGE 05P-21, Hitachi Koki Co. Ltd., Tokyo, Japan). The samples precipitated after centrifugation were weighed, and their swelling ratios, = (the weights of the samples precipitated by centrifuge)/(the weight of the dry starch, 0.345 g), were estimated. The amount of amylose per unit volume in the supernatant made by centrifugation was estimated by the method of Chrastil (*31*) because the time soaked in NaOH solution of the sample in this method is much shorter than that of McCready and Hassid's method (*28*), and the amount of leached out amylose for many samples can be estimated. The standard curve was made by using only amylose (from potato starch, Sigma-Aldrich Co., Missouri).

Determination of Annealing Temperature (T_a). The general definition of annealing is that starch is kept at a temperature above the glass transition temperature and below the gelatinization temperature, T_{gel}, of the starch granule in sufficient water (*2*−*4*). In the present study, T_a was selected in the wide temperature range from T_{o1} (48 °C), which is much lower than T_{gel} (=62 °C, T_{p1}), to T_{c1} (73 °C), which is much higher than T_{gel}, to understand in detail the effect of T_a on the rheological behavior of the starch gelatinization. At first, T_{o1} (48 °C), T_{p1} (62 °C, T_{gel}), and T_{c1} (73 °C) were chosen as T_a (**Figure 1**). Temperatures 55, 58, 60, 65, 68, and 70 °C were chosen as the other T_a's to investigate the intermediate state (**Figure 1**). Nine totally different T_a's were used, and annealing time was 15 min for each annealing. Rice starch suspension without annealing was shown as "control" or "25 °C" in this paper.

RESULTS AND DISCUSSION

Effect of Annealing Temperature (T_a) on the Complex Shear Moduli, G' and G'', Obtained by the Dynamic Viscoelastic Measurements of Rice Starch Suspension on Heating. Figure 2 shows the temperature dependence of (**a**) the storage modulus, G', and (**b**) the loss modulus, G'', for 10 wt % rice starch suspension with and without annealing at different temperatures for 15 min. In a temperature range from about 60 to 70 °C for the nonannealed rice starch suspension, the control, G' and G'' sharply increased from 1×10^0 Pa to 1×10^3 Pa and from 1×10^0 Pa to 1×10^2 Pa, respectively. It was reported by Eliasson (*30*) that the large increase of G' for the nonannealed starch suspensions on heating is caused by the small increase of the amount of leached out amylose and the swelling ratio of the starch suspension. It is suggested that this large increase of G' and G'' observed in the present study was also caused by the small increase of the amount of leached out amylose and the swelling ratio because a temperature at which leached out amylose and swelling ratio start to increase was almost the same as the onset temperature (T_o) of G' and G'', a temperature at which G' and G'' start to increase, as we will discuss later. Further, the storage and the loss moduli, G' and G'', were almost constant values, 1×10^3 Pa for G' from 70 to 90 °C and 1×10^2 Pa for G'' from 70 to 80 °C. The storage modulus, G', in the temperature range from 90 to 97 °C decreased from 1×10^3 to 1×10^2 Pa. The loss modulus, G'', in the temperature range from 80 °C to about 88 °C increased from 1×10^2 Pa to 2×10^2 Pa, and G'' in the temperature range from 88 to 97 °C decreased to approximately 3×10^1 Pa. This decrease of G' and G'' observed in the temperature range from 90 to 97 °C was induced by melting of amylose−lipid complex because the removal of lipid in the starch granule makes the higher temperature DSC peak disappear (*32*, *33*).

The changes of G' and G'' on heating for rice starch suspension annealed at a temperature from T_{o1} (48 °C) to T_{c1} (73 °C) were classified into three types in terms of annealing temperature, T_a (T_{a1}, 48 and 55 °C; T_{a2}, 58, 60, and 62 °C; and T_{a3}, 65, 68, 70, and 73 °C), as follows.

T_{a1} (T_a = 48 and 55 °C) is much lower than the gelatinization temperature, T_{gel}: The effect of annealing at a temperature close to T_{o1} (T_a = 48 and 55 °C) on the rheological behavior of the

starch suspension was hardly observed. Only a small effect, the increase in T_o of G' and G'' compared with that of the control was observed.

T_{a3} (T_a = 65, 68, 70, and 73 °C) is much higher than T_{gel}: In the temperature range from 50 to 65 °C, which is lower than T_{gel} (=T_{p1}, 62 °C), G' and G'' for the samples annealed at a temperature from about T_{p1} (62 °C, T_{gel}) to T_{c1} (73 °C) (T_a = 65, 68, 70, and 73 °C) showed $1-4 \times 10^2$ and $1-4 \times 10^1$ Pa at 50 °C and gradually increased to 1×10^3 Pa at 90 °C and to 2×10^2 Pa at about 88 °C, respectively. These larger values of G' and G'' for the samples annealed at T_{a3} than those for the control in the temperature range from 50 to 65 °C were caused by the starch gelatinization because T_{a3} is a much higher temperature than T_{gel}. In the temperature range from 65 to 90 °C, G' and G'' for the samples annealed at T_{a3} were smaller than those of the control and those of the samples annealed at T_{a1} and 58 °C. In the temperature range from 90 to 97 °C, G' and G'' decreased to 1×10^2 Pa and to about 3×10^1 Pa, respectively. Since the values of G' and G'' were almost the same as those of the control in the temperature range from 90 to 97 °C, this decrease of G' and G'' was caused by the melting of amylose−lipid complex. The annealing effect on the melting of amylose−lipid complex was not observed.

T_{a2} (T_a = 58, 60, and 62 °C) is almost the same as T_{gel}: The storage and the loss moduli, G' and G'', of the samples annealed at a temperature from about T_{o1} (48 °C) to T_{p1} (=62 °C, T_{gel}) (T_a = 58, 60, and 62 °C) began to increase at about 68 °C, and the onset temperature, T_o, of G' and G'' for these annealed samples shifted to higher temperatures with increasing T_a compared with that of the control. In the temperature range from about 68 to 90 °C, G' and G'' gradually increased from about 1×10^0 to 1×10^3 Pa and from 1×10^0 to 2×10^2 Pa, respectively, and they showed smaller values than those of the control in this temperature range. As for the reason for these smaller values of G' and G'' in the temperature range from about 68 to 90 °C, the possibility of the degradation of amylose and amylopectin by the enzyme contained in the starch samples is excluded, although it is well-known that the decrease of the viscosity of starch suspension is caused by the enzymatic degradation (*34*−*37*). The experimental finding that G' and G'' in the temperature range from 90 to 97 °C for the samples annealed at T_{a2} were almost the same as those of the nonannealed sample, the control, supports this conclusion. Although T_{a2} (T_a = 58, 60, and 62 °C) lies in the optimum temperature ranges of the highest enzyme activities (*34*, *37*), the enzymatic degradation did not occur. Therefore, G' and G'' in the temperature range from about 68 to 90 °C for the samples annealed at T_{a2} showed smaller values than those of the control, not because of the enzymatic degradation but because of annealing.

To clarify the effects of annealing temperature (T_a) on the rheological behavior of the starch suspension, it is essential to understand the origin of (a) the smaller values of G' and G'' for the samples annealed at T_{a2} (T_a = 58, 60, and 62 °C) and T_{a3} (T_a = 65, 68, 70, and 73 °C) than those of the control in the temperature range from 65 to 90 °C and (b) the increase in T_o of G' and G'' for those annealed at T_{a2}. Thus, we will discuss in detail the origin of these two behaviors later based on the other two measurements, the estimation of the amount of leached out amylose, and the swelling ratio for rice starch suspension annealed at a temperature range from T_{o1} (48 °C) to T_{c1} (73 °C) on heating.

Effect of Annealing Temperature (T_a) on the Thermal Characteristics of Rice Starch Suspension. Figure 3 shows

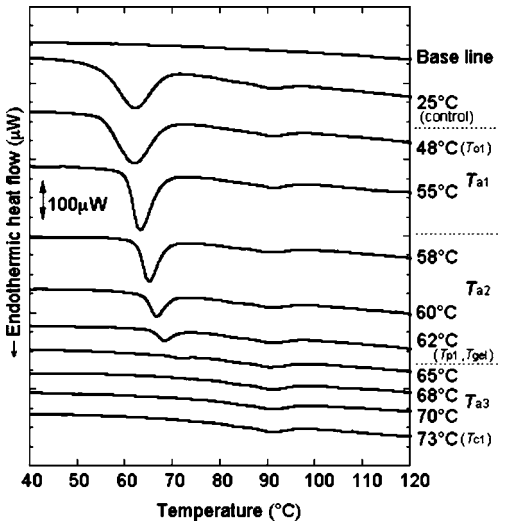

Figure 3. DSC heating curves of 10 wt % rice starch suspension with and without annealing at different temperatures for 15 min. Heating rate is 1 °C/min.

DSC heating curves of 10 wt % rice starch suspension with and without annealing at different temperatures for 15 min. **Figure 4** shows the effects of annealing temperature (T_a) on the thermal characteristics of (**a**) the lower temperature DSC peak, T_{o1}, T_{p1}, T_{c1}, and ΔH_1, and (**b**) the higher temperature DSC peak, T_{o2}, T_{p2}, T_{c2}, and ΔH_2 for 10 wt % rice starch suspension. The changes of T_{o1}, T_{p1}, T_{c1}, and ΔH_1 for the samples annealed at a temperature from T_{o1} (48 °C) to T_{c1} (73 °C) were classified into three types in terms of annealing temperature, T_a (T_{a1}, 48 and 55 °C; T_{a2}, 58, 60, and 62 °C; and T_{a3}, 65, 68, 70, and 73 °C) (**Figure 4a**), just as in the previous section. All the thermal characteristics for the melting of amylose−lipid complex, T_{o2}, T_{p2}, T_{c2}, and ΔH_2 for rice starch were unchanged by annealing at all the T_a's (**Figure 4b**) because amylose−lipid complex cannot melt during annealing at all the T_a's. This is the reason G' and G'' for all the samples including the control in the temperature range from 90 to 97 °C are the same.

T_{a1} (T_a = 48 and 55 °C) is much lower than the gelatinization temperature, T_{gel}: T_{o1} and T_{p1} for the samples annealed at a temperature close to T_{o1} (T_a = 48 and 55 °C) shifted to higher temperatures compared with those of the nonannealed rice starch, the control, and T_{c1} unchanged by annealing. The gelatinization enthalpy, ΔH_1, of the sample annealed at 48 °C was slightly larger than that of the control. The width and the height of the lower temperature DSC peak for the sample annealed at 55 °C became narrower and higher in comparison with those of the control (**Figure 3**). It was reported that T_{o1}, T_{p1}, T_{c1}, and ΔH_1 of the annealed rice starches increased, and the lower temperature DSC peak of those samples became narrower and higher (*16*, *38*). This similar tendency was observed in the present study. The amorphous and thermally unstable portion in the starch becomes somewhat more crystalline by annealing, and the disintegration of this changed portion appears as the partial gelatinization, which is observed as the increase in ΔH_1 for the samples annealed at 48 °C, and the decrease of the width and the increase of the height of the lower temperature DSC peak for the sample annealed at 55 °C. The crystalline and thermally stable portion in the starch is not disintegrated at a temperature below T_{a1}, but this portion can begin to be disintegrated at a temperature above T_{a1} so that T_{o1} of the samples annealed at T_{a1} shifted to higher temperatures.

T_{a3} (T_a = 65, 68, 70, and 73 °C) is much higher than T_{gel}:

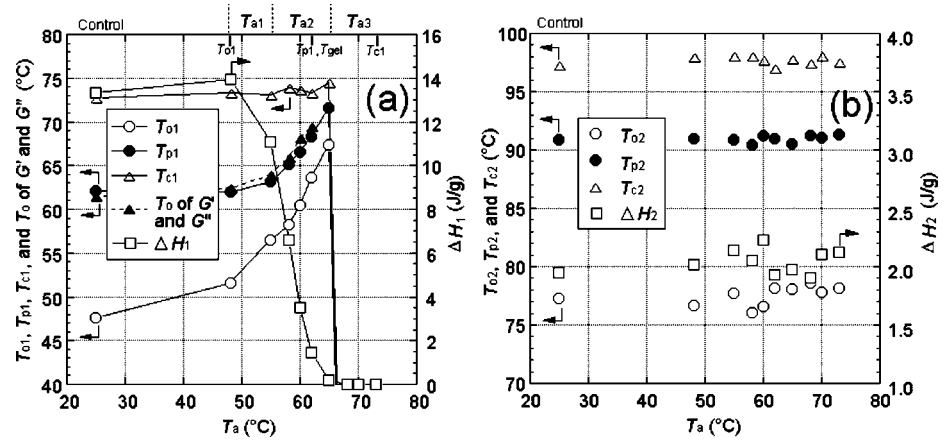

Figure 4. Effects of annealing temperature (T_a) on the thermal characteristics of (**a**) the lower temperature DSC peak: T_{o1}, T_{p1}, T_{c1}, and ΔH_1, and (**b**) the higher temperature DSC peak: T_{o2}, T_{p2}, T_{c2}, and ΔH_2 for 10 wt % rice starch suspension. Heating rate is 1 °C/min. The onset temperature (T_o) of G' and G'', a temperature at which G' and G'' start to increase, is also shown in **Figure 4a** for comparison.

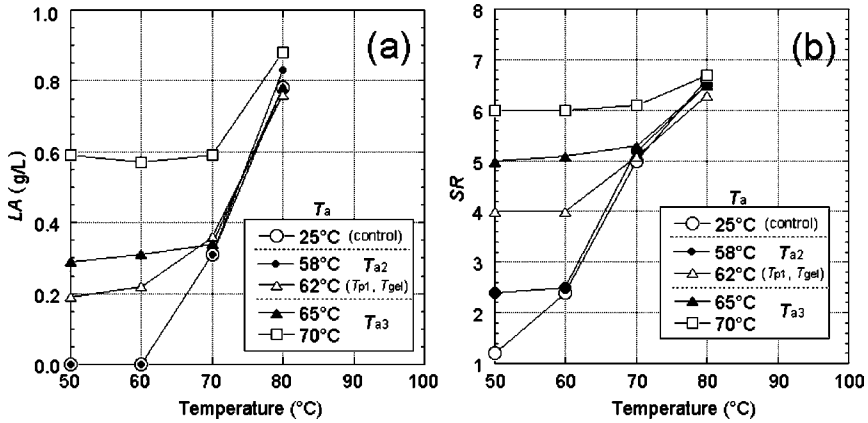

Figure 5. Temperature dependence of (**a**) the amount of leached out amylose and (**b**) the swelling ratio for 3 wt % rice starch suspension with and without annealing at different temperatures for 15 min. The amount of amylose per unit volume in the supernatant made by centrifugation (1500*g* for 20 min at 25 °C) was estimated by the method of Chrastil (*31*). The swelling ratio was estimated as "(the weight of the sample precipitated by centrifuge)/(the weight of dry starch)". Heating rate is 1 °C/min. T_a and T_{gel} are annealing temperature and the gelatinization temperature, respectively.

The lower temperature DSC peak of the samples annealed at a temperature from about T_{p1} (=62 °C, T_{gel}) to T_{c1} (73 °C) (T_a = 65, 68, 70, and 73 °C) disappeared completely, which means that the amylopectin portion has melted at T_{a3}, and the partial gelatinization has already occurred on annealing. It is suggested that the melting of amylopectin for the samples annealed at T_{a3} increases the viscosity and resulted in the larger values of G' and G'' for the samples annealed at T_{a3} than those for the control in the temperature range from 50 to 65 °C, even at lower temperatures than T_{gel}, 62 °C.

T_{a2} (T_a = 58, 60, and 62 °C) is almost the same as T_{gel}: T_{o1} and T_{p1} for the samples annealed at a temperature from about T_{o1} (48 °C) to T_{p1} (=62 °C, T_{gel}) (T_a = 58, 60, and 62 °C) shifted to higher temperatures compared with those of the control. The increase of T_{o1} and T_{p1} with increasing T_a means that the temperature at which the annealed samples are gelatinized became higher than that of the control, which was consistent with the increase in the onset temperature, T_o, of G' and G'' for the same samples (**Figure 4a**). Further, ΔH_1 of these three annealed samples decreased with increasing T_a compared with that of the control. The increase of T_{o1} and T_{p1}, and this decrease of ΔH_1 for the samples annealed at T_{a2} are similar to the results in the reported papers (*39*−*41*). The thermally unstable portion in the starch is disintegrated at a temperature below T_{a2}, and this is also the partial gelatinization. The remaining more crystalline and stable portion can be disintegrated at a temperature above T_{a2} so that T_{o1} of the samples annealed at T_{a2} shifted to higher temperatures compared with that of the control. Since the portion disintegrated by annealing does not contribute to ΔH_1 , and only the thermally stable portion which is not disintegrated at T_{a2} contributes to ΔH_1, the enthalpy of the samples annealed at T_{a2} became smaller compared with that of the control.

Effect of Annealing Temperature (T_a) on the Amount of Leached Out Amylose and Swelling Ratio of Starch Suspension on Heating. Figure 5 shows the temperature dependence of (**a**) the amount of leached out amylose and (**b**) the swelling ratio for 3 wt % rice starch suspension with and without annealing at different temperatures for 15 min. It was reported that the increase of G' and G'' for the nonannealed starch suspensions was caused by the increase of leached out amylose and swelling ratio on heating (*30*). It is suggested that the increase of leached out amylose and swelling ratio for the nonannealed sample, the control, observed in the present study also causes the increase of G' and G'' because the temperature at which leached out amylose and swelling ratio start to increase was almost the same as T_o of G' and G'', the temperature at which G' and G'' start to increase, as shown in **Figures 2** and **5**. The amount of leached out amylose and the swelling ratio of the annealed samples were almost constant at a temperature lower than each T_a, and they increased at a temperature higher than each T_a. The effects of annealing temperature, T_a, on

leached out amylose and swelling ratio of the starch suspension on heating were not classified into three types such as the classification of the effects of T_a on the rheological and thermal properties of the starch suspension on heating. In the case of the samples annealed at T_{a2} (T_a = 58, 60, and 62 °C), it is suggested that the increase in T_o of G' and G'' with increasing T_a and the smaller values of G' and G'' than those of the control in the temperature range from 65 to 90 °C are caused by the partial gelatinization. The samples annealed at T_{a2} consist of leached out amylose and the starch granules that have not been disintegrated by annealing because the starch gelatinization of those annealed samples is not completed as shown in the results obtained by the DSC measurements (**Figures 3** and **4a**). The leached out amylose prevents further amylose from leaching out. Therefore, the increase of the gelatinization temperature, T_{gel}, and the smaller values of G' and G'' for the samples annealed at T_{a2} were observed in the temperature range from 65 to 90 °C. For the samples annealed at T_{a3} (T_a = 65, 68, 70, and 73 °C), G' and G'' were larger than those of the control in the temperature range from 50 to 65 °C as shown in **Figure 2** because the starch gelatinization is almost completed, i.e., the disappearance of the lower temperature DSC peak (**Figures 3** and **4a**), and the amount of leached out amylose is enough to make a starch gel. In the temperature range from 65 to 90 °C, G' and G'' of the samples annealed at T_{a3} were smaller than those of the control, and they were almost the same as those of the samples annealed at T_{a2}. It is suggested that these smaller values of G' and G'' for the samples annealed at T_{a3} than those for the control in the temperature range from 65 to 90 °C are caused by the same origin to explain the rheological behavior for those annealed at T_{a2}, the prevention of further leaching out of amylose from the starch granule.

Conclusion. The effects of annealing on the rheological behavior of 10 wt % rice starch suspension are classified into three types in terms of annealing temperature (T_a): T_{a1}, 48 and 55 °C, which are much lower than the gelatinization temperature, T_{gel} (=62 °C); T_{a2}, 58, 60, and 62 °C, which are almost the same as T_{gel}; and T_{a3}, 65, 68, 70, and 73 °C, which are much higher than T_{gel}. The annealing at T_{a1} did not affect significantly the physicochemical properties of the starch suspension, and the storage modulus, G', begins to increase at about 65 °C and remains constant about 1×10^3 Pa at 90 °C. When the starch suspension is annealed at T_{a2}, G' begins to increase at 68 °C; the temperature at which G' starts to increase, T_o of G', shifts to higher temperatures compared with that of the nonannealed sample, the control. Moreover, G' gradually increases from 1×10^0 to 1×10^3 Pa in the temperature range from 68 to 90 °C, and it is smaller than that of the control in this temperature range. For the samples annealed at T_{a2}, the increase in T_o of G' with increasing T_a and the smaller value of G' in the temperature range from about 68 to 90 °C mean that the starch gelatinization delays, and the partial gelatinization occurs during annealing at T_{a2}. It is suggested that the samples annealed at T_{a2} are composed of leached out amylose and the starch granules, and only the small amount of leached out amylose is enough to prevent further amylose from leaching out. The storage modulus, G', of the samples annealed at T_{a3} shows $1-4 \times 10^2$ Pa at 50 °C and gradually increases to 1×10^3 Pa at 90 °C. In the temperature range from 50 to 65 °C, G' of those annealed at T_{a3} is larger than that of the control, but it is smaller than that of the control in the temperature range from 65 to 90 °C. This increase of G' in the temperature range from 50 to 65 °C is caused by the starch gelatinization being almost completed during annealing at T_{a3}, and the small amount of leached out amylose made by the gelatinization makes a starch gel. The origin of the smaller value of G' for the samples annealed at T_{a3} than that of the control in the temperature range from 65 to 90 °C is the same as those annealed at T_{a2}, preventing further leaching out of amylose from the starch granule.

The classification of the annealing effects obtained in the present study is helpful in controlling the physicochemical properties of starch foods during the processing and storage in industry even for the starch from a different origin which has different rheological and thermal properties.

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LITERATURE CITED

(1) Stute, R. Hydrothermal modification of starches: The difference between annealing and heat/moisture-treatment. *Starch* **1992**, *44*, 205−214.

(2) Jacobs, H.; Delcour, J. A. Hydrothermal modifications of granular starch, with retention of the granular structure. *J. Agric. Food Chem.* **1998**, *46*, 2895−2905.

(3) Tester, R. F.; Debon, S. J. J. Annealing of starch. *Int. J. Biol. Macromol.* **2000**, *27*, 1−12.

(4) Larsson, I.; Eliasson, A. C. Annealing of starch at an intermediate water content. *Starch* **1991**, *43*, 227−231.

(5) Franco, C. M. L.; Ciacco, C. F.; Tavares, D. Q. Effect of the heat-moisture treatment on the enzymatic susceptibility of corn starch granules. *Starch* **1995**, *47*, 223−228.

(6) Collado, L. S.; Corke, H. Heat-moisture treatment effects on sweetpotato starches differing in amylose content. *Food Chem.* **1999**, *65*, 339−346.

(7) Watson, S. A.; Sanders, E. H. Steeping studies with corn endosperm sections. *Cereal Chem.* **1961**, *38*, 22−33.

(8) Krueger, B. R.; Knutson, C. A.; Inglett, G. E.; Walker, C. E. A differential scanning calorimetry study on the effect of annealing on gelatinization behavior of corn starch. *J. Food Sci.* **1987**, *52*, 715−718.

(9) Matsumoto, F.; Abe, N. Studies on the cookery with wheat flour: 7. Formation of the choux paste (1). *J. Home Econ. Jpn.* **1962**, *13*, 240−244 (in Japanese).

(10) Nishimura, K.; Imazuya, N.; Nakai, S. Optimum preparative method for storing cream puff paste without deterioration. *Food Sci. Technol. Int. Tokyo* **1998**, *4*, 18−24.

(11) Wiegel, V. E. Verlauf der Warmverkleisterung von Kartoffelstärke insbesondere bei verschiedener thermischer Vorgeschichte. *Kolloid-Z.* **1933**, *62*, 310−324.

(12) Hjermstad, E. T. Potato starch properties by controlled heating in aqueous suspension. U.S. Patent 3,578,497, 1971.

(13) Yamamoto, K.; Kaji, M.; Onogaki, T. Effects of warm water treatment on gelatinization properties of air-classified potato starches. *J. Jpn. Soc. Starch Sci.* **1983**, *30*, 276−283 (in Japanese).

(14) Kuge, T.; Kitamura, S. Annealing of starch granules: Warm water treatment and heat-moisture treatment. *J. Jpn. Soc. Starch Sci.* **1985**, *32*, 65−83 (in Japanese).

(15) Hoover, R.; Vasanthan, T. The effect of annealing on the physicochemical properties of wheat, oat, potato and lentil starches. *J. Food Biochem.* **1994**, *17*, 303−325.

(16) Jacobs, H.; Eerlingen, R. C.; Clauwaert, W.; Delcour, J. A. Influence of annealing on the pasting properties of starches from varying botanical sources. *Cereal Chem.* **1995**, *72*, 480−487.

(17) Jacobs, H.; Eerlingen, R. C.; Delcour, J. A. Factors affecting the visco-amylograph and rapid visco-analyzer evaluation of the impact of annealing on starch pasting properties. *Starch* **1996**, *48*, 266−270.

(18) Schierbaum, F.; Kettlitz, B. Studies on rye starch properties and modification: 3. Viscograph pasting characteristics of rye starches. *Starch* **1994**, *46*, 2−8.
(19) Eerlingen, R. C.; Jacobs, H.; Block, K.; Delcour, J. A. Effects of hydrothermal treatments on the rheological properties of potato starch. *Carbohydr. Res*. **1997**, *297*, 347−356.
(20) Gomes, A. M. M.; Mendes da Silva, C. E.; Ricardo, N. M. P. S.; Sasaki, J. M.; Germani, R. Impact of annealing on the physicochemical properties of unfermented cassava starch ("*Polvilho Doce*"). *Starch* **2004**, *56*, 419−423.
(21) Adebowale, K. O.; Afolabi, T. A.; Olu-Owolabi, B. I. Hydrothermal treatments of Finger millet (*Eleusine coracana*) starch. *Food Hydrocolloids* **2005**, *19*, 974−983.
(22) Sekine, M.; Otobe, K.; Sugiyama, J.; Kawamura, Y. Effects of heating, vacuum-drying and steeping on gelatinization properties and dynamic viscoelasticity of various starches. *Starch* **2000**, *52*, 398−405.
(23) Li, J. Y.; Yeh, A. I. Relationships between thermal, rheological characteristics and swelling power for various starches. *J. Food Eng*. **2001**, *50*, 141−148.
(24) Jayakody, L.; Hoover, R. The effect of lintnerization on cereal starch granules. *Food Res. Int*. **2002**, *35*, 665−680.
(25) Srichuwong, S.; Sunarti, T. C.; Mishima, T.; Isono, N.; Hisamatsu, M. Starches from different botanical sources: 1. Contribution of amylopectin fine structure to thermal properties and enzyme digestibility. *Carbohydr. Polym.* **2005**, *60*, 529−538.
(26) Tester, R. F.; Morrison, W. R. Swelling and gelatinization of cereal starches: 1. Effects of amylopectin, amylose, and lipids. *Cereal Chem*. **1990**, *67*, 551−557.
(27) Sasaki, T.; Matsuki, J. Effect of wheat starch structure on swelling power. *Cereal Chem*. **1998**, *75*, 525−529.
(28) McCready, R. M.; Hassid, W. Z. The separation and quantitative estimation of amylose and amylopectin in potato starch. *J. Am. Chem. Soc.* **1943**, *65*, 1154−1157.
(29) Biliaderis, C. G.; Page, C. M.; Maurice, T. J.; Juliano, B. O. Thermal characterization of rice starches: A polymeric approach to phase transitions of granular starch. *J. Agric. Food Chem.* **1986**, *34*, 6−14.
(30) Eliasson, A. C. Viscoelastic behaviour during the gelatinization of starch: 1. Comparison of wheat, maize, potato and waxy-barley starches. *J. Texture Stud.* **1986**, *17*, 253−265.
(31) Chrastil, J. Improved colorimetric determination of amylose in starches or flours. *Carbohydr. Res*. **1987**, *159*, 154−158.
(32) Kugimiya, M.; Donovan, J. W.; Wong, R. Y. Phase transitions of amylose-lipid complexes in starches: A calorimetric study. *Starch* **1980**, *32*, 265−270.
(33) Hoover, R.; Vasanthan, T. Studies on isolation and characterization of starch from oat (*Avena nuda*) grains. *Carbohydr. Polym*. **1992**, *19*, 285−297.
(34) Özbek, B.; Yüceer, S. α-Amylase inactivation during wheat starch hydrolysis process. *Process Biochem*. **2001**, *37*, 87−95.
(35) Afoakwa, E. O.; Sefa-Dedeh, S. Changes in rheological properties and amylase activities of trifoliate yam, *Dioscorea dumetorum*, starch after harvest. *Food Chem.* **2002**, *77*, 285−291.
(36) González, C. F.; Fariña, J. I.; Figueroa, L. I. C. A critical assessment of a viscometric assay for measuring *Saccharomycopsis fibuligera* α-amylase activity on gelatinised cassava starch. *Enzyme Microb. Technol*. **2002**, *30*, 169−175.
(37) Apar, D. K.; Özbek, B. α-Amylase inactivation during rice starch hydrolysis. *Process Biochem*. **2005**, *40*, 1367−1379.
(38) Tester, R. F.; Morrison, W. R. Swelling and gelatinization of cereal starches: 2.Waxy rice starches. *Cereal Chem*. **1990**, *67*, 558−563.
(39) Nakazawa, F.; Noguchi, S.; Takahashi, J.; Takada, M. Thermal equilibrium state of starch-water mixture studied by differential scanning calorimetry. *Agric. Biol. Chem.* **1984**, *48*, 2647−2653.
(40) Knutson, C. A. Annealing of maize starches at elevated temperatures. *Cereal Chem.* **1990**, *67*, 376−384.
(41) Seow, C. C.; Teo, C. H. Annealing of granular rice starches: Interpretation of the effect on phase transitions associated with gelatinization. *Starch* **1993**, *45*, 345−351.

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