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Study on the granular characteristics of starches separated from Chinese rice cultivars

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ABSTRACT

Granule size distribution, the relative crystallinity, morphology and thermal degradation of starches from 10 different non-waxy rice cultivars were measured in present study. The relationships between granular structure and thermogravimetric parameters of tested starches were evaluated using Pearson correlation analysis. The range of median size for rice starches was 6.23–7.81 μm. The relative crystallinity of 10 non-waxy rice starches ranged from 20.4% to 33.4%. The range of activation energy from different rice starches was between 155.6 and 201.5 kJ/mol. The Pearson correlation results showed that the relative crystallinity was positively correlated ($r = 0.6750$, $p < 0.05$) with the percentage of branch chains with DP12–23. Furthermore, the activation energy of the rice starches showed a positive correlation ($r = 0.7903$, $p < 0.01$) with relative crystallinity.

1. Introduction

Starch is a biopolymer present in seeds, roots and stems of various plant sources, such as corn, rice, cassava and potato. Traditionally, starch is widely used in food industry as a thickening and gelling agent; whereas as a biodegradable and safe materials, starch is also used in industrial applications. However, the food and the thermoplastic materials industries are carried out at relatively high temperatures. In these processes, the thermal degradation parameters of starches have a direct influence on their physical properties and technological applications (Ma, Chang, Yu, & Wang, 2008; Ramis et al., 2004). Although starch from different botanical origin has identical structural units, different starches (even if starches are the same botanical origin) also exhibit various compacted structure and physicochemical properties. All the starches existed in the form of granules, their granular characteristic including granule size, specific surface area and crystal properties, have a great influence on the processing techniques and applications. Chiotelli and Le Meste (2002) reported that B-granules (smaller than 10 μm in diameter) had a higher affinity for water than the A-granules (generally larger than 10 μm in diameter) at room temperature, resulting in faster hydration. The granule size had a greater effect on reactivity when modified with acetic anhydride, smaller granule size fraction showed higher degree of substitute due to larger specific surface area (Huang, Schols, Jin, Sulmann, & Voragen, 2007). A-type crystals were found to be less soluble than the B-type, as indicated by preferential solubilization, the solubility decreased with the increase of chain length (Crochet, Beauxis-Lagrave, Noel, Parker, & Ring, 2005). Guinesi et al. studied the thermal degradation kinetic of starches from different sources, but found no significant differences between values of kinetic triplet of starches (Guinesi et al., 2006). There is no systematic research to evaluate the effect of granular characteristic of starch from the same botanical origin on the thermal degradation.

Rice starches were isolated from different rice cultivars in China. Their composition, rheological properties, thermal properties and amylopectin structure were previously reported. In the present work, they were further characterized by particle size analysis, crystal structure analysis and thermogravimetric analysis. The starch granular characteristics, including amylose content, relative crystallinity, granule size and the kinetic parameters activation energy (E), pre-exponential factor, (A), and their correlation were investigated, in order to reveal relationship between granular characteristics and the thermogravimetric parameters for further application in food industry.

2. Materials and methods

2.1. Materials

Ten rice cultivars, collected from different regions in China, were the samples previously prepared by Wang et al. (2010), who also reported on the amylose content and amylopectin chain length distribution of different rice starch. The amylose content

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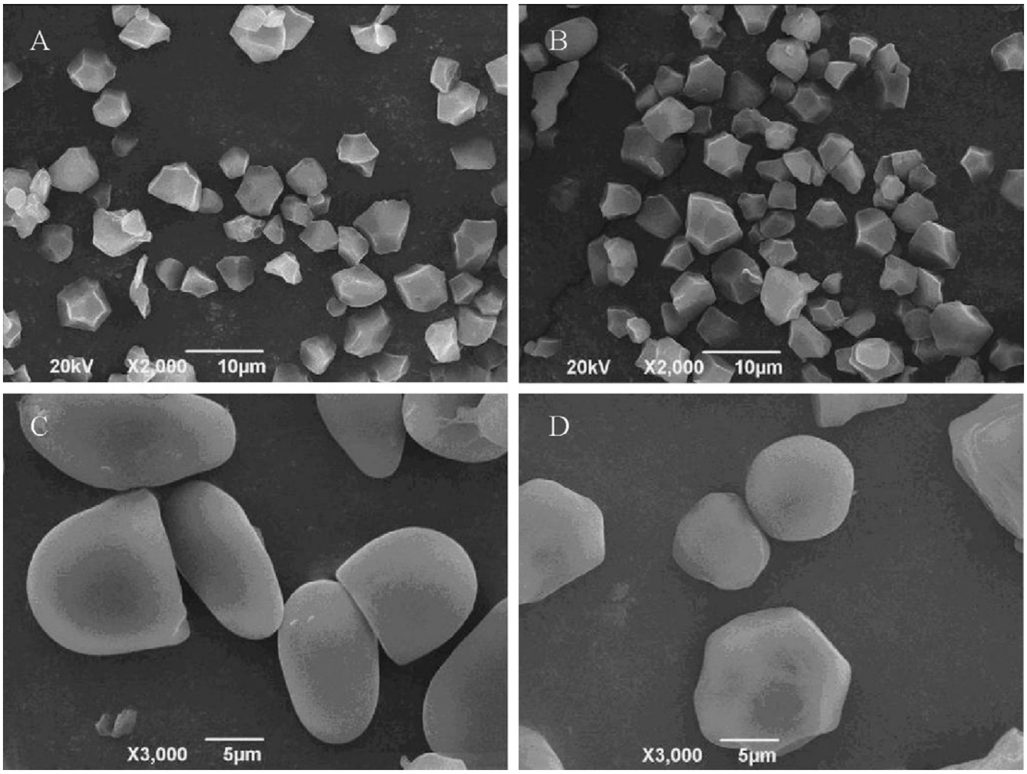

Fig. 1. SEM micrographs of rice starches: A, Exiang No.1; B, Tianjinxiaozhan; C, Tapioca; D, Maize starch (tapioca and maize starch as control).

of different rice starch ranged from 18.1% to 31.6%. Ten rice cultivars were collected from five regions in China: three cultivars, Exiang No.1, Honglianyou No.6, and Liangyoupeijiu, from Hubei Province; other three cultivars, Liangyoupeijiu, Zhongjian No.2, and 98112jing, from Hunan Province; the two cultivars, Tongjin611 and Jiyujinjing from Jilin Province; one cultivar, Tianjinxiaozhan from Tianjin municipality; other one cultivars, Heiyouzhan, from Guangzhou city. Exiang No.1, Honglianyou No.6, Liangyoupeijiu (from Hubei), Liangyoupeijiu (from Hunan), Zhongjian No.2 and Heiyouzhan were type *Indica* rice; while 98112jing, Tongjin611, Jiyujing and Tianjinxiaozhan were *Japonica* type rice. All cultivars were freshly-reaped seeds in 2005. Maize starch and tapioca were obtained from starch factory (Wu Han, China).

2.2. Scanning electron microscopy (SEM)

Scanning electron micrographs of samples were obtained with a scanning microscope (JSM-6390/LV, Japan), operating at an acceleration voltage of 20 kV. Micrographs were taken at 2000×, 3000× magnification, and the diameter of the starch granules was determined. Starch samples were suspended in ethanol to obtain a 1% suspension. One drop of the starch–ethanol solution was applied on an aluminum stub, dried for removing the ethanol, and then vacuum coated with gold–palladium (60:40) for SEM.

2.3. Size distribution

A laser particle size analyzer BT-9300H (Bettersize, China) was used to determine the size distribution of starch particle. A mount of starch was dispersed in ethanol regularly for the analysis of starches size distribution.

2.4. X-ray diffraction

Starch sample were equilibrated in a relative humidity (5% RH) at room temperature. X-ray diffraction analysis was performed with a D/max-RA III X-ray diffractometer (Rigaku Corporation, Tokyo, Japan), operated at 40 kV and 40 mA with the Cu Kα radiation. The sample was scanned through the 2θ (diffraction angle) from 3° to 50° at the speed of 8°/min. The degree of crystallinity was quantitatively estimated following the method of Ribotta, Cuffini, León, & Añón (2004). The crystallinity of starch was integrated on the JADE 5.0 software (Materials Data. Inc).

2.5. Thermogravimetry (TG)

Thermogravimetry (TG) and differential thermogravimetry (DTG) were performed in TG209C (Netzsch company, Germany). The TG/DTG curves of the starches were carried out under dynamic nitrogen atmosphere (60 mL/min), platinum crucible, sample mass around 7 mg and heating rates of 5, 10, 15 and 20 °C/min from 30 to 700 °C (Zhang, Xue, Mo, & Jin, 2006).

2.6. Statistical analysis

The data were subjected to correlation analysis and Pearson correlation coefficients were calculated using SAS for windows, version 9.0, TS (Inc., Cary, NC, USA) and the significance level was $P < 0.05$.

3. Results and discussions

3.1. Morphological characteristics

The scanning electron micrographs of native rice starches and other starches were shown in Fig. 1. The results indicated that the morphology of starches from the different rice cultivars was the same. It was suggested that starch from the same botanical origin possessed the same packing structure. The rice starch granules were angular and polygonal, with characteristic dimensions in the range 3–8 μm, which was the same with the previous reports

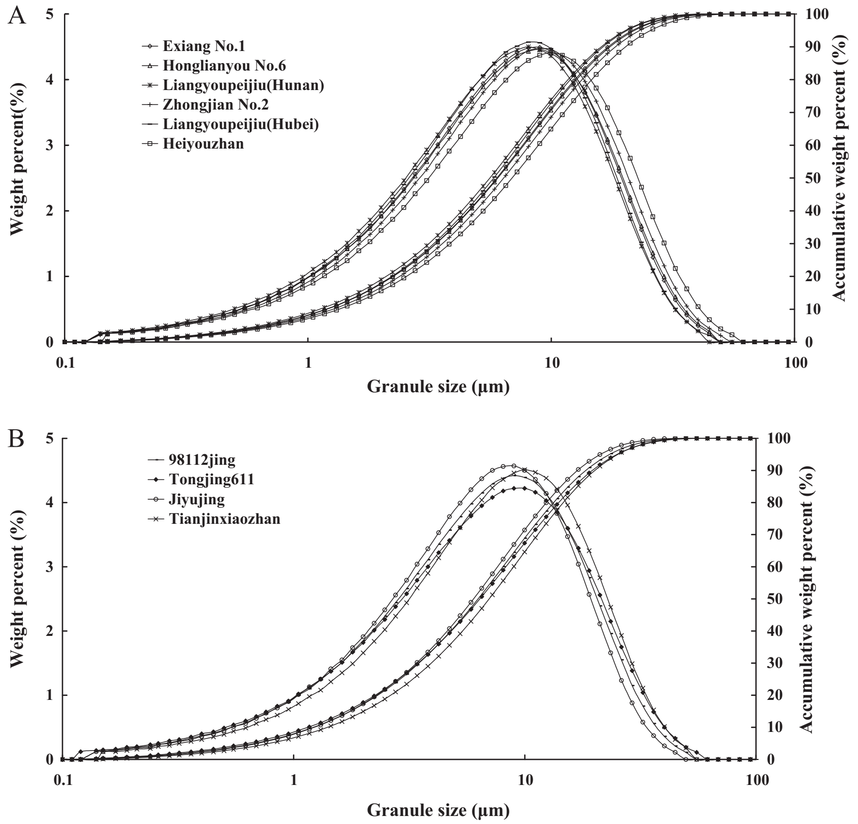

Fig. 2. The size distribution of rice starch granules from different Chinese rice cultivars: A. *Indica* rice; B. *Japonica* rice.

(Guinesi et al., 2006; Raina, Singh, Bawa, & Saxena, 2007). The tapioca starch appeared to be round, oval, while maize starch was observed to be polygranular, but their characteristic dimensions were larger than rice starches (Wang, Yin, Zhang, Xie, & Sun, 2008). The starch from different botanical origin of starch appeared to be especial shape and characteristic dimensions (Singh, Singh, Kaur, Sodhi, & Gill, 2003). Conversely, it explained further that the morphological properties of starch could be an index to estimate the botanical origin.

3.2. Granule size distribution

The granule size distributions of different rice starches were presented in Fig. 2. The granule size distribution curves of different rice starches exhibited a unimodal shape with a mode diameter of about 10 μm. The size, shape and distribution showed no significant distinctions between various rice starches. The range of median size for *Indica* and *Japonica* rice starch were around 6.23–7.69 μm and 6.68–7.81 μm, respectively. The granule size of rice starches was comparable with a previous observation by Li and Yeh (2001), which reported an average granule size of 6.4 μm for Taiwan rice starch. It was thus clear that the granule distribution of starch only related to botanical origin.

3.3. X-ray diffraction

The X-ray diffraction patterns of starches from different rice cultivars were shown in Fig. 3. The crystallinity level calculated from the ratio of area of crystalline diffraction peak and total diffraction peaks area were given in Table 1 (Ribotta et al., 2004). Rice starches exhibited strong diffraction peaks at 2θ with values of around 15.1°, 17.1°, 18.2°, 23.2° and 29.4°. These results indicated that the crystal type of native non-waxy rice starch was a characteristic A-type. However, no significant differentia was observed between the X-ray diffraction patterns of different rice starches. As shown in Table 1, the relative crystallinity of different rice starches ranged from 20.4% to 33.4%, which was lower than the reported range of 29.2–39.3% for various rice starches (Ong & Blanshard, 1995). In the tested rice cultivars, Honglianyou No.6 and Heiyouzhan showed the higher value of relative crystallinity than did the other starches. The relative crystallinity of *Indica* type starch was higher than that of *Japonica* type. The differences in relative crystallinity among the starches could not be attributed to differences in crystallite size (since the sharpness in X-ray pattern was identical in all starches) or to amylose content (since Honglianyou No.6 and Heiyouzhan with the highest amylose content (31.6%) and medium values (23.6%) respectively, exhibited the similar higher relative crystallinity (33.4% and 32.5%)). Ong and Blanshard (1995) also reported similar relative crystallinities for 11 non-waxy rice starch varieties. No significant differences were found among the non-waxy varieties crystallinity ($p < 0.05$) despite their different amylose:amylopectin ratios. Therefore, the higher relative crystallinity of Honglianyou No.6 and Heiyouzhan could be due to interplay of the following factors: (1) higher extent of interaction between double helices, (2) better orientation of double helices in the crystalline areas and (3) more longer branch chain (forming double helices) distribution in amylopectin (Hoove & Ratnayake, 2002). The amylopectin structure model maintained it to be a cluster (Robin, Mercier, Charbonniere, & Guilbot, 1974) with polymodal chain length distribution (Hizukuri, 1986). The semi-crystalline nature of starch was ascribed to double helices formed by amylopectin branches with polymodal chain length distribution. The crystallinity was shown as the amount of crystalline domains that were sufficiently large and regularly arranged to diffract X-ray. The

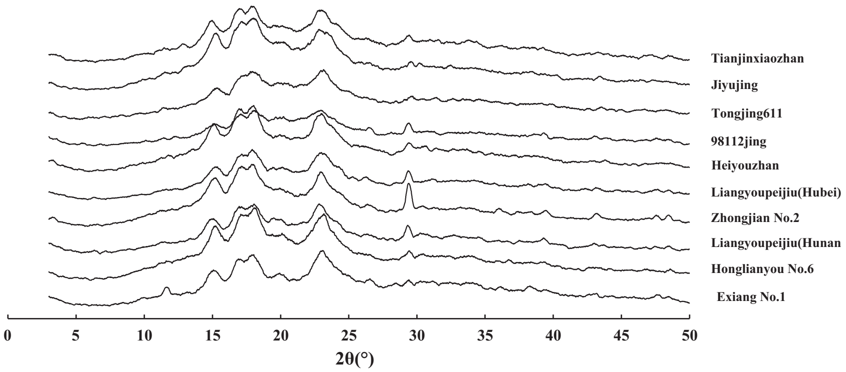

Fig. 3. The X-ray diffraction patterns of starch separated from different rice cultivars grown in China.

relative crystallinity was directly related with the interaction of double helices and the orientation of double helices. Although these 10 rice starches were from the same botanical origin, the interaction of double helices and the orientation in crystalline of starches were different. The interaction of double helices was corresponding with the higher activation energy. Therefore, rice starch with the higher activation energy possessed the more crystalline domains (the higher relative crystallinity) (Tables 1 and 2) in crystalline lamellae. The better orientation of double helices in crystalline areas was, the more regular of crystalline domains was to diffract X-ray. The action of amylopectin chain length in crystallinity would be discussed later. Furthermore, the intra- and inter-molecular hydrogen bonds were responsible for the highly ordered crystalline structure (Xu, Vesselin, & Milford, 2004).

3.4. *Calculation of the activation energy and pre-exponential factor*

The thermogravimetric curves were used to examine the differences in thermal stability caused by structural distinctions to determine the weight loss of the material on heating. The TGA and obtained kinetic parameters for the native starch of different rice cultivars grown in China were shown in Fig. 4 and Table 2, respectively. The native rice starch showed a two-stage weight loss below 600 °C, the first minor loss between 25 °C and 200 °C was related to the water elimination. The second step between 230 °C and 380 °C was related to depolymerization and decomposition of starch in a non-oxidative process. There were two crystal structures with different compositions and properties; one was (crystal structure) from the ordered packing between starch molecular chains by the interaction of hydrogen bonds, the other was from (between) the ordered packing including starch molecular chains and water molecules (Aggarwal & Dollimore, 1998; Guinesi et al., 2006; Liming, Weiguang, Xi, Ying, & Wenyuan, 2009). The first weight loss corresponded with the destruction of starch-water structure, while the second was related to the destruction of starch-starch structure.

The decomposition temperature of native rice starches ranged from 230 °C to 257 °C, the decomposition temperature was observed to be the highest for Exiang No.1 and the lowest for 98112jing. Factors such as botanical source, surface area, chemical modification and degree of substitute mainly governed the decomposition of starch (Aggarwal & Dollimore, 1998; Li et al., 2010; Ma, Chang, Zheng, Yu, & Ma, 2010; Stojanovic, Katsikas, Popovic, Jovanovic, & Jeremic, 2005). The range of activation energy (E) from different rice starches was between 155.6 and 201.5 kJ/mol. Honglianyou No.6 showed the highest value, whereas the lowest was observed in 98112jing. The differences of E might be associated to intermolecular interaction between individual granules that were aggregated in the starch (Guinesi et al., 2006). In the present study, the activation energy of *Japonica* rice (except Jiyujing) starches showed the lower values than *Indica* rice starches. It was inductive that *Indica* rice starch granules might be compacter than *Japonica* rice starch granules, because *Indica* starch needed more energy to decompose granules.

Pre-exponential factor, (A) was the pre-exponential constant in the Arrhenius equation, an empirical relationship between temperature and rate coefficient. In present study, the $\ln A$ of rice starch molecule was between 6.4 and 8.4. Honglianyou No.6 showed the highest value in experimental samples. Generally, A of *Japonica* rice (except Jiyujing) starches was lower than *Indica* rice starches. It implied that the higher processing temperature for *Indica* rice was needful in food and no-food manufacture.

3.5. *Correlation analysis*

A correlation analysis was conducted between starch structural data and thermogravimetric properties for the starches (Table 3).

Table 1
Granular parameters and the relative crystallinity of starches from different rice cultivars.

Samples	Type	Median Size (μm)	Mean volume diameter (μm)	Specific surface area (m^2/g)	Relative crystallinity (%)	Amylose (DW%)*
Exiang No.1	*Indica*	6.66	8.75	0.7884	25.1	18.1 ± 0.1
Honglianyou No.6	*Indica*	6.71	8.86	0.7908	33.4	31.6 ± 0.2
Liangyoupeijiu (Hunan)	*Indica*	6.23	8.18	0.8328	26.5	27.4 ± 0.2
Zhongjian No.2	*Indica*	7.1	9.36	0.7496	23.2	20.7 ± 0.2
Liangyoupeijiu (Hubei)	*Indica*	6.45	8.41	0.7927	30.3	29.6 ± 0.1
Heiyouzhan	*Indica*	7.69	10.24	0.7003	32.5	23.6 ± 0.3
98112jing	*Japonica*	6.99	9.29	0.7708	20.4	22.4 ± 0.1
Tongjin611	*Japonica*	7.11	9.72	0.8109	22.5	25.7 ± 0.3
Jiyujing	*Japonica*	6.68	8.69	0.7724	26.8	23.5 ± 0.2
Tianjinxiaozhan	*Japonica*	7.81	10.23	0.6778	21.9	24.2 ± 0.1

* Wang et al. (2010).

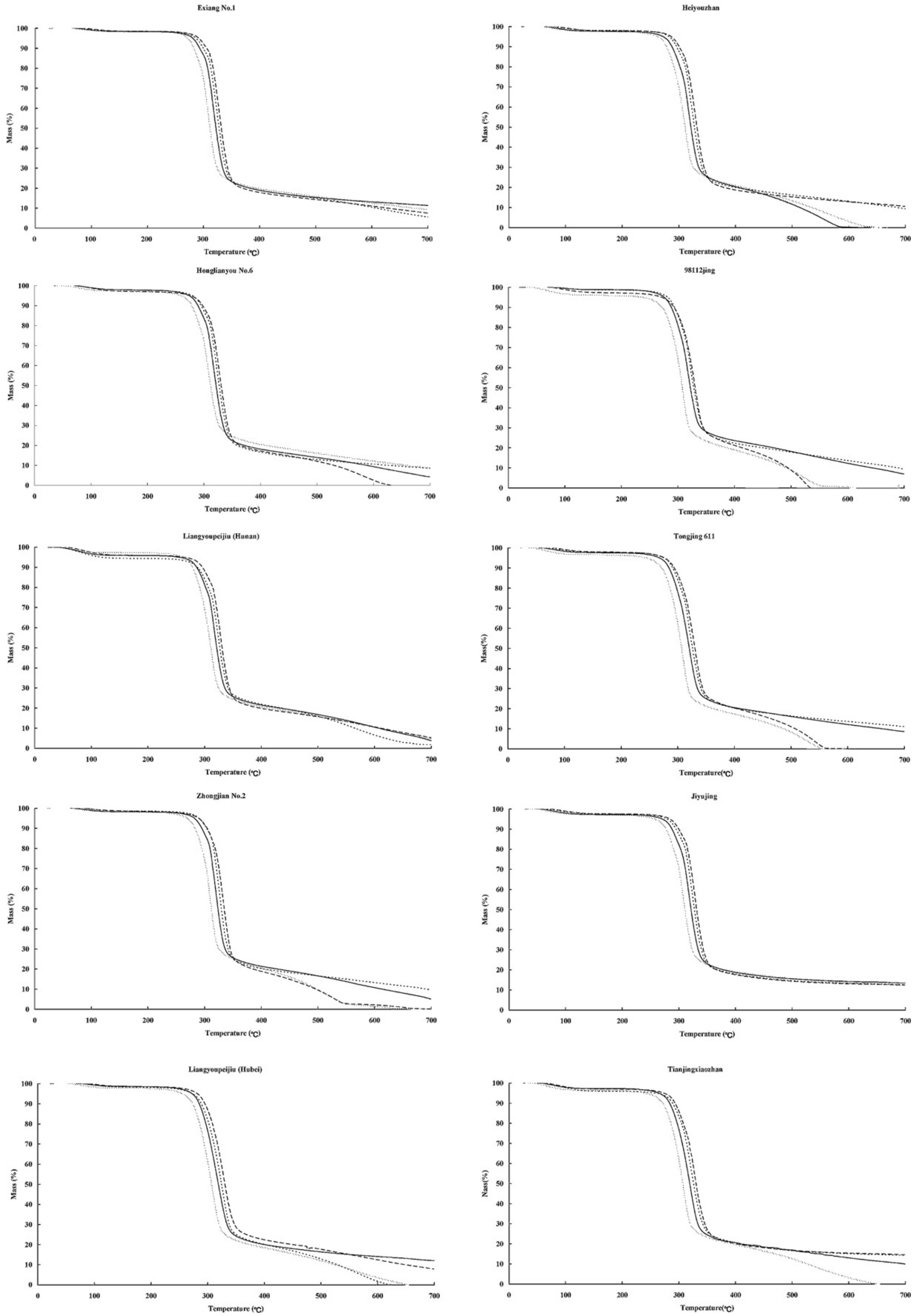

Fig. 4. TG curves to rice starches under nitrogen atmosphere at 5 °C/min (⚄), 10 °C/min (–), 15 °C/min (…), 20 °C/min (– –).

Table 2
Reaction limits taken from TG curves to obtain kinetic parameters *E* and *A* regarding the second step of thermal degradation to rice starches.

Sample	Heating rate (°C/min)	Temperature range (°C)	Mass loss (%)	*E* (kJ/mol)	$\ln A$ (min^{-1})
Exiang No.1	5	257–356	74.1	190.6 ± 9.8	7.7
	10	257–360	74.3		
	15	258–368	75.2		
	20	265–362	74.8		
Honglianyou No.6	5	243–339	70.1	201.5 ± 12.7	8.4
	10	251–357	69.8		
	15	261–360	70.3		
	20	269–371	70.5		
Liangyoupeijiu (Hunan)	5	253–349	67.1	185.1 ± 13.7	7.5
	10	265–357	66.5		
	15	272–361	67.3		
	20	278–368	67.8		
Zhongjian No.2	5	248–338	69.7	170.8 ± 12.3	6.9
	10	256–350	69.7		
	15	268–358	69.9		
	20	271–362	69.5		
Liangyoupejiu (Hubei)	5	231–339	73.1	181.2 ± 3.6	6.6
	10	251–357	72.8		
	15	254–361	72.5		
	20	261–368	72.7		
Heiyouzhan	5	243–341	71.2	179.3 ± 10.1	7.2
	10	250–355	70.8		
	15	267–374	70.5		
	20	271–377	70.6		
98112jing	5	230–334	69.2	155.6 ± 9.8	6.4
	10	243–372	68.7		
	15	258–368	68.7		
	20	260–374	68.5		
Tongjin611	5	242–338	71.2	156.7 ± 9.7	6.4
	10	253–350	70.9		
	15	257–353	71		
	20	261–355	69.8		
Jiyujing	5	249–344	71.3	181.4 ± 15.3	7.3
	10	255–264	71.1		
	15	261–364	71		
	20	266–367	69.9		
Tianjingxiaozhan	5	240–347	67.7	157.2 ± 4.2	6.4
	10	261–252	67.8		
	15	264–356	68.1		
	20	272–361	68.2		

Highly significant positive correlations ($r = 0.7903$, $p < 0.01$) were seen between relative crystallinity and activation energy. Because the coefficient was large, they did suggest that higher relative crystallinity might result in activation energy shifting to higher values. The amylopectin branch chain length distribution was as mentioned in Wang et al. (2010). The positive correlation ($r = 0.6750$, $p < 0.05$) between relative crystallinity and the amount of longer amylopectin chains with DP12–23 found in this study were in contrast to studies by Vandeputtea, Vermeylena, Geeromsb, & Delcoura (2003), who found the relative amount of chains with DP12–22 increased RC. It was also suggested that the DP12–23 chains formed double helices within crystalline lamellae and was significant contributing for crystal structure. And the relative crystallinity of rice was significantly positive correlating with $\ln A$ ($r = 0.6526$, $p < 0.05$). There was no significant correlation between amylose and crystallinity among 10 non-waxy rice starches ($p < 0.05$). The role of amylose in crystallinity was complicated. Although, some authors proposed that amylose disrupted the amylopectin crystallite formation (Cheetham & Tao, 1998; Jenkins & Donald, 1995), there was NMR evidence that the amylose formed double helices (and

Table 3
Relationship between the granule structure and thermogravimetric parameters.

	AC	D_{50}	MD	RC	*E*	$\ln A$	DP12–23
AC	1	−0.3047	−0.2807	0.5708	0.3152	0.2215	0.5087
D_{50}		1	0.9843**	−0.2025	−0.5535	−0.4185	−0.0825
MD			1	−0.2241	−0.5939	−0.4401	−0.0867
RC				1	0.7903**	0.6526*	0.6750*
E					1	0.9222**	0.5084
$\ln A$						1	0.4433
DP12–23							1

AC, amylose content; D_{50}, Median size; MD, mean volume diameter; RC, relative crystallinity; *E*, activation energy; *A*, pre-exponential factor; DP12–23, the percentage of branched chains with DP12–23.

* $P < 0.05$.

** $P < 0.01$.

potentially crystalline arrays) (Shi, Capitani, Trzasko, & Jeffcoat, 1998; Tester, Debon, & Sommerville, 2000). In present work, Honglianyou No.6 and Liangyoupeijiu (from Hubei) starch with higher amylose content (31.6% and 29.6%) showed higher relative crystallinity, indicating that amylose might also participate in crystals. And that the effect of branch chain length distribution of amylopectin on the relative crystallinity had different reports. Cheetham and Tao (1998) suggested that the proportion of short chains (DP10–13) in maize amylopectin played an important role in the formation of crystallinity, with a higher proportion of DP10–13 resulting in lower crystallinity. An opposite result was obtained in another study (Chung, Liu, Lee, & Wei, 2011) that the short A chains (DP6–12) in amylopectin was positively correlated with relative crystallinity. Amylopectin had generally been considered responsible for starch crystallinity, while amylose disrupts the crystalline packing of amylopectin (Cheetham and Tao, 1998; Hizukuri, 1985). Vandeputtea et al. (2003) found the relative amount of chains with DP12–22 increased relative crystallinity. Therefore, the relative crystallinity in rice starches varied with branch chain length distribution of amylopectin. In this regard, further research was needed to be learnt how amylose and amylopectin were tightly packed in the starch granules and the effect of branch chain length distribution of amylopectin on the relative crystallinity.

4. Conclusion

The present study demonstrated that the relative crystallinity of rice starches, as measured by X-ray diffractometer, depends upon amylopectin chain length (forming double helices within crystalline lamellae) distribution. Furthermore, the relative crystallinity values increased with the rise of activation energy of the investigated starches. The activation energy was corresponding to the energy of starch granule decomposition, reflecting the aggregation extent between starch molecule chains. The relative crystallinity expressed the amount of crystal structure in starch. It was concluded that the energy needed in starch thermal degradation during N_2 mainly destroyed the crystal structure of starch. The relationship observed in the granular and thermogravimetric properties of starches might provide a crucial basis for understanding the effect of the starch structure at industrial processing.

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