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Physicochemical characteristics and fine structure of high-amylose wheat starches isolated from Australian wheat cultivars

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Abstract

High-amylose starch is a source of resistant starch (RS) which have great impact on human health like dietary fiber. Nowadays, high-amylose wheat has been produced by genetic backcrossing, which enhances apparent amylose content and generates altered amylopectin. In this study, the high-amylose wheat starches isolated from various high-amylose wheat cultivars grown in Australia were characterized for understanding their physicochemical properties and fine structure of starch. The physicochemical characteristics of the high-amylose wheat starches are significantly different among the cultivars. Amylose contents of these cultivars were in a range of 28.0–36.9%, which is significantly higher than that of the normal wheat starch (25.6%). The high-amylose wheat starches also had higher blue value but lower λ_{max} than the normal wheat starch. Gelatinization temperature of the high-amylose wheat starches is higher than that of the normal wheat starch but transition enthalpy is lower. X-ray diffraction showed that the high-amylose wheat starch had C-type crystals close to A-type crystal. Pasting properties of the high-amylose wheat starches were varying depending on the cultivars. However, almost high-amylose wheat starches had lower peak and final viscosities and higher setback viscosity than did the normal wheat starch. Fine structure of amylose and amylopectin was different among the high-amylose wheat cultivars and related to the physicochemical properties of starch. These results help to understand well the characteristics of the high-amylose wheat starches before application for food processing.

Keywords: High-amylose wheat; Starch structure; Amylose content; Physicochemical property

1. Introduction

Wheat (*Triticum aestivum* L.) is among the oldest and most extensively grown of all crops. It is a main cereal cultivated throughout the world along with rice, barley, maize, rye, sorghum, oats and millet. Nowadays, wheat cultivars have been developed for different qualities in accordance with the development of genetic recombination. In wheat, starch is a major component located at endosperm and has significant effects on quality of end-use products. Starch is composed of two glucose polymers, amylose and amylopectin, with the ratio of amylose/amylopectin ranging between 25–28% and 72–75%, respectively. Amylose and amylopectin are synthesized in wheat grains by biosynthetic activities of enzymes. The isoforms of granule-bound starch synthase (GBSS) are responsible for the biosynthesis of amylose fraction, whereas amylopectin synthesis is more complicated with concerted activities of the soluble starch synthase together with branching and de-branching enzymes. Therefore, the new physicochemical and biological techniques have been recently developed to enhance or

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reduce the amylose content of wheat starch by giving impact on synthase enzymes. Nakamura, Yamamori, Hirano, Hidaka, and Nagamine (1995) reported that waxy (amylose-free) wheat was completely produced by using the traditional hybridizations between the Wx-D1 single null line BaiHuo and the Wx-A1/Wx-B1 double null line "Kanto 107" resulting in progeny that lacked all isoforms of GBSS and had no starch amylose. Other methods also produced waxy wheat by elimination of Wx protein genes in the single or double null Wx protein wheats (Kiribuchi-Otobe, Nagamine, Yanagisawa, Ohnish, & Yamaguchi, 1997; Yasui, Matsuki, Sasaki, & Yamamori, 1997). In contrast, three kinds of starch granule proteins (SGP-1, -2 and -3) are also found to relate to amylose/amylopectin synthesis. Yamamori, Fujita, Hayakawa, Matsuki, and Yasui (2000) reported that the lack of SGP-1 in mutant wheat enhances apparent amylose content and generates altered amylopectin. The waxy and high-amylose wheats which have the specific structure and unique characteristics of starches (Hung, Maeda, & Morita, 2007) contributed to improve the quality of food products. The benefits of waxy starch application in bakery products are retardation of staling and extended shelf life of breads, and formation of a new texture of breads with the soft, viscous and glutinous breadcrumbs (Hayakawa, Tanaka, Nakamura, Endo, & Hoshino, 2004; Morita et al., 2002a; Morita et al., 2002b). Likewise, the high-amylose wheat was found to have the unique functionality which also improves the nutritional value of the end-use products. The high-amylose wheat was applied for breadmaking to increase amount of resistant starch in bread during storage (Hung, Yamamori, & Morita, 2005) and was used to improve the texture of noodle (Morita et al., 2003). Although there were several studies on structure and physicochemical properties of high-amylose wheat starches (Hung et al., 2007; Yamamori et al., 2000), the information does not represent for all cultivars because each cultivar was produced by a different method. Therefore, it is necessary to characterize new wheat cultivars which were produced by genetic modification or backcrossing to understand well their structure and characteristics and use them effectively. In this study, the high-amylose wheat starches isolated from various high-amylose wheat cultivars grown in Australia were characterized for understanding their physicochemical properties and fine structure of starch.

2. Materials and methods

2.1. Materials

Six high-amylose wheat cultivars (Diamondbird, Pelsart, Banks, Lang, SM1028 and SM1126) which were grown and cultivated at Horsham, Victoria, Australia in the 2004/05 season were used in this study. The wheats were milled on a Buhler experimental mill and starches were isolated using a dough-washing method. A normal wheat starch was isolated from Cameria, a commercial bread wheat supplied by Nisshin Flour Co., Ltd. (Kobe, Japan).

2.2. Scanning electron microscopy (SEM)

Appearances of high-amylose wheat starch granules were observed by SEM. The preparation and operation procedures were carried out as previously described (Hung & Morita, 2005) as follows. An amount of starch was suspended in 95% ethanol for a few minutes and then sprinkled on a double-sided adhesive tape mounted on aluminum stub. After drying by vacuum aspiration for several hours, the samples were coated with Pt/Pd and photographed using a SEM apparatus (Hitachi model S-800, Tokyo, Japan) at an accelerating potential of 10 kV.

2.3. Starch–iodine absorbance spectra and amylose content

Iodine absorption spectra of starch were measured according to the method of Takeda, Takeda, and Hizukuri (1983) with a slight modification (Hung & Morita, 2005). The starch (10 mg, db) was suspended in 0.2 ml of 99% ethanol and 1 ml of distilled water. The suspension was heated at 100 °C for 5 min, cooled to room temperature. Then 0.5 ml of 1 M NaOH was added, followed by heating in a boiling water bath for 10 min with shaking to completely dissolve all starch granules. The suspension was adjusted to pH 6.5 with 1 M HCl and diluted to 10 ml with distilled water. An aliquot (0.4 ml) of the solution was added to 0.4 ml of 0.2% iodine solution and made up to 10 ml with distilled water. The mixture was kept at room temperature for 1–2 h. Then an absorbance curve was measured between 450 and 800 nm with a spectrophotometer (UV-160A, Shimadzu, Osaka, Japan). Blue value of iodine–starch complexes at 680 nm was also measured according to the procedure of Takeda et al. (1983). Amylose contents of the high-amylose wheat starches were determined according to the approved method 61-03 (American Association of Cereal Chemists, 2000) with a slight modification (Hung & Morita, 2005). A calibration curve was established using a mixture of amylose and amylopectin fractionated from high-amylose wheat starch.

2.4. Differential scanning calorimetry

Thermal characteristics of the high-amylose wheat starches were determined using a differential scanning calorimeter (DSC-60, Shimadzu Co., Kyoto, Japan). Sample (3.0 ± 0.1 mg) was mixed with 10 μl of distilled water in an aluminum vessel. The vessel was sealed and kept at room temperature for more than 30 min for equilibration. The heating condition was from 30 to 120 °C at a rate of 10 °C/min. The initial, peak and completion temperatures and transition enthalpy were recorded and automatically calculated using a TA-60WS program (Shimadzu Co., Kyoto, Japan).

2.5. X-ray diffractions

X-ray diffraction patterns of the high-amylose wheat starches were analyzed using an X-ray diffractometer (Rigaku Co., Ltd., Rint-2000 type, Tokyo, Japan) operated at 40 kV and 80 mA. Diffractograms were carried out from 4° 2θ to 40° 2θ with a scanning speed of 8°/min and scanning step of 0.02°.

2.6. Pasting properties

Pasting properties of starch suspension (8%, w/v) were determined using a visco-amylograph (Brabender, Germany) as described previously (Hung & Morita, 2005). The starch was suspended in distilled water and heated from 30 to 93 °C at the rate of 1.5 °C/min. The paste was further heated at 93 °C for 15 min and then cooled to 30 °C at the same rate of 1.5 °C/min followed by keeping at 30 °C for 15 min. The amylograms of the pastes were recorded.

2.7. Amylose and amylopectin structures

Amylose and amylopectin of the high-amylose wheat starches were fractionated based on the method of Klucinec and Thompson (1998). The starches (10 g) were firstly dispersed in 200 ml of 90% (v/v) of dimethyl sulfoxide (DMSO) in water by heating the mixture in a boiling water bath for 3 h with constant stirring. The nongranular starch was recovered by addition of 4 volumes of ethanol and centrifuged at 6500*g* for 15 min at 4 °C. The pellet was washed by suspending them in 50 ml of ethanol followed by re-centrifugation. The washing was repeatedly done with ethanol and finally with acetone as the some procedure and then the nongranular starch was dried in a forced-air oven at 40 °C for 24 h. Amylose and amylopectin were then separated from the nongranular starch based on the different soluble activity of amylose and amylopectin in a mixed solution of 1-butanol and isoamyl alcohol. The separation steps were repeated several times for purification. The amylose and amylopectin were recovered and dried at an air-dried oven at 40 °C overnight.

Iodine absorption spectra and blue value of amylose and amylopectin were measured as described above for starch. Isoamylolysis of amylose and amylopectin was done according to the procedure of Hizukuri (1985). The starch (30 mg) was suspended in 2 ml of water by boiling in a water bath for 5 min. After cooling to room temperature, the solution was mixed with 0.5 ml of 1 M NaOH and completely dissolved with shaking. The solution was then adjusted to pH 7.0 with 1 M acetic acid followed by addition of 0.1 M sodium acetate buffer (pH 3.5). Isoamylase (1770 U) was added and the solution was incubated at 45 °C for 2.5 h. After incubation, the mixture was neutralized to pH 6.5–7.0 with 0.1 M NaOH and heated at 100 °C for 3 min to inactivate enzymes. The number-average degrees of polymerization (DP_n) and the average chain length (CL) after isoamylolysis were determined by the method of Hizukuri, Takeda, Yasuda, and Suzuki (1981). The average number of chains per molecule (NC) = [(DP_n/CL) − 1] (Hizukuri et al., 1981; Suzuki, Hizukuri, & Takeda, 1981).

2.8. Statistical analysis

All tests were performed at least in triplicate. Analysis of variance (ANOVA) was performed using Duncan's multiple-range test to compare treatment means at $P < .05$ using SPSS software (SPSS Inc., USA).

3. Results and discussion

3.1. Appearance of starch granules

The appearance and granular size of six cultivars of high-amylose wheat starches are shown in Fig. 1. All high-amylose wheat cultivars also contained two kinds of granules, large and small granules, like normal wheat as reported previously by Evers (1971). The shape of high-amylose wheat starch granules was also both spherical and oval as normal wheat starch. However, surface of the granules was damaged and cracked. The rough and porous surface of the large granules was clearly observed. This observation was also reported by Yamamori et al. (2000) for the high-amylose wheat cultivar produced and grown in Japan. Thus, the genetically backcrossed wheats were not only modified the amylose content of starch but also modified the appearance of starch granules. The abnormal starch granules might influence on the physicochemical properties of these wheat starches.

3.2. Iodine absorbance spectra of starch and amylose content

Amylose content of the six high-amylose wheat cultivars ranged from 28.0% to 36.9% which is significantly higher than that of the normal wheat starch (25.6%) (Table 1). Among the six cultivars, starch of Diamondbird had the lowest amylose content (28.0 ± 2.3%) and starch of SM1028 had the highest amylose content (36.9 ± 2.6%), whereas there were no significant difference in amylose content of other high-amylose wheat starch (34.0–36.1%). The higher amylose contents of the high-amylose wheat starches were illustrated by the higher blue values of these high-amylose wheat starches than that of the normal one. In contrast, the maximum starch–iodine absorbance (λ_{max}) of the high-amylose wheat starches was significantly lower than that of the normal wheat starch. This result is contrastable with the high-amylose null SGP-1 wheat cultivars produced and grown in Japan as previously reported by Yamamori et al. (2000) and Hung et al. (2007). The λ_{max} of starch was reported to relate to degree of polymerization and average chain length of amylose and amylopectin (Banks, Greenwood, & Khan, 1971; Fales, 1980). Thus, the lower λ_{max} of the high-amylose wheat starches in this

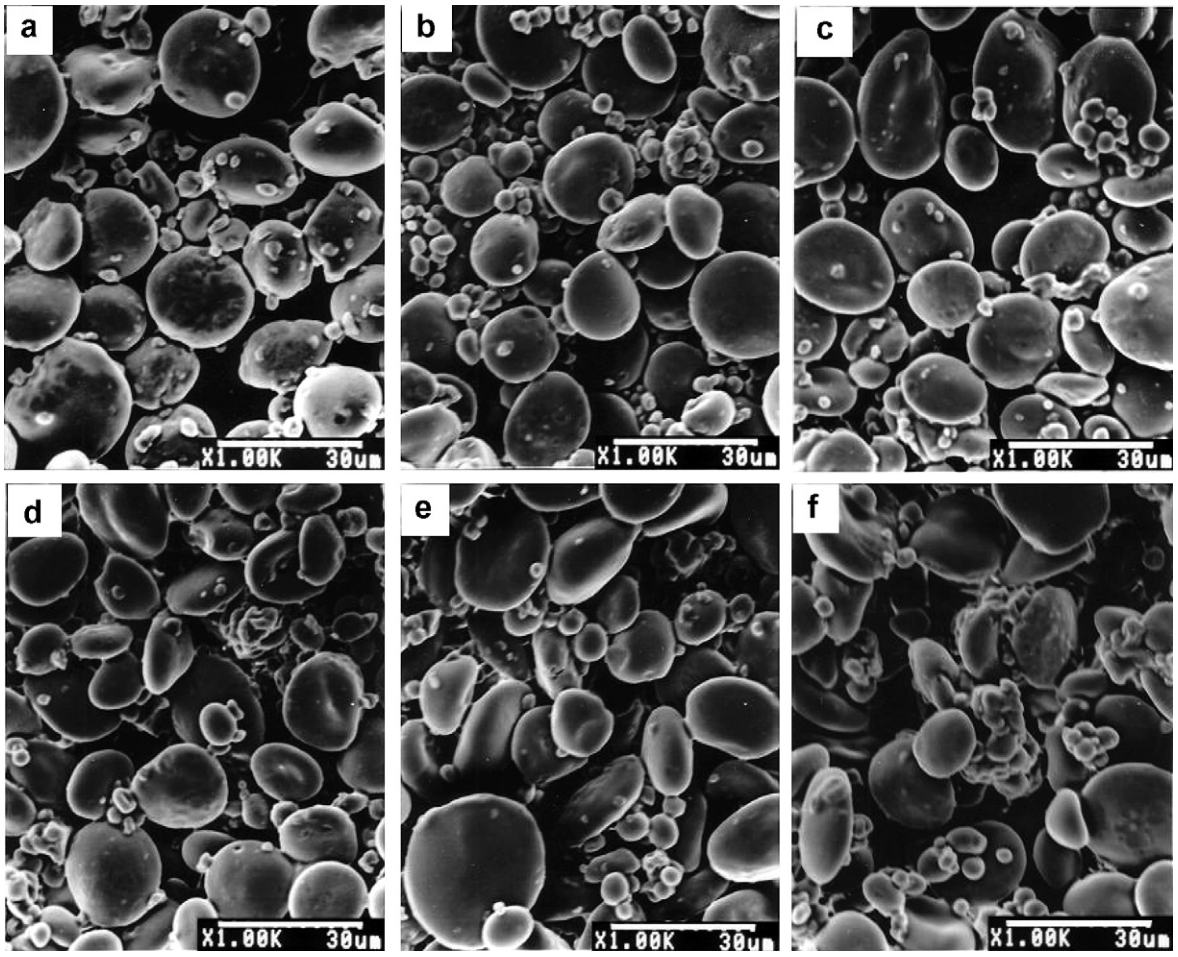

Fig. 1. SEM images of several Australian high-amylose wheat starches. (a) Diamondbird; (b) Pelsart; (c) Banks; (d) Lang; (e) SM1028; (f) SM1126.

Table 1
Absorbance of starch–iodine complex and amylose contents of several Australian high-amylose wheat starches[a]

Sample	λ_{max} (nm)	Blue value	Amylose content (%)
Normal wheat	596.8 ± 1.0	0.34 ± 0.01	25.6 ± 1.0
Diamondbird	571.8 ± 1.5	0.38 ± 0.01	28.0 ± 2.3
Pelsart	581.2 ± 2.0	0.40 ± 0.01	34.3 ± 0.1
Banks	585.3 ± 2.0	0.42 ± 0.01	36.1 ± 1.3
Lang	573.0 ± 1.8	0.41 ± 0.03	35.3 ± 2.8
SM1028	576.5 ± 1.5	0.42 ± 0.01	36.9 ± 2.6
SM1126	581.8 ± 2.0	0.40 ± 0.01	34.0 ± 2.9

[a] The data show means of triplicate data.

study might be due to the large branching chains existed in molecules of amylose and amylopectin.

3.3. Thermal characteristics

Thermal characteristics of the high-amylose wheat cultivars are shown in Table 2. DSC data show that the gelatinization temperatures of starches isolated from these high-amylose wheat cultivars were not significantly different except for starch of Diamondbird, which had lower gelatinization temperature than other cultivars. The starches from the high-amylose wheat cultivars in this study had higher gelatinization temperature than the normal wheat starch. This result is opposite to the result reported previously by Hung et al. (2007), which studied on the high-amylose cultivars cultivated in Japan with null SGP-1. The high-amylose wheat starch with null SGP-1 exhibited lower gelatinization temperature than did the normal wheat starch. The high-amylose wheat starch with null SGP-1 was found to have the altered structure of amylopectin resulting in low starch crystallinity. However, the lower gelatinization temperature might be related to changes of three parameters: polymorphous structure of crystal, thickness and free energy of the surface of face side of crystals (Wasserman et al., 2006). Therefore, the lower gelatinization of starch might not be correlated to the increase in amylose content of starch. From these results, it can be seen that the crystalline structure of starch granules of the high-amylose wheat starches in this study was not changed though the amylose contents increased. The transition enthalpy of the high-amylose wheat starches was lower than that of the normal wheat starch, which is agreed with the result of previous study (Hung et al., 2007). Thus, the high-amylose content of starch requires low energy for gelatinization caused by large amorphous

Table 2
Thermal properties of several Australian high-amylose wheat starches[A,B]

Sample	First peak				Second peak			
	T_{i1} (°C)	T_{p1} (°C)	T_{c1} (°C)	ΔH_1 (J/g)	T_{i2} (°C)	T_{p2} (°C)	T_{c2} (°C)	ΔH_2 (J/g)
Normal wheat	55.1a	61.4b	66.9a	6.9c	92.4a	98.3a	102.5a	1.0d
Diamondbird	55.6b	60.8a	67.3a	5.8ab	93.1ab	99.7ab	103.5a	0.9cd
Pelsart	56.5c	62.0c	68.2b	6.2abc	93.2ab	99.5ab	103.6a	0.6bc
Banks	57.0d	62.6d	69.0b	6.5bc	94.5ab	101.3b	106.9b	0.9cd
Lang	57.5d	62.5d	69.0b	5.7ab	95.0ab	100.2ab	104.4ab	0.5ab
SM1028	57.2d	62.2cd	68.4b	6.0abc	96.3b	100.1ab	104.0ab	0.3ab
SM1126	57.4d	62.3cd	68.4b	5.3a	96.5b	100.1ab	104.4ab	0.3a

[A] T_{i1}, T_{p1}, T_{c1}, initial, peak and complement of the first peak; T_{i2}, T_{p2}, T_{c2}, initial, peak and complement of the second peak; ΔH_1, ΔH_2, enthalpies of the first and second peaks.
[B] The same letter in the same column is not significantly different ($P < .05$), $n = 3$.

area in starch granules. The data also show the second peak existed during gelatinization of starch. This peak corresponds to the melting of amylose–lipid complexes as described by Eliasson (1980). The previous study reported that the high-amylose wheat starch with null SGP-1 exhibited lower melting temperature of amylose–lipid complex but higher transition enthalpy than those of the normal wheat starch (Hung et al., 2007). However, the high-amylose wheats cultivars in this study had no significant difference in melting temperature of amylose–lipid complex but lower transition enthalpy than the normal wheat starch. These results might be due to low amount of lipid existed in these high-amylose starches as compared to the starch from the cultivar with null SGP-1.

3.4. X-ray diffraction patterns

X-ray diffraction patterns of the high-amylose wheat starches are given in Fig. 2. Starches of Pelsart and SM1126 showed the A-type crystal with the major peaks at 5.8, 5.2, 4.8, 4.4 and 3.8 Å like the normal wheat starch, whereas starches of other four high-amylose wheat cultivars had modified structure and exhibited the C-type crystal which is close to A-type crystal with unclear peaks at 5.2 and 4.8 Å. Thus, the X-ray diffraction patterns indicate that the internal structure of starch granules of the high-amylose wheat cultivars in this study was not altered as similar as the high-amylose null SGP-1 wheat cultivar produced and grown in Japan (Hung et al., 2007; Yamamori et al., 2000) though the amylose content of starches from these high-amylose wheat cultivars was also enhanced. Bochanikova et al. (2003) previously reported that the high-amylose wheat starches showed a typical A-type polymorphous structure, irrespective of the increased amylose content. The unchanged A-type polymorphous structure of the high-amylose wheat starches was apparently opposite behaviors of maize and pea starches, which exhibited that the increase in amylose content in granules is accompanied by a transformation of their crystalline lattice. Thus the increase in amylose content of starch granules was not correlated to the change in the crystal pattern of starch.

3.5. Pasting properties

Pasting properties of starch paste reflect their amylose content and affect quality of the end-use products. The high-amylose starch exhibited the lower peak viscosity, breakdown and final viscosity (Hung et al., 2007). However, there were cultivars showing high scores in both amylose content and peak viscosities (Miura & Tanii, 1994). In this study, pasting and peak viscosity temperatures of all high-amylose wheat starches were not significantly different (Table 3). However, the peak and final viscosities of these starches were significantly different depending on each cultivar. In general, starches from all high-amylose wheat cultivars had lower peak and final viscosities than the normal wheat starch except for starch of Diamondbird, which exhibited higher peak and final viscosities than the normal wheat starch. Among them, the starch of SM1126 had the lowest peak and final viscosities followed by starches of SM1028 and Banks, which were typical properties of the high-amylose starches. All high-amylose wheat starches except for Diamondbird had no breakdown during heating. Thus, the pasting properties of starch depended on the cultivars. However, the high-amylose wheat starches generally had the unique characteristic of the low peak and final viscosities of starch paste. The low peak viscosity and breakdown of the high-amylose wheat starches were reported to be favorable for pasta noodle with improvement of the texture quality of noodle (Morita et al., 2003).

3.6. Fine structure of amylose and amylopectin

Fine structure of amylose and amylopectin fractionated from starches of the high-amylose wheat cultivars is shown in Tables 4 and 5. Blue values of amylose and amylopectin of these starches were a range of 0.458–0.773 and 0.093–0.200, respectively. This result shows that the amylose and amylopectin were already separated from each other. However, the blue values of the amylose fraction of the high-amylose wheat cultivars were significantly lower than that of the other cereals or tube, which was in a range of 1.40–1.47 for rice (Takeda & Hizukuri, 1986), 1.220–1.253 for barley (Tang, Ando, Watanabe, Takeda, &

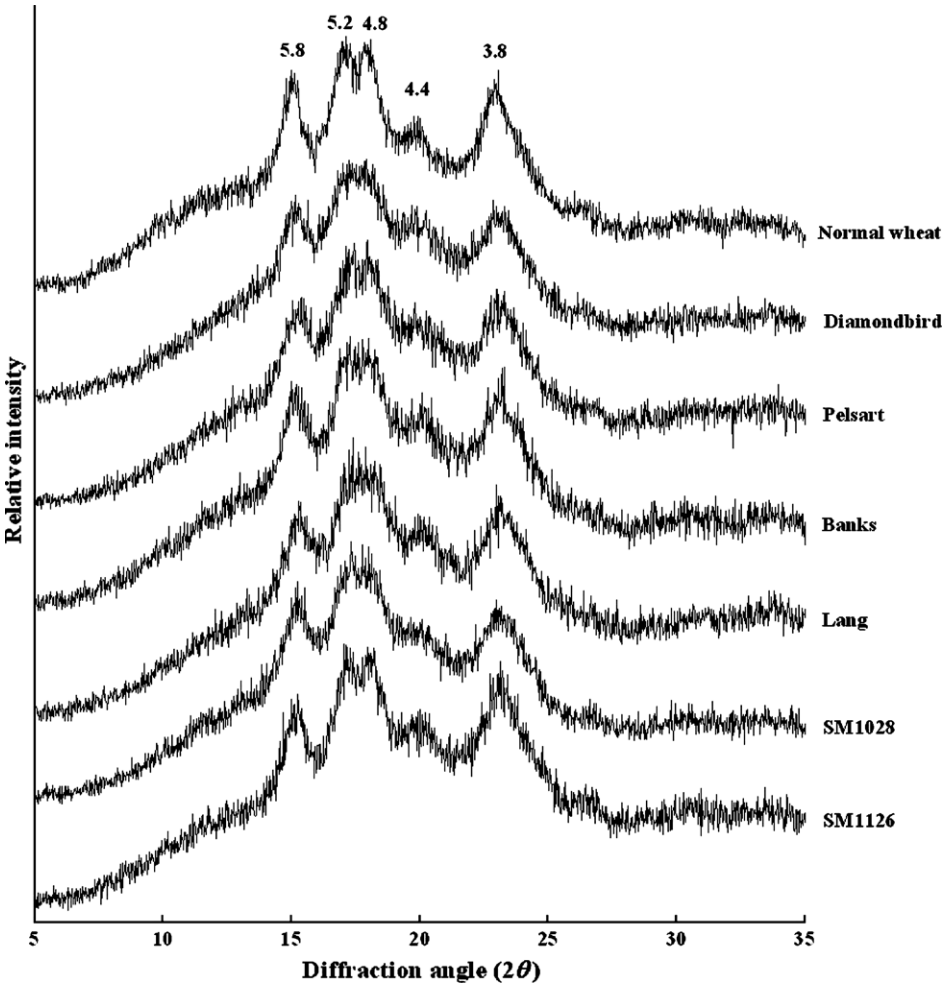

Fig. 2. X-ray diffraction patterns of several Australian wheat starches.

Table 3
Pasting properties of several high-amylose wheat starches (8%, w/v)[A,B]

Sample	T_g (°C)	T_p (°C)	V_p (BU)	V_f (BU)	BD (BU)	SB (BU)
Normal wheat	88.0a	93.0a	140e	400e	10b	260b
Diamondbird	87.0a	93.0a	240f	670g	20c	450c
Pelsart	88.5a	93.0a	120d	360d	0a	240b
Banks	88.5a	93.0a	90c	310c	0a	280b
Lang	87.0a	93.0a	140e	420f	0a	280b
SM1028	87.0a	93.0a	70b	290b	0a	220b
SM1126	87.0a	93.0a	30a	100a	0a	70a

[A] T_g, T_p, gelatinization and peak temperatures; V_p, V_f, peak and final viscosities; BD, breakdown; SB, setback.
[B] The same letter in the same column is not significantly different ($P < .05$).

Mitsunaga, 2001) and 1.19–1.23 for kudzu (Hung & Morita, 2007). This result was due to the higher number of chains existed in the amylose molecules of the high-amylose wheat starches (14.0–26.8 chains per molecule). λ_{max} of the amylose fraction of these starches was in a range of 618.2–625.5, which is significantly higher than that of the amylopectin fraction (530–565.5). This result indicates that the iodine–amylose complex of linear chains had higher maximum absorbance than that of the branching chains. Average degrees of polymerization (DP_n) of amylose molecules of the high-amylose wheat starches were in a range of 854–1155 glucose molecules, which were significantly lower than that of amylopectin (1278–3841 glucose molecules). In contrast, average chain length of the amylose molecules was longer than that of the amylopectin molecules. Amylopectin molecules of the high-amylose wheat cultivars in this study had smaller DP_n but longer chain length than the normal wheat reported by Hizukuri and Maehara (1990). Among the high-amylose wheat starches, SM1126 cultivar exhibited small DP_n and less number of chains of the

Table 4
Fine structure of amylose fractionated from several Australian high-amylose wheat starches[A,B]

Sample	Blue value	λ_{max} (nm)	DP	CL	NC
Diamondbird	0.570b	641.8b	1124ab	41a	26.8d
Pelsart	0.773e	651.8c	1078ab	72d	14.0a
Banks	0.624d	652.5c	904ab	56bc	15.1ab
Lang	0.627d	641.5b	854a	50ab	16.0ab
SM1028	0.596c	636.0b	1155b	64cd	17.0b
SM1126	0.458a	618.2a	943ab	45ab	19.8c

[A] DP, average degree of polymerization; CL, average chain length; NC, average number of chains per molecule.
[B] Values showing the same letter in the same column is not significantly different ($P < .05$), $n = 3$.

Table 5
Fine structure of amylopectin fractionated from several Australian high-amylose wheat starches[A,B]

Sample	Blue value	λ_{max} (nm)	DP	CL	NC
Diamondbird	0.110b	530.0a	2899b	29a	98.9b
Pelsart	0.093a	530.0a	3373bc	29a	113.9b
Banks	0.142c	530.0a	3049b	30ab	99.6b
Lang	0.163d	530.0a	3841c	32b	120.0b
SM1028	0.140c	530.0a	3085b	29a	106.4b
SM1126	0.200e	565.5b	1278a	30ab	41.9a

[A] DP, average degree of polymerization; CL, average chain length; NC, average number of chains per molecule.
[B] Values showing the same letter in the same column is not significantly different ($P < .05$), $n = 3$.

amylopectin molecules but large number of chains of the amylose molecules resulting in low peak and final viscosities and transition enthalpy of this starch as compared to other ones. Consequently, the data show that fine structure of amylose and amylopectin of the high-amylose wheat cultivars has changed with large number of chain in amylose molecules and small degree of polymerization of amylopectin molecules though internal structure of granule might not be changed.

4. Conclusion

In this study, the physicochemical characteristics and fine structure of starches from different high-amylose wheat cultivars were investigated. Amylose contents of these cultivars were a range of 28.0–36.9%, which is significantly higher than that of the normal wheat starch (25.6%). The high-amylose wheat starches also had higher blue value but lower λ_{max} than the normal wheat starch. The typical characteristics of these high-amylose wheats were higher gelatinization temperature and lower transition enthalpy than those of the normal wheat starch. The high-amylose wheat starches also showed lower peak and final viscosities and higher setback viscosity than the normal wheat starch. X-ray diffraction showed that the high-amylose wheat starch structure had only minor change in crystalline structure with still remaining the A-type crystal of starch granules. Fine structure of amylose and amylopectin was different among the high-amylose wheat cultivars and related to their physicochemical properties. The data show that fine structure of amylose and amylopectin of the high-amylose wheat cultivars has changed with large number of chain in amylose molecules and small degree of polymerization of amylopectin molecules though internal structure of granule might not be changed.

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