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Functionality of starch granules in milling fractions of normal wheat grain

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Abstract

Normal wheat grains were milled into nine fractions (A–I) ranging from the surface layer to the center of a grain with a modified machine used for polishing brewers' rice. Starch granules were isolated from the fractions in this study. Amylose content and functionality of the starches, hydrolysability with enzyme, gelatinization and retrogradation properties, swelling power and freeze-thaw stability of gels of starches were investigated. The results indicated that the apparent amylose content was similar among wheat fraction starches, but the structures and the functionalities of the starches differed with location of the grain. Starch granules from the surface were hydrolyzed and gelatinized easily, and swelled little compared with those from the center. The gel from the starches of the center was more stable in freeze-thawing than those from the surface, and more difficult to retrograde. These findings may provide important information to utilize the classified wheat flours produced by the new method.

Keywords: Wheat grain; Fractions; Starch; Functionality

1. Introduction

Wheat grain is one of the world's three most important cereals. Most wheat grain is used as wheat flour. The flour has the unique characteristic of forming gluten, and is used for the making of bread, noodles, cakes and the other foods according to gluten content. Wheat flour is now produced by a combination of roll milling and sieving of wheat grain. Many varieties of wheat grains and many processing procedures are necessary in order to produce commercial wheat flour for various uses. A new method of milling barley grain with a modified machine used to polish brewers' rice has been developed (Mitsunaga, Shimizu, Inaba, Yoshida, & Hayashi, 1994; Tang, Ando, Watanabe, Takeda, & Mitsunaga, 2000). The classified wheat flours with the new method differ from those with the conventional method. The flours are ground to the size of starch granules. The approximate composition, mineral content and properties of protein differ with the fraction. The classified wheat flours may have different processing and cooking characteristics, with different uses for the different fractions even in one variety of wheat grain (Ando, Sugi, Watanabe, Morita, & Mitsunaga, 2002). The main component of wheat flour is starch. The properties of starch are important to the quality of final products and being studied (Ando, Tang, Watanabe, & Mitsunaga, 2002; Lee, Swanson, & Baik, 2001; Noda, Tohnooka, Taya, & Suda, 2001; Sasaki & Matuski, 1998). However, studies of classified wheat flours are still few, and there is thus little knowledge about the classified wheat flours. To address this issue, in this study we determined the amylose contents of starches and the functionality, hydrolysability with enzyme, gelatinization and retrogradation properties, swelling power and freeze-thaw stability of gels of starches from normal wheat fractions, and analyzed the relationships among functionalities.

2. Experimental

2.1. Materials

Mature wheat grain (*Triticum aestivum* L. 'Norin No. 61', thousand grain weight 30 g) sown in the autumn of 1999 in Shiga prefecture, Japan was used. Wheat flour

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was prepared using a modified milling machine with a roller for brewers' rice as described previously (Tang et al., 2000; Tang, Ando, Watanabe, Takeda, & Mitsunaga, 2001a). The size class of the grains was calculated as: (scraped grain weight/whole grain weight)×100 (%). The fractions are indicated as A (100–90%), B (90–80%), C (80–70%), D (70–60%), E (60–50%), F (50–40%), G (40–30%), H (30–20%) and I (20–0%) from the surface layer to the center of the grain. Numbers in parentheses show the starting and ending percentages of the grains from which the flour fraction was obtained. Starch granules were prepared from the fractions by the modified alkali method as described previously (Tang et al., 2000, 2001a). Beta-amylase (from barley) was purchased from Sigma Chemical Co. (St. Louis, MO). Isoamylase (from *Pseudomonas amyloderamosa)* was the product of Hayashibara Biochemical Laboratories, Inc. (Okayama, Japan). Other chemicals were of the highest grade commercially available.

2.2. Iodine absorption spectra of starch

Iodine absorption spectra of starch were measured by the method reported previously (Takeda, Takeda, & Hizukuri, 1983). The blue value (BV) of starches was absorbance at 680 nm. Apparent amylose content was equal to: [BV (starch)/BV (amylose)]×100, assuming the amylose BV to be 1.2 (Takeda et al., 1983).

2.3. Susceptibility of starch granules to enzymes

Hydrolysis of starch granules with enzymes was done as reported previously (Tang, Watanabe, & Mitsunaga, 2002b). To 25 mg of sample was added, successively, 1 ml of 0.1 M acetate buffer (pH 4.8), 100 units of beta-amylase and 700 units of isoamylase. The reaction was initiated at 37 °C for 0–30 h. The supernatant was analyzed for soluble carbohydrate by the phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Percent hydrolysis was expressed as milligrams of maltose released per 100 mg of dry starch. Appropriate controls without the enzymes were prepared.

2.4. Gelatinization properties of starch granules

Gelatinization properties of the prepared starch granules were measured by sensitive DSC-8240D (Rigaku Denki, Co. Ltd, Tokyo). The samples with a starch-to-water ratio of 5–7 mg to 15 μl were sealed hermetically into an aluminum pan of 30 μl. Distilled water was used as a reference material. The temperature was raised from 25 to 140 °C at a heating rate of 5 °C/min (Tang, Watanabe, & Mitsunaga, 2002a).

2.5. Swelling power of starches

Swelling power (g/g) was evaluated by a modified version of the method reported previously (Tang et al., 2002b). Starch (0.1 g) was weighed in glass tubes with coated screw caps to which 5 ml of a 0.1% $AgNO_3$ solution was added. The tubes were placed in a shaking water bath at 70 °C for 10 min and then transferred into a boiling water bath. After gelatinizing perfectly, the tubes were cooled in cold water (20 °C) for 5 min and centrifuged at 1700×*g* for 4 min. The supernatant was removed carefully and swelling power was determined as sediment weight (g/g).

2.6. Stability of starch gel with freeze-thaw

Stability of starch gel was tested for freeze-thaw using the procedure of Wu and Seib (1990) and Zheng, Han, and Bhatty (1998). Starch granules (0.2 g) were weighed in glass tubes with coated screw caps, to which 5 ml of distilled water was added, and boiled for 1 h in a water bath with rapid stirring for 1 min in every 10 min. After cooling to 20 °C, the tubes containing starch pastes were stored at 4 °C for 24 h. After determining free water in the samples, the tubes were put into a freezer at −20 °C for 22 h and thawed at 30 °C in a water bath for 2 h. After each cycle, triplicate tubes were centrifuged for 15 min at 1500×*g*, and the amount of liquid separated was determined by weight.

2.7. Retrogradation of dispersed starch

The turbidity of non-granular starch was measured using the method reported previously (Tang et al., 2002b). The 0.5% starch dispersion in 20% (v/v of water) dimethyl sulfoxide was transferred into 1.5 ml glass cuvettes. The contents of the cuvettes were degassed at 20 °C (for 40 min) before the measurement of absorbance. The cuvettes containing dispersion were immersed in an ice water bath for 5 min, and absorbance was remeasured. The absorbance of the dispersion was measured every 5 min for 15 min while standing at 20 °C.

2.8. Statistical analysis

Statistical analysis of all the data was performed using Microsoft Excel.

3. Results and discussion

3.1. Starch content and iodine absorption spectra of starch

The starch content of wheat fractions and the absorbance of the starch–iodine complex are shown in Table 1. The starch content in the wheat fractions varied from 21.6 to 69.9%, as measured by the modified alkali method, and increased significantly from the surface to the center of the grain ($P<0.0001$). The results correspond with sugar content of wheat fractions as measured by the AOAC method (Ando et al., 2002). The variability occurs because of more dietary fibers, lipids, proteins, and minerals are

Table 1
Starch content in milling wheat fractions and properties of the starches

Fraction	Starch (%)[a]	λ_{max}[b]	BV[c]	Amylose (%)[d]
A	21.6±1.2	623±0.2	0.424±0.019	35.4
B	37.4±2.1	619±0.0	0.417±0.006	34.8
C	41.5±1.4	618±1.8	0.425±0.010	35.4
D	48.3±2.3	614±0.2	0.432±0.005	36.0
E	55.3±3.5	616±0.0	0.424±0.003	35.4
F	60.8±1.0	615±0.5	0.416±0.008	34.7
G	61.2±2.6	611±2.8	0.421±0.006	35.1
H	64.4±3.4	613±3.2	0.415±0.001	34.6
I	69.9±2.5	612±0.5	0.428±0.000	35.7

Values are the mean±SD of three separate measurements.
[a] Isolated by alkali method (Tang et al., 2000).
[b] Maximum absorption wavelength of starch–iodine complex.
[c] Blue value of starch–iodine complex at 680 nm.
[d] Apparent amylose content=(BV/1.2)×100 (Takeda et al., 1983).

found on the surface than in the center of the grain. The BV was 0.415–0.432, and was similar among the different fractions. The apparent amylose content calculated by the BV was 34.6–36.0%, and not significantly different among the fractions. The results were similar to those of normal wheat starches (Ando et al., 2002) and normal barley starches (Tang et al., 2001b). However, the λ_{max} of the starch–iodine complex was 611–623 nm and decreased significantly from the surface to the center of grain ($P<0.002$). This result suggests that the starch molecules may contain longer non-branched structures in the starches from the surface than in those from the center of the grain, or have a difference in distribution of molecular weights for the different fractions of starches (Takeda, Hizukuri, & Juliano, 1986).

3.2. Susceptibility of starch granules to enzymes

The starch granules from normal wheat fractions were hydrolyzed for 30 h by the combination of beta-amylase and isoamylase (Table 2). The carbohydrate solubilized by the enzyme in the starch supernatant amounted to 0.5–2.3% of the weight of starch by the phenol–sulfuric acid method without the addition of enzymes, and significantly decreased from the surface to the center of the grain ($P<0.02$). It was not clear that the solubilized carbohydrates resulted from damaged starch granules, or from the dietary fiber and other sugars adsorbed onto the starch granules. However, the values were similar to those reported previously (Tester & Morrison, 1992; Vasanthan & Bhatty, 1996; Zheng et al., 1998). It is thought that the level of damaged starch granules in this study did not influence the susceptibility of the starch granules to hydrolysis by the enzymes. The starch granules were hydrolyzed at a relatively fast rate for the initial 2 h, followed by a slower rate after 2–8 h. The degree of hydrolysis reached 50–65% in the first 8 h, but increased only another four points by 22 h in all fractions. Similar observations on hydrolysis have been reported in barley and quinoa starches (Tang et al., 2002a,b; Vasanthan & Bhatty, 1996). Different rates in the early and the later stages of hydrolysis can be understood by considering that the enzymes initially attack the more amorphous regions of the starch granules, whereas the less accessible crystalline regions are hydrolyzed at a slower rate. Also, the degree of hydrolysis for the fraction starches decreased significantly from the surface to the center of the grains for all stages of measurements ($P<0.0001$). The differences among the fractionated starches may be attributed to differences in the structures of the starch in the granules.

3.3. Gelatinization properties of starch granules

Differential scanning calorimetry (DSC) thermal curves (data not shown) of the starch granules displayed two endotherms for all of the fractions. The transition temperatures and enthalpy changes of the starches are given in Table 3. The first endotherm depends on crystallinity of the starch granule, and the second endotherm relates to the amylose–lipid complex (Czuchajowska, Klamczynski, Paszczynska, & Baik, 1998). In the first endotherms, onset (T_{o_1}), peak (T_{p_1}), final (T_{f_1}) temperatures, transition temperature range (ΔT_1) and enthalpy change (ΔH_1) were 51.2–53.6, 58.2–59.2, 64.0–64.9, 10.7–13.6 °C and 8.6–10.5 J/g, respectively. The T_{p_1} and T_{f_1} were similar among fractions. But the T_{o_1} ($P<0.05$) and ΔH_1 ($P<0.001$) tended to

Table 2
Enzyme hydrolysis of starch granules in wheat fractions

Fraction	Hydrolyzed rate (%)						
	0 h[a]	2 h	5 h	8 h	22 h	26 h	30 h
A	2.3±0.3	42.7±0.1	60.4±0.1	65.9±0.4	67.6±1.0	69.0±0.1	69.6±0.7
B	1.0±0.1	41.9±0.2	51.5±0.1	59.3±0.7	64.9±0.4	65.6±1.2	65.8±0.0
C	0.7±0.2	40.6±0.1	50.6±0.5	60.1±0.1	64.5±1.1	65.0±2.1	64.8±0.2
D	1.3±0.2	39.1±0.5	45.8±0.1	57.4±0.6	59.2±0.3	59.6±0.2	61.0±0.5
E	1.1±0.2	40.0±0.1	43.8±1.0	57.0±0.2	58.3±1.0	58.3±0.1	59.0±1.0
F	0.8±0.5	39.9±0.2	43.1±2.6	54.3±1.5	54.5±1.4	57.1±0.9	57.2±2.6
G	0.5±0.1	38.6±0.1	42.7±1.3	50.4±0.8	51.4±2.0	52.5±0.2	53.1±0.5
H	0.5±0.2	39.3±0.1	41.8±1.0	49.7±1.1	52.0±1.3	52.1±1.4	53.7±0.4
I	0.5±0.2	37.4±1.0	39.0±0.2	49.3±0.3	52.5±0.2	52.5±0.6	52.4±0.7

Values are the mean±SD of three separate measurements.
[a] No enzymes.

Table 3
Gelatinization properties of starch granules in wheat fractions

Fraction	T_o[a]	T_p[b]	T_f[c]	ΔT[d]	ΔH[e]
Peak-1					
A	51.2±2.0	58.3±0.6	64.8±0.6	13.6	8.6±0.6
B	51.9±0.4	58.8±0.1	64.6±0.2	12.7	9.2±0.0
C	51.5±0.7	58.2±0.1	64.2±0.2	12.7	9.4±0.5
D	51.9±0.3	58.9±0.2	64.7±0.3	12.8	9.4±0.5
E	53.5±0.1	59.2±0.1	64.9±0.3	11.4	9.2±0.5
F	53.1±0.6	59.2±0.2	64.2±0.4	11.1	9.4±0.1
G	52.3±0.3	58.4±0.3	64.2±0.3	11.9	9.6±0.5
H	52.5±0.5	58.6±0.2	64.0±0.1	11.5	10.2±0.5
I	53.6±0.4	58.9±0.0	64.3±0.2	10.7	10.5±0.1
Peak-2					
A	95.3±1.4	114.6±0.7	116.4±0.4	21.1	1.6±0.4
B	92.5±0.6	114.3±0.0	116.5±0.2	24.0	1.8±0.5
C	91.0±0.0	115.0±0.0	116.2±1.6	25.3	1.9±0.4
D	92.2±1.0	115.2±0.8	116.6±0.5	24.4	1.7±0.4
E	97.2±0.5	115.4±1.7	119.7±2.0	22.5	1.2±0.4
F	93.1±2.2	114.6±0.2	116.5±0.9	23.4	1.3±0.2
G	91.7±0.6	114.4±2.0	116.9±0.0	25.2	1.3±0.5
H	92.1±2.0	114.4±0.5	116.7±0.4	24.6	1.1±0.3
I	92.5±1.7	114.3±0.7	116.3±0.7	23.8	1.3±0.5

Values are the mean ±SD of three separate measurements.
[a] Onset temperature (°C).
[b] Peak temperature (°C).
[c] Final temperature (°C).
[d] Transition temperature range ($T_f - T_o$).
[e] Enthalpy change.

increase from the surface to the center of grain, while the ΔT_1 ($P < 0.002$) decreased. The values of transition temperatures in this study were similar to those of Norin No. 61 reported previously (Noda et al., 2001), but the enthalpy changes were lower, probably due to the cultivation environment. These results were supported by the susceptibility of the starch granules to enzyme hydrolysis (Table 2). The starches with difficult gelatinizations showed a lower rate of hydrolysis. In the second endotherms, onset (T_{o_2}), peak (T_{p_2}), final (T_{f_2}) temperatures, and transition temperature range (ΔT_2) were 92, 114, 116 and 24 °C, respectively. The starches had higher transition temperatures and broader endotherms, compared with those of barley, but were similar to those of adzuki starch (Tang et al., 2002a). The enthalpy changes (ΔH_2) of the starches were 1.1–1.9 J/g, and tended to decrease from the surface to the center ($P < 0.05$). The values agreed with those of wheat starches reported previously (Noda et al., 2001).

3.4. Swelling power of starches

The swelling power of starch depends on the capacity of starch molecules to hold water via hydrogen bonding (Lee & Osman, 1991). When the hydrogen bonds between starch molecules are broken after complete gelatinization, they are replaced by hydrogen bonds with water. The amylose content and the proportion of outside-chains of amylopectin are thought to be the major factors stabilizing the gel structure to retain water (Tang et al., 2002b). Swelling

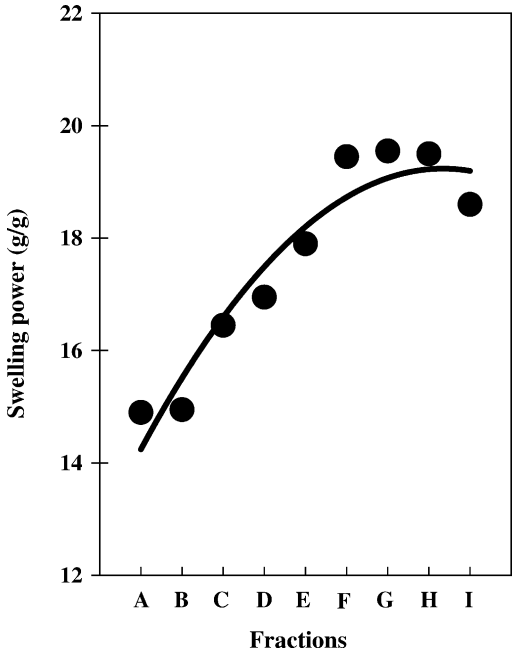

Fig. 1. Swelling power of starch in wheat fractions.

power has no significant correlation with particle size, but is highly correlated with the amylose content and the chain length distribution of amylopectin (Sasaki & Matsuki, 1998). In this study, the swelling power for the wheat fraction starches was measured at around 95 °C (Fig. 1). The values of swelling power were 14.9–19.6 g/g starch, and tended to increase from the surface to the center of the grain ($P < 0.002$). The values were similar to those of normal wheat (Sasaki & Matsuki, 1998) and barley starches (Tang et al., 2002b) previously reported. Also, these results correspond with the susceptibility to the enzymes and gelatinization properties of wheat starch granules in this

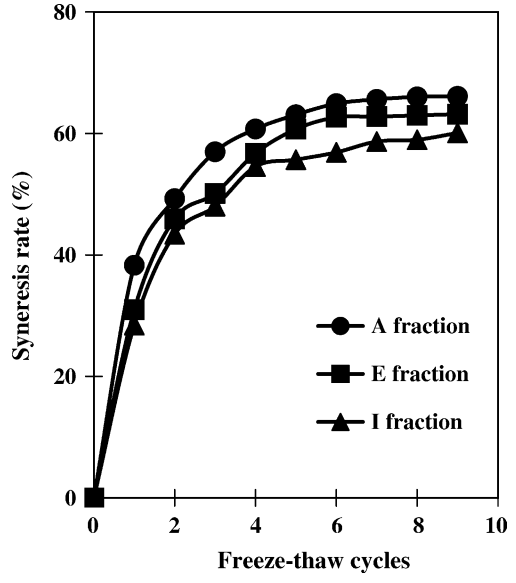

Fig. 2. Syneresis of starch gel in wheat fractions.

Table 4
Syneresis (wt%/time) of starch gels with freeze-thaw in wheat fractions

Fraction	Syneresis-I[a]	Syneresis-II[b]
A	19.0±2.4	1.5±0.2
B	18.4±1.8	1.6±0.4
C	18.4±1.7	1.4±0.0
D	18.3±1.9	1.5±0.2
E	16.7±2.7	2.2±0.8
F	16.3±3.1	2.2±1.0
G	16.8±2.7	2.1±0.3
H	16.3±2.6	2.2±0.6
I	16.0±1.8	2.0±0.5

Values are the mean of three separate measurements.
[a] Syneresis-I is average syneresis (wt%/time) of starch gels in the first three freeze-thaw cycles.
[b] Syneresis-II is average syneresis (wt%/time) of starch gels from the fourth to the ninth freeze-thaw cycle.

Table 5
Turbidity of 0.5% dispersions of non-granular starches in 20% DMSO–water (v/v)

Fraction	Degassed at 20 °C for 40 min	Ice-water for 5 min	20 °C for 15 min
A	0.252±0.0372	0.804±0.038	0.076±0.018
B	0.202±0.008	0.757±0.008	0.074±0.034
C	0.174±0.004	0.692±0.033	0.064±0.035
D	0.148±0.005	0.633±0.004	0.053±0.037
E	0.144±0.003	0.613±0.000	0.035±0.007
F	0.114±0.023	0.587±0.007	0.034±0.009
G	0.108±0.030	0.629±0.037	0.032±0.003
H	0.094±0.030	0.617±0.000	0.039±0.005
I	0.085±0.007	0.444±0.015	0.043±0.000

Values are the mean ± SD of three separate measurements.

study. Thus, in analogy with barley fraction starches (Tang et al., 2000, 2001a,b), it is thought that wheat fraction starches differ in their structure and functionality by their location in the grain.

3.5. Stability of starch gel on freeze-thawing

In order to compare the stability of gel from wheat fraction starches, syneresis rate of starch gel was observed by freeze-thaw tests (Fig. 2). All starch gels showed a faster syneresis in the early freeze-thaw cycles. Syneresis rates were 48.0–57.0% in the initial three cycles, but increased only to 60.1–66.1% in the ninth cycle. The results for the initial cycles differed from those of waxy barley (WB and WB-X) starches as reported previously (Wu & Seib, 1990), probably due to the amylose content. Also, syneresis of gel for wheat fraction starches decreased significantly from the surface to the center in all the cycles ($P<0.0001$). The mean of syneresis of the initial three cycles was calculated with Syneresis-I, and the mean of syneresis from the fourth to ninth cycles with Syneresis-II (Table 4). Syneresis-I in A-fraction starch gels was 19.0%/cycle, and 16.0%/cycle in I-fraction starch gels. Syneresis-II was 1.4–2.2%/cycle, and tended to increase from the surface to the center. In this way, the correlations between the stability of starch gel and other functionalities can be analyzed quantitatively because the syneresis rate is represented by two average rates.

3.6. Retrogradation of dispersed starch

To observe the retrogradation behavior of wheat fraction starches, the turbidity of starch dispersions in DMSO–water solvent was determined at different temperatures (Table 5). The turbidity was 0.085–0.252 after degassing for 40 min at 20 °C, 0.444–0.804 after cooling for 5 min in the ice water bath, and 0.032–0.076 after rewarming for 15 min at room temperature, respectively. The values decreased from the surface to the center of the grain with the three steps of temperature ($P<0.01$). The turbidities for starch dispersions from H and I fractions of the center were similar to those for

Table 6
Correlation among functionalities of wheat fraction starches

	λ_{max}[a]	Rate-I	ΔT_1	ΔH_1	Swelling	Syneresis-I	Syneresis-II
Rate-I[b]	0.9379						
ΔT_1[c]	0.7340	0.8971					
ΔH_1[d]	−0.8013	−0.8114	−0.7447				
Swelling[e]	−0.8636	−0.8715	−0.8255	0.6735			
Syneresis-I[f]	0.7818	0.8961	0.9692	−0.7445	−0.9068		
Syneresis-II[g]	−0.6087	−0.7243	−0.8322	0.4880	0.8259	−0.9173	
Turbidity[h]	0.8187	0.9127	0.8913	−0.8319	−0.7576	0.8435	−0.5811

[a] Maximum absorption wavelength of starch–iodine complex.
[b] Rate-I are the average hydrolysis weight proportion of starch granules per hour with enzymes for 5 h.
[c] Transition temperature range (T_f-T_o) of peak 1.
[d] Enthalpy change of peak 1.
[e] Swelling power of starches.
[f] Syneresis-I is average syneresis (wt%/time) of starch gels in the first three freeze-thaw cycles.
[g] Syneresis-II is average syneresis (wt%/time) of starch gels from the fourth to the ninth freeze-thaw cycle.
[h] Ice-water for 5 min.

normal barley starches reported previously (Tang et al., 2002b) for the three steps of temperature. However, the turbidities for the starch dispersions from the surface and middle of wheat grain were higher in the steps of degassing and cooling than those for normal barley starches, but lower in the step of rewarming. It was thus thought that normal wheat starches were easier to retrograde, compared with normal barley starches. Starch dispersions from the center of wheat grain were also difficult to retrograde, relative to those from the surface. The observations agreed with other functionalities of the starch of normal wheat fractions. The findings suggest that the structures of wheat starches differ among fractions, and are supported by those of barley starches (Tang et al., 2000, 2001a,b).

3.7. Correlation between functionalities

All significant correlations among the functionalities of starch from normal wheat fractions are listed in Table 6 ($P<0.05$). The maximum absorption wavelength (λ_{max}) of starch–iodine complex is related with the chain length of starch molecules. A molecule with a longer chain length has a greater λ_{max}. While hydrolysis-I, ΔT_1 and ΔH_1 dependent on the structures of starch granule, swelling power, turbidity, and syneresis-I and -II for a non-granular starch are controlled directly by the amylose–amylopectin ratio and molecular structure of starch. In this study, the λ_{max} was positively correlated with hydrolysis-I, ΔT_1, syneresis-I and turbidity in ice water, but negatively correlated with ΔH_1, swelling power and syneresis-II. Starch granules that were hydrolyzed easily were also easy to gelatinize, but swelled little. The gel from the starches was also unstable in freeze-thaw, and easier to retrograde. These findings are supported by the general opinion about the functionality and structure of starch.

4. Conclusion

The present results indicated that the functionalities of wheat fraction starches differed with location in the grain, and add to our study on barley. Starch granules from the surface of normal wheat grain were easily hydrolyzed and gelatinized, and only swelled slightly, compared with starch granules from the center of the grain. Gels formed by the starches at the center of the grains were more stable to freeze-thawing than those from the surface, and retrograded less. These findings provide important information for the production of wheat flours by the new method, and in their use. However, classified wheat flours need to be examined further to establish whether there is universality for the present results.

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