

Effect of Wheat Starch Structure on Swelling Power

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ABSTRACT

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Starches were isolated from the endosperm of 12 wheat samples with a wide swelling power range in the wholemeal. Starch amylose content (24.8–34.2%) correlated negatively with the swelling power of isolated starch (18.3–26.9), but starch lipid content showed no such correlation. Higher proportions of long chains (DP \geq 35) in amylopectins contributed to

increased starch swelling. Native starch gelatinization temperatures and enthalpy measured by differential scanning calorimetry correlated positively with swelling power, which also correlated significantly with the regelatinization enthalpy of retrograded starches stored at 5°C for two and four weeks.

Starch is the main component of wheat flour and its properties are important to the quality of final products such as white salted noodles (Oda et al 1980; Toyokawa et al 1989a,b). When starch is heated with excess water, the starch granule swells and its volume increases. These changes are responsible for the unique characteristics of some wheat flour products. The swelling power of starch, flour, and wholemeal of wheat correlate positively with the eating quality of white salted noodles and peak paste viscosity monitored on the Rapid Visco Analyser (Crosbie 1991, McCormick et al 1991, Crosbie et al 1992, Yun et al 1996). The swelling power test is very simple and was recently recognized as an indicator of noodle quality. Testing using wholemeal is particularly convenient in preliminary experiments because it requires neither the quantity of wheat grains nor the long time to produce flour or isolate starch.

Swelling power of starch was reported to be inhibited by amylose and lipids (Tester and Morrison 1990, 1992; Morrison et al 1993; Wang and Seib 1996), but comparatively little work has been done on other starch chemistry that may affect swelling power. In this study, we investigated the effects of starch structure on swelling power. We selected a wide range of samples whose wholemeal differed in swelling power, then we isolated starches and evaluated amylose and lipid contents, distribution of the amylopectin chain length, and granule size. We also analyzed relationships between swelling power and gelatinization and retrogradation behavior of starch granules to determine the effects of starch crystalline properties on swelling.

MATERIALS AND METHODS

Twelve samples with a wide range of wholemeal swelling power were selected (Fig. 1). Seeds from two individuals from each cultivar, Norin 31 (A and B), Chinese Spring (C and D), Norin 61 (E and F), Haruhikari (G and H), Chihokukomugi (I and J), and Kanto 107 (K and L) were used. Wheat samples were grown sheltered from rainfall at the National Agriculture Research Center (NARC) in 1994 and harvested individually.

Wholemeal and Starch

Bulk grains from each individual plant were ground using a Cyclotec mill (Tecator, Sweden) with a 0.5-mm sieve. Starch was isolated from degermed grains by the method of Sulaiman and Morrison (1990) and freeze-dried.

Swelling Power Measurement

Swelling power was determined using 0.32 g of wholemeal or 0.16 g of isolated starch by the modified method of McCormick et al (1991), in which 0.1% AgNO₃ was used instead of distilled water to inhibit α -amylase activity (Yasui et al 1996). Wholemeal or isolated starch was weighed into glass tubes with coated screw caps to which 5 mL of 0.1% AgNO₃ was added. The tubes were placed in a shaking water bath at 70°C for 10 min and transferred into a boiling water bath. After boiling for 10 min, the tubes were cooled in cold water for 5 min and centrifuged at 1,700 \times g for 4 min. The supernatant was removed carefully and swelling power was determined as sediment weight (g/g).

Chemical Analysis

After defatting, the amylose content of isolated starch was determined by amperometric titration (Fukuba and Kainuma 1977). Lipids in starch were extracted using water-saturated *n*-butanol at 100°C (Morrison et al 1980). Lipid content was determined as total fatty acid methyl esters (FAME) using gas-liquid chromatography (AOAC 1995). α -Amylase activity in wholemeal and isolated starch was determined by the method of McCleary and Sheehan (1987) using an α -amylase assay kit (Megazyme International Ireland Ltd., Ireland); one unit of activity was defined as a Ceralpha Unit.

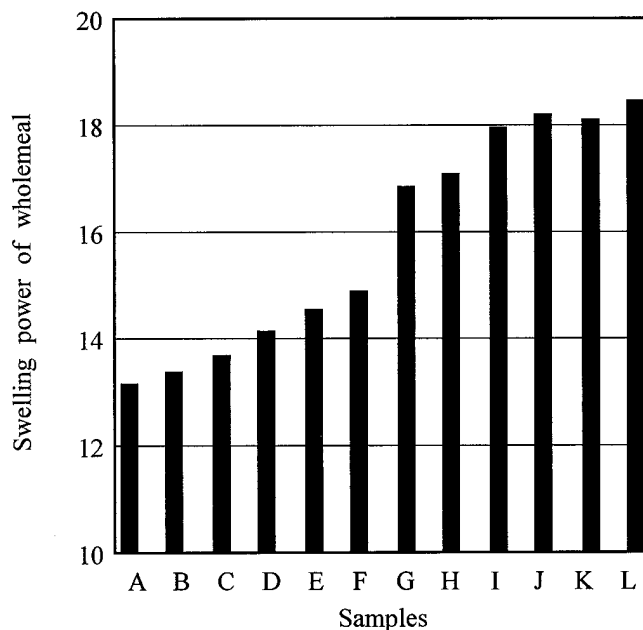


Fig. 1. Swelling power of wholemeal in 12 samples: Seeds from two individuals from each cultivar, Norin 31 (A and B), Chinese Spring (C and D), Norin 61 (E and F), Haruhikari (G and H), Chihokukomugi (I and J), and Kanto 107 (K and L).

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The chain length distribution of amylopectin in starch was analyzed with high-performance anion-exchange chromatography (Dionex, CA) equipped with a pulsed-amperometric detector. After isoamylase debranching, sample solution was eluted at 1 mL/min with a linear gradient of 100–500 mM sodium acetate in 100 mM NaOH using the method of Nagamine and Komae (1996). Unit chains of amylopectin were classified into three groups based on the degree of polymerization (DP): 6–12, 13–34, or ≥ 35 (Matsuki et al 1996). The peak area ratio (%) of the three groups was then calculated.

Granule size distribution in freeze-dried starches was determined using a laser diffraction particle size analyzer (LS100, Coulter Electronics, FL) after starch was dispersed in 99.5% 2-propanol.

Differential Scanning Calorimetry Measurements

Differential scanning calorimetry (DSC) measurements were conducted (SSC 5200 with a DSC 120U, Seiko Electronics, Tokyo, Japan) and calibrated with indium. For gelatinization studies, 50 mg of 10% starch suspension (w/w) was weighed into silver pans. After sealing, pans were scanned at a rate of 1°C/min from 40 to 120°C (Kohyama and Nishinari 1991). For retrogradation studies, 50 mg of 30% starch suspension (w/w) was weighed into silver pans. Pans were heated at 100°C for 15 min, immediately cooled to 5°C, and stored at 5°C for two or four weeks, and scanned at a rate of 1°C/min from 5 to 120°C.

TABLE I
Swelling Power of Starch, Amylose, and Lipid Content and Mean Particle Size of Starch

| Sample | Swelling Power | Amylose (%) | Lipid (%) | Mean Particle Size (μm) |
|--------|---------------------|-------------|-----------|--------------------------------------|
| A | 19.2ac ^a | 32.5cde | 0.66ed | 19.5cd |
| B | 19.6ac | 33.0a–d | 0.65e | 17.9e |
| C | 18.9bc | 33.8ab | 0.72a–c | 20.6b |
| D | 18.3c | 34.2a | 0.71b–d | 22.1a |
| E | 18.4c | 32.7b–d | 0.75a | 19.7c |
| F | 18.6c | 33.5a–c | 0.77a | 19.4cd |
| G | 22.9d | 32.2d–f | 0.68c–e | 18.7de |
| H | 23.4d | 30.5g | 0.65de | 16.5f |
| I | 21.2abd | 31.0fg | 0.67c–e | 19.8bc |
| J | 21.8ad | 31.3e–g | 0.77a | 19.5cd |
| K | 26.8e | 24.8i | 0.75ab | 22.8a |
| L | 26.6e | 27.6h | 0.69bc–e | 22.3a |

^a Values followed by the same letter in the same column are not significantly different ($P < 0.05$).

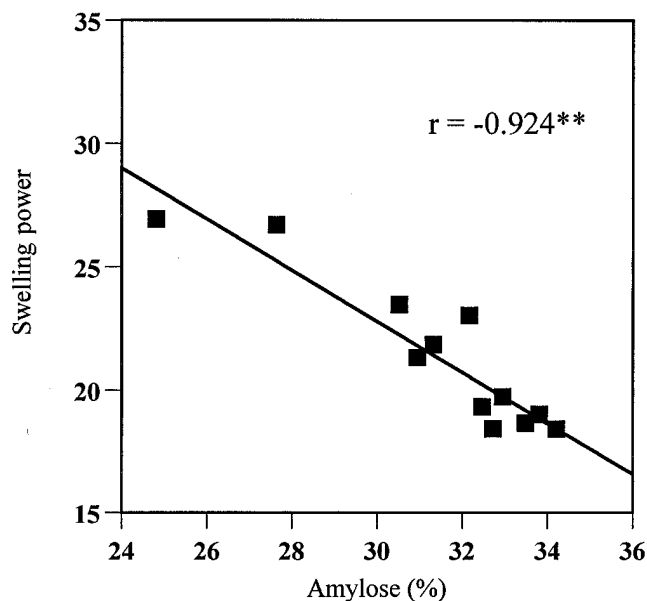


Fig. 2. Relationship between swelling power and amylose content of starch.

Statistical Analysis

All samples were analyzed in duplicate. The general linear model and multiple regression analysis (SAS Institute, Cary, NC) were used to analyze data. Analysis of variance was conducted using Tukey's studentized range test at the 5% level.

RESULTS AND DISCUSSION

Starch Particle Size

The mean diameter of isolated starch granules ranged from 16.5 to 22.8 μm (Table I). No significant correlation was found between swelling power and particle size, which agrees with the relationship observed by Tester and Karkalas (1996) using oat starches. Kulp (1973), however, reported that the swelling power of small granules at 95°C was higher, and Campbell et al (1996) suggested a highly positive correlation between starch granule diameters and gelatinization temperature. Wheat starch can be separated into A- and B-granule fractions based on granule size. The large A-granule range is ≈ 15 –40 μm and the small B-granule range is ≈ 1 –10 μm . Careful fractionation of starch granules and chemical and physical studies of each fraction are needed to clarify the relationship between particle size and swelling power.

Amylose and Lipid Contents

The swelling power of wholemeal (Fig. 1) and isolated starches ranged from 13.1 to 18.5 and 18.3 to 26.8 (Table I). The amylose content of starch was 24.8–34.2% and correlated highly with swelling power of starch (Fig. 2). The lipid content was 0.65–0.77% as

TABLE II
Chain Length Distribution of Amylopectin

| Sample | Chain Length Distribution ^a | | |
|--------|--|----------|--------------|
| | DP 6–12 | DP 13–34 | DP ≥ 35 |
| A | 25.3ac ^b | 58.0b–d | 16.7c |
| B | 25.5ab | 57.6c–e | 16.9c |
| C | 25.5ab | 58.6a | 15.9e |
| D | 25.6ab | 58.5ab | 15.9e |
| E | 25.8b | 58.2a–c | 16.0de |
| F | 25.7ab | 57.5de | 16.8c |
| G | 24.9cd | 58.6ab | 16.5cd |
| H | 25.3a–c | 57.8c–e | 16.9c |
| I | 24.8d | 57.7c–e | 17.5b |
| J | 24.4de | 57.9c–e | 17.7ab |
| K | 24.6d | 57.5e | 17.9ab |
| L | 24.1e | 57.8c–e | 18.1a |

^a Sum of peak-area ratios (%) of group with degree of polymerization (DP).

^b Values followed by the same letter in the same column are not significantly different ($P < 0.05$).

TABLE III
Thermal Analysis^a of Starch Gelatinization

| Sample | T_o | T_p | T_c | ΔH | $\Delta H(\text{AP})$ |
|--------|--------------------|--------|----------|------------|-----------------------|
| A | 52.4a ^b | 56.7a | 61.6eg | 7.2 | 10.7 |
| B | 52.5a | 56.9a | 63.6afh | 7.7 | 11.5 |
| C | 52.6ad | 57.9cd | 63.4cfg | 8.2 | 12.4 |
| D | 52.7ad | 58.3c | 63.9adfh | 8.2 | 12.5 |
| E | 51.5f | 57.0a | 61.3e | 8.3 | 12.3 |
| F | 52.1af | 57.2ad | 62.3ceh | 9.2 | 13.8 |
| G | 53.4cd | 59.8b | 66.2bd | 8.1 | 11.9 |
| H | 54.5b | 59.8b | 66.0b | 9.5 | 13.7 |
| I | 53.6c | 58.6c | 63.4cgh | 9.3 | 13.5 |
| J | 53.6c | 58.1c | 62.4cef | 8.7 | 12.7 |
| K | 55.0b | 59.4b | 63.9acd | 10.0 | 13.3 |
| L | 55.9e | 60.8e | 65.5ab | 10.2 | 14.1 |

^a T_o , T_p , T_c = onset, peak, and final gelatinization temperature (°C). ΔH and $\Delta H(\text{AP})$ = gelatinization enthalpy and calculated enthalpy on the basis of amylopectin content (J/g of dry matter).

^b Values followed by the same letter in the same column are not significantly different ($P < 0.05$).

total FAME (Table I) and fatty acid composition was similar to each other. No correlation was found between lipid content and swelling power of starch ($r = -0.071$) in these starches. Tester et al (1990, 1992) suggested that starch swelling is a property of amylopectin, and amylose acts as a diluent. The inverse correlation between swelling power and amylose content agreed with their results. In barley and wheat, starch amylose content was reported to correlate with lipid content, and lipid-complexed amylose inhibited the swelling power of starch (Tester and Morrison 1990, 1992, 1993; Morrison et al 1993; Tester et al 1993). However, no significant correlation was found between lipid and amylose content ($r = -0.076$) in these starches.

Because amylase activity affects swelling and gelatinization properties of starch, the measurement of activity in native samples is required. Wholemeal had an amylase activity range of 0.048–0.098 U/g;

isolated starch had a range of 0.000 to 0.005 U/g. No sample exhibited high amylase activity.

Amylopectin Structure

The peak area ratios of DP 6–12, DP 13–34, and DP ≥ 35 were 24.1–25.8%, 57.5–58.6%, and 15.9–18.1%, respectively (Table II). The peak area ratio of the longer chain (DP ≥ 35) correlated positively with swelling power of starch ($r = 0.846$) and the shorter chain (DP 6–12) ratio correlated negatively ($r = -0.802$). This showed that starches with higher swelling power tended to contain higher proportions of long chains in amylopectin and the difference in amylopectin molecular structure was related to variations in starch swelling properties. Tester et al (1990, 1996) studied the contribution of the molecular size and unit chain distribution of amylopectin to the swelling power of cereals using gel-permeation chromatography (GPC). Since they recognized no difference in the amylopectin structure within groups, no correlation was found.

Gelatinization and Retrogradation Properties

Gelatinization properties using DSC, onset (T_o), peak (T_p), final (T_c) gelatinization temperature, gelatinization enthalpy (ΔH), and gelatinization enthalpy calculated on the basis of amylopectin content ($\Delta H/AP$) are shown in Table III. There were positive correlations between swelling power and T_o , T_p , and ΔH (Fig. 3).

Gelatinization temperature and enthalpy in retrograded starches are shown in Table IV. The retrograded starches developed more recrystallinity after four weeks than after two weeks. Starches stored for two periods showed similar T_o , T_p , and T_c values. The ΔH of retrograded starches after two and four weeks correlated positively with swelling power (Fig. 4). Starch with a higher swelling power tended to have a larger ΔH after retrogradation.

The gelatinization enthalpy reflected both crystalline order and the level of amylopectin double-helical order (Cooke and Gidley 1992). Gelatinization temperature was a parameter of crystallite perfection (Tester and Morrison 1990, Shi and Seib 1992). On the other hand, a difference in the amylopectin fine structure affect gelatinization properties. The higher proportion of longer chains contributes to higher gelatinization temperature, because these chains form long double helices that require higher temperature to dissociate (Yuan et al 1993). Sanders et al (1990) reported that longer chain in amylopectin would result in longer crystallites and it would take more thermal energy to break the kinetic barrier of longer crystallites due to the increased crystalline order.

Starch retrogradation occurs when starch molecules begin to reassociate in an ordered structure (Atwell et al 1988). The chain distribution of amylopectins affects the retrogradation rate. Kalic-

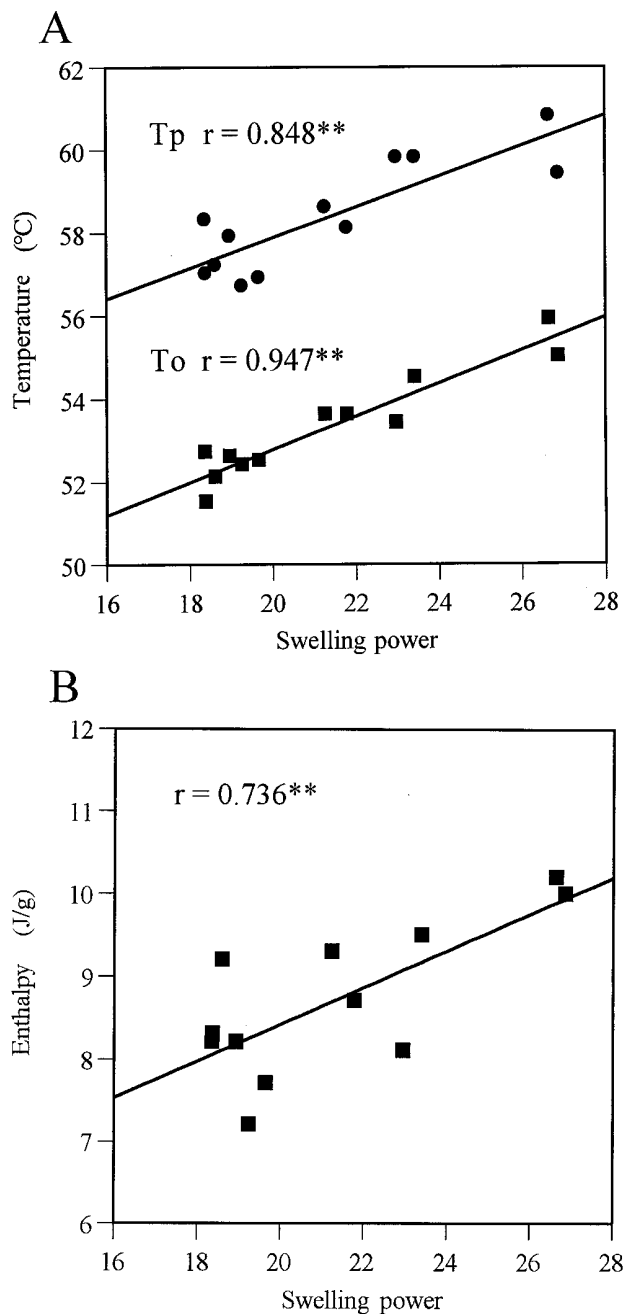


Fig. 3. Relationship between swelling power and gelatinization temperature of starch (A) and relationship between swelling power and enthalpy (B). T_o , T_p , = onset and peak gelatinization temperature; ΔH = gelatinization enthalpy (J/g of dry matter).

TABLE IV
Thermal Analysis^a of Retrogradation for Starches Stored at 5°C for Two and Four Weeks

| Sample | Two Weeks | | | Four Weeks | | |
|--------|-----------|-------|---------------------|------------|-------|------------|
| | T_o | T_p | ΔH | T_o | T_p | ΔH |
| A | 30.1 | 41.9 | 7.6c–e ^b | 31.1 | 41.3 | 8.6d–f |
| B | 30.5 | 41.9 | 7.5de | 31.2 | 41.2 | 8.3fg |
| C | 29.9 | 42.4 | 7.4ef | 31.0 | 41.3 | 8.1g |
| D | 31.7 | 42.4 | 7.5de | 32.6 | 41.6 | 8.3fg |
| E | 30.5 | 42.7 | 7.3ef | 31.6 | 41.6 | 8.3fg |
| F | 30.9 | 42.1 | 7.0f | 31.8 | 41.6 | 8.5e–g |
| G | 31.0 | 42.4 | 8.5a | 31.6 | 42.6 | 9.0cd |
| H | 30.6 | 42.7 | 8.1ab | 30.9 | 42.4 | 9.3bc |
| I | 31.5 | 41.8 | 7.9bc | 31.9 | 41.9 | 8.8de |
| J | 29.8 | 42.2 | 7.9bc | 31.7 | 41.7 | 9.7a |
| K | 30.6 | 42.1 | 8.0b | 32.0 | 42.2 | 9.5ab |
| L | 30.3 | 42.1 | 7.8b–d | 31.9 | 42.1 | 9.8a |

^a T_o , T_p , T_c , = onset, peak, and final gelatinization temperature (°C). ΔH = gelatinization enthalpy (J/g of dry matter).

^b Values followed by the same letter in the same column are not significantly different ($P < 0.05$).

hevsky et al (1990) reported that a reduced retrogradation rate in cereal amylopectins was due to their shorter average chain length, as compared to pea, potato, and canna amylopectins. Using waxy maize, rice, and barley, Shi and Seib (1992) showed that gelatinization enthalpy after retrogradation was proportional to the mole fraction of DP 14–24 unit chains and negatively proportional to that of DP 6–9. Yuan et al (1993) reported that the presence of longer DP chains and a higher proportion of longer B chains accelerated retrogradation. Increased portion of shorter chains results in increasing area of noncrystalline branched regions due to a number of branch points (Shi and Seib 1992).

Consequently, the chain distribution of amylopectin could affect the crystallinity of both native starch and retrograded starch. In these samples, gelatinization temperature, enthalpy, and regelatinization enthalpy after four weeks correlated with the longer chain (DP \geq 35) ratio. The findings suggest that a higher proportion of longer chain might form more stable crystallites and more regions of crystallites in the both native and retrograded starch. If values of gelatinization enthalpy reflect only the content of amylopectin, $\Delta H/AP$ would have similar values. In practice, the values of $\Delta H/AP$ varied, thus the variation of DSC parameters reflects the molecular structure of amylopectin.

The swelling power of starch depends on the water-holding capacity of starch molecules by hydrogen bonding (Lee and Osman 1991). Hydrogen bonds stabilizing the structure of the double helices in crystallites are broken during gelatinization and are replaced by hydrogen bonds with water, and swelling is regulated by the crystallinity of the starch (Tester et al 1996). It is considered from the relationship of swelling power, DSC parameters, and chain distribution of amylopectin in this work, that the crystalline region of the starches with a higher portion of longer chain is stabilized due to longer double helices and may form more hydrogen bonds with water when they are heated in excess water than that of starches with shorter chains.

Multiple regression analysis using stepwise forward regression method showed that swelling power was described as:

$$Y = -1.037X_1 + 53.904 \quad (R^2 = 0.855) \quad (1)$$

$$Y = -0.817X_1 - 1.370X_2 + 81.545 \quad (R^2 = 0.887) \quad (2)$$

where Y = swelling power, X_1 = amylose content, and X_2 = DP 6–12.

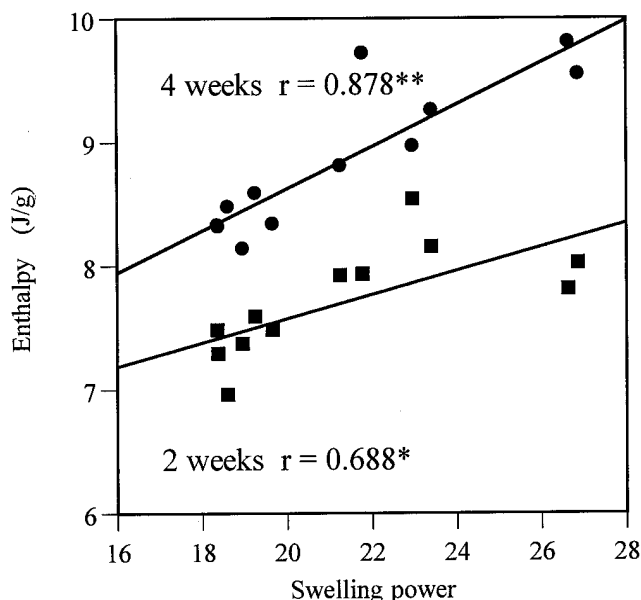


Fig. 4. Relationship between swelling power of starch and enthalpy of retrograded starches stored at 5°C for two and four weeks.

Amylose content and amylopectin structure accounted for 88.7% of the total variation of swelling power of starch, with amylose content having a greater influence than amylopectin chain length.

CONCLUSION

Swelling power of wheat starch correlated negatively with amylose content. An analysis of the amylopectin structure showed that starch with a higher swelling power tends to contain higher proportions of longer chains (DP \geq 35). Gelatinization enthalpy (ΔH) and gelatinization temperature (T_o , T_p) correlated positively with swelling power. The enthalpy of retrograded starches correlated positively with swelling power. This suggested that factors contributing to granule crystallinity influence differences in starch swelling properties. The molecular characteristics of amylopectin (longer chains with DP \geq 35) that favor recrystallization also appear to positively influence starch swelling. No correlation was found between lipid content and swelling power.

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