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Correlation between Wheat Starch Annealing Conditions and Retrogradation during Storage

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Abstract

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The effects of annealing conditions on the degree of retrogradation (DR) in wheat starch were analysed by differential scanning calorimetry (DSC) and X-ray diffraction (XRD). Three annealing parameters were varied, namely moisture (50–90%), time (0.5–48 h), and temperature (35–50°C). These factors had different impacts on the DR. The results suggest that 25% (w/v) annealed wheat starch samples stored at 4°C for 7 days had smaller DR values than native wheat starch under the same storage conditions. XRD indicated that annealed wheat starches stored at 4°C for 7 days had fewer recrystallisations than native wheat starch. Optimised annealing conditions can be used to control the structural and mechanical properties of certain starch-based products, such as in the production of bread, since retrogradation results in staling and reduced digestibility.

Keywords: annealed starch; degree of retrogradation; X-ray diffraction; differential scanning calorimetry

Many starch-based foods are prepared through thermal processing (baking) with relatively limited moisture (10–80%) and are consumed in glassy states (Chung *et al*. 2005). The thermal treatment also influences flavour and palatability. The physical aging of these low moisture starchy foods is an important storage behaviour (Ambigaipalan *et al*. 2013) and affects their long-term stability, freshness, and quality. Staling is influenced by "retrogradation" (Lai *et al.* 2000), a process by which gelled starch expels water from the polysaccharide network. Gelatinisation is the transition of starch from a stable equilibrium state to a disordered non-equilibrium state (Chung & Lim 2006), resulting in the development of an amylose gel network. This takes place relatively quickly, allowing the structure to remain unchanged during storage. Relaxation of this gelled structure occurs in processed starchy products during their storage (Kumar Varma *et al*. 2014). As a result, the gelatinised starches recrystallise. Retrogradation of starch is affected by many factors, including storage temperature, starch concentration, presence of additives, and physical modification of the starch (Hoover 1995).

Annealing is a simple heat-treatment process that puts the starch at a temperature just above its glass transition temperature and below the onset of gelatinisation temperature with excess water for a period of time (Tester & Debon 2000). Eerlingen *et al.* (1997) proposed that annealing causes a decrease in swelling power by alteration of the interaction between crystallites and the amorphous matrix. Other researchers suggested that retrogradation of starch gel was associated with the swelling power and solubility of starch (Koo *et al*. 2000). The effect of annealing on the molecular structures and physicochemical properties of starches of different botanical origins has been reported by Jayakody and Hoover (2008), when the authors concluded that crystalline starches become perfect and that the molecular chains of starches move and interact (Jayakody & Hoover 2008). The influence of an-

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nealing on the gelatinisation properties, retrogradation, and susceptibility to enzymatic hydrolysis of starch from the tropical breadfruit (*Artocarpus communis*) has been reported (Siswoyo & Morita 2010). The gelatinisation temperature of breadfruit starch is lower than that of maize starch but higher than that of potato starch, and the gelatinisation and retrogradation of breadfruit starch paste are weaker (Zhang *et al.* 2010). The authors concluded that annealing the breadfruit starch at different temperatures caused an increase in the peak temperature of gelatinisation (as determined by DSC), slowed retrogradation during storage, and retarded enzyme hydrolysis. While DSC can monitor these changes during retrogradation, the mechanism by which annealing affects retrogradation – how it changes the starch molecular structure – is not well understood.

Bread and other baked products made from wheat flour rely on the unique properties of wheat starch (Gray & Bemiller 2003). Staling through retrogradation leads to economic losses for both the industry and the consumer. Therefore, the objective of this study was to investigate how different annealing conditions may influence retrogradation of wheat starch-based foods. A considerable amount of evidence indicates that the major factor in such staling is the crystallisation of the starch component (Hellman *et al.* 1954; Gudmundsson 1994). Based on the idea that the aging process in starch gels is analogous to the staling of bread, DSC and XRD were used (Manzocco *et al.* 2002; Hoover *et al.* 2010) to test the effects of annealing conditions on starch retrogradation.

MATERIAL AND METHODS

Material. Native wheat starch (NWS) was purchased from Sigma Co. (St. Louis, USA). Biochemical grade sodium azide was purchased from Sinopharm Chemical Reagent Co. (SCRC, Shanghai, China). All other reagents used were of analytical grade from SCRC.

Methods

Annealed starches. Moisture content, annealing temperature, and annealing time were varied during the annealing process according to Tester *et al.* (1998). Briefly, wheat starch (3 g) was heated in different amounts of water (50–90% w/v) at 8–25°C lower than the onset gelatinisation temperature (T_0) for each mixture and for various times (0.5–48 h). Sodium azide was added as a bacteriostatic agent. After annealing, samples were centrifuged and washed three times with acetone, ethyl alcohol, and distilled water, successively. Annealed starches were then collected by suction filtration using a Hirsch funnel and subjected to vacuum drying in an oven at 25°C (Valentina *et al.* 2005). This temperature was chosen to avoid additional annealing of some samples.

*Preparation of samples for DSC and XRD***.** The water-to-starch ratio of the samples (native wheat starch and annealed starch) was 4 : 1 for DSC. Native starch and annealed starch samples were prepared for XRD analysis by heating to gel them at the same concentration (25% w/v water). The gelatinised starches were freeze-dried before analysis.

Degree of retrogradation monitored by DSC. The degree of retrogradation (DR) was measured by DSC (Liu *et al.* 2007) using a Perkin-Elmer DSC 8000 equipped with a Thermal Analysis Data Station (TADS). Samples of native wheat starch (NWS) and annealed wheat starch (AS; 100 mg) were weighed directly in a 4 ml centrifugal tube, to which 300 μl distilled water was then added. About 7–10 mg of the prepared specimen was sealed in an aluminium pan. The sample was equilibrated at 4°C for one night before DSC analysis. An empty pan was used as a reference. The sample was heated from 20°C to 120°C at a rate of 10°C/minute. The enthalpy of gelatinisation (ΔH_g) was calculated by the TADS and was expressed as J/g of dry starch.

The gelatinised starches were stored at 4°C for 7 days. After 7 days, the samples of retrograded native starch (RNS) and retrograded annealed starch (RAS) were scanned again by DSC to measure retrogradation enthalpies (ΔH_r) (White *et al.* 1989). The DR for each starch sample was calculated using the following formula:

$$\text{Retrogradation (\%)} = [\Delta H_r/\Delta H_g] \times 100$$

where: ΔH_g (J/g) – gelatinisation enthalpy; ΔH_r (J/g) – retrogradation enthalpy

All results are the average of three samples.

Crystalline texture detected by X-ray diffraction. Gelatinised native starch (GNS) and gelatinised annealed starch (GAS) were stored at 4°C for 7 days. Freeze-dried samples were ground into powder with mortar and pestle and passed through a 200 mesh sieve. The prepared samples were analysed using a Rigaku TTR-III X-ray diffractometer equipped with a copper tube operating at 40 kV and 50 mA producing CuK_α radiation of 1.54 Å wavelength. Diffractograms were recorded over a range of 3° and 50° (2θ) at 0.02° intervals. The speed of the X-ray goniometer

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and recording paper was 1°/min and 10 mm/min, respectively (Karim *et al.* 2000; Rudi *et al.* 2006; Wu *et al.* 2009). The degree of crystallisation was calculated by the following equation (Du *et al.* 2011):

$$\text{Crystallinity (\%)} = A_c/(A_c + A_a) \times 100$$

where: A_c – crystalline area on the X-ray diffractogram; A_a – amorphous area on the X-ray diffractogram

Statistical analyses. All the experiments were repeated three times and the results were reported as the mean and standard deviation. Analysis of variance (one-way ANOVA) was performed and Tukey's HSD tests at 95% ($P < 0.05$) confidence level were carried out to determine any significance of differences between data points using the SPSS statistical package (Soetaredjo 2012).

RESULTS AND DISCUSSION

DR of native wheat starch (NWS) and annealed starch (AS). DSC showed that the gelatinisation enthalpy (ΔH_g) of NWS was 8.08 J/g and its retrogradation enthalpy (ΔH_r) after 7 days of storage at 4°C was 3.08 J/g. The degree of retrogradation (DR) of NWS was calculated as about 38%, consistent with other results (Liu *et al.* 2007). Annealing affects these properties, but each annealing parameter must be varied against each other annealing parameter. Starch samples were annealed under different conditions, stored and subjected to the same rheological measurements as the NWS (Tables 1–3).

The effects of sample moisture content and annealing temperature on the retrogradation of wheat starch are illustrated in Table 1. The annealing time was fixed at 48 h to allow for macromolecular mobility and to decrease effects of time. Annealed starches had higher ΔH_g values and lower ΔH_r values than those of NWS, resulting in reduced DR values. Increased gelatinisation enthalpy with annealing implied increased molecular structure in the annealed starch granules (Koo *et al.* 2000). Wheat starch at 70% moisture and annealed at 40°C for 48 h had the lowest degree of retrogradation (17.65%). Reports have shown that the rigidity of swollen starch granules is the major factor determining the rheological properties of a starch paste or gel (Koo *et al.* 2000). It is hypothesised that even though excess water causes leaching of amylose, which would increase the DR, annealing should increase the rigidity of the swollen granules, which would reduce the DR (Lii *et al.* 1996; Tsai *et al.* 1997). The DR values of wheat starch samples annealed at 35 and 50°C varied slightly, while annealing at 40 or 45°C resulted in larger changes in DR values. For each temperature, 60–80% moisture content resulted in the lowest DR values. Compared to NWS, annealing reduced the degree of retrogradation of wheat starch. Furthermore, both

Table 1. Gelatinisation enthalpy (ΔH_g), retrogradation enthalpy after storage (ΔH_r), and degree of retrogradation (DR) as determined by DSC of wheat starch samples annealed at different moisture contents (in %) and at different temperatures for 48 hours

Annealing condition	ΔH_g (J/g)	ΔH_r (J/g)	DR (%)	Annealing condition	ΔH_g (J/g)	ΔH_r (J/g)	DR (%)
35°C				**40°C**			
50	10.24 ± 0.46	2.44 ± 0.16	23.83 ± 0.50[c]	50	11.44 ± 0.37	3.12 ± 0.08	27.27 ± 0.38[d]
60	9.52 ± 0.23	1.92 ± 0.09	20.17 ± 0.46[a]	60	9.72 ± 0.35	2.20 ± 0.08	22.63 ± 0.53[b]
70	9.24 ± 0.08	2.08 ± 0.02	22.51 ± 0.12[b]	70	8.84 ± 0.26	1.56 ± 0.06	17.65 ± 0.62[a]
80	10.00 ± 0.09	2.40 ± 0.04	24.00 ± 0.18[c]	80	10.00 ± 0.23	2.12 ± 0.07	21.20 ± 0.78[b]
90	9.20 ± 0.05	2.24 ± 0.02	24.35 ± 0.34[c]	90	11.36 ± 0.28	2.88 ± 0.06	25.35 ± 0.98[c]
45°C				**50°C**			
50	9.96 ± 0.34	2.88 ± 0.06	28.92 ± 0.56[b]	50	9.20 ± 0.14	2.00 ± 0.06	21.74 ± 0.59[a]
60	9.48 ± 0.21	2.44 ± 0.08	25.74 ± 0.61[a]	60	9.20 ± 0.20	2.24 ± 0.07	24.35 ± 0.77[b]
70	8.88 ± 0.19	2.32 ± 0.05	26.13 ± 0.51[a]	70	8.56 ± 0.07	2.12 ± 0.03	24.77 ± 0.51[b]
80	10.28 ± 0.36	3.04 ± 0.09	29.57 ± 1.02[b]	80	8.96 ± 0.24	2.16 ± 0.06	24.11 ± 0.58[b]
90	9.76 ± 0.32	2.76 ± 0.06	28.28 ± 0.87[b]	90	10.48 ± 0.36	2.80 ± 0.08	26.72 ± 0.92[c]

The average values ± standard deviation ($n = 3$); for each temperature set, values in the same column followed by different letters differed significantly ($P < 0.05$)

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Table 2. Gelatinisation enthalpy (ΔH_g), retrogradation enthalpy after storage (ΔH_r), and degree of retrogradation (DR) as determined by DSC of wheat starch samples of different moisture contents (in %) annealed at 50°C for different lengths of time (in h)

Annealing condition	ΔH_g (J/g)	ΔH_r (J/g)	DR (%)	Annealing condition	ΔH_g (J/g)	ΔH_r (J/g)	DR (%)
50%				**60%**			
0.5	8.52 ± 0.23	2.20 ± 0.08	25.82 ± 1.55^{bc}	0.5	8.84 ± 0.16	1.92 ± 0.08	21.72 ± 1.12^{ab}
4	9.40 ± 0.12	3.48 ± 0.05	37.02 ± 0.92^{d}	4	8.64 ± 0.24	2.84 ± 0.05	32.87 ± 1.36^{c}
12	9.12 ± 0.17	2.12 ± 0.06	23.24 ± 1.08^{ab}	12	8.56 ± 0.15	1.80 ± 0.06	21.03 ± 1.08^{a}
24	8.32 ± 0.12	2.20 ± 0.08	26.44 ± 1.34^{c}	24	8.44 ± 0.16	1.88 ± 0.06	22.27 ± 1.02^{ab}
48	9.20 ± 0.14	2.00 ± 0.06	21.74 ± 0.59^{a}	48	9.20 ± 0.20	2.24 ± 0.07	24.35 ± 0.77^{b}
70%				**80%**			
0.5	9.08 ± 0.26	2.16 ± 0.08	23.79 ± 1.50^{a}	0.5	8.76 ± 0.19	2.48 ± 0.06	28.31 ± 0.86^{b}
4	8.56 ± 0.32	2.52 ± 0.09	29.44 ± 1.04^{b}	4	8.56 ± 0.22	2.48 ± 0.09	28.97 ± 1.01^{b}
12	7.52 ± 0.28	2.08 ± 0.08	27.66 ± 1.12^{b}	12	7.36 ± 0.27	1.92 ± 0.06	26.09 ± 1.06^{a}
24	7.76 ± 0.16	1.92 ± 0.05	24.74 ± 0.98^{a}	24	7.48 ± 0.35	1.84 ± 0.12	24.60 ± 0.92^{a}
48	8.56 ± 0.07	2.12 ± 0.03	24.77 ± 0.51^{a}	48	8.96 ± 0.24	2.16 ± 0.06	24.11 ± 0.58^{a}
90%							
0.5	8.52 ± 0.26	2.80 ± 0.06	32.86 ± 1.26^{c}				
4	7.32 ± 0.32	2.00 ± 0.08	27.32 ± 0.92^{b}				
12	7.52 ± 0.21	1.88 ± 0.08	25.00 ± 1.19^{ab}				
24	7.72 ± 0.19	1.88 ± 0.12	24.35 ± 1.21^{a}				
48	10.48 ± 0.36	2.80 ± 0.08	26.72 ± 0.92^{ab}				

The average values ± standard deviation (n = 3); the values in the same column followed by different letters differ significantly ($P < 0.05$)

annealing temperature and moisture content of the starch sample affected retrogradation behaviour.

The combined effects of moisture content and annealing time on wheat starch rheology are reported in Table 2. A fixed temperature of 50°C was chosen according to the result of Table 1, since the DR values for wheat starches annealed at 50°C for 48 h were similar between 60 and 80% moisture content. Starch mixtures of different moisture contents that were annealed for 30 min had DR values ranging from 21.7% to 32.9%. As water content increased from 50% to 60%, the DR values tended to decrease for each incubation time below 48 hours. However, as moisture content increased from 70% to 90%, the DR values increased with increasing water content, resulting in a more degraded sample after storage. It is clear that lower moisture content results in more stable starch. Likewise, a clear benefit of longer annealing times can be seen. At 30 min, the annealing time is relatively short and does not allow for sufficient intermolecular rearrangement, especially at low or high moisture content. Some water improves the flow of the starch molecules, but excess water may increase the leaching of amylose, especially at shorter annealing times (Guo 1997). Our results showed that the lowest moisture content and the longest annealing time (50% moisture and 48 h) resulted in the most stable starch.

The interaction between annealing temperature and annealing time on wheat starch rheology is described in Table 3. On the basis of the above results (Table 2), moisture content was kept at 60%. These results further showed that wheat starch was more stable with increased annealing time. There is also an interesting interplay between moderate incubation times and warmer temperatures. Using this data on annealing starch with a moisture content of 60%, ANOVA between temperature and time was performed (Table 4). The results of comparison between different temperatures and different times are listed in Table 5. The ANOVA results also suggested that the significance of different temperature and time was less than 0.05 (Table 5). The correlation coefficient between temperature and time was 98.60% (Table 4).

X-ray diffraction pattern of retrograded native starch (RNS) and retrograded annealed starch (RAS) samples. The X-ray patterns of cereal (such

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Table 3. Gelatinisation enthalpy (ΔH_g), retrogradation enthalpy after storage (ΔH_r), and degree of retrogradation (DR) as determined by DSC of wheat starch samples with 60% moisture content annealed at different temperatures (in °C) and for different lengths of time (in h)

Annealing condition	ΔH_g (J/g)	ΔH_r (J/g)	DR (%)	Annealing condition	ΔH_g (J/g)	ΔH_r (J/g)	DR (%)
0.5 h				**4 h**			
35	8.44 ± 0.18	2.00 ± 0.08	23.70 ± 0.83^{ab}	35	10.52 ± 0.52	3.12 ± 0.16	29.66 ± 1.34^{b}
40	9.48 ± 0.21	2.48 ± 0.06	26.16 ± 0.92^{b}	40	8.64 ± 0.41	2.08 ± 0.12	24.07 ± 1.15^{a}
45	8.92 ± 0.99	2.24 ± 0.07	25.11 ± 1.31^{b}	45	8.16 ± 0.22	2.44 ± 0.16	29.90 ± 1.27^{b}
50	8.84 ± 0.16	1.92 ± 0.08	21.72 ± 1.12^{a}	50	8.64 ± 0.24	2.84 ± 0.05	32.87 ± 1.36^{c}
12 h				**24 h**			
35	9.16 ± 0.31	2.12 ± 0.09	23.14 ± 0.98^{a}	35	9.52 ± 0.29	2.32 ± 0.08	24.37 ± 0.96^{a}
40	9.52 ± 0.21	2.56 ± 0.12	26.89 ± 1.02^{b}	40	9.92 ± 0.33	2.32 ± 0.13	23.39 ± 0.88^{a}
45	9.60 ± 0.26	3.04 ± 0.15	31.67 ± 1.39^{c}	45	11.32 ± 0.56	3.84 ± 0.21	33.92 ± 1.41^{b}
50	8.56 ± 0.15	1.80 ± 0.06	21.03 ± 1.08^{a}	50	8.44 ± 0.16	1.88 ± 0.06	22.27 ± 1.02^{a}
48 h							
35	9.52 ± 0.23	1.92 ± 0.09	20.17 ± 0.46^{a}				
40	9.72 ± 0.35	2.20 ± 0.08	22.63 ± 0.53^{b}				
45	9.48 ± 0.21	2.44 ± 0.08	25.74 ± 0.61^{d}				
50	9.20 ± 0.20	2.24 ± 0.07	24.35 ± 0.77^{c}				

The average values ± standard deviation (n = 3); the values in the same line followed by different letters differ significantly (P < 0.05)

as maize, wheat, and rice) starches and retrograded starches are A-type patterns and B-type patterns, respectively (Imad & Juliane 2001; Thais *et al.* 2012). The C-type pattern is usually observed in legume seed starches and is an intermediate between A and B types (Tester *et al.* 1998). The XRD pattern of native wheat starch had several strong peaks (Figure 1 – curve a) at 2θ = 15°, 17°, 18°, and 23° (Fu *et al.* 2013). The NWS had an A-type crystalline structure, as reported by Guo (1997), and a degree of crystallization of about 29.87%. This NWS was gelatinised and was immediately freeze-dried (stored for 0 days, GNS) or stored for 7 days (GNS7) before XRD. In the GNS, there were few crystallizsation peaks, with only one peak at 2θ = 16.6° which then weakened at 2θ = 15°, 17°, 18°and 23°, indicating that the typical A-type crystallinity seen in NWS was largely lost (Figure 1– curve b). The GNS after 7 days of storage at 4°C had lower peaks at 2θ = 17° and 20° – Figure 1 – curve c), similar to reported characteristic peaks of B-type XRD pattern. The peaks in the GNS0 and GNS7 indicated that B-type crystallinity appeared

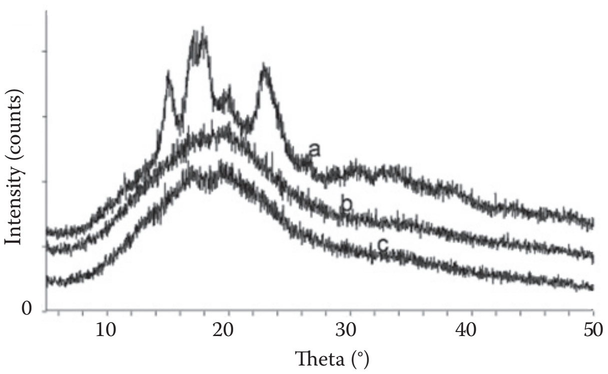

Figure 1. The X-ray patterns of native wheat starch and retrograded native wheat starch stored at 4°C for 0 or 7 days

a – native wheat starch (NWS); b – gelatinised NWS, retrograded 0 days (GNS); c – gelatinised NWS, retrograded 7 days (GNS7)

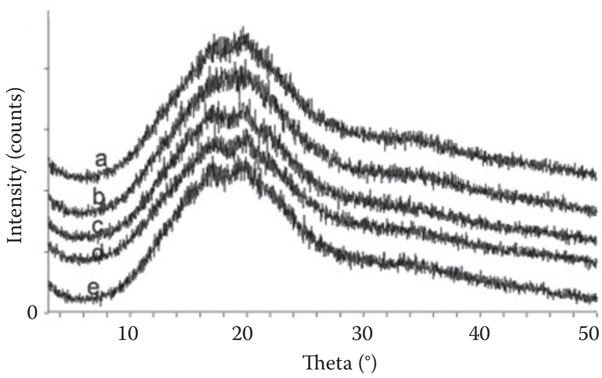

Figure 2. The X-ray patterns of wheat starches with different water contents after annealing at 50°C for 48 h and storage at 4°C for 7 days

a – 50%; b – 60%; c – 70%; d – 80%; e – 90% moisture content

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Table 4. ANOVA of the effects of temperature and time on the degree of retrogradation of wheat starch at 60% moisture

Factors/interactions	Type × SS	DF	MS	*F*-value	Significance
Calibration model	786.451	19	41.392	227.400	0.000
Temperature	214.758	3	71.586	393.278	0.000
Time	232.387	4	58.097	319.172	0.000
Temperature × time	339.306	12	28.275	155.340	0.000

R^2 = 99.10%; R^2(adj) = 98.60%; DF – degrees of freedom; MS – mean square

Table 5. Comparison between different temperatures and time

(I) Temperature	(J) Temperature	(I–J) ΔMean	Significance	(I) Temperature	(J) Temperature	(I–J) ΔMean	Significance
35	40.00	–0.4820*	0.004	45.00	40.00	4.1580*	0.000
	45.00	–4.6400*	0.000		50.00	4.2493*	0.000
	50.00	–0.3907*	0.016	50.00	40.00	–0.0913	0.561
(I) Time	(J) Time	(I–J) ΔMean	Significance	(I) Time	(J) Time	(I–J) ΔMean	Significance
0.50	4.00	–4.5683*	0.000	4.00	12.00	3.5125*	0.000
	12.00	–1.0558*	0.000		24.00	3.0717*	0.000
	24.00	–1.4967*	0.000		48.00	5.9142*	0.000
	48.00	1.3458*	0.000	12.00	24.00	–0.4408*	0.015
24.00	48.00	2.8425*	0.000		48.00	2.4017*	0.000

*ΔMean at 0.05 was significant

and increased with longer storage time. The degree of crystallinity of native wheat starch gelatinised and stored for 7 days was about 10.26%. After documenting the XRD patterns of NWS, gelatinised NWS (GNS) and GNS stored for 7 days (GNS7), XRD patterns were generated for starches annealed at different moisture contents, different temperatures and for different lengths of time.

To see how the moisture content of starch affects the crystallinity after storage, samples were annealed at 50°C for 48 h prior to 7 days of storage at 4°C. The XRD patterns of stored starches annealed at ≤ 70% moisture content had less obvious peaks than those of stored samples annealed at ≥ 80% moisture content (Figure 2). The crystalline structure of retrograded annealed starches (RAS7) increased with increased

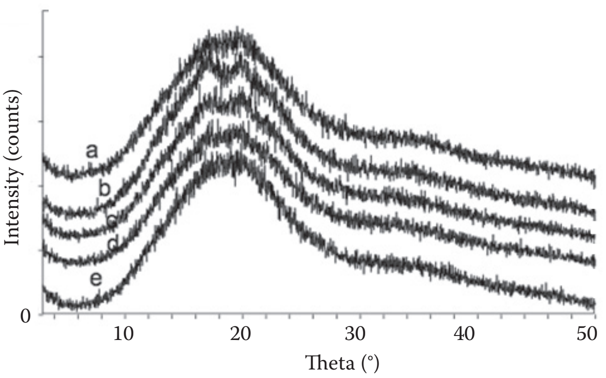

Figure 3. The X-ray diffraction patterns of wheat starches annealed at 60% water content and at 50°C for various times after storage at 4°C for 7 days

a – 0.5 h; b – 4 h; c – 12 h; d – 24 h; e – 48 h

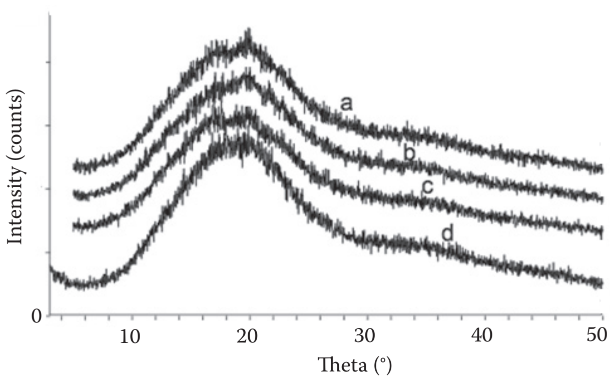

Figure 4. The X-ray diffraction patterns of wheat starch with fixed water content (60%) after annealing at various temperatures for 48 h and storage at 4°C for 7 days

a – 35°C; b – 40°C; c – 45°C; d – 50°C

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Table 6. The crystallinity after storage as determined by XRD of wheat starch samples of different moisture contents annealed at different temperatures and for different lengths of time

Fixed annealing condition 48 h 50°C		Fixed annealing condition 60% 50°C		Fixed annealing condition 60% 48 h	
Moisture content (%)	crystallinity (%)	annealing time (h)	crystallinity (%)	annealing temperature (°C)	crystallinity (%)
50	5.92 ± 0.02[a]	0.5	6.03 ± 0.08[a]	35	5.93 ± 0.08[a]
60	6.18 ± 0.03[b]	4	7.82 ± 0.13[e]	40	6.16 ± 0.04[b]
70	7.16 ± 0.09[c]	12	7.05 ± 0.03[c]	45	7.09 ± 0.04[d]
80	9.14 ± 0.06[e]	24	7.38 ± 0.05[d]	50	6.81 ± 0.03[c]
90	8.69 ± 0.13[d]	48	6.81 ± 0.03[b]		

The average values ± standard deviation ($n = 3$); the values in the same line followed by different letters differ significantly ($P < 0.05$)

moisture content and showed new peaks, other than B-type crystalline peaks, in samples above 60% moisture. From 50% to 80% moisture content, the degree of crystallinity increased incrementally with each increase of moisture content, from 5.92% to 9.14% (Table 6). The RAS annealed at 90% moisture content has a degree of crystallinity of 8.69% (Figure 2). All annealed starches showed less crystallinity, and thus less retrogradation, than the native wheat starches stored for the same length of time.

To see how annealing time affects the crystalline structure of wheat starch after storage, samples were annealed at 50°C for 0.5–48 h prior to 7 days of storage at 4°C (Figure 3). The changes in XRD patterns appeared to be consistent with the extension of annealing time (Figure 3 and Table 6). The changes in diffraction patterns showed that the increasing annealing time decreased recrystallisation. Although the starch annealed for 4 h showed a higher crystallinity than starches annealed for other periods of time, it was still less than in unannealed starch at 10.26%.

To see how annealing temperature affects the crystalline structure of wheat starch after storage, samples with 60% moisture content were annealed at 35–50°C for 48 h prior to 7 days of storage at 4°C (Figure 4). While all annealed starches retrograded to B-type patterns, different temperatures resulted in differing amounts of recrystallisation, with the most at 35°C (5.93%) and the least at 50°C (7.09%), indicating that annealing at 50°C results in less retrogradation (Figure 4 and Table 6).

CONCLUSIONS

The process of annealing decreased the DR of wheat starch, as observed by DSC. Both low and high annealing temperatures induced fewer changes than annealing in the middle range of temperatures. When starch was annealed at 70% moisture content or below, it showed a lower DR, and was thus more stable, than starches of above 80% moisture content. Increased annealing time also increased wheat starch stability. ANOVA of the interaction between annealing temperature and time suggested that time influenced DR the most at lower temperatures and that this influence diminished as the temperature increased, up to 50°C. In addition, XRD patterns of stored samples further demonstrated that the three annealing factors affected the degree of retrogradation and crystallinity. This study has investigated the effects of annealing on the degree of retrogradation of wheat starch, but further studies should focus on the rate of retrogradation in order to fully explore the relationship between annealing and starch stability. These findings on the abrogation of wheat starch retrogradation are important for improving the storage of starchy foods through the development of various thermal processes.

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