Carbohydrate Polymers 78 (2009) 268–274

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Physicochemical changes of starch in refrigerated dough during storage

Yu Zhang, Senay Simsek *

Department of Plant Sciences, North Dakota State University, P.O. Box 6050, Dept#7670, 1250 Bolley Dr. Fargo, ND 58108-6050, USA

ARTICLE INFO

Article history:
Received 6 March 2009
Received in revised form 24 March 2009
Accepted 25 March 2009
Available online 2 April 2009

Keywords:
Refrigerated dough
Starch
Physicochemical properties

ABSTRACT

Refrigerated dough is a flour-based, unbaked product that is stored between 4 and 7 °C. Maintaining the dough quality during storage is very crucial. Starch functionalities have important effects on flour and dough quality. The objective of this research was to determine the physicochemical changes of starch in refrigerated dough during extended storage. Two wheat flours with different amylose/amylopection ratio were used in this study. The relative percentage of amylopectin decreased up to 10.51%. Overall, we observed that the pasting properties and thermal properties of starch were changing during the storage: peak viscosity decreased up to 23.06%, breakdown viscosity decreased up to 57.70%, and setback viscosity decreased up to 41.25% compared to flour samples during 34 days refrigerated storage. We detected the variation in starch granular morphology. These results showed that physicochemical properties of starch changes during refrigerated storage, which may have significant impacts on end-product quality.

1. Introduction

The refrigerated dough industry is considered one of the fastest growing segments of the ready-to-use grain-based industry. Refrigerated dough products account for more than $1.6 billion of sales per year in the USA. The items in this category include canned refrigerated biscuits, canned refrigerated croissants and sweet rolls (Courtin, Gys, & Delcour, 2006). In today's market, in-store bakeries use frozen dough or par-bake products, which offer good quality for fresh-bread lovers, but to improve further the quality of these products, refrigerated dough is considered a convenient and economical choice for the production of fresh-bread products. The high quality of bakery products made with refrigerated doughs should be characterized by a long shelf life with good organoleptic properties. However, during refrigerated storage, a yellowish liquid separates from the dough forming a syrup that leaks out of the product to produce a deleterious phenomenon known as "dough syruping" (Courtin, Gys, Gebruers, & Delcour, 2005; Simsek & Ohm, 2009). It has been hypothesized that dough syruping is due to the action of natural enzymes named endoxylanases. These enzymes modify the water holding capacity of Arabinoxylans (AX), which are polysaccharides present in the dough (Gys, Courtin, & Delcour, 2003). Our previous studies also showed that rheological properties and protein fractions of dough also changed during the refrigeration storage.

Starch is the major storage polysaccharide of higher plants, such as wheat. It has unique physical and chemical properties and nutritional quality compared to other carbohydrates. Starch granules are composed of two constituent polymers: a basically linear polysaccharide (α-1,4 linked glucose) named amylose and a highly branched polysaccharide named amylopectin (α-1,4 linked glucose and α-1,6 linked glucose) (Whistler & BeMiller, 1997). Wheat endosperm contains mainly two types of starch granules: the large, lenticular (A type) and small, spherical (B type). Wheat flour consists of 70–80% dry matter of starch. Normal wheat starch contains an average of about 25% amylose. High amylose starch has greater than 40% amylose.

When starch is primarily composed of amylopectin, it is defined as waxy starch. Hexaploid partial waxy wheats are characterized by slight reduction in amylose content due to the absence of waxy-protein, granule-bound starch synthase (GBSS), alleles at one or two of the three *Wx* loci (Nakamura, Yamamori, Hirano, & Nagamine, 1995). The potential use of wheat with reduced amylose content is a current focus of interest among wheat breeders, geneticists and cereal scientists (Graybosch, 1998). Partial waxy wheats are sources of flours with optimal quality characteristics in certain Asian wet noodle products. Partial waxy wheats were shown to enhance Japanese Udon noodle quality, which generally decreases with an increase in flour amylose content (Oda, Yasuda, Okazaki, Yamauchi, & Yokoyama, 1980). In addition, partial waxy wheats are essential to the development of waxy wheats with acceptable agronomic performance (Graybosch, 1998).

The refrigerated dough market is a relatively new market. There was very limited peer-reviewed research conducted in this area, especially about the role of flour fractions on the quality of refrigerated dough products. In frozen dough, the roles of starch and starch structural and functional properties during storage had been

* Corresponding author. Tel.: +1 701 231 7737; fax: +1 701 231 8474.
E-mail address: senay.simsek@ndsu.edu (S. Simsek).

0144-8617/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.carbpol.2009.03.041

studied. Lu and Grant (1999b) used fractionation and reconstitution of wheat flour components to investigate the roles of starch and others in breadmaking quality of frozen dough. They found that the starch fraction contributed significantly to frozen dough quality. Also, they found that starch isolated from frozen dough made with different wheat flour varied in enthalpy (ΔH) largely, but all the samples showed a significant increase of ΔH with increased frozen storage time (Lu & Grant, 1999a). This could be explained by the way water interacts with the starch during the freezing process and the rate of water migration in the frozen dough during frozen storage (Kulp, 1995). Low-temperature Scanning Electron Microscopy (SEM) was used to examine the effects of storage on the structure of frozen bread doughs. Starch granules were no longer associated with gluten fibrils in the dough structure (Kulp, 1995). Onset temperature of starch gelatinization measured by Differential Scanning Calorimetry (DSC) increased during frozen storage (Autio & Sinda, 1992). The increase of onset temperature may be attributed to the delay in the rate of diffusion of water into the starch granules or the increased crystallinity of the granules. In frozen dough, the amylose–amylopectin ratios were negatively correlated with frozen storage time (Wolt & D' Appolonia, 1984). It was uncertain whether this relationship reveals any fundamental functional effect or whether it simply reflected a higher degree of retrogradation of the soluble starch polymers in frozen doughs. No studies on the role of starch in refrigerated dough have been done yet. Understanding the changes in the physical and functional properties of starch during storage would be helpful to predict the end-product quality.

The objective of this research is the determination of physicochemical properties of starch in refrigerated dough during storage using two kinds of flours, which differ in their amylose–amylopectin ratios.

2. Experimental

2.1. Materials

Glenn and Parshall, two wheat varieties of Hard Red Spring Wheat (HRSW), were obtained from North Dakota State University (NDSU) Casselton Research Extension Center in 2007. Glenn is one of the most commonly grown varieties in North Dakota, the main HRSW growing area in the United States. It is normal hexaploid wheat. Parshall is a partial waxy hexaploid HRSW cultivar, developed by North Dakota State University. Parshall would provide information on the role of amylose reduction in refrigerated dough products.

They were milled using a Buhler lab mill in the NDSU-HRSW quality laboratories. Test weight, wheat protein, falling number, flour ash, flour protein, wet gluten, and farinograph absorption were determined using standard AACC methods 55-10, 46-30, 56-81B, 08-01, 46-30, 38-12A, and 54-21 (AACC, 2000).

2.2. Preparation of refrigerated dough

In order to avoid confounding factors arising from the presence of other ingredients, a lean dough formula was used. The dough was prepared by using 100 g of flour (14% moisture basis), 1.8 g of salt, and a certain amount of water, containing 0.06% w/v of sodium azide (Mallinckrodt baker Inc. Paris, KY) to prevent microbial spoilage, to reach the desired moisture content previously determined according to the specific farinograph absorption test (Table 1). Dough was mixed in a 100 g pin mixer (National Manufacturing, Lincoln, NE) for the optimum mixing time, sheeted, molded, and stored in plastic containers for 34 days at 6 °C. The dough samples on storage day 1, 6, 16 and 34 were lyophilized and ground (Simsek & Ohm, 2009). They are named as G1, G6, G16, G34 and P1, P6, P16 and P34, respectively. Glenn and Parshall flour are named as GF and PF.

2.3. α-Amylase activity measurement

The α-amylase activity (CU/g) of two flour samples and all dough samples was determined using enzymatic digestion assay kits (Megazyme International, Wicklow, Ireland).

2.4. Starch isolation and preparation

Starch isolation: Starch was isolated from the flour samples by the dough washing method according to Lu and Grant (1999a). Two percent NaCl solution helped to separate the starch from gluten. The starch was washed with water, centrifuged at 2000g for 15 min. The purified starch was freeze-dried, ground using mortar and pestle, and stored in plastic bags.

2.5. Analysis of extracted starch samples with High Performance Liquid Chromatography (HPLC)

Percentages of amylose and amylopectin of the starch were determined using a method developed for HPLC by Grant and Ostenson (Grant, Ostenson, & Rayas-Duarte, 2002). The extracted starch was completely dissolved in 1 M KOH/Urea solution and then analyzed with an Agilent 1200 series HPLC (Agilent Technologies, Wilmington, DE), equipped with an auto sampler. A refractive index detector and PC with ChemStation (HP ChemStation for LC Rev. A.04.01) were used for control and integration. The amylose: amylopectin ratio was determined by HPLC analysis using a Waters Ultrahydrogel linear column (Waters Co., Milford, MA).

2.6. Analysis of extracted starch samples with Rapid Visco Analyzer (RVA)

Pasting properties of the starch samples were evaluated using RVA (Newport Scientific, Narrabeen, Australia) interfaced with a computer equipped with Thermocline and Thermoview software (Newport Scientific). The method used was modeled after that of Chakraborty et al. (2004) with minor modifications. Starch (3 g, 14% moisture basis) was added to pre-weighed deionized distilled water in an RVA canister. The rate of heating and cooling in the Std 1 profile was 12 °C per minute, idle temperature was 50 °C, and the total run time was 13 min. Parameters recorded were peak viscos-

Table 1
Properties of Hard Red Spring Wheat varieties, Glenn and Parshall, used in this study.

Variety	Test weight (kg/hl)	Wheat protein (%)[a]	Falling number (s)	Flour ash (%)[b]	Flour protein (%)[b]	Wet Gluten (%)[b]	Farinograph absorption (%)[c]	α-Amylase activity (CU/g)	Amy/AP ratio[d]
Glenn	83.0	14.3	429	0.49	13.6	34.8	66.2	0.115	0.32
Parshall	80.8	13.5	465	0.51	12.8	32.3	64.2	0.119	0.28

[a] The analysis was expressed on 12% moisture basis.
[b] The analysis was expressed on 14% moisture basis.
[c] Water absorption to reach 500 farinograph units (FU) line and based on 14% moisture basis.
[d] Amylose/amylopectin ratio.

ity (PV), hot paste viscosity (HPV), breakdown (BKD), cold paste (CPV) and setback (STB) viscosity. All measurements were reported in Rapid Visco Units (RVU).

2.7. Analysis of extracted starch samples with Differential Scanning Calorimetry (DSC)

The DSC analysis of the flour and starch was done using a Perkin-Elmer DSC-7 (PerkinElmer Life and Analytical Sciences, Inc., Waltham, MA) with a thermal analysis data station after minor modifications to the method of White, Abbas, Pollak, and Johnson (1990). The samples (3.5 mg, as is) were weighed into aluminum pans, and deionized water (0.8 μL) was added. Then, the pans were sealed and kept at room temperature overnight. The reference was an aluminum pan with deionized water (0.8 μL). Each sample was heated under nitrogen gas from 10 to 100 °C at 10 °C per minute. All analyses were carried out in triplicate. Enthalpy of gelatinization (ΔH), onset (T_o), peak (T_p), and conclusion (T_c) temperatures were computed automatically. The gelatinization temperature range was computed as $[(T_c - T_o)]$.

2.8. Analysis of extracted starch samples with X-ray powder diffraction

Relative degree of crystallinity of starch samples was investigated by X- ray powder diffraction (Philips vertical Multi-Purpose Diffractometer PW3040) operating at 50 kV and 40 mA (Cu-K_α radiation of 0.154 nm). The diffracted intensity was measured from 5° to 35° as a function of 2θ. The degree of crystallinity of the sample was defined by the intensity ratio of the diffraction peaks and of the sum of all measured intensity using PANalytical software X'Pert HighScore v. 2.2c. The constant background intensity, arising from imperfections of the sample, the X-ray optics of the instrument, sample fluorescence and scatter, was subtracted from the total intensity. The standard reference material was Respirable Alpha Quartz (NBS 1878, 95.5% crystallinity) which determined the constant background. All backgrounds were determined by using exactly the same automatic setting (Granularity = 20, Use smoothed input data = yes, Bending factor = 6).

2.9. Analysis of extracted starch samples with Scanning Electron Microscopy (SEM)

Starch samples were sprinkled onto carbon tape attached to aluminum mounts. Loose particles were removed using short bursts of compressed nitrogen gas. The sample then was coated with gold using a Hummer II sputter coater (Technics/Anatech Ltd., Alexandria, VA, USA). Images were obtained using a JEOL JSM-6490LV Scanning Electron Microscope (SEM) (JEOL, Peabody, MA, USA). Magnification, accelerating voltages, and micron bars are listed on each photo.

2.10. Statistical analysis

DSC analysis was done in triplicate. All other analyses were done in duplicate. The mean and standard deviations were calculated and reported in tables and figures.

3. Results and discussion

3.1. Flour characteristics

Two different Hard Red Spring Wheat (HRSW) varieties, Glenn and Parshall, with different amylose contents were used in this study. The physical and chemical properties of flours from Glenn and Parshall were summarized in Table 1. Glenn had slightly higher protein and wet gluten. Parshall had higher falling number. Both flours showed extremely low α-amylase activity, indicating grain soundness. Since Glenn has normal starch characteristics and Parshall is partial waxy wheat, the major difference between these two flours was amylose content, which was reported further in the part of HPLC analysis (Table 1). These two flours were chosen based on their different amylose/amylopectin ratio, which is an important structural characteristic of starch.

3.2. α-Amylase activity and pasting properties of starch from refrigerated dough

The α-amylase activity of the dough samples was lower than their parental flour samples. Freeze drying could be the reason for the reduction of enzyme activity in the dough samples. The α-amylase activity of all Glenn dough samples was similar to each other, and it was the same case in α-amylase activity of Parshall dough samples (Table 2). Based on these results we concluded that refrigeration storage did not have much influence on α-amylase activity.

RVA pasting properties of samples were shown in Table 2. The pasting properties of starch determined by RVA are directly related to its microstructure. Amylose, which contributes to higher gel consistency upon cooling, may have caused the initial rigidity to the swollen starch granules (Tsai, Li, & Lii, 1997). Since the amylose

Table 2
Pasting characteristics of starch samples determined by Rapid Visco Analyzer (RVA) and α -amylase activity of flour and ground dough samples.[a]

Sample name[c]	RVA pasting characteristics[b]						α-Amylase activity (CU/g)
	PV[d]	BKD[d]	STB[d]	HPV[d]	CPV[d]	PT (min)	
GF	270.3 ± 5.01	75.3 ± 1.19	127.5 ± 5.64	194.7 ± 3.82	322.2 ± 9.47	7.0 ± 0.00	0.115
G1	234.7 ± 10.34	72.3 ± 0.69	131.5 ± 12.41	162.4 ± 9.65	293.9 ± 22.07	7.0 ± 0.00	0.079
G6	226.6 ± 1.57	46.2 ± 0.25	55.5 ± 1.07	180.4 ± 1.82	235.8 ± 2.88	7.0 ± 0.00	0.078
G16	225.9 ± 0.88	63.9 ± 1.25	103.0 ± 4.76	162.1 ± 2.13	265.1 ± 2.63	7.0 ± 0.00	0.077
G34	238.9 ± 3.20	41.8 ± 2.32	95.5 ± 1.94	197.1 ± 5.52	292.6 ± 7.46	7.0 ± 0.00	0.083
PF	288.3 ± 2.88	73.5 ± 0.69	134.9 ± 0.19	214.9 ± 3.57	349.8 ± 3.39	7.0 ± 0.00	0.119
P1	251.8 ± 9.15	71.8 ± 1.00	126.7 ± 0.94	179.9 ± 10.16	306.7 ± 11.10	7.0 ± 0.00	0.091
P6	234.8 ± 4.64	54.7 ± 1.57	86.2 ± 6.96	180.2 ± 3.07	266.4 ± 10.03	7.0 ± 0.00	0.088
P16	243.9 ± 2.51	39.4 ± 1.88	102.3 ± 1.25	204.6 ± 0.63	306.9 ± 1.88	6.3 ± 0.05	0.090
P34	221.8 ± 3.01	31.1 ± 0.19	79.3 ± 0.44	190.7 ± 3.20	270.0 ± 3.64	6.9 ± 0.05	0.097

[a] Mean and standard deviation of duplicates.
[b] RVA = Rapid Visco Analyzer; PV = peak viscosity; BKD = breakdown; STB = setback; HPV = hot paste viscosity; CPV = cold paste viscosity; PT = peak time.
[c] GF = flour from Hard Red Spring Wheat (HRSW) variety Glenn; G1 = dough prepared using HRSW Glenn and stored 1 day; G6 = dough stored 6 days; G16 = dough stored 16 days; G34 = dough stored 34 days. PF = flour from HRSW variety Parshall; P1 = dough prepared using HRSW Parshall and stored 1 day; P6 = dough stored 6 days; P16 = dough stored 16 days; P34 = dough stored 34 days.
[d] The unit is expressed as RVU.

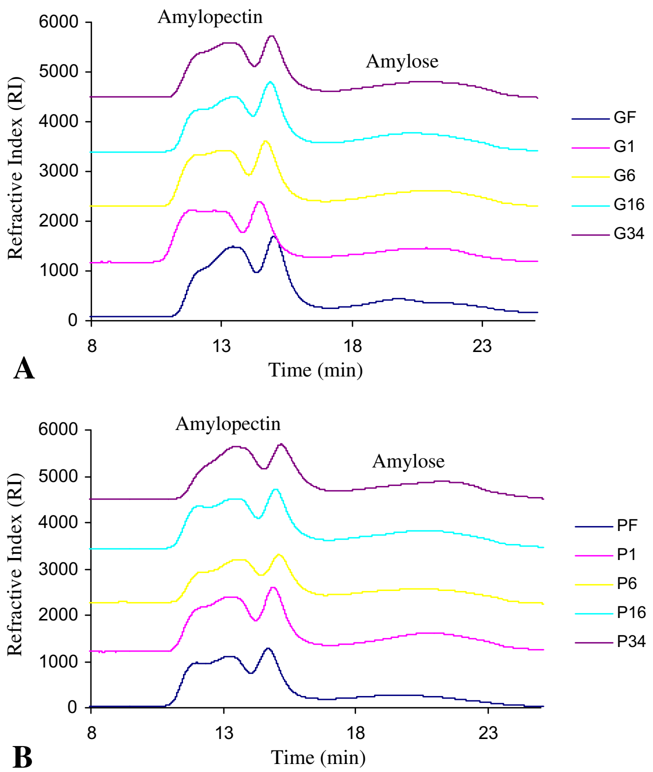

Fig. 1. Analysis of starch by High Performance Liquid Chromatography (HPLC) (GF = flour from Hard Red Spring Wheat (HRSW) variety Glenn; G1 = dough prepared using HRSW Glenn and stored 1 day; G6 = dough stored 6 days; G16 = dough stored 16 days; G34 = dough stored 34 days. PF = flour from HRSW variety Parshall; P1 = dough prepared using HRSW Parshall and stored 1 day; P6 = dough stored 6 days; P16 = dough stored 16 days; P34 = dough stored 34 days). Chromatograms show elution patterns of starch from flour and dough samples for Hard Red Spring varieties (A) Glenn and (B) Parshall.

content of Glenn- flour was about 2–3% higher than Parshall-flour, peak viscosity (Chakraborty et al., 2004), setback and paste viscosity of starch from Parshall-flour were higher than that from Glenn-flour. Breakdown viscosity was a little lower in starch from Parshall-flour. The peak time was the same for starch from Glenn-flour and Parshall-flour. However, the dough samples of these samples did not show the same trend over storage since a lot of factors influenced the starch properties, such as water hydration during dough mixing, water distribution and loss (dough syruping) during storage, and enzymatic degradation of amylopectin.

All the RVA parameters changed dramatically during refrigeration storage, except peak time, which indicated that storage had profound effects on starch pasting properties. Viscosities decreased during the storage for both samples. The RVA profile of starch extracted from G34 showed: peak viscosity decreased by 11.54%, breakdown viscosity decreased by 44.50%, and setback viscosity decreased by 25.07% compared with Glenn-flour starch. The RVA profile of starch extracted from P34 showed: peak viscosity decreased by 23.06%, breakdown viscosity decreased by 57.70%, and setback viscosity decreased by 41.25% compared with Parshall-flour starch (Table 2). So, the water binding capacity of starch and starch paste stability decreased during the refrigeration storage.

3.3. Changes in molecular weight distribution

Three peaks were shown in the HPLC chromatograms (Fig. 1). The first two peaks corresponds amylopectin and the following peak represents amylose (Simsek, Tulbek, Yao, & Schatz, 2009). Parshall-flour exhibited 2.2% lower amylose content than Glenn-flour: Glenn had 24.2% amylose while Parshall had 22.0% amylose. The term "partial waxy", characterized by a slight reduction in amylose content, was first defined by Nakamura, Yamamori, Hirano, and Hidaka (1993).

Based on the changes in apparent molecular weight of starch, we concluded that amylopectin became soluble during the storage, due to the internal α-amylase activity and formation of free water during storage. Compared the flour starch and the starch from dough on day 34, the percent of amylopectin of G34 decreased by 6.33%, while the percent of amylopectin of P34 decreased by 10.51%. Also, the amylose/amylopectin ratio of Glenn changed from 0.32 to 0.41 and the amylose/amylopectin ratio of Parshall changed from 0.28 to 0.43 during storage. So, the average molecular weight of starch became smaller during the storage. Since α-amylase activity of Parshall flour and dough samples was higher than that of Glenn-flour and dough samples, it turned out that Parshall's amylose content was even higher than Glenn on day16 and day 34 (Table 3).

3.4. Thermal properties and crystalline structure

Starch undergoes an irreversible order–disorder transition called gelatinization when heated in the presence of water. Various changes can be observed: granules swelling, absorption of water, loss of crystallinity and leaching amylose. Gelatinization behavior is influenced by starch granule structure, size, molecular alignment, or hydrogen bonding (White et al., 1990).

DSC has been used widely and extensively to study the gelatinization of starch (Donovan, Lorenz, & Kulp, 1983; Hayakawa, Tanaka, Nakamura, Endo, & Hoshino, 1997; Yoo & Jane, 2002). In our study, there were not significant changes of T_c, T_o, and T_p in the storage for flour and dough starch samples (Table 4). However, starch ΔH decreased during storage. A similar result was reported

Table 3
Analysis of starch fractions using High Performance Liquid Chromatography (HPLC).[a]

Fraction[b]		Glenn					Parshall				
		GF	G1	G6	G16	G34	PF	P1	P6	P16	P34
AP (%)	Peak 1	46.7	47.6	45.6	41.6	44.7	47.5	46.6	45.5	43.3	43.0
	Peak 2	29.1	26.6	26.9	29.6	26.3	30.5	27.9	27.4	26.8	26.7
	Total	75.8	74.2	72.5	71.3	71.0	78.0	74.6	73.0	70.2	69.8
Amy (%)		24.2	25.8	27.5	28.7	29.0	22.0	25.4	27.0	29.8	30.2
Amy/AP ratio		0.32	0.35	0.38	0.40	0.41	0.28	0..34	0.37	0.42	0.43

[a] GF = flour from Hard Red Spring Wheat (HRSW) variety Glenn; G1 = dough prepared using HRSW Glenn and stored 1 day; G6 = dough stored 6 days; G16 = dough stored 16 days; G34 = dough stored 34 days; PF = flour from HRSW variety Parshall; P1 = dough prepared using HRSW Parshall and stored 1 day; P6 = dough stored 6 days; P16 = dough stored 16 days; P34 = dough stored 34 days.
[b] AP = amylopectin; Amy = amylose.

Table 4
Thermal properties of starch samples determined by Differential Scanning Calorimetry (DSC).[a]

Sample name [c]	Thermal properties[b]				
	T_o (°C)	T_p (°C)	T_c (°C)	$T_c - T_o$ (°C)	ΔH (J/g)
GF	56.6 ± 0.08	61.53 ± 0.00	66.32 ± 0.37	9.72 ± 0.46	8.92 ± 0.35
G1	56.04 ± 0.31	61.12 ± 0.12	66.15 ± 0.18	10.11 ± 0.13	11.11 ± 0.17
G6	56.47 ± 0.20	61.70 ± 0.17	66.90 ± 0.18	10.43 ± 0.07	10.93 ± 0.17
G16	56.89 ± 0.25	62.12 ± 0.14	66.84 ± 0.24	9.75 ± 0.01	10.93 ± 0.35
G34	56.19 ± 0.09	61.02 ± 0.49	65.82 ± 0.91	9.63 ± 0.82	10.74 ± 0.22
PF	55.56 ± 0.06	61.03 ± 0.00	66.86 ± 0.02	11.30 ± 0.04	10.20 ± 0.20
P1	55.69 ± 0.21	61.19 ± 0.15	66.74 ± 0.15	11.06 ± 0.11	10.23 ± 0.49
P6	55.61 ± 0.39	61.13 ± 0.09	66.49 ± 0.14	10.88 ± 0.87	10.18 ± 0.18
P16	56.23 ± 0.15	61.27 ± 0.09	66.31 ± 0.03	10.08 ± 0.12	9.74 ± 0.30
P34	56.34 ± 0.40	61.29 ± 0.26	66.17 ± 0.41	9.82 ± 0.17	9.32 ± 0.57

[a] Mean and standard deviation of triplicate analysis.
[b] T_o, T_p, T_c = gelatinization onset, peak and conclusion temperatures; $T_c - T_o$ = gelatinization temperature range; ΔH = gelatinization enthalpy.
[c] GF = flour from Hard Red Spring Wheat (HRSW) variety Glenn; G1 = dough prepared using HRSW Glenn and stored 1 day; G6 = dough stored 6 days; G16 = dough stored 16 days; G34 = dough stored 34 days; PF = flour from HRSW variety Parshall; P1 = dough prepared using HRSW Parshall and stored 1 day; P6 = dough stored 6 days; P16 = dough stored 16 days; P34 = dough stored 34 days.

in previous work. ΔH is a result of a breakdown of crystalline order and molecular order (double helices) during gelatinization (Cooke & Gidley, 1992).

For flour starch, Parshall (partial waxy) displayed higher enthalpy of gelatinization compared to Glenn (normal starch). This was in agreement with a previous report (Chakraborty et al., 2004). However, starch extracted from G1 started to have higher enthalpy than starch from P1. That meant the thermal properties changed during refrigeration storage. The dough samples displayed the same trend from day 6 to day 34. Parshall flour and dough starch displayed gelatinization temperatures comparable to Glenn – slightly higher. It was reported that DSC thermal properties were more significantly affected by the degree of crystallinity than the granule size of starch.

X-ray diffraction patterns showed the typical A-type patterns for cereal starch (Paris, Bizot, Emery, Buzare, & Buleon, 1999). It was found that even if the samples were tightly packed, X-rays still can penetrate to the aluminum-well substrate, producing the undesired high peaks observed at higher angles (Fig. 2). A scan range of 5–35° 2θ was selected to avoid the aluminum peaks. Also, the contribution from the starch material at high angles yielded little valuable information. The peaks in the range between 5° and 35° 2θ were used to calculate the relative degree of crystallinity.

The crystal structures were derived from studies on amylose (Imberty, Buleon, Tran, & Perez, 1991). In fact, the amylopectin crystallizes within the granule. Its side chain branches interwine to form the double helices which are the basis of the crystals. The observed difference in gelatinization properties is perhaps reflective of the differences in amylopectin structure.

For starch from Glenn-flour, G1, G6, G16 and G34, the calculated degree of crystallinity was 8.38%, 10.86%, 9.76%, 9.28%, 8.12% respectively. For starch from PF, P1, P6, P16 and P34, the calculated degree of crystallinity was 8.04%, 8.84%, 9.65%, 8.15%, and 8.54% respectively. Those numbers were comparatively lower than value previously reported (Chakraborty et al., 2004) since the methods were different. Starch samples from Parshall- and Glenn-flour and from dough did not exhibit significantly different polymorph patterns and relative crystallinity, which reaffirms that the mutation for the *Wx*-B1 gene does not significantly reduce amylose content (Kim, Johnson, Graybosch, & Gaines, 2003). *d*-Spacing at 2θ was a major difference between the waxy and non-waxy starches. It corresponded to the amyose-lipid complex (Zobel, Young, & Rocca, 1988). This *d*-spacing in Parshall was not missing, but the height of the peak was lower than that of Glenn.

ΔH of the starches was consistent with their degree of crystallinity (Yoo & Jane, 2002). Glenn-flour and dough starch samples both had higher ΔH and crystallinity than Parshall. A large amount of energy was required for gelatinization because of the higher crystallinity. However, DSC and X-ray diffraction techniques do not measure the same property of starch. The X-ray diffraction method detects the crystalline part of starch but does not determine the level of molecular order. DSC, on the other hand, determines the breakdown of crystalline order and molecular order (double helices) during gelatinization (Cooke & Gidley, 1992).

3.5. Starch granule morphology

Starch is seen as discrete granules within the endosperm of the wheat kernel. Wheat endosperm contains mainly two types of

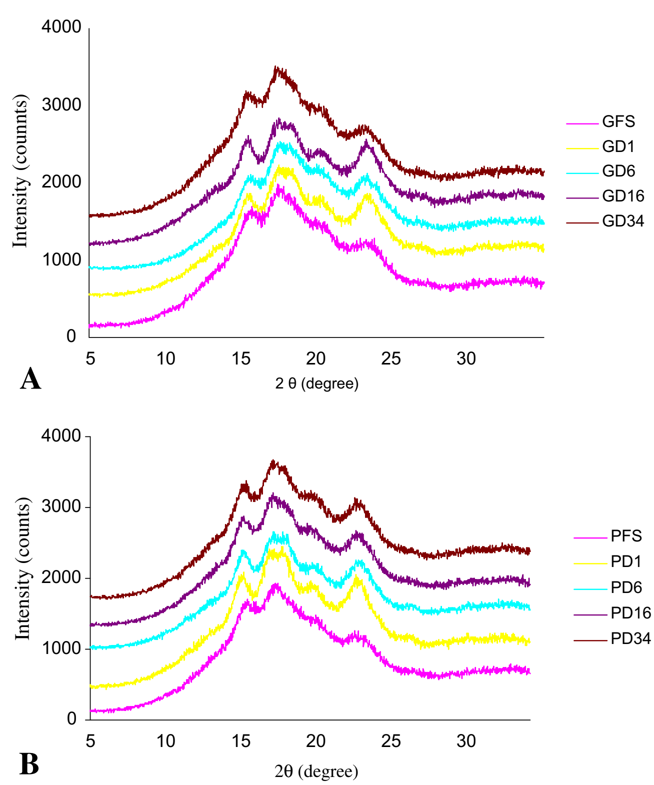

Fig. 2. X-ray diffraction patterns of starch from flour and dough samples for Hard Red Spring varieties (A) Glenn and (B) Parshall (GF = flour from Hard Red Spring Wheat (HRSW) variety Glenn; G1 = dough prepared using HRSW Glenn and stored 1 day; G6 = dough stored 6 days; G16 = dough stored 16 days; G34 = dough stored 34 days. PF = flour from HRSW variety Parshall; P1 = dough prepared using HRSW Parshall and stored 1 day; P6 = dough stored 6 days; P16 = dough stored 16 days; P34 = dough stored 34 days).

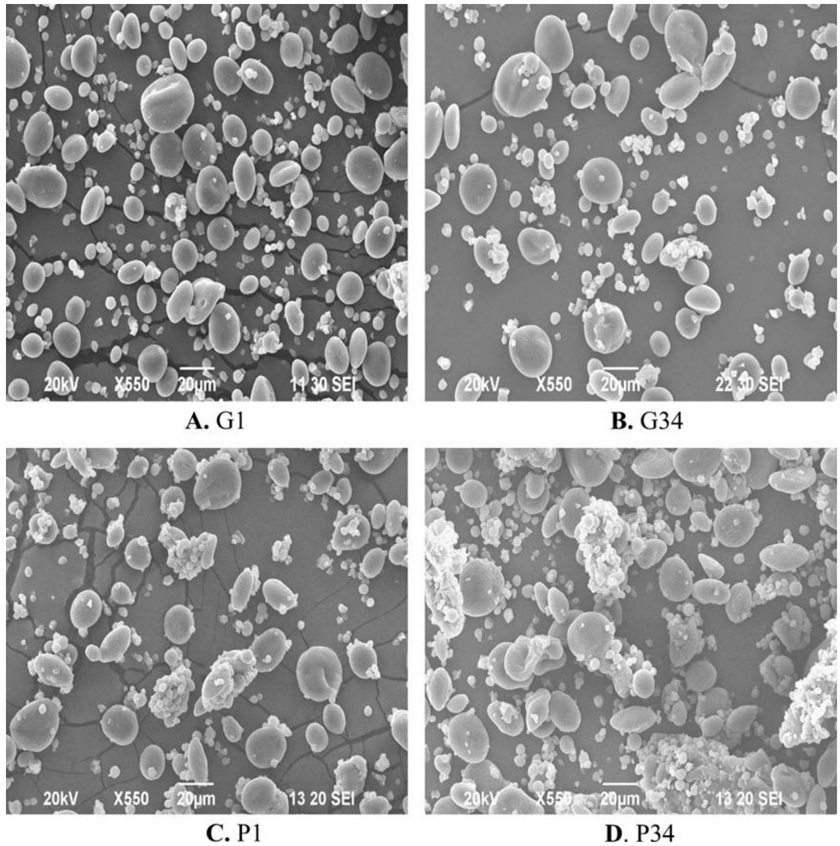

Fig. 3. Scanning Electron Microscopy (SEM) images of starch from dough samples. (A) G1 = starch from dough prepared using Hard Red Spring (HRSW) variety Glenn and stored 1 day; (B) G34 = starch from dough prepared using HRSW variety Glenn and stored 34 days; (C) P1 = starch from dough prepared using HRSW variety Parshall and stored 1 day; (D) P34 = starch from dough prepared using HRSW variety Parshall and stored 34 days.

granules: a larger type, mostly about 20–35 micrometers (μm) across (A-starch), being lenticular in shape, and a smaller spherical shape, ranging from 2 to 8 μm in diameter (B-starch) (Cornell, 2004). It was also reported that no detectable differences were found in the granule-size distribution and granule morphology between waxy and other wheat flour (Yoo & Jane, 2002). The ultra-structure of the starch granules in frozen dough was observed as: starch granules were intact after 24 h of frozen storage, but they were damaged internally and obviously after 24 weeks of storage (Berglund, Shelton, & Freeman, 1991).

Table 5
Starch granule-size distribution using Scanning Electron Microscopy (SEM).[a]

	Sample name[b]									
	GF		G1		G6		G16		G34	
	Length	Width	Length	Width	Length	Width	Length	Width	Length	Width
Large granules	25.00	22.50	29.17	24.17	24.17	19.17	25.00	23.33	29.17	26.67
	21.67	16.67	27.50	17.50	25.00	21.67	22.08	19.17	23.33	20.00
	20.83	16.67	23.33	18.33	25.83	22.50	25.00	21.67	23.33	21.67
Small granules	5.00	4.17	5.83	5.00	5.83	5.83	7.08	5.83	5.00	4.17
	4.58	4.58	5.42	5.42	7.50	6.67	7.08	5.00	5.00	4.58
	4.17	3.75	5.83	5.83	7.50	6.67	6.67	5.00	5.00	5.00
	PF		P1		P6		P16		P34	
	Length	Width	Length	Width	Length	Width	Length	Width	Length	Width
Large granules	25.83	20.83	29.17	22.50	26.67	21.67	22.08	19.58	26.25	23.33
	25.83	24.17	28.33	24.17	25.83	22.50	17.50	16.67	26.67	23.33
	22.92	20.00	26.67	25.00	27.50	22.50	20.34	19.05	26.25	21.25
Small granules	5.83	5.00	8.33	6.67	5.42	5.00	5.00	5.00	4.17	4.17
	6.25	5.00	5.83	5.83	6.67	5.42	5.00	4.58	5.42	4.17
	6.67	6.25	5.00	4.17	7.08	7.08	6.67	5.00	5.00	4.17

[a] The unit of length and width is μm.
[b] GF = flour from Hard Red Spring Wheat (HRSW) variety Glenn; G1 = dough prepared using HRSW Glenn and stored 1 day; G6 = dough stored 6 days; G16 = dough stored 16 days; G34 = dough stored 34 days. PF = flour from HRSW variety Parshall; P1 = dough prepared using HRSW Parshall and stored 1 day; P6 = dough stored 6 days; P16 = dough stored 16 days; P34 = dough stored 34 days.

The granular sizes of flour and dough starch samples measured in our study were in accordance with the above range (Large granules: 20–30 μm; small granules: 4–8 μm). Glenn and Parshall flour and dough starch did not have difference in granule-size distribution and granule morphology (Table 5). But, from day 16, the granular surface changed as the crackers showed up, especially in large granules, indicating α-amylase degraded the starch granules, preferentially large granules (Fig. 3).

4. Conclusions

Starch properties changed in many aspects during refrigeration storage in both normal and partial waxy wheat starches. We have detected more noticeable changes in starch properties in RVA profiles compare to the X-ray and DSC data. When we compared Parshall (partial waxy) with Glenn (normal starch), Glenn had less change on pasting properties and molecular weight distribution of starch component during storage, which may be a indication that Glenn might have better stability over storage as the raw material of refrigerated dough.

Previously, it has been reported that water holding capacity of dough decreased during refrigerated storage and a dark-yellowish liquid known as syrup was formed (Courtin et al., 2006; Simsek & Ohm, 2009). Therefore, we believe that formation of this free water has effect on starch properties. Water plays many significant roles in determining the properties of starch. Water below the gelatinization (melting + dissolution) temperature acts essentially as a plasticizer (Bizot et al., 1997), while, at higher temperatures, it becomes a solvent (Moates, Noel, Parker, & Ring, 1997). Water is essential to the crystallinity of starch based glucans as it permits rearrangements by plasticization of amorphous areas and the build up of crystalline hydrate lattices of different stoichiometries depending on the polymorphic type (Imberty et al., 1991). In frozen dough study, after 24 weeks of frozen storage, water separated into pools causing less free water to be distributed throughout the doughs, which resulted in significant change of DSC thermal properties (Lu & Grant, 1999a).

In our study, water was also an important factor. In refrigerated dough system and during its storage, the temperature was below gelatinization temperature. However, as water diffused to the dough surface and dough essentially lost water binding ability, the properties of starch changed during storage. Also, due to the consistent existence of α-amylase activity – even if low levels – significantly changed the apparent molecular weight of starch during storage (Table 3), thus resulting in the changes of its functional properties.

Acknowledgements

This work was supported by grant #FAR0013155 (BD0007-16) from the North Dakota Agricultural Product Utilization Commission and North Dakota State University Agricultural Experiment Station and North Dakota Wheat Commission. We would like to thank Kristin Whitney for her help in preparation of the dough samples and Angelo Ostenson and Angel Ugrinov for their help during analysis of starch samples.

References

AACC (2000). *Approved methods of the AACC* (10th ed.). St. Paul, MN: American Association of Cereal Chemists.

Autio, K., & Sinda, E. (1992). Frozen doughs: Rheological changes and yeast viability. *Cereal Chemistry, 69*, 409–413.

Berglund, P. T., Shelton, D. R., & Freeman, T. P. (1991). Frozen bread dough ultrastructure as affected by duration of frozen storage and freeze–thaw cycles. *Cereal Chemistry, 68*, 105–107.

Bizot, H., LeBail, P., Leroux, B., Davy, J., Roger, P., & Buleon, A. (1997). Calorimetric evaluation of the glass transition in hydrated, linear and branched polyanhydroglucose compounds. *Carbohydrate Polymers, 32*, 33–50.

Chakraborty, M., Matkovic, K., Grier, D. G., Jarabek, E. L., Berzonsky, W. A., McMullen, M. S., et al. (2004). Physicochemical and functional properties of tetraploid and hexaploid waxy wheat starch. *Starch-Starke, 56*, 339–347.

Cooke, D., & Gidley, M. J. (1992). Loss of crystalline and molecular order during starch gelatinization – origin of the enthalpic transition. *Carbohydrate Research, 227*, 103–112.

Cornell, H. (2004). The functionality of wheat starch. In A. C. Eliasson (Ed.), *Starch in food* (pp. 221–238). Cambridge, UK: Woodhead Publishing Limited.

Courtin, C. M., Gys, W., & Delcour, J. A. (2006). Arabinoxylans and endoxylanases in refrigerated dough syruping. *Journal of the Science of Food and Agriculture, 86*, 1587–1595.

Courtin, C. M., Gys, W., Gebruers, K., & Delcour, J. A. (2005). Evidence for the involvement of arabinoxylan and xylanases in refrigerated dough syruping. *Journal of Agricultural and Food Chemistry, 53*, 7623–7629.

Donovan, J. W., Lorenz, K., & Kulp, K. (1983). Differential scanning calorimetry of heat-moisture treated wheat and potato starches. *Cereal Chemistry, 60*, 381–387.

Grant, L. A., Ostenson, A. M., & Rayas-Duarte, P. (2002). Determination of amylose and amylopectin of wheat starch using high performance size-exclusion chromatography (HPSEC). *Cereal Chemistry, 79*, 771–773.

Graybosch, R. A. (1998). Waxy wheats: Origin, properties, and prospects. *Trends in Food Science & Technology, 9*, 135–142.

Gys, W., Courtin, C. M., & Delcour, J. A. (2003). Refrigerated dough syruping in relation to the arabinoxylan population. *Journal of Agricultural and Food Chemistry, 51*, 4119–4125.

Hayakawa, K., Tanaka, K., Nakamura, T., Endo, S., & Hoshino, T. (1997). Quality characteristics of waxy hexaploid wheat (*Triticum aestivum* L.): Properties of starch gelatinization and retrogradation. *Cereal Chemistry, 74*(57), 6–580.

Imberty, A., Buleon, A., Tran, V., & Perez, S. (1991). Recent advances in knowledge of starch structure. *Starch-Starke, 43*, 375–384.

Kim, W., Johnson, J. W., Graybosch, R. A., & Gaines, C. S. (2003). Physicochemical properties and end-use quality of wheat starch as a function of waxy protein alleles. *Journal of Cereal Science, 37*, 195–204.

Kulp, K. (1995). Biochemical and biophysical principles of freezing. In K. Kulp, K. Lorenz, & J. Brummer (Eds.), *Frozen and refrigerated dough and batters* (pp. 63–89). St. Paul, MN: American Association of Cereal Chemists.

Lu, W., & Grant, L. A. (1999a). Effects of prolonged storage at freezing temperatures on starch and baking quality of frozen doughs. *Cereal Chemistry, 76*, 656–662.

Lu, W., & Grant, L. A. (1999b). Role of flour fractions in breadmaking quality of frozen dough. *Cereal Chemistry, 76*, 663–667.

Moates, G. K., Noel, T. R., Parker, R., & Ring, S. G. (1997). The effect of chain length and solvent interactions on the dissolution of the B-type crystalline polymorph of amylose in water. *Carbohydrate Research, 298*, 327–333.

Nakamura, T., Yamamori, M., Hirano, H., & Hidaka, S. (1993). Decrease of waxy (*Wx*) protein in 2 common wheat cultivars with low amylose content. *Plant Breeding, 111*, 99–105.

Nakamura, T., Yamamori, M., Hirano, H., & Nagamine, T. (1995). Production of waxy (amylose-free) wheats. *Molecular Genetics and Genomics, 248*, 253–259.

Oda, M., Yasuda, Y., Okazaki, S., Yamauchi, Y., & Yokoyama, Y. (1980). A method of flour quality assessment for Japanese noodles. *Cereal Chemistry, 57*, 253–254.

Paris, M., Bizot, H., Emery, J., Buzare, J. Y., & Buleon, A. (1999). Crystallinity and structuring role of water in native and recrystallized starches by C-13 CP-MAS NMR spectroscopy 1: Spectral decomposition. *Carbohydrate Polymers, 39*, 327–339.

Simsek S., & Ohm J. B. (2009). Structural changes of arabinoxylans in refrigerated dough. *Carbohydrate Polymers*. doi:10.1016/j.carbpol.2008.12.012.

Simsek, S., Tulbek, M. C., Yao, Y., & Schatz, B. (2009). Starch characteristics of dry peas (*Pisum sativum* L.) grown in the USA. *Food Chemistry, 115*, 832–838.

Tsai, M. L., Li, C. F., & Lii, C. Y. (1997). Effects of granular structures on the pasting behaviors of starches. *Cereal Chemistry, 74*, 750–757.

Whistler, R. L., & BeMiller, J. N. (1997). Starch. In R. L. Whistler & J. N. BeMiller (Eds.), *Carbohydrate chemistry for food scientists* (pp. 117–152). St. Paul, MN: American Association of Cereal Chemists.

White, P., Abbas, I., Pollak, L., & Johnson, L. (1990). Intrapopulation and interpopulation variability of thermal-properties of maize starch. *Cereal Chemistry, 67*, 70–74.

Wolt, M. J., & D' Appolonia, B. L. (1984). Factors involved in the stability of frozen dough. II. The effect of yeast type, flour type, and dough additives on frozen-dough stability. *Cereal Chemistry, 61*, 213–221.

Yoo, S. H., & Jane, J. (2002). Structural and physical characteristics of waxy and other wheat starches. *Carbohydrate Polymers, 49*, 297–305.

Zobel, H. F., Young, S. N., & Rocca, L. A. (1988). Starch gelatinization – an X-ray-diffraction study. *Cereal Chemistry, 65*, 443–446.