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Model Approach to Starch Functionality in Bread Making

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We used modified wheat starches in gluten−starch flour models to study the role of starch in bread making. Incorporation of hydroxypropylated starch in the recipe reduced loaf volume and initial crumb firmness and increased crumb gas cell size. Firming rate and firmness after storage increased for loaves containing the least hydroxypropylated starch. Inclusion of cross-linked starch had little effect on loaf volume or crumb structure but increased crumb firmness. The firming rate was mostly similar to that of control samples. Presumably, the moment and extent of starch gelatinization and the concomitant water migration influence the structure formation during baking. Initial bread firmness seems determined by the rigidity of the gelatinized granules and leached amylose. Amylopectin retrogradation and strengthening of a long-range network by intensifying the inter- and intramolecular starch−starch and possibly also starch−gluten interactions (presumably because of water incorporation in retrograded amylopectin crystallites) play an important role in firming.

KEYWORDS: Modified starch; bread; crumb structure; crumb firmness; bread staling

INTRODUCTION

The transformation of dough to bread is a complex, ill-understood process in which several irreversible structural transformations take place. In general, a series of physical phenomena, as well as chemical and biochemical reactions, are involved (*1*). As a result, a light, porous, and readily digestible product is formed.

The foam structure of fermenting dough consists of a dispersion of discrete gas cells in a continuous starch−protein matrix. The setting of the bread crumb structure ends (and hence the transformation from dough to bread crumb is complete) when the bread crumb becomes gas continuously (*2, 3*). This has been attributed to the rupture of the matrix as a result of the sharp increase in dough viscosity induced by starch gelatinization (*4*). In addition, the stability of gas cell walls during baking is strongly related to strain-hardening properties of the gluten (*5*). During baking, the polymerization of glutenin molecules (starting at about 50−60 °C) (*3, 6–10*) may contribute to a rapid increase in tensile strength in the membrane surrounding the gas cell, which may initiate its rupture. Apart from the formation of the continuous gas phase, and in many instances related to it, the most apparent phenomena during baking are gelatinization of starch, activation and inactivation of yeast and enzymatic activities, protein polymerization, volume expansion, and crust formation (*11*). However, at present, the importance of the properties of the starch fraction for the crumb structure and firmness formation remains largely unclear.

Chemical modification (hydroxypropylation and cross-linking) is a useful tool to modify starch properties. X-ray diffraction studies showed that hydroxypropylation mainly occurred in the amorphous regions of the starch granule (*12*) and, hence, mainly affects amylose and the amorphous regions of amylopectin. Hydroxypropylation facilitates the penetration and absorption of water into the starch granules thereby increasing the initial rate of plasticization of the amorphous regions of modified granules (*13*). The increased water absorption in these regions promotes swelling. Thus, the crystalline phase is (more easily) disrupted and melts at a lower temperature than in the case of unmodified starch (*14–16*). In contrast, cross-linking stabilizes the granular structure (*13*). It restricts the swelling, moisture migration, and leaching during gelatinization in a pasta-making process (*15*).

Earlier studies also incorporated chemically modified starch in bread-making recipes and investigated the resulting bread firmness or firming (*17–20*). However, in these studies, non-wheat starches were used, such as cross-linked waxy barley starch (*17, 18*) and modified tapioca starch (*19*). Early on, Hoseney et al. (*21*), for a series of starches, showed that except for barley and rye starches nonwheat starches have poorer bread-making characteristics than wheat starch. In line with the above, it was stated that only 20% of the wheat flour can be substituted by modified tapioca starch (*19*). Furthermore, the absence of amylose in waxy barley starch affects crumb structure, since breads made with 100% waxy barley starch have been reported to shrink (*22*) or even collapse (*23*) after baking.

The purpose of this study was to investigate the role of the starch fraction during bread making and storage. In our experimental setup, we use a modified wheat starch based model

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system (gluten−wheat starch blends as a model for flour). The kind (i.e., hydroxypropylation and cross-linking) and extent (a rather limited level of modification) of the wheat starch chemical modifications were chosen on the basis of their different expected impact on starch swelling and rigidity characteristics. The properties of the starch fraction were investigated, were related to model bread crumb properties, and were discussed with respect to model bread crumb structure and firmness.

MATERIALS AND METHODS

Materials. Wheat starch and gluten [protein content ($N \times 5.7$) 78.0%] were obtained from Tate & Lyle (Aalst, Belgium). Moisture contents (starch: 14.7% and commercial gluten: 6.7%) were determined on the basis of weight loss at 130 °C for 120 min of ca. 1.0 g of accurately weighed samples (AACC method 44−15A (*24*)). The close-packing concentration of the wheat starch, determined as described earlier (*25*), was 5.2% starch dry matter (dm). All chemicals and reagents were of at least analytical grade and were obtained from Sigma-Aldrich (Bornem, Belgium).

Starch Hydroxypropylation (*15, 26*). Wheat starch (1200 g) was suspended in a solution prepared by adding 15.6 g NaOH and 180 g Na_2SO_4 in 1420 mL of demineralized water. Suspensions were placed in stoppered bottles with magnetic stirrers in a water bath at 38 °C. Propylene oxide (40 or 80 mL) was added, and the starches were incubated for 24 h under continuous stirring. Next, the starches were washed with demineralized water until the wash water was sulfate-free (as checked with 1.0 M $BaCl_2$), and then the starches were air-dried. They are further referred to as HP40 and HP80. Their hydroxypropyl group content was determined colorimetrically (*27*) and was ca. 25 and 40 μmol hydroxypropyl groups per 100 mg starch (dm) for HP40 and HP80, respectively. Control starch (HPcon) was prepared using the same procedure except that no propylene oxide was added.

Starch Cross-Linking (*28*). Wheat starch (1500 g) was suspended in 3500 mL 0.1 M NaOH. Next, sodium trimetaphosphate (STMP) solution (5.0% (w/v), 50 or 150 mL) was added. These levels of STMP resulted in starches that had been treated with 0.10 and 0.31 mol STMP/100 mol anhydroglucose, respectively. The suspensions were incubated for 18 h in a water bath at 40 °C under continuous stirring. After incubation, the suspensions were neutralized to pH 6.5 with 6.0 M HCl. Cross-linked starches were washed several times with demineralized water (1500 mL) and were air-dried. They are further referred to as CL50 and CL150. Control starch (CLcon) was prepared as described above except that no cross-linking agent was added. Phosphorus content was estimated by analysis of ash content [with addition of 1.0 mL 10.0% (w/v) zinc acetate solution per gram starch (dm) to limit loss of phosphorus during ashing (*47*)] relative to the control samples spiked with 10 and 20 μmol NaH_2PO_4 per gram starch (dm) and was ca. 3.5 and 7.5 μmol phosphate per gram starch (dm) for CL50 and CL150, respectively.

Rapid Viscosity Analysis. Rapid viscosity analysis (RVA) was with a Rapid Visco Analyzer (Model RVA-4D; Newport Scientific, Sydney, Australia) interfaced with a computer equipped with Thermocline software. Starch suspensions [11.0% dm (w/v), total weight 25.0 g] were subjected to a temperature increase from ambient temperature to 40 °C (0−1 min) followed by a linear temperature increase from 40 to 95 °C at 3.95 °C/min, a holding step (5 min at 95 °C), a cooling step with a linear temperature decrease from 95 to 50 °C (6.4 °C/min), and a final isothermal step at 50 °C (23.5 min). RVA parameters were onset temperature, peak viscosity (PV), peak time (PT), hot paste viscosity (HPV, i.e., the minimum viscosity value read after the peak), cold paste viscosity (CPV, i.e., viscosity after 50 min, i.e., at the end of the run), and breakdown (i.e., the difference between the PV and the HPV). All viscosity readings were in Poise (P; 0.1 Ns/m^2), a unit of dynamic viscosity. The viscograms measured by RVA were excellently reproducible with experimental error on viscosity readings less than 3%.

Differential Scanning Calorimetry. Differential scanning calorimetry (DSC) measurements were performed with a DSC Q1000 (TA Instruments, Newcastle, United Kingdom). Control and modified starch samples (5−6 mg) were accurately weighed into aluminum sample pans, and following water addition [starch (dm)/water, 1:2 (w/w)], were equilibrated at room temperature. Dough samples [based on gluten−starch blends (16/84) with 100% of the starch fraction either the modified starch or the control starch] (18−22 mg) were similarly analyzed but without water addition. The retrogradation of amylopectin in the model bread samples was analyzed after 0, 3, and 7 days of storage using 30−40 mg crumb without water addition into DSC high-pressure pans (Mettler Toledo, Norwalk, CT). For each starch/storage time combination, crumbs of four to five different loaves were analyzed. The pans were sealed, were equilibrated at 0 °C in the DSC, and were heated from 0 to 150 °C at a heating rate of 4 °C/min. The system was calibrated before analysis with indium, and an empty pan was used as reference. The onset, peak, and conclusion temperatures (T_o, T_p, and T_c, respectively) and enthalpies corresponding to the gelatinization of the starch or the melting of recrystallized amylopectin were evaluated from the thermograms using Universal Analysis (TA Instruments). The enthalpy was expressed in J/g of sample (dm).

Model Bread-Making Procedure. Model doughs and breads were produced at 100 g scale according to the straight-dough bread-making procedure of Finney (*29*). The model flours consisted of wheat starch and gluten in a ratio of 84/16 calculated on dry matter (dm) content. Water absorption (59.5% with the assumption of a 14% moisture content of the gluten and starch) and optimal mixing time were determined on the basis of the properties of the control gluten−starch blend (consisting of gluten and native, unmodified wheat starch) as determined by an experienced baker and were kept constant for all samples. To evaluate the impact of the modifications, 65% of the starch fraction in the reconstituted gluten−starch blends was replaced by the modified starches. The gluten−starch blend (100 g, 14% moisture base), fresh yeast (5.3 g), salt (1.5 g), and sugar (6.0 g) were mixed for 180 s with water in a 100 g pin mixer (National Manufacturing, Lincoln, NE). Dough was fermented at a relative humidity of 95% and 30 °C for 90 min with intermediate punching at 52 and 77 min. The third punch and molding were performed after 90 min of fermentation. Finally, dough was proofed (36 min, 30 °C) and baked at 215 °C for 24 min in a rotary oven (National Manufacturing). After cooling to ambient temperature (240 min), the loaves were weighed, and their volume was measured by rapeseed displacement. The coefficients of variation for the specific loaf volumes and weights were lower than 2.5% and 1.0%, respectively. Moisture loss during storage (at ambient temperature) was prevented by keeping the loaves in sealed plastic bags. This way, for each starch, 15 loaves were prepared.

Moisture Content. Moisture contents of the crumb and the crust of the loaves were measured as outlined above.

Digital Image Analysis. For each bread type, digital image analysis of the crumb was performed on three breads. For each loaf, four slices (16 mm thickness) were cut from the center and were analyzed. A single 40 × 40 mm field of view (FOV) was evaluated for each image (*30*). This FOV captured the crumb area of the center of each slice. Images were collected using a flatbed scanner (Vuego Scan Brisa 610S, Acer Peripherals, Shizou, China) and supporting software (iPhoto Plus 4.0, Ulead Systems, Tapei, Taiwan). Images were scanned full scale in 256 gray levels at 300 dots per inch each comprising 470 columns by 471 rows of pixels. They were processed using the image processing toolbox of Matlab 6.1 (The Mathworks Inc., Natick, MA). A threshold method was used for image segmentation (conversion to a binary image) according to Otsu (*31*), since this method yields consistent binary images (*32*). Crumb cell detection was conducted on the binary images and mean cell areas, numbers of cells/cm^2, and cell to total area ratios were determined.

Firmness. Bread firmness was measured as described by AACC Method 74−09 (*24*) using a TA.XT2 instrument (Texture Technologies, New York) with a 40 mm diameter cylindrical probe, for 25% of compression, at a test speed of 1.0 mm/min. The average values are reported on the basis of three different loaves. For each loaf, at least four slices (25 mm thickness) were cut from the center and were analyzed. In an additional computation, the measurements for crumb firmness were divided by loaf specific weight to express firmness on a weight basis and, thus, to correct for loaf volume differences. The uncorrected data is referred to as the "measured firmness", while the corrected values are referred to as the "intrinsic firmness".

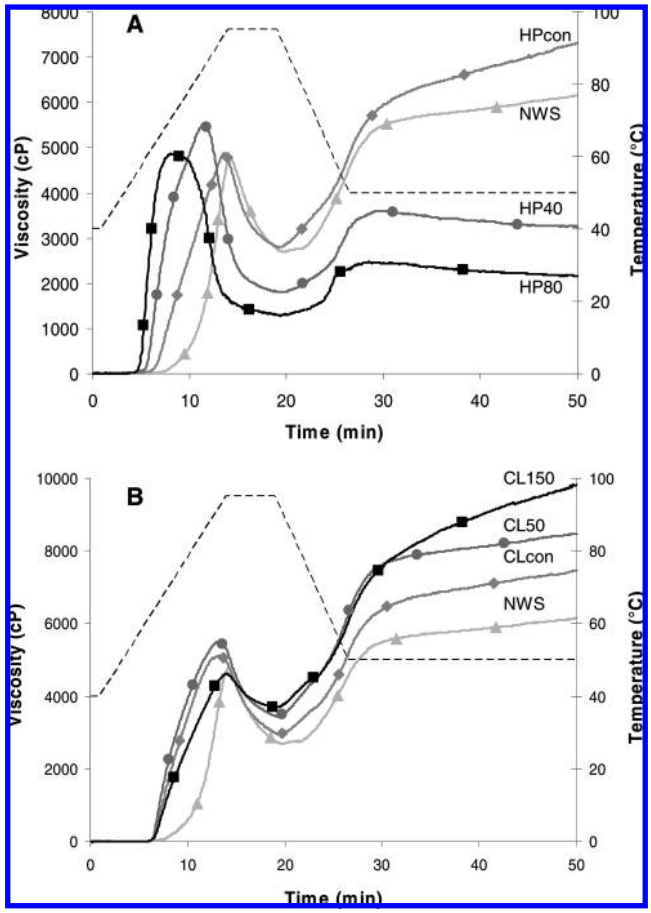

Figure 1. RVA viscograms of wheat starch slurries (11% starch dm) (dashed line, temperature profile). (**A**) Hydroxypropylated (HP) starch and control samples: HP80 (■), HP40 (●), HPcon (◆), native wheat starch (NWS, ▲). (**B**) Cross-linked (CL) starch and control samples: CL150 (■), CL50 (●), CLcon (◆), native wheat starch (NWS, ▲). Sample codes are defined in the Methods section.

Statistical Analyses. For statistical analyses, *t* test (PROC ANOVA) was used (significance level $P < 0.05$). Statistical analyses were conducted using the Statistical Analysis System software 8.1 (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

1. Characterization of the Chemically Modified Wheat Starches. *1.1. Thermal, Pasting, and Gelation Properties in Excess Water.* Wheat starches with different swelling and gelatinization properties were obtained by hydroxypropylation and cross-linking. The concentrations of modifying agents applied were more limited than those used in literature for durum wheat (*15*) or tapioca starches (*19*). Preliminary experiments showed that, in the case of cross-linking, a more enhanced degree led to undesired loaf volumes. The modified wheat starches were characterized using RVA and DSC. **Figure 1** shows the RVA profiles of native and modified wheat starches. **Table 1** lists the corresponding gelatinization enthalpies and DSC gelatinization transition temperatures (T_o, T_p, and T_c).

DSC analysis of the modified starches showed that hydroxypropylation decreased T_o by about 2 °C, T_p by about 2 and 3 °C, and T_c by about 7 and 10 °C for HP40 and HP80, respectively. This is in line with earlier reports (*15, 16, 19*). The decrease in gelatinization temperatures was more pronounced for T_c than for T_p and T_o with T_o being the least affected (**Table 1**). Only the most hydroxypropylated sample (HP80) showed a decreased gelatinization enthalpy, which is in line with the lower enthalpy values for hydroxypropylated starch reported before (*12, 15, 16*). **Figure 1** also shows that both degrees of hydroxypropylation impacted the pasting behavior of the starches. The results are in line with those of Delcour et al. (*15*) for durum wheat starch and with those of Gunaratne and Corke (*16*) for starches from different sources. The unmodified starch sample had a PV of 4770 cP, a PT of 14.2 min, and a CPV of 6150 cP (**Figure 1**). The PV of both hydroxypropylated starches occurred earlier (PT of 11.5 and 8.2 min for HP40 and HP80, respectively) and, hence, at lower temperatures. In addition, higher PV values of 5500 and 4850 cP for HP40 and HP80, respectively, were found, which demonstrates a more pronounced swelling of the starch granules. Indeed, since the overall viscosity of the starch paste (as measured with RVA) is determined both by the swollen granules and the composition of the continuous phase, swelling, amylose leaching, and starch granule rupturing play a key role in determining the viscosity during heating. Hydroxypropyl groups are mainly introduced in the amorphous regions (*13*) and facilitate the penetration and absorption of water into the starch granules (*12*). Hence, starch swelling starts sooner, and the granules swell faster and to higher PV and are more easily disrupted (as indicated by the higher breakdown viscosity). Furthermore, in the case of HP80 with the higher degree of hydroxypropylation, the faster swelling is presumably partially countered by a more rapid granular disruption resulting in a lower PV than the HP40 sample. This accelerated granular swelling also led to a reduced energy requirement (measured as ΔH in DSC analysis) to disrupt the starch structure, which is in line with the notion of gelatinization as a swelling-driven process (*33–35*). Finally, the CPV was decreased to a large extent for both hydroxypropylated starch samples (CPV of 3250 and 2160 cP for HP40 and HP80, respectively) indicating that the formation of amylose−amylose junction zones and the subsequent gelation were hindered. This can be attributed to the sterical hindrance posed by the hydroxypropyl groups.

Cross-linked starches generally had slightly higher T_o and T_p than the native wheat starch (**Table 1**). However, their gelatinization temperature range was smaller because of a decrease in T_c by 6−7 °C. The most cross-linked sample (CL150) showed a somewhat increased gelatinization enthalpy. The differences between the cross-linked starches became evident in the RVA (**Figure 1**). The PV was increased for the CL50 sample (5500 cP) but was slightly decreased for the most cross-linked starch (4620 cP). CPV was increased for both cross-

Table 1. Onset (T_o), Peak (T_p), and Conclusion Temperatures (T_c) and Melting Enthalpies (ΔH) of Native, Hydroxypropylated, and Cross-Linked Wheat Starches[a]

starch sample	T_o (°C)	T_p (°C)	T_c (°C)	ΔH [J/g starch (dm)]
native	53.9 ± 0.2 d	59.0 ± 0.1 d	79.1 ± 2.2 a	12.4 ± 0.2 b
HPcon[b]	55.6 ± 0.2 c	60.0 ± 0.1 c	77.1 ± 0.9 ab	12.1 ± 0.3 b
HP40[c]	51.5 ± 0.3 e	55.8 ± 0.2 e	75.9 ± 1.3 ab	12.5 ± 0.7 b
HP80[c]	51.6 ± 0.1 e	57.1 ± 0.2 f	75.3 ± 0.7 b	10.3 ± 0.8 c
CLcon[b]	57.0 ± 0.2 b	59.9 ± 0.1 c	72.7 ± 0.9 c	13.3 ± 0.1 ab
CL50[d]	58.4 ± 0.1 a	60.7 ± 0.2 b	73.0 ± 1.3 bc	13.1 ± 0.1 ab
CL150[d]	59.0 ± 0.3 a	61.5 ± 0.2 a	72.4 ± 2.1 bc	14.1 ± 0.3 a

[a] Values followed by different letters in the same column indicate significantly different means at $P < 0.05$. [b] HPcon, CLcon: wheat starch samples subjected to the reaction conditions specified in Materials and Methods without the respective modification agents. [c] HP40, HP80: hydroxypropylated wheat starch with varying degree of modification as defined in Materials and Methods. [d] CL50, CL150: cross-linked wheat starch with varying degree of modification as defined in Materials and Methods.

Table 2. Onset (T_o), Peak (T_{p1} and T_{p2}), and Conclusion Temperatures (T_c) and Melting Enthalpies (ΔH) of Control Model Doughs (Based on Gluten/Starch Mixtures) Using Native, Hydroxypropylated, and Cross-Linked Wheat Starches[a]

incorporated starch[b]	T_o (°C)	T_{p1} (°C)	T_{p2} (°C)	T_c (°C)	ΔH_{AP} [J/g dough (dm)]
native starch	62.1 ± 0.4 b	69.2 ± 0.3 b	92.8 ± 0.8 b	102.8 ± 1.1 a	5.2 ± 0.1 a
HP40[c]	59.3 ± 0.7 c	65.7 ± 0.3 c	90.0 ± 0.4 a	102.7 ± 0.9 a	5.5 ± 0.2 a
HP80[c]	56.8 ± 0.1 d	66.1 ± 0.5 c	90.5 ± 0.4 a	104.2 ± 0.5 a	5.1 ± 0.2 a
CL50[d]	65.1 ± 0.3 a	71.3 ± 0.2 a	91.9 ± 0.5 b	103.5 ± 0.6 a	5.3 ± 0.1 a
CL150[d]	65.2 ± 0.3 a	71.0 ± 0.5 a	92.4 ± 0.8 b	103.3 ± 1.1 a	5.3 ± 0.2 a

[a] Values followed by different letters in the same column indicate significantly different means at $P < 0.05$. [b] 100% of total starch fraction. [c] HP40, HP80: hydroxypropylated wheat starch with varying degrees of modification as defined in Materials and Methods. [d] CL50, CL150: cross-linked wheat starch with varying degrees of modification as defined in Materials and Methods.

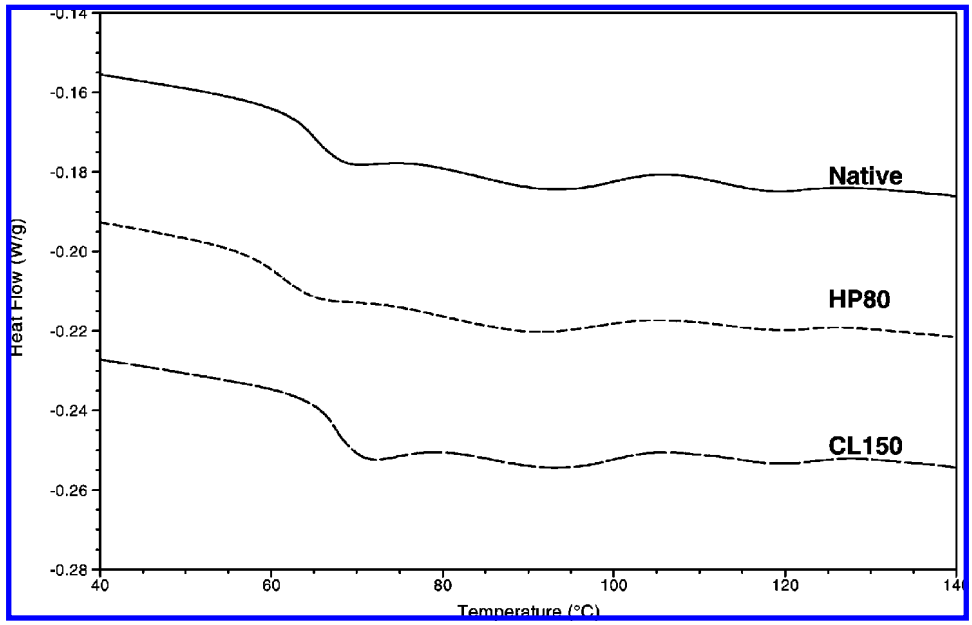

Figure 2. DSC thermograms of model doughs consisting of gluten (16%) and (native or modified wheat) starch (84%). Solid line: native wheat starch; short dashed line: hydroxypropylated starch (HP80); long dashed line: cross-linked starch (CL150).

linked starch samples (8470 and 9820 cP for CL50 and CL150, respectively). In the theory of Steeneken (*36*), the high viscosity of the CL50 and CL150 samples can be explained by the increased rigidity of the swollen starch conveyed by the covalent cross-linking of the starch polymers with a concomitant decreased shear/temperature induced disintegration of the granules. With increasing degree of cross-linking (CL150), granule swelling was more restricted and the viscosity was governed by the granule rigidity and solubility of the starch.

Although the alkaline reaction conditions during the preparation of the modified starches also affect starch properties, as indicated by the results of the HPcon and CLcon samples (**Table 1** and **Figure 1**), the impact of the chemical modification was clearly dominant. Indeed, HPcon and CLcon showed a more rapid viscosity development and higher CPV values than the native wheat starch, but this did not interfere with the interpretation of the destabilizing (in the case of HP40 and HP80) and stabilizing (in the case of CL50 and CL150) effects of the chemical modification. Likewise, the higher T_o, T_p, and T_c of the HPcon and CLcon samples may be explained by annealing phenomena during the incubation (at alkaline conditions) but, again, this had little if any effect on the DSC characteristics of the chemically modified starch. Furthermore, the results for HPcon were in agreement with the findings reported by Gunaratne and Corke (*16*). Therefore, we are confident that the changes in bread properties following incorporation of the modified starches can be related to the chemical modifications.

1.2. Starch Gelatinization in Model Bread Doughs. The rather limited moisture content (ca. 43% dough moisture content), but also the presence of other ingredients and constituents, such as sugar and gluten, shifts the starch gelatinization range in bread-making conditions to higher temperatures (*37*). **Table 2** summarizes the DSC gelatinization parameters of gluten−starch model doughs made either with the native or the modified wheat starches (100% of the starch fraction). Without addition of extra water to the dough samples, two peaks ($T_p \sim 70$ and ~90 °C) are detected below 100 °C (**Figure 2**). Starch gelatinization is often interpreted in terms of water absorption, accompanied by swelling in specific regions of the granule, and loss of crystalline order. According to the theory of Donovan (*33*) as stated by Jacobs et al. (*38*), at low water content, less water is absorbed in the amorphous parts and only a fraction of the crystallites within the granule is cooperatively disrupted giving rise to a first endotherm. Redistribution of water in the granules then occurs and the remaining crystallites melt at a higher temperature (second endotherm). In the more recent liquid-crystalline structural model of starch, the two peaks are attributed to moisture-mediated disorganization of starch crystalline structures (helix−helix dissociation) and to disentanglement of the amylopectin side chains (helix−coil transition), respectively (*39*).

In general, the effect of starch modification on the DSC thermograms (particulary on T_o and T_p) was similar when analyzing the pure starch samples (see above) or the model doughs. However, in the latter case, no impact was seen on T_c

Table 3. Loaf Volume and Weight and Crumb Firmness after 0 (d0), 3 (d3), and 7 (d7) Days of Storage of Gluten−Starch Based Model Breads[a]

			firmness (*N*)		
incorporated starch	volume (cm^3)	weight (g)	d0	d3	d7
native starch	659 ± 21 a	138.1 ±1.1 a	2.54 ± 0.31 a	11.05 ± 0.91 b	16.83 ± 1.19 a
HP40	555 ± 31 bc	138.5 ± 0.6 a	1.89 ± 0.32 b	14.31 ± 0.86 a	24.85 ± 1.89 c
HP80	529 ± 11 c	139.2 ± 1.1 a	0.98 ± 0.11 c	7.08 ± 0.35 c	16.54 ± 1.34 a
CL50	591 ±11 b	137.9 ± 0.8 a	3.16 ± 0.41 a	15.03 ± 0.47 a	21.34 ± 1.60 bc
CL150	623 ± 20 a	139.3 ± 0.8 a	3.26 ± 0.52 a	11.98 ± 1.10 b	17.81 ± 1.50 ab

[a] This is with incorporation of native wheat starch (100%) or hydroxypropylated (HP) and cross-linked (CL) wheat starch (65% of total starch fraction with various degrees of modification). Values followed by different letters in the same column indicate significantly different means at $P < 0.05$.

Table 4. Crumb Grain Characteristics, Determined by Digital Image Analysis, of Gluten−Starch Based Model Breads[a]

incorporated starch	mean cell area (mm^2)	# cells/cm^2	cell to total ratio (%)
native starch	0.35 ± 0.01 b	90.1 ± 6.6 a	38.9 ± 1.7 a
HP40	0.54 ± 0.05 a	91.9 ± 8.1 a	44.0 ± 1.5 b
HP80	0.51 ± 0.02 a	90.2 ± 2.5 a	43.4 ± 1.5 b
CL50	0.35 ± 0.04 b	93.8 ± 7.0 a	39.3 ± 4.0 a
CL150	0.37 ± 0.03 b	90.7 ± 7.1 a	38.1 ± 2.7 a

[a] This is with incorporation of native wheat starch (100%) or hydroxypropylated (HP) and cross-linked (CL) wheat starch (65% of total starch fraction with various degrees of modification). Values followed by different letters in the same column indicate significantly different means at $P < 0.05$.

and the melting enthalpy. This is possibly due to the impact of the limited moisture content on starch gelatinization, while the similar ΔH values can probably also be attributed to the rather limited extent of modification. Incorporation of hydroxypropylated starch decreased T_o (by 3−5 °C) and T_p (T_{p1} by ∼3 °C; T_{p2} by 2 °C) for HP40 and HP80, respectively. DSC analyses of the cross-linked starch-containing model doughs showed higher T_o values (increase of about 3 °C) for both degrees of cross-linking. The melting of the amylopectin crystallites was not influenced.

2. Properties of the Model Breads. Table 3 lists loaf volumes and weights for the different model breads. The loaf volume of the control model breads containing 100% of native wheat starch was 659 cm^3. Inclusion of hydroxypropylated starches (65% of the starch fraction) decreased the loaf volume by approximately 16% and 20% for the HP40 and HP80 model breads, respectively. Presumably, the viscosity increase of the hydroxypropylated starch at lower temperatures prematurely ended the oven rise resulting in lower bread volumes. The inclusion of cross-linked starch (65% of the starch fraction) had a minor impact on the bread volume with a slight decrease by approximately 5−10% for CL150 and CL50, respectively. The breads, with and without modified starches, differed neither in weight after bread making (ca. 140 g) nor in moisture contents of the crumb (ca. 43−44%) and crust. While crumb moisture decreased (by ca. 2−3%), that of crust increased during storage indicating water migration from the crumb to the crust in agreement with Baik and Chinachoti (*40*). Finally, while incorporation of hydroxypropylated wheat starch led to a more sticky bread crumb, cross-linked wheat starch resulted in a drier feeling crumb. Similar observations were reported by Miyazaki et al. (*19*) for hydroxypropylated and cross-linked tapioca starch containing breads.

3. Bread Crumb Cellular Properties: Role of Starch in Dough/Crumb Transformation. Crumb parameters were determined using digital image analysis and are listed in **Table 4**. The native wheat starch containing model bread crumb had a mean cell area of 0.35 mm^2, a cell density of 90.1 cells per cm^2, and a cell to total surface area of 38.9%. The inclusion of hydroxypropylated starch in the recipe (HP40 and HP80) resulted in bread with larger mean cell area (0.54 and 0.50 mm^2, respectively) and larger cell to total area ratio (44.0 and 43.9%, respectively) than the control. No difference was observed between the number of cells per unit area of the native and hydroxypropylated starch containing crumb samples. Inclusion of cross-linked starch in the bread model recipe did not impact the bread cellular properties (**Table 4**). Therefore, the number of gas cells per cm^2 seemed unaffected by differences in starch properties, and this was the case for the gas cell dimensions particularly upon incorporation of the starch with earlier and more pronounced swelling.

These findings point to an important role of starch in determining crumb texture and loaf volume. During baking, gluten continuous dough is converted into gas and gluten continuous bread. The leavening gas gradually takes more volume until the changing gluten properties destabilize the gas cell wall (*5*) and the system becomes gas continuous (*3*) thus ending the oven rise. We believe that, in this transition, the starch fraction, more in particular when it gelatinizes, is a crucial factor as well. As discussed above (sections 1.1 and 1.2), hydroxypropylation hastened the gelatinization-related swelling and water uptake of the granules. It can be reasoned that when the starch takes up water sooner in the process, proportionally more water is withdrawn from the gluten phase, which, as a result, loses flexibility. Because of the less flexible and weaker gluten network, the continued gas expansion then induces the rupture of the cell walls resulting in both lower loaf volume and a higher level of coalesced cells as seen upon incorporation of hydroxypropylated starch. Cross-linked and native starch granules, on the other hand, led to comparable bread crumb structures and loaf volumes. This shows that cross-linking under the conditions of the bread-making process did not greatly affect water uptake by the granules or granule swelling. Indeed, considering the limited level of water available to the starch in the oven and, hence, its limited swelling capabilities, it is tempting to speculate that, with the cross-linked starches used, the additional potential restriction of swelling induced by the derivatization may not have had effect.

4. Role of Starch in Establishing Initial Crumb Firmness. Table 3 lists the measured firmness data of the different model breads for different storage times. Large-scale deformation firmness measurements are largely influenced by the volume and density of the bread loaves. Given the considerable differences in loaf volume (**Table 3**), we preferred to correct the measured crumb firmness data (**Table 3**) for the different loaf volumes by dividing the crumb firmness values by the respective loaf density. This way, a value, which we define as the intrinsic firmness, was calculated and is shown in **Figure 3**. As indicated below, these calculations had generally little effect on the conclusions of this study.

The measured firmness of the control model bread after cooling was 2.54 N. Despite the higher bread density of the

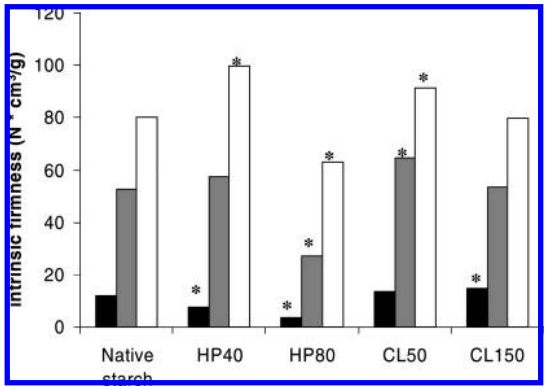

Figure 3. Model bread crumb intrinsic firmness data after 0 (black bars), 3 (gray bars), and 7 (white bars) days of storage. Intrinsic firmness was defined as the firmness of the sample divided by the density of the respective loaf to correct for the difference in loaf volume. The gluten−starch based model breads were made with either native wheat starch (100% of total starch fraction) or with hydroxypropylated (HP) and cross-linked (CL) wheat starch (65% of total starch fraction with various degrees of modification). *: significantly different compared to the native wheat starch containing model bread ($P < 0.05$).

hydroxypropylated starch containing model breads, their initial measured firmness was decreased to a large extent, that is, by approximately 25% and 60% for HP40 and HP80, respectively. A similar trend was seen for the initial intrinsic firmness, which was decreased by 37% and 70% for HP40 (to 7.6 N × cm^3/g) and HP80 (to 3.7 N × cm^3/g), respectively. The incorporation of the cross-linked starches increased initial measured firmness by 25−30%. Likewise, the intrinsic firmness was increased as well but only by 12−20% [to 13.5 N × cm^3/g (CL50) and 14.6 N × cm^3/g (CL150)]. For cross-linked tapioca starch containing breads, a higher initial firmness has been observed as well (*19*).

These observations can probably to a large extent be explained by different amylose properties or leached amylose content. Many studies indicated that amylose is an essential structuring element in fresh bread crumb and that it may also be a major factor determining initial crumb firmness. Baking experiments with waxy starch showed that amylose is necessary for obtaining a good loaf of bread with good crumb characteristics (*22, 23, 41*). It is generally believed that amylose is already gelled to a certain extent in fresh bread and that it forms a network in which swollen and deformed starch granules are embedded and interlinked (*42, 43*). Furthermore, Hug-Iten et al. (*44*) reported an amylose-rich region in the center of the gelatinized starch granules after baking attributed to amylose−amylopectin phase separation (*45*). In the view of Hug-Iten et al. (*44*), gelation/crystallization of this amylose fraction contributes to the rigidity of the starch granule remnants in the bread.

The reduced firmness of the hydroxypropylated starch containing model breads can be linked to the decreased CPV observed when the HP samples were analyzed in the RVA indicating that similar short-term phenomena, such as amylose gelation, occur in both systems. In this context, it is relevant that amylose-leaching experiments (performed as described by Vandeputte et al. (*25*)) indicated that hydroxypropylation markedly increased the level of leached amylose molecules (results not shown). Considering that hydroxypropylation primarily occurs in the amorphous regions of the starch granule and, hence, affects amylose, substitution with hydroxypropyl groups presumably reduced the gel-forming properties of the amylose polymers (formation of junction points) to a large extent. In this view, the reduced gelation properties temporarily lead to a weaker gel structure and, consequently, to a softer bread crumb and lower CPV in the RVA. However, Miyazaki et al. (*19*) ascribed the softer fresh crumb of hydroxypropylated tapioca starch containing bread to the more swollen and dispersed state of the modified starch granules after baking. They postulated that, during baking, the hydroxypropylated starch granules disperse and combine with the gluten matrix, while the native starch granules remain as distinct granules (remnants) entangled within the gluten network. In their view, in the former case, a smaller force is needed to compress the crumb.

The increased CPV values of the CL starch samples also correlated well with the higher initial crumb firmness and, hence, the higher rigidity of cross-linked starch granules.

5. Role of Starch in Crumb Firming during Storage. During storage, intrinsic firmness of the control model bread increased from 12.1 N × cm^3/g (d0) to 52.7 N × cm^3/g (d3) and 80.3 N × cm^3/g (d7) (**Figure 3**). Hence, firmness increased the most during the first 3 days of storage. The firming rate of the hydroxypropylated starch containing model breads depended on the extent of the modification. The HP40 containing model breads had a faster firming rate than the native wheat starch containing model breads (**Table 3**, **Figure 3**) as evidenced by an intrinsic firmness increase of approximately 49.8 N × cm^3/g and 92.0 N × cm^3/g after 3 and 7 days of storage, respectively. This resulted in higher measured (+50%) and intrinsic (+25%) firmness values after 7 days of storage compared to the control model breads. The firming rate of the HP80 containing model breads was lower particularly during the first 3 days of storage (intrinsic firmness increase of 23.2 N × cm^3/g) but was only slightly lower when considering the 7 day storage time (intrinsic firmness increase of 59.1 N × cm^3/g) than that of the native wheat starch model breads. This led to model breads with similar measured firmness but significantly lower intrinsic firmness (−20%) after 7 days of storage than measured for the control model breads. For both hydroxypropylated starches, the firmness increased more between 3 and 7 days of storage than the control model breads did. It would thus seem that the impact of the hydroxypropylation is to be primarily interpreted in terms of effects on kinetics. However, these results are somewhat in contrast to the findings of Miyazaki et al. (*19*) who, for hydroxypropylated tapioca starch containing breads, observed a lower firming rate. However, the storage time in their study was only 3 days and, with the HP80 model breads, we also observed a lower firming rate during this time period.

Incorporation of cross-linked starch generally had no large impact on firming during storage. The firming rate was slightly higher than or similar to that of the native wheat starch containing model bread as demonstrated by the intrinsic firmness increase after 7 days of storage of 77.9 N × cm^3/g (CL50) and 65.1 N × cm^3/g (CL150). This resulted in similar measured and intrinsic firmness data after 3 and 7 days of storage for the CL150 and native wheat starch containing model breads, while the CL50 model breads had a slightly higher measured (+25%) and intrinsic (+15%) firmness after 7 days of storage.

Rearrangements in the starch fraction, particularly amylopectin retrogradation, are generally accepted as greatly contributing to bread firming. DSC analysis of bread crumb showed that, during storage, an endothermic peak, originating from the melting of retrograded amylopectin, emerged around 60 °C. This can be attributed to the melting of retrograded amylopectin. The corresponding T_o, T_p, and T_c and the melting temperature range did not differ much between the different model breads (results not shown). Likewise, rather small differences in the melting enthalpies of retrograded amylopectin were generally observed (**Figure 4**). Incorporation of HP80 led to a significantly lower

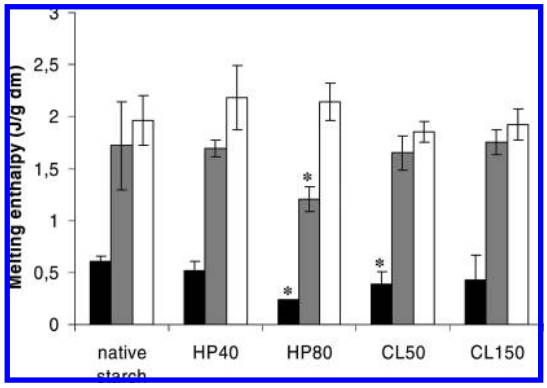

Figure 4. Amylopectin retrogradation in gluten−starch based model breads with incorporation of native wheat starch (100%) or hydroxypropylated (HP) and cross-linked (CL) wheat starch (65% of total starch fraction with various degrees of modification) after 0 (black bars), 3 (gray bars), and 7 (white bars) days of storage. Melting enthalpy of retrograded amylopectin was determined by DSC and is expressed as J/g crumb (dm). *: significantly different compared to the native wheat starch containing model bread ($P < 0.05$).

melting enthalpy after 0 and 3 days of storage and, hence, to lower levels of retrograded amylopectin than in the native wheat starch containing model breads. However, after 7 days of storage, the melting enthalpy of the former model bread did not differ from that of the control model bread. Model HP40 bread had a somewhat lower melting enthalpy after cooling, but it did not differ significantly from the native wheat starch containing breads after 3 and 7 days of storage in this respect. In line with these observations, the increase in melting enthalpy of retrograded amylopectin between 3 and 7 days of storage was higher for the hydroxypropylated starches. This indicates that the sterical hindering by the hydroxypropyl groups impacted the reassociation kinetics of the side chains of the amylopectin fraction. However, hydroxypropylation mainly affects the amorphous parts of the granules and, hence, can be expected to have little if any effect on the amylopectin side chains which make up the crystallites of both the native and retrograded starches. Alternatively, the enhanced swelling and facilitated disruption of hydroxypropylated granules may promote amylose−amylopectin phase separation resulting in more concentrated amylose and amylopectin-enriched regions. The latter would then be able to retrograde more during storage.

Incorporation of the cross-linked starch slightly decreased the melting enthalpy after cooling but had little if any effect on the level of retrograded amylopectin during further storage.

The fact that crumb firms and retrograded amylopectin levels increase during storage point to a role of amylopectin retrogradation in crumb firming. However, when comparing the data in **Figure 3** and **4** and **Table 3**, it is equally clear that crumb firmness and retrogradation DSC characteristics do not correlate well under these conditions. Although after the first 3 days of storage the HP80 sample had both a lower retrograded amylopectin melting enthalpy and a lower crumb firmness than the control model breads, the situation was completely different for the other samples and other storage times. Indeed, after 7 days of storage, the level of retrograded amylopectin was similar for the different model breads, while their crumb firmness differed significantly. This implies that amylopectin retrogradation and bread firming are not the same thing. Therefore, DSC monitoring of the retrograded amylopectin melting peak is not a suitable method to predict bread firmness during storage. Likewise, no correlation between CPV values and staling parameters (such as firmness and retrograded amylopectin level) was found. This can be explained by the different phenomena contributing to these parameters, such as gelation and retrogradation, as well as by the different time frames under consideration.

Although starch retrogradation cannot be solely responsible for the crumb firming, our results nevertheless indicate that the properties of the starch fraction are very important in establishing firmness not only in the fresh bread as discussed above (section 4) but also during storage. In this context, a different impact on bread firmness after 7 days of storage and on firming rate was observed depending on both the extent and the kind of modification. Hence, other factors are needed to explain crumb firming during storage. In this respect, we believe that the establishment of a long-range permanent (starch) network can be expected to be necessary for building firmness during bread storage. In our view, such a network depends on inter- and intramolecular starch−starch and possibly also starch−gluten interactions. This does not imply that the starch fraction in bread is 100% continuous. Rather, we visualize distinct areas with a more continuous starch fraction, where the swollen granules and the leached and semileached amylose molecules can interact with each other and with the proteins of the (permanent) gluten network. These interactions, in their turn, evidently depend on the status of the starch molecules and (remnants of) granules in the bread as defined by their swelling and rigidity properties, the level and extent of amylose leaching, and the granule surface area. In this context, Martin and Hoseney (*46*), on the basis of their results, referred to the importance of the effective starch granule surface area exposed to gluten in the buildup of firmness during storage. Over time, this network would develop further and become more rigid, presumably by losing the plasticizing water, which is gradually incorporated in the crystallites of the retrograding amylopectin.

The above listed properties of starch in bread are impacted differently by hydroxypropylation and cross-linking. In the case of the hydroxypropylated starches, the increased amylose leaching and the increased swelling (resulting in an increased granule surface area) during baking lead to a high number of interactions (starch−starch associations or entanglements and possibly also starch−gluten interactions) and an extensive network. Because of their high number, the strengthening of these interactions during storage contributes to a large extent to the (fast) crumb firmness increase. Moreover, the association rate is probably also influenced by the sterical hindering posed by the hydroxypropyl moieties. In this respect, phenomena contributing to initial firmness (e.g., amylose gelation) may now play a role in firming during storage. In the case of the cross-linked starches, the reduced swelling (with a reduced granule surface area) and amylose leaching would result in fewer interactions between the biopolymers in the crumb. However, because of the limited moisture conditions in the dough, swelling of native wheat starch during baking is limited as well. As indicated above, with the cross-linked starches used, the additional potential restriction of swelling induced by the derivatization has presumably only a limited effect.

Finally, the crumb firmness after storage is also influenced to some extent by the initial firmness. This can particularly be seen for the CL50 model breads. Both their initial firmness and their firmness after storage were higher compared to the native wheat starch samples.

In conclusion, incorporation of chemically modified starch in the recipe of gluten−starch model breads demonstrated the importance of starch swelling, gelatinization, and rigidity properties in determining the textural properties, porosity, and gas cells and the staling rate of the bread crumb. Our experi-

mental approach allowed proposing different hypotheses regarding the role of starch and gluten in determining crumb texture, crumb firmness, and firming during storage. First, incorporation of hydroxypropylated starch, with enhanced swelling and gelatinization properties and subsequent increased water uptake at lower temperatures, resulted in model breads with coarser crumb and larger gas cells. In our view, the water uptake by the gelatinizing starch granules results in a loss of flexibility of the gluten protein phase leading to destabilization of the gas cell walls, gas cell coalescence, and ultimately their rupture. Because starch swelling is limited during baking because of the limited level of water available to the starch in the oven, additional restriction of swelling such as induced by cross-linking may presumably not have had effect. Second, initial crumb firmness, much as the CPV in RVA experiments, seems governed by the rigidity of the gelatinized granule (remnants) as well as by the properties of the leached amylose. Finally, crumb firming and firmness during storage seem determined by amylopectin retrogradation and a strengthening of the interactions in the starch−starch and possibly also by the gluten−starch networks and the crumb firmness after baking.

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