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Structural Changes of Starch during Baking and Staling of Rye Bread

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ABSTRACT: Rye sourdough breads go stale more slowly than wheat breads. To understand the peculiarities of bread staling, rye sourdough bread, wheat bread, and a number of starches were studied using wide-angle X-ray diffraction, nuclear magnetic resonance (^{13}C CP MAS NMR, ^{1}H NMR, ^{31}P NMR), polarized light microscopy, rheological methods, microcalorimetry, and measurement of water activity. The degree of crystallinity of starch in breads decreased with hydration and baking to 3% and increased during 11 days of storage to 21% in rye sourdough bread and to 26% in wheat bread. ^{13}C NMR spectra show that the chemical structures of rye and wheat amylopectin and amylose contents are very similar; differences were found in the starch phospholipid fraction characterized by ^{31}P NMR. The ^{13}C CP MAS NMR spectra demonstrate that starch in rye sourdough breads crystallize in different forms than in wheat bread. It is proposed that different proportions of water incorporation into the crystalline structure of starch during staling and changes in starch fine structure cause the different rates of staling of rye and wheat bread.

KEYWORDS: *rye sourdough bread staling, starch retrogradation, amylose, amylopectin, XRD, MAS NMR*

INTRODUCTION

Rye sourdough bread is a traditional and popular bread in northwestern Russia, the Baltic states, Finland, northern Germany, and Denmark. In the Baltic states, rye bread is industrially produced by mixing sourdough with flour, baker's yeast, and scalded flour, followed by leavening, molding, proofing, and baking. Rye bread is a recommended dietary product because it is a good source of nutritionally important substances, including B-complex vitamins and dietary fiber. Complex technology and relatively short storage time causes the limitation of consumption of rye bread outside the traditional areas of production.

The main factor that limits the storage time of breads is staling, which is mainly a physical process in bread and other starch-containing foods that reduces their palatability. Staling is not simply a drying-out process due to evaporation; packing bread hermetically does not prevent staling. Although the precise mechanisms of staling remain unknown, most theories are related to migration of moisture within the microstructures of bread and structural changes in starch structure during storage. During baking the temperature inside the bread reaches 99 °C, during which starch granules swell, gelatinize, and partly liquidize.[1] After cooling, the crystalline structure of starch during storage slowly recovers.[2] This process is termed starch retrogradation. Retrogradation is technologically important because it produces significant changes in properties such as springiness, softness, and moistness of the crumb, which are important in the sensory perception of bread.[3] The rate of bread staling depends on the recipe used and the storage conditions, mainly temperature and humidity. During storage, water migration from the crumb to crust occurs, which leads to a decrease in water concentration and activity in the crumb[4] and softening of the crust.[5] However, water migration is not the main reason for bread staling and starch retrogradation. It is supposed that staling is caused mainly by retrogradation of amylopectin.[6] In starch, the short-chain nonbranched fraction of amylopectin molecules is organized as small crystallites formed from double helices.[7,8]

Linear molecules of amylase are apparently present in an amorphous state in the starch granule.[9,10] Amylopectin chains are primarily responsible for the crystallinity of starch. It was suggested that the branching points and intercluster connections of amylopectin are located in the more amorphous region.[11]

The crystalline structure of starch has been classified according to X-ray diffraction patterns as A-, B-, or C-type.[9] Typical cereal starches such as wheat and rye starches belong to the A-type, in which crystals contain double helices that are densely packed in a monoclinic lattice. The B-polymorph, characteristic of potato starch, has double helices packed in a hexagonal lattice with 27% hydration.[12,13] Mixed C-type starch is observed in legume starches.[2]

Also, starch−protein complexes are considered to be very important in the development of the firmness of the crumb during staling.[14] In contrast to wheat gluten, the rye proteins (secalins, prolamins) cannot take part in the formation of

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dough structure as a starch−gluten complex due to the lack of low molecular weight glutenin subunits, and it is thus impossible for intermolecular disulfide bonds to form.[15] In the case of rye bread, water-extractable arabinoxylans play an important structural role, binding water and forming viscous dough.[16,17] The low pH of rye sourdough increases the extractability and swelling properties of arabinoxylans and inactivates amylase activity. The processes mentioned above contribute to an increase in water-binding capacity.[18] These peculiarities might be why rye bread prepared with sourdough stales more slowly than wheat breads.[19]

Also, scalding, a common procedure during bread processing in several countries, might affect the rate of staling. Scalding is the mixture of flour and hot water, which is allowed to cool.[6] During scalding the starch is gelatinized and hydrolyzed partly by endogenous enzymes or added malt. Siljeström and coauthors[20] showed that retrogradation of starch in bread made from malted whole grain wheat flour was slower that that in bread made from unmalted whole grain wheat flour.

The aim of this work is to study and characterize the structural changes of starch in rye sourdough bread during baking and staling by comparing rye and wheat starch as well as bread structures during processing.

MATERIALS AND METHODS

Dough Ingredients. Lactic acid bacteria strains *Lactobacillus plantarum* L-73 and *Lactobacillus brevis* L-62 (freeze-dried) were kindly provided by Lallemand Inc. (Montreal, Canada) and used for the production of sourdough. Fresh baker's yeast (sp. *Saccharomyces cerevisiae*), used for bread dough leavening, was obtained from a local market.

Dark rye flour (type 1370) and wheat flour (W550) were obtained from the Tartu Grain Mill Ltd. (Estonia). Fermented (red) and unfermented (white) rye malts were obtained from Eesti leivalinnase Ltd. (Võru, Estonia). Sugar, margarine, milk powder, and salt were obtained from a local market.

Starches. Wheat, corn, and potato starches, amylose from potatoes, and amylopectin from maize were obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Dimethyl sulfoxide (DMSO-d_6) was also obtained from Sigma-Aldrich.

Rye starch was isolated from fine-ground rye flour by alkaline extraction.[21] Rye flour was suspended in tap water and 0.5% NaOH in a ratio of 2:7:7, stirred for 60 min, and centrifuged at 3000 rpm for 10 min. The sediment was washed with water and centrifuged again. The procedure was repeated two times, neutralized with 0.1 N HCl, and centrifuged. The upper grayish layer was removed, and the white starch was washed with water and centrifuged again. The isolated starch was air-dried overnight and sieved trough a 250 μm sieve.

Baking. Rye sourdough breads were prepared in a laboratory from sourdough starter culture, rye flour, scalded flour, and yeast suspension. Sourdough was prepared according to method described by Mihhalevski and coauthors[22] by mixing 225 g of the dark rye flour and a bacterial suspension made from a mixture of *L. brevis* L-62 and *L. plantarum* L-73 (in a ratio of 1:1, 5×10^6−5×10^7 cfu mL^{-1}) in 0.5% of NaCl to a 1:1 ratio in Stomacher 400 circulator (Seward Ltd., UK) bags for 15 min at 100 rpm and then incubated in the stomacher bags in an Environmental Test Chamber (Sanyo, Japan) at 32 °C during 24 h.

Scalded flour was made by mixing 239.5 g of rye dark flour and 16.5 g of red rye malt into 810 mL of warm (55 °C) tap water in a 2 L temperature-controlled kettle equipped with a refrigerated/heating circulator (Julabo F25, Seelbach, Germany). After 15 min of mixing, rye white malt was added. This mixture was heated to 67 °C, and after saccharification during 40 min, it was cooled to 27 °C.

Sourdough (450 g) was mixed with scalded flour (1100 g), rye dark flour (1200 g), 30 g of yeast suspension (20% dry weight), and 120 g of 24% salt solution during 20 min in a 5 L Bear Teddy dough mixer (Varimixer, Shreveport, LA, USA) at 100 rpm. Dough fermentation occurred in the Environmental Test Chamber at 32 °C during 110 min. Dough was molded into 400 g portions, put into baking molds (8.5 × 15 × 9.5 cm), proofed in the Environmental Test Chamber at 32 °C during 45 min, and baked in a Self Cooking Center (Metos System Rational, Weikersheim, Germany) as follows: 10 min, 230 °C; 15 min, 200 °C; 15 min, 180 °C; 10 min, 150 °C. The bread was taken from the oven and cooled to room temperature in a laminar flow cabinet under UV light (Telstar, Terrassa, Spain), sealed aseptically into plastic bags, and stored at room temperature.

Wheat bread was made by mixing 50 mL of yeast suspension (20% DM) with sugar (14 g), wheat flour (300 g), margarine (10 g), milk powder (15 g), salt (5 g), and water (110 mL). Molded dough pieces were proofed for 20 min at 32 °C and baked for 30 min at 220 °C.

Microbaking. Starch−water or flour−water mixtures with 42% water content (similar to wheat and rye dough water content) were prepared for baking simulations. A small amount of dough or starch−water suspension was baked according to a temperature profile that simulates the baking profile inside the bread. The temperature profile was recorded during bread baking using a Data Logger (Onset, Cape Cod, MA, USA). Microbaking trials were carried out using three different methods: (i) in the 3 mL microcalorimeter vials in a thermostat; (ii) between plates (25 mm, gap of 2 mm) of a dynamic rheometer, Physica MCR 301 (Anton Paar, Ostfildern, Germany); (iii) between the object-plate and cover-glass, glued by silicone to hermetically seal the sample, in a thermostat VEB MLW U2C (Prüfgeräte-Werk, Medingen, Sitz Freital, Germany).

To determine the heat flows after baking, vials were placed into an isothermal microcalorimeter TAM III (TA Instruments, New Castle, DE, USA) at 20 °C for 200 h. The power−time (P−t) curves of samples were measured in 15 min intervals starting at 1 h after microbaking.

The storage modulus (G', kPa) of dough and starch samples during microbaking in the rheometer was measured using an oscillation test at a frequency of 1 Hz, a normal force of 1 N, and a strain of 0.01−10%.

Sample Preparation. Fresh-baked hot breads (0 h) were sliced in a laminar flow cabinet under UV light, packed aseptically in plastic bags, sealed hermetically, and stored at 22 °C and 95% relative humidity for 11 days for further analysis. After slicing at 0 h (immediately after removal from the oven) and storing for 5 h or 11 days, the bread samples were flash cooled in liquid nitrogen, freeze-dried, and ground (particle size = 0.25 mm).

The total starch in the dough and bread samples was determined using an amyloglucosidase/α-amylase assay kit from Megazyme International Ltd. (Bray, Ireland). Three milliliters of thermostable α-amylase (3% in 3-(N-morpholino)propanesulfonic acid (MOPS) buffer, 50 mM, pH 7.0) and 200 μL of ethanol (80%) were added to sample (100 mg). The tube with mixture was placed in a boiling water bath for 6 min with stirring after 2 and 4 min. Sodium acetate buffer (4 mL, 200 mM, pH 4.5) and amyloglycosidase (100 μL) were added to the mixture, and the sample was incubated at 50 °C for 30 min. After incubation, the sample was diluted to 100 mL and centrifuged (3000 rpm, 10 min). After the addition of 10 μL of supernatant to 3 mL of glucose determination reagent (GOPOD) and incubation at 50 °C for 20 min, the mixture's absorbance was measured at 510 nm.

Moisture content was measured at 105 °C using a Halogen Moisture Analyzer HR 83 (Mettler Toledo, Urdorf, Switzerland). Water activity was measured using a Water Activity Meter FA-st LAB (GBX, Bourg de Peage, France).

XRD Analysis. The crystallinity of flours, starches, and freeze-dried and ground bread samples was studied by wide-angle X-ray diffraction (XRD) measurements using an X-ray diffractometer Ultima IV (Rigaku, Tokyo, Japan). The diffractometer settings were as follows: line detector D/tex Ultra, copper tube operating at 40 kV and 40 mA, and irradiation of the sample with Cu Kα radiation (1.541 Å) using a Ni filter to restrain Kβ radiation. Diffractograms were acquired at 25 °C over a 2θ range of 6−50° with a measurement speed of 5°/min. The step size was 0.02°.

The degree of crystallinity (DC, %) was determined according to method described by Ribotta and coauthors[23]

$$\text{DC, \%} = \frac{I_C}{I_C + I_A} \times 100 \quad (1)$$

where I_C is the integrated area of the crystalline phase and I_A is the integrated area of the amorphous phase. Data analysis was performed using the programs EVA and TOPAS 4.2 (Bruker).

NMR. ^{13}C CP MAS NMR spectra were recorded on a Bruker AVANCE II 600 MHz spectrometer using a 14.4 T external magnetic field and custom-built MAS probe for 4 mm rotors. The sample spinning speed was 15.0 kHz, and typically 10000 accumulations were averaged to obtain a reasonable signal-to-noise ratio. For all samples we applied a contact time of 0.5 ms for cross-polarization and a relaxation delay of 5 s between accumulations. All spectra were referenced to solid adamantane resonance lines at 29.46 and 38.48 ppm.[24]

^{1}H and ^{13}C NMR spectra of amylose (9 mg dissolved in 300 mg of DMSO-d_6) and amylopectin (7 mg dissolved in 300 mg of DMSO-d_6) model compounds were obtained in a Bruker AVANCE III 800 MHz spectrometer at a temperature of 313 K. To fit the obtained NMR spectra, the TOPSPIN program from Bruker was used.

^{31}P NMR spectra of starches dissolved in DMSO-d_6 were recorded on a Bruker AVANCE III 800 spectrometer at a frequency of 324 MHz (ambient temperature). H_3PO_4 (85%) resonance (at 0 ppm) and egg yolk phosphatidylcholine were used as external references.

Microscopy. Starch and dough samples were observed using a polarized light Nikon Eclipse E200 microscope (Tokyo, Japan) with 400× magnification and analyzed using the picture image analysis program ACT-2U. The micrographs were made before heating, after heating, and during storage for 7 days.

RESULTS

Microbaking. The effects of baking and storage on dough and starch water suspensions were studied using microbaking techniques. The contents of starch were 76.11 ± 1.01 g/100 g DM for wheat flour and 65.96 ± 1.77 g/100 g DM for rye flour.

Lenticular and spherical granules with a diameter of 2−40 μm were observed on microscope slides of hydrated rye starch, wheat starch, amylopectin, and rye dough (Figure 1). The rye starch granules were larger than those of amylopectin and wheat starch, observed also by Gomand and coauthors.[25] The form of a Maltese cross indicates orderly arrangement of the crystalline areas within each granule.[9] The pictures after heating to 99 °C showed losses of the Maltese cross pattern, which indicates that disordering processes related to starch gelatinization occurred. In the case of amylopectin isolated from maize, the structures containing Maltese cross formations were restored, whereas in the case of baked rye and wheat starch the longish structures appeared on the micrographs after cooling. During storage, the intensity of brightness of those structures increased, probably due to the crystallization processes. The longish structures were not observed on micrographs of pure amylopectin or amylose. The effect can be related to leakage of amylose from the starch granule. The increase in intensity of those structures from starch during storage might be related to the change of crystallinity and rheological properties during storage of bread. Hug-Iten and coauthors[26] suggested that the reorganization of the intragranular amylose fraction enhances the rigidity of starch granules on bread staling.

Changes in the viscoelastic properties of starches and dough were studied using baking between rheometer plates. The viscoelastic profiles of rye and wheat starch showed significant differences during the first stages (Figure 2). With an increase in temperature, the storage modulus (G') increase of pure wheat starch started at 40 °C and that of pure rye starch at 45 °C. With further increase of temperature over 55 °C the G' decreased. This is probably due to liquidation of the starch gel. In the case of wheat and rye dough, the increase in G' was

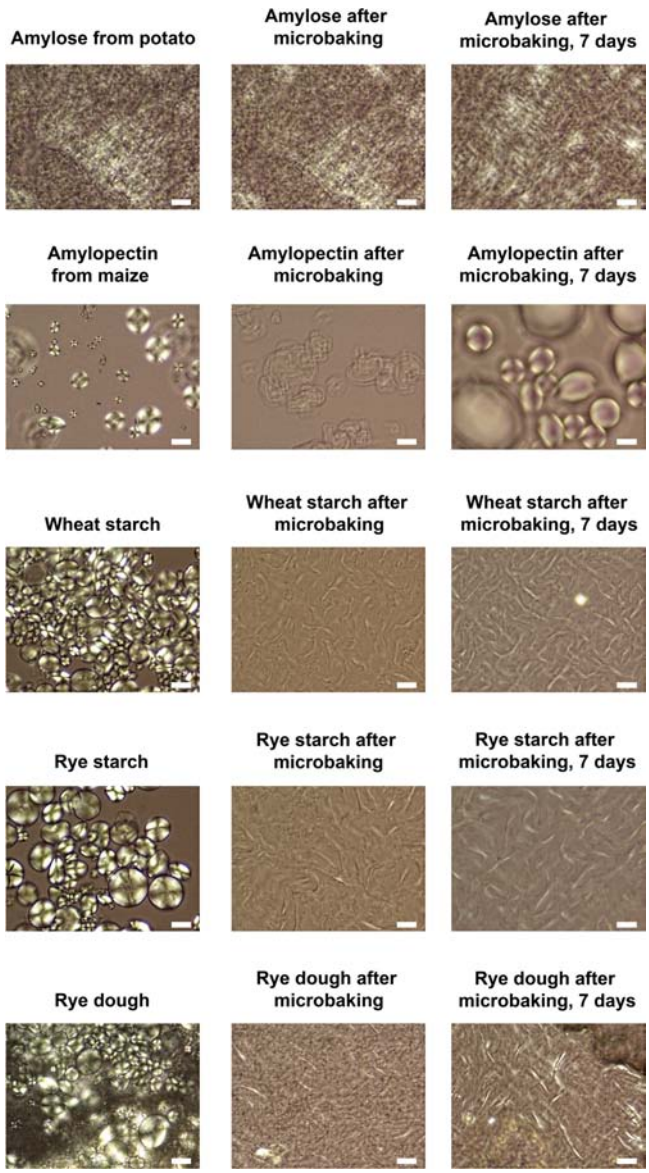

Figure 1. Polarized light micrographs of potato amylose−, maize amylopectin−, wheat starch−, rye starch−water suspensions and rye dough before and after microbaking and storage. Bar = 20 μm.

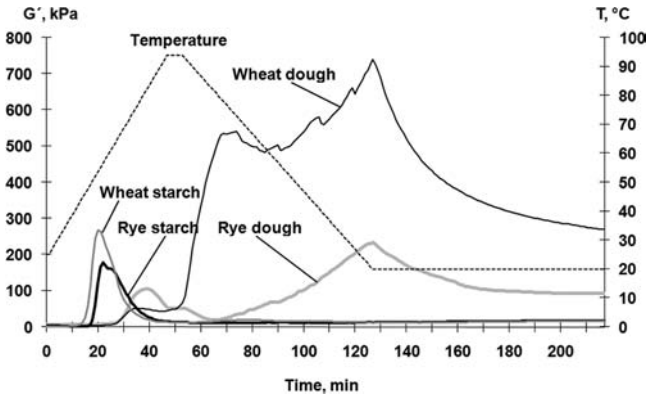

Figure 2. Viscoelastic properties of starch−water suspensions and dough during baking and cooling between rheometer plates.

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observed at temperatures around 65 °C, whereas the storage modulus of pure starch decreased. The difference can be related to the effects of the dough matrix protecting starch from hydration and damage.

The behavior of wheat and rye dough was different during holding at 95 °C. In the case of rye dough the G' rather decreased, whereas in the case of wheat dough G' increased to 550 kPa. The difference may be related to the formation of a gluten network in wheat dough. Upon further cooling from 80 to 20 °C the G' increased for both doughs to equal extent, about 250 kPa. The process can be related to gel formation from liquidized starch, protein, or arabinoxylans. The increase in G' was not observed in pure starch samples. The decrease in G' of both doughs during holding at 20 °C may be explained by continuous mechanical stress to the samples.

Power−time curves were measured in starch suspensions and dough samples heated to simulate baking. The heat flow (μW/g of starch) was lower in rye starch and rye dough than in wheat starch and dough during the period of 2−48 h after baking. This might be related to different rates or intensities of phase transition processes (crystallization and glass transition) in rye and wheat dough after baking. Silverio and coauthors[27] suggested that the heat flow during the first 5−10 h is related to the amylose crystallization.

Wide-Angle X-ray Diffraction. Figure 3 provides wide angle X-ray diffractograms of rye, wheat, corn, and potato

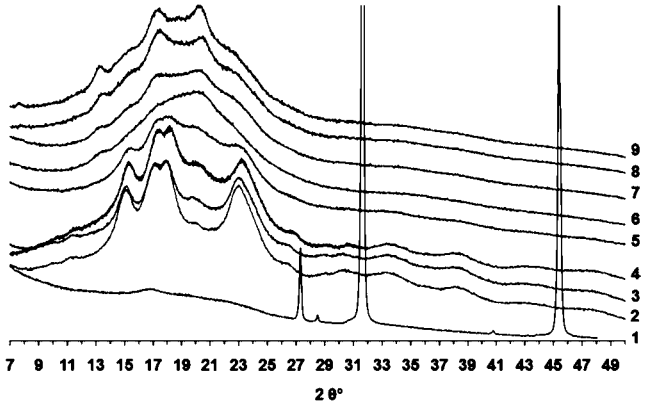

Figure 3. X-ray diffractograms of starch fractions, starches, flour, dough, and breads (1, amylose from potato; 2, amylopectin from maize; 3, rye starch; 4, rye flour; 5, rye dough; 6, rye bread, 0 h; 7, rye bread made from mix, sourdough, 11 days; 8, rye bread made from *L. plantarum*, 22 days; 9, rye bread made from *L. brevis*, 22 days).

starches, maize amylopectin, and potato amylose. A high degree of similarity is observed between rye starch, wheat starch, and amylopectin isolated from high-amylopectin maize. Peaks at 15.3, 17, 18.5, and 23.1° (2θ) correspond to the A-type starch pattern[8,28] and are clearly present in the spectra of Figure 3. The native corn starch sample (40% amylose) has an XRD pattern similar to B-type starches characteristic of potato starch (not shown), having peaks around 17, 20, and 22−23° (2θ).[29,30]

The peaks characteristic to amylose preparation from potato had 2θ values of 27.4, 31.7, and 45.5° (Figure 3) and additionally 56.5, 66.3, and 75.3° (2θ) (not shown).

The A-type rye and wheat starch pattern became weaker after flour hydration during dough making and disappeared after baking (Figure 3). The XRD spectra of fresh rye and wheat breads (not shown) were almost identical and showed both the maximum at 20.1° and small peaks at 13.4°. The peaks at 13.4° and 20° (2θ) are reported to be typical for cocrystallized Va-type (anhydrated) amylose as single helices with alcohols or fatty acids.[31,32,3] Those structures may correspond to bright longish structures seen in Figure 1. With bread aging, the peaks around 15, 17, and 23° (2θ) reappear and a new peak appears at 7° (2θ) (Figure 3) in all bread samples studied. The typical A-starch double XRD pattern at 17 and 18° (2θ) in flour and dough changed after baking and storage into a single peak at 17° (2θ) for both wheat (not shown) and rye breads. The peaks at 15.0, 17.0, 22.2, and 24.0° (2θ) are characteristic of the B-type crystalline phase, which might include water in the crystalline structure.[23] Diffraction peaks that appear at 8, 13, and 20° (2θ) characterize a 6-fold single-helix structure (V6), whereas a 7-fold helical polymorph is characterized by peaks at 7, 12.5, and 18.5°, and the V8 structure is characterized by peaks at 17 and 22° (2θ).[33]

The crystallinity of wheat and rye flour, starch, dough, and bread was calculated from their diffractograms. The degree of crystallinity of starch in fresh rye bread (Table 1) increased

Table 1. Degree of Crystallinity of Starch Fractions, Starch, and Bread Samples

sample	degree of crystallinity, %	
amylopectin from maize	25	
amylose from potato	41	
corn starch	25	
potato starch	29	
wheat starch	20	
rye starch	20	
	rye	wheat
flour	18	23
dough	14	20
bread, 0 h	3	3
bread, 5 h	9	9
bread, 11 days	21	26

from 3 to 21% in 11-day-old bread and in wheat bread increased from 3 to 26%. The degree of crystallinity of the bread starch fraction increased during rye bread staling up to 60% for rye bread and up to 80% for wheat bread. Using hetero- (*L. brevis*) or homofermentative species (*L. plantarum*) for sourdough fermentation did not affect the rye bread XRD pattern after storage (Figure 3).

Proton-Decoupled ^{13}C NMR and ^{31}P NMP Spectra of Starches. The chemical structure of rye and wheat starch was studied using ^{13}C and ^{31}P NMR. In linear amylose molecules only six peaks corresponding to the different glucose carbons appear in the proton-decoupled ^{13}C NMR spectra (Figure 4A). In the spectra of amylopectin, rye starch, and wheat starch we observe several smaller signals at all six carbon positions in addition to the C1−C6 signals of linear amylose and the linear part of amylopectin. For example, in addition to the main signal for the C4 glucose unit in rye starch, five smaller signals with comparable intensities are visible (Figure 4B). The peaks belong to the carbons of amylopectin in glucose units at the terminal ends, branching units, and units linked through (4→1)-α, (1→4)-α, (6→1)-α bonds to the branching glucose molecules. Using 2D methods (^{1}H−^{1}H COSY, HSQ, HMBC) these six signals are sorted out. These primed signals in Figure 4 belong to (1→4)-linked α-D-glucopyranosyl units with free 4-OH groups attached through C4 from branched amylopectin

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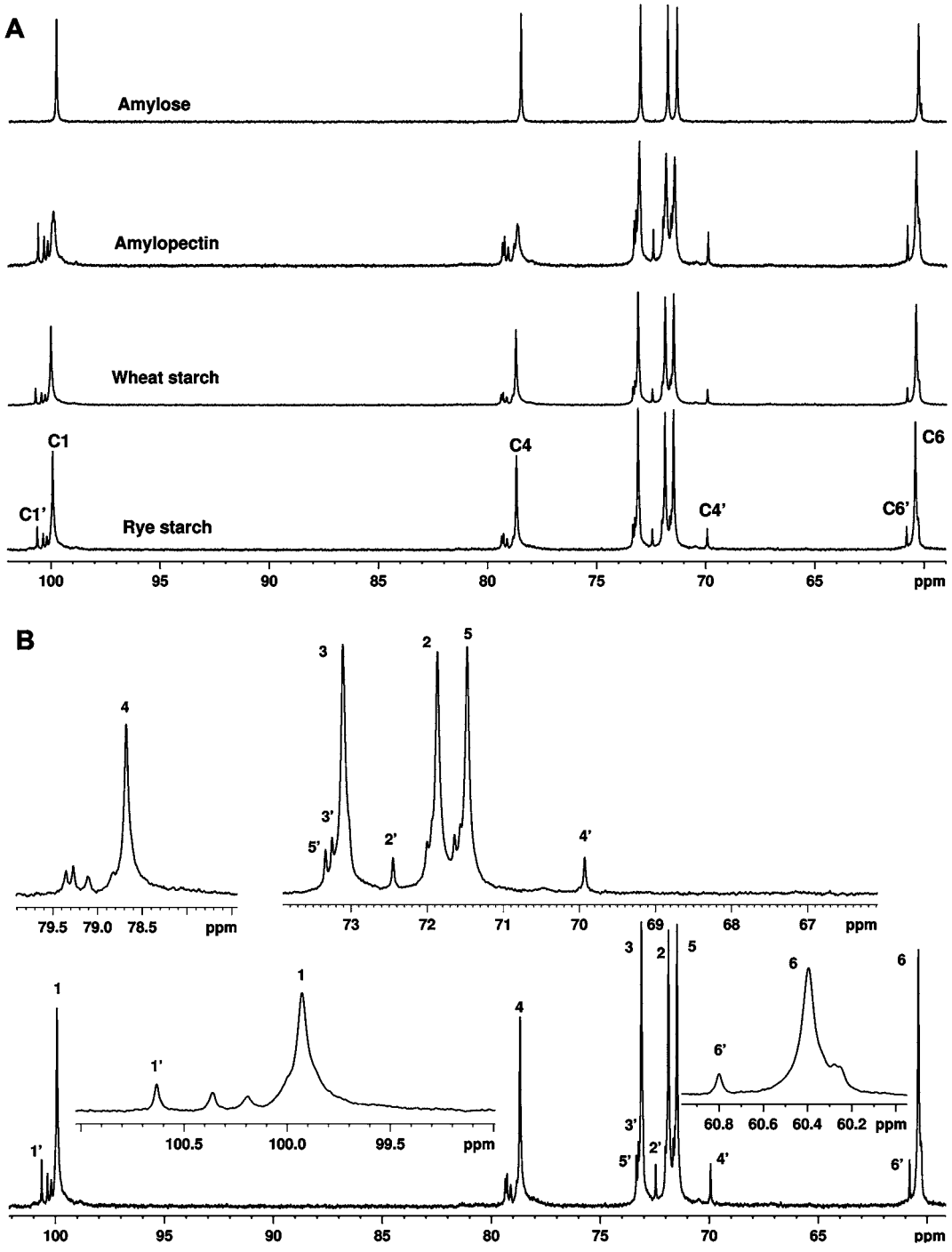

Figure 4. ^{13}C NMR spectra of amylose, amylopectin, and wheat and rye starches (A) and ^{1}H-decoupled 200 MHz ^{13}C NMR spectra of rye starch (B) in DMSO-d_6 solution at 313 K. Carbon signals from end glucose units of branches are primed.

end units. Exceptional positions a of the 4′ carbon signal at 70 ppm and a corresponding 4′ proton signal at 3.08 ppm (data not shown) are useful reference signals for the determination of degree of branching in starches. There are two different types of C6 carbons in starch glucose units: free side-chain CH_2OH groups and (1→6)-α-CH_2O-bridge groups. Despite this, ^{13}C signals of both types resonate within a narrow 0.4 ppm interval at 60 ppm. The only outstanding ^{13}C signal in this region is C6 from a terminal glucose unit with a free 4-OH group. Diastereotopic protons at this C6 have a comparatively strong chemical shift difference.

The ^{13}C NMR spectra of rye and wheat starch (Figure 4A) are very similar, and only some differences in the intensities of carbons corresponding to terminal glucose units (C′) compared those of the linear part (C) are observed. This can be caused by differences in amylose content and/or branching degree and chain lengths of amylopectin. By comparison of the signal intensity of primed carbons to all carbons of amylopectin, it can be calculated from the C1 signal that the percentage of terminal glucose units with a free 4-OH group in amylopectin was 10% and that in wheat and rye starches was 8%. The

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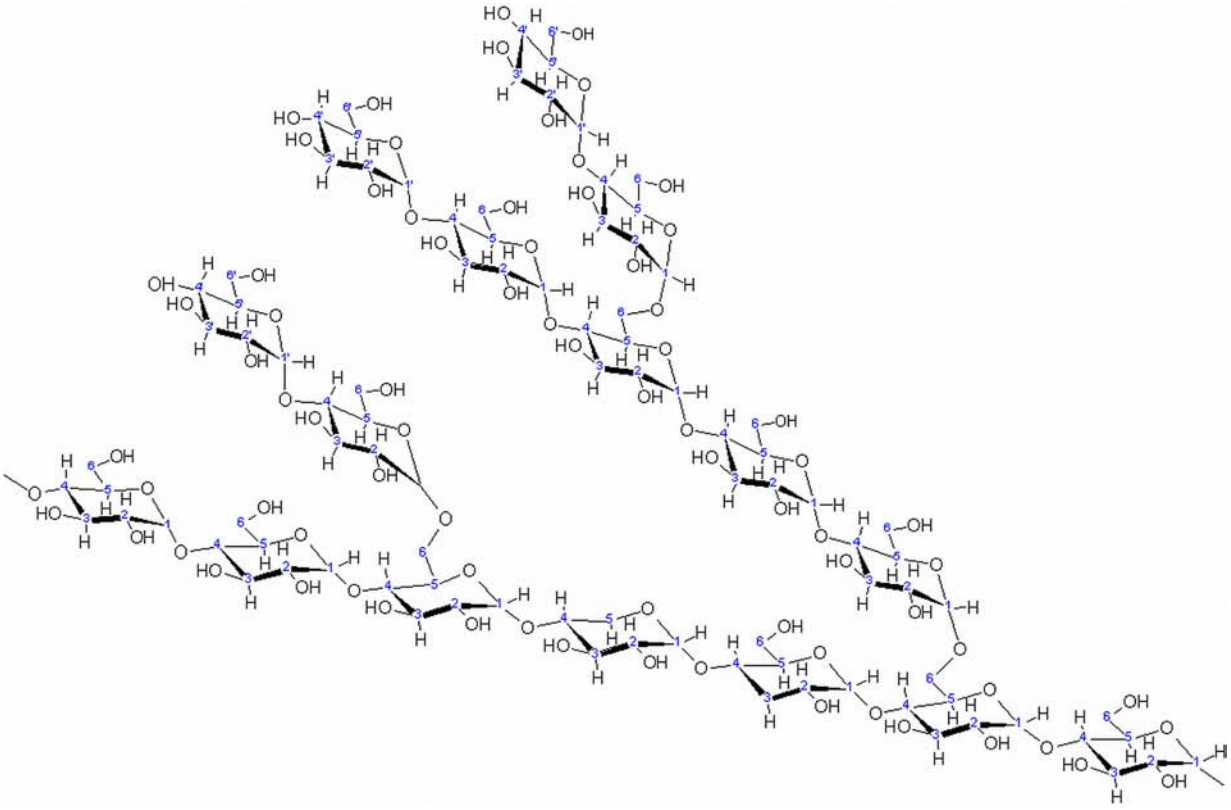

Figure 5. Schematic structure of amylopectin amorphous lamella region with ^{13}C and ^{1}H NMR signals. Primed numbers correspond to the carbon atoms of the end glucose units.

spectra suggest that, like amylose content, amylopectin structures (Figure 5) in rye and wheat starch are very similar. **^{31}P NMR spectra** of different starches are given in Figure 6. The significant difference observed between wheat and rye

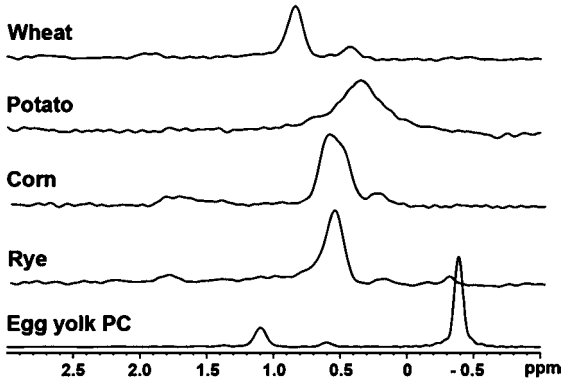

Figure 6. ^{31}P NMR spectra of starches (dissolved in DMSO-d_6). Egg yolk PC, egg yolk phosphatidylcholine (as reference).

starches can be caused by different compositions of the starch granule membrane, the phospholipids, and phosphate monoesters[34] or incorporated amylose−lipid complexes of rye and wheat starch.[11,35] Most of the cereal starch lipids in amylose−lipid complexes are lysophospholipids. Wheat starch contains 70% lysophosphatidylcholine, 20% lysophosphatidylethanolamine, and 10% lysophosphatidylglycerol.[36] Finnie and coauthors[37] reported that wheat starch contains phosphatidylcholine (70%), lysophosphatidylcholine (12%), phosphatidylethanolamine (8%), and phosphatidylglycerol (5%) located on the surface of wheat starch. Other phopholipids (lysophosphatidylglycerol, phosphatidylinositol) were reported to form 1−2% of the total phospholipid content in starch surface.

^{13}C Cross-Polarization Magic Angle Spinning Nuclear Magnetic Resonance (^{13}C CP MAS NMR). ^{13}C CP MAS NMR was used in parallel with XRD to study the changes in starch structure during baking. Although the lines of starch in solid phase ^{13}CP MAS NMR spectra (Figure 7B) are much broader than those dissolved in DMSO (Figure 4), significant changes in peak intensities corresponding to amylopectin carbons C1 (90−110 ppm) and C6 (63−60 ppm) during bread processing and storage are observed. Several changes in the C2, C3, and C5 (69−78 ppm) region, especially at 73.8−74.4 ppm, corresponding to the C3 and C4 (82 ppm) area are also observed.

Decomposition of the C1 and C6 resonances (Figure 7B) was carried out to better interpret differences and changes in the crystalline, semicrystalline, or amorphous structure of starch during rye bread baking and storage. The C1 region at 110−99 ppm was decomposed to five peaks (A−F), and the C6 region at 63−59 ppm was decomposed to three peaks (C6-H, C6-I, C6-J). peak A (103 ppm) in the C1 region is typical of the V-type single helix[38−41] with eight glucose cycles per turn[31] and the amorphous content (junction points of amylopectin double helices).[38−41] Peak A dominates in both the amylose and bread spectra. For amylopectin, the proportion of peak A was about 30% and that in starch and breads was 40−50%. The difference can be related to 22−25% amylose content in starch. In addition, rye and wheat starches and flour spectra displayed three peaks the in C-1 region: 101.5 (peak B), 100.5 (peak C), 99.5 (peak D) ppm are common to amylopectin (Figure 7) and correspond to three nonidentical sugar residues.[41] Peaks C and

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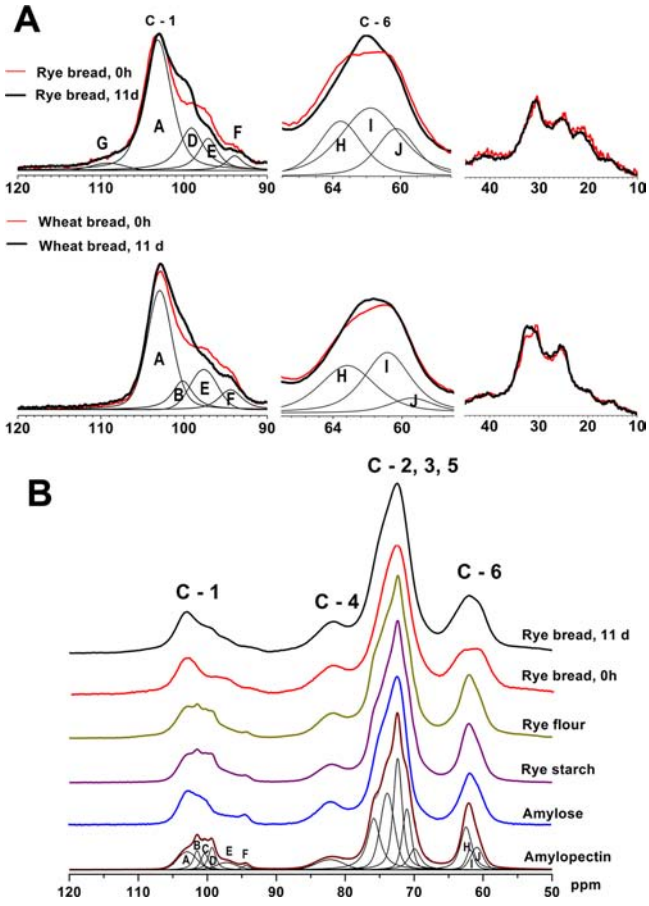

Figure 7. ^{13}C CP MAS NMR spectra of rye and wheat bread (A) and amylopectin with decomposition of resonances, amylose, rye starch, flour, and bread (B). Interpretations of the individual components: C1-A (102.9−103.2 ppm), amorphous region of amylopectin (branching points) and V-type single helix; C1-B (101.4−101.5 ppm), double helices; C1-C (100.1−100.5 ppm), double helices; C1-D (99.1−99.8 ppm), double helices; C1-E (96.4−97.7 ppm), glucose units near α-(1→6) linkages within the branched regions; C1-F (93.8−94.7 ppm), associated with constrained linkages; C1-G (106.9−109.1 ppm), C1 of rye cellulose.

D are characteristic of crystalline B-type double helices and correspond to two nonidentical sugar residues,[41] peak E can be related to the glucose units near α-(1→6) linkages within the branched regions, and peak F can be associated with constrained linkages.[11,31,38,41]

During baking of wheat and rye dough, the change in relative intensity of line A during baking and storage is not observed. Common to both of these breads during baking is an observed decrease in signal intensity in the region related to the crystalline structure (102−99 ppm, peaks B, C, and D) and an increase in relative intensity of the broad peak in the range of 98−96 ppm, which corresponds to a noncrystalline region. During staling, the area of this region decreased and that of the crystalline region (101−99 ppm) increased (Figure 7A). During staling of rye and wheat bread, the rye bread displayed a maximum at 99.3 ppm, whereas wheat bread displayed its maximum at 100.1 ppm. This suggests that these breads display differences in starch retrogradation.

NMR spectra showed changes during baking and storage also in the C6 region. Rye and wheat flour and starch all had a dominating peak at 62 ppm (Figure 7A). Upon baking, the maximum of the peak split and shifted from 62 to 61 ppm. During staling, the C6 peak became sharper again and obtained a maximum intensity at 62 ppm in both rye and wheat breads (Figure 7A). The effect can be explained by loss and reformulation of crystalline structure during baking and staling, respectively.

Additionally to the C1−C6 region, peaks at 173.6, 171.5, 160.6, 129.6, 106.1−109, 40.2, 31.5, 30.7, 25.1, and 21.6 ppm are observed in the MAS NMR spectra of bread (Figure 7A). The peak at 106.1−109 ppm (C1-G) is observed only in rye flour and bread, not in starch. The C1-G line recorded at 108 ppm in flour peaked at 109.1 ppm in rye bread and then shifted to 107 ppm during storage. This is probably explained by the presence of cellulose (1−3%)[42,43] or mixed-linkage (1→3)(1→4)-β-D-glucans (1.5−2.5%)[44,45] in dark rye flour.

DISCUSSION

The main fundamental questions are (1) what are the mechanisms causing the staling of bread and (2) what causes the differences in staling between wheat bread and rye sourdough bread. The recrystallization of starch is one of most important components affecting staling.[3,6]

Supposing that after gelatinization of starch during baking, the amylose and amylopectin start to crystallize in hydrated forms (for example, Vh or B-type) that contain about 30% water.[11] A considerable amount of water (15 g/100 g of bread) can be removed from the crumb amorphous phase into the crystalline structures from starch during bread storage. This reduction in water content in the amorphous bread structures can explain the dry mouthfeel in aged breads. According to XRD analysis an increase in the degree of crystallization occurs (Table 1). Taking into account a crystallinity change of 18% during staling, it can be calculated that about 3 g of water/100 g of bread can be bound into the crystalline structures of starch. That makes all together about 7.5% of water in bread, which might cause both the observed dry mouthfeel and an increase in bread firmness during storage.

The main question is whether the starch crystallizes out in hydrated forms in amounts sufficient to explain the staling. Comparison of X-ray spectra (Figure 3) as well as ^{13}C MAS NMR spectra (Figure 7) of flour and 11-day-stored bread revealed clear differences in the patterns of flour and bread. This can partly be explained by recrystallization of the starch in hydrated crystalline forms.[3,41] The XRD spectra of aged bread resembles more water-containing B-type potato than A-type wheat and rye starch spectra, suggesting that the B-type is preferred in aged bread. The development during storage of a B-type pattern in the crumb has been suggested also by Primo-Martin and coauthors.[3]

A water content >43% leads to the development of a B-type pattern, whereas a water content <29% leads to an A-type pattern.[46] The water content in rye bread was 42% and that in wheat bread, 39%. This difference in water content can partly explain the different patterns of C1 region in ^{13}C MAS spectra.

The decrease in water activity expected during crystallization in chemically bound forms is not being observed in this study. This might be explained by slow sensitivity of water activity to soluble compound concentration in the noncrystalline phase and parallel processes such as glass transition that could occur during staling. Incorporation of water from the bread crumb into the crystalline structures of starch during bread storage will increase the concentration of solubles in bread and can also initiate the transition from a rubber into a glassy state, which

might explain the increase in firmness and storage module during bread staling.[47]

The structural differences during starch retrogradation of rye and wheat bread are relatively small, probably because of the high similarity of the chemical structures of wheat and rye starch. Significant differences in the ^{13}C NMR spectra of rye and wheat starch dissolved in DMSO were not observed. Analyzing the well-resolved C-1 and C-4 areas in starch spectra, we observed five additional lines with almost equal intensity besides all of the major lines corresponding to the amylase type structure. We are able to designate only terminal glucose unit carbons; three others belong to carbons linked directly to the branching glucose unit of amylopectin, and one belongs to the branching unit itself.

As the percentage of branching glucose units is calculated from ^{13}C NMR spectra of amylopectin is 10%, the branching region in amylopectin takes $5 \times 10 = 50\%$ of the glucose units. Taking into account that the average chain lengths of rye and wheat amylopectin are 20−22 and 23, respectively,[25] about 2.5 medium glucose cycle chains per 10 branching glucose cycles can be calculated according to the structure postulated in Figure 5.

Overall, the results show that regardless of the type of rye and wheat, the A-type starch chemical structure is very similar, and they transform after baking into hydrated crystalline forms during storage. However, some differences in the staling process were observed. According to ^{13}C NMR the major crystallites that formed during staling were different and the relative crystallinity of starch in rye sourdough bread is less and increases more slowly than in wheat bread. This can be related to differences in starch structures (phospholipids, granule size), protein matrix, content of water, and pH, as well as the scalding of flour.

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