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Rheological and Nuclear Magnetic Resonance (NMR) Study of the Hydration and Heating of Undeveloped Wheat Doughs

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The undeveloped doughs of two wheat flours differing in technological performance were characterized at the supramolecular level, by fundamental small-deformation oscillatory rheology and shear viscometry, and at the molecular level, by nuclear magnetic resonance (NMR) spectroscopy. For the harder variety, the higher storage moduli indicated lower mobility of the protein/water matrix in the 0.001−100 s range. Conversely, ^{1}H NMR indicated higher molecular mobility in the sub-microsecond range for protein/water, whereas starch was found to be generally more hindered. It is suggested that faster protein/water motions are at the basis of the higher structural rearrangement indicated by tan δ for the harder variety. Rheological effects of heating−cooling reflect mainly starch behavior, whereas ^{1}H NMR spectra and relaxation times give additional information on component mixing and molecular mobility. The heated softer variety dough formed a rigid lattice and, although a similar tendency was seen for the hard variety, all of its components remained more mobile. About 60% of starch crystallizes in both varieties, which may explain their similar rheological behaviors upon cooling.

KEYWORDS: Undeveloped doughs; hydration; thermal treatment; dynamic rheological properties; NMR; magic angle spinning (MAS); spectroscopy

INTRODUCTION

The breadmaking capability of wheat flours is largely dependent on the unique viscoelastic properties conferred to wheat doughs by the multiprotein gluten complex (*1*). Dough is essentially a cohesive three-dimensional network of gluten (protein) in which starch granules and gas cells are embedded. The macroscopic rheological behavior of dough has been widely studied (*2*−*10*). However, even when fundamental rheological methods are used, the interpretation of rheological phenomena in terms of molecular architecture is difficult to achieve, in part due to the complex interplay of several factors: flour composition, water content, temperature, hydration, and degree of energy input. In this way, a more complete understanding of the rheological properties of doughs, at the molecular level, would certainly enable an informed control and tailoring of the macroscopic properties.

An increasingly popular approach to gain insight on the molecular level characteristics of dough and its components has been to use spectroscopic methods, namely, nuclear magnetic resonance (NMR). Low-resolution ^{1}H NMR has been used to monitor water and component mobility (*11*−*13*), and recent examples are the study of flour hydration and aging based on proton T_2 (spin−spin), T_1 (spin−lattice), and $T_{1\rho}$ (T_1 in the rotating frame) relaxation times (*14*) and the study of water mobility in biscuit dough, viewed by proton T_2 (*15*). NMR imaging has been used to investigate baked bread structure (*16*), and ^{1}H cross-relaxation NMR has been applied to monitor solid-like components in aged bread, starch and gluten, in an attempt to follow and understand retrogradation (*17*). High-resolution solid-state NMR enables, in principle, a clearer distinction of each of the components of dough. ^{13}C cross-polarization and magic angle spinning (CP/MAS) detect the most rigid components of dough, in their relative natural abundance, whereas the complementary ^{13}C single-pulse excitation (SPE) experiment selects the signal arising from the most mobilized components. Previous results have indicated no significant changes in either type of spectrum as a function of flour hydration or aging (*14*). Using ^{13}C NMR methods presents, however, significant disadvantages due to the required long acquisition times under MAS conditions. This may promote undesirable changes in the sample (both by sample spinning and by heating), and water loss may be difficult to avoid. ^{1}H MAS enables rapid acquisitions to be employed, but care should be taken when in the study of dynamical heterogeneous systems because the signal of more hindered groups may not be resolved, appearing as broad underlying spectral component(s). ^{1}H MAS and ^{1}H high-resolution (HR)-MAS of dough and bread (*10*, *14*, *18*) have shown significant promise, allowing resolution enhancement for all components and compositional differences to be detected,

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for instance, for samples from different geographical origins. The increasing need to correlate the molecular level information provided by spectroscopy, for example, NMR with rheological measurements (supramolecular level), has been recognized in recent technical developments that enable spectroscopic measurements to be carried out under conditions of mechanical stress, as discussed in a recent review relating to gluten (*20*).

The present work describes the characterization of the dough of two wheat varieties differing in grain hardness and water absorption capability by rheological methods in tandem with NMR, in an attempt to establish a bridge between macroscopic and molecular level information. With this purpose in mind, we have characterized the doughs' rheological behavior using small-amplitude oscillatory measurements and viscosimetry, governed by material structure at the supramolecular level, and the molecular level dynamics and component mixing by ^{1}H MAS NMR and relaxation time measurements.

Although the importance of mixing time and work input on dough properties and breadmaking quality has long been recognized (*21*−*25*), in this work, we have intentionally avoided the mixing phenomenon effects by preparing the so-called undeveloped doughs following the concept reported earlier (*26*, *27*). The extension of this work to the study of developed dough is intended.

MATERIALS AND METHODS

Flour Samples. The two Portuguese cultivars of *Triticum aestivum* spp. *vulgare* used in this study, 'Amazonas' and 'Sorraia', were grown at the National Plant Breeding Station (ENMP-Elvas, Portugal). Grain hardness was evaluated by near-infrared reflectance (NIR) using the AACC approved method 39-70A (*28*). Wheat grains were tempered to 14% moisture during 24 h, and straight-grade flours were obtained by milling using a Cyclotec mill (Tecator, Sweden) equipped with a 0.5 mm sieve. AACC approved methods (*28*) were used to determine flour moisture (method 44-16), ash (method 08-17), total protein (method 46-11A, N × 5.7), and crude fat content (method 30-25). Total starch, damaged starch, and amylose content were determined as previously described (*29*). The SDS sedimentation test was performed according to the method of Dick and Quick (*30*).

Technological Characterization of the Doughs. The water absorption capability of the flours and dough consistency were evaluated by a Chopin consistograph (Chopin AS, France) using AACC approved method 54-50 (*28*). Alveographic parameters of tenacity (*P*), extensibility (*L*), and the energy needed to change the shape of the dough (strength, *W*) were determined at fixed hydration using a Chopin alveograph. Dough development time (DT), maximum height (MH), curve height after 3 min (H3), and breakdown in resistance (%BDR) were measured with a 10 g bowl mixograph (National Manufacturing Co.) according to AACC approved method 54-40A (*28*). Dough for the mixograph test was prepared according to the method of Martinant et al. (*31*), that is, taking into consideration the different water absorption capacities of both flours.

Dough Preparation for Rheology and NMR Analysis. Defatted flours were obtained by extraction with chloroform (3 × 200 g of flour/500 mL in each extraction). The flour suspension was filtered on sintered glass, and the residual solvent was evaporated by air-drying at room temperature. Undeveloped dough samples were prepared at 50% (w/w) total water content, taking into account the moisture content of each flour. For NMR analysis, water was substituted by D_2O so that dynamic range problems caused by the water peak in the ^{1}H MAS spectra are minimized; this uses the assumption that the hydration and thermal behavior of flour in deuteriated water does not differ significantly from that in protonated water.

Samples were prepared on the basis of the method described by Campos et al. (*27*), with some modifications. Ice water powder was prepared by freezing small drops of water in liquid nitrogen and then pulverizing them in a mortar and pestle device, previously immersed in liquid nitrogen, until a fine powder was produced. A suitable amount of flour was weighed and collected in a crucible immersed in liquid nitrogen to keep the temperature below the melting temperature of ice. Ice powder was weighed and blended with flour (1:1) in the crucible (still in liquid nitrogen). The ice/flour powder blend was mixed to form a uniform mixture of ice and flour, transferred onto a stoppered glass container, and left at room temperature for 20 h.

Rheological Analysis. Rheological analysis was performed under shear deformation using a controlled-stress rheometer (AR-1000, TA Instruments, New Castle, DE), fitted with a parallel plate geometry (stainless steel wrinkled plate, 4 cm diameter, 2 mm gap). The sample was carefully transferred to the rheometer measuring device, and the excess of sample was cut with a blade. Sample edges were covered with a low-viscosity mineral oil (d = 0.84 g/mL, Sigma-Aldrich Química SA, Sintra, Portugal) to minimize water loss. Preliminary experiments indicated negligible contributions to the rheological parameters from the oil itself. After the rheometer had been loaded, doughs were allowed to rest for 1 h before measurements were taken, in order to relax from any residual stresses. Techniques employed included small strain harmonic tests and shear flow at constant applied stress. Stress sweep tests were performed to assess the linear viscoelastic strain limits. Frequency sweep tests (0.005−50 Hz, 20 °C, and 0.02% strain amplitude) were performed before and after temperature sweep tests to evaluate the effect of heating/cooling on the dough samples. Temperature sweep tests were performed by heating the sample from 20 to 80 °C, holding at 80 °C for 10 min, and cooling to 20 °C at the same rate (0.5 Hz, 2 °C/min). Structure development was assessed by time sweep experiments (20 °C, 0.5 Hz). Accurate temperature control (±0.1 °C) was achieved by a Peltier system at the bottom measuring plate. Peak hold steps were performed at constant shear stresses between 100 and 150 Pa for 30 min.

The experiments were replicated at least three times, and the average values of the rheological parameters were calculated. The calculated standard deviations for the viscoelastic properties determined for the unheated systems were below 8%.

NMR Analysis. ^{13}C and ^{1}H spectra were obtained in a Bruker DRX 400 spectrometer operating at 400 MHz for proton, using a 4 mm double-bearing MAS probe and a 4 mm diameter rotor. The ^{13}C spectra CPMAS were recorded using 90° pulses of 4−5 μs, contact time of 1 ms, and spinning rates (SR) of 5−6 kHz. ^{13}C single-pulse excitation (SPE) spectra were recorded using 90° pulses of 4−5 μs and short recycle times (5 s) to select for the signals of the more mobile carbons. The ^{1}H NMR spectra were recorded using 90° pulses of 6 μs, recycle times of 3−5 s, and SR of 5−6 kHz. Proton spin−lattice relaxation times, T_1, were obtained using the inversion−recovery pulse sequence with 12−14 interpulse delay times (τ) in the 0,01−15 s range. T_1 values were calculated for each peak in the ^{1}H MAS spectrum, by fitting the experimental $I(\tau)$ curve to the equation

$$I(\tau) = I_0[1 - 2\exp(-\tau/T_1)]$$

where I_0 is the signal intensity at τ zero.

Proton spin−spin relaxation times, T_2, were obtained using the Carr−Purcell−Meiboom−Gill (CPMG) pulse sequence with 12−14 interpulse delay times (τ) in the 0,01−60 ms range. Calculations assumed either mono- or biexponential decays

$$I(\tau) = I_0\exp(-\tau/T_2) I(\tau) = I_{0A}\exp(-\tau/T_{2A}) + I_{0B}\exp(-\tau/T_{2B})$$

where T_{2A} and T_{2B} refer, respectively, to the more rigid (fast relaxing) spin population and to the more mobile (slow relaxing) spin population.

Samples were heated in situ in the MAS rotor, in a stepwise manner, from 20 to 80 °C and subsequently cooled to 20 °C, with equilibrium time intervals at target temperatures of a minimum of 15 min. A tight fit ceramic cap was used, and the packed rotors were weighed before and after each heating−cooling cycle. The extent of water loss was considered not to be significant under the conditions used because mass variations were not larger than ±0.001 g. Care was taken to keep the heating and cooling rates constant between experiments. For the hydration and heating−cooling NMR experiments on the doughs, only duplicates of the experiments could be performed for each variety, due to the long experimental times and technical difficulty of spinning dough

Table 1. Flour Composition[a]

	AMA	SOR
moisture, % w/w	12.53 ± 0.01	13.84 ± 0.01
protein, % w/w	12.0 ± 0.1	12.6 ± 0.2
ash, % w/w	0.564 ± 0.004	0.637 ± 0.002
crude fat, % w/w	1.30 ± 0.01	1.55 ± 0.09
total starch, % w/w	79.8 ± 0.3	83.0 ± 0.5
damaged starch, % w/w	3.2 ± 0.2	6.6 ± 0.1

[a] Mean ± standard deviation values were obtained for triplicate measurements and refer to dry weight basis, with the exception of moisture.

Table 2. Flour Technological Performance Parameters

	AMA	SOR
alveographic parameters		
W (× 10^4 J), dough strength	318	398
P (mm), tenacity	70	156
L (mm), extensibility	125	80
P/*L*	0.56	1.99
mixographic parameters[a]		
DT (s)	204	186
MH (mm)	90	95
H3 (mm)	80	83
%BDR%	11.1	12.8
consistograph parameters		
maximum dough pressure (mb)	2715	3768
dough water requirement for fixed consistency (2200 mb) (mL/100 g of flour)	53.5	58.2
hardness	23	84
SDS (mm)	107	99

[a] Dough development time (DT), maximum height (MH), curve height after 3 min (H3), and breakdown in resistance (%BDR).

samples. As will be discussed below, it was generally observed that, although absolute values of relaxation times do vary, the relative differences discussed are reproduced.

RESULTS AND DISCUSSION

Flour Composition and Technological Properties. The two varietal samples, 'Amazonas' (AMA) and 'Sorraia' (SOR), were chosen on the basis of their different hardnesses and water absorption capacities, AMA corresponding to a soft wheat cultivar and SOR to a hard wheat cultivar, both having similar protein contents (**Table 1**) and SDS sedimentation volumes (**Table 2**). The sedimentation test depends on protein content and protein quality (*32*), and the similar SDS volumes indicate that both flours have similar strengths in protein. In agreement, the mixing time, which often reflects differences in protein quality, is also similar for both flours.

The Chopin alveograph enabled dough properties to be studied in biaxial extension (**Table 2**). At constant hydration, both flours showed high dough strengths ($W > 300 \times 10^{-4}$ J) but different tenacities and extensibilities: SOR flour showed higher tenacity (*P*) and lower extensibility (*L*) than the AMA flour sample. The results obtained from the consistograph tests showed that the hard wheat flour SOR required a higher water level to achieve a dough of satisfactory consistency (**Table 2**), compared to the soft wheat flour AMA, with a similar protein content. Similar results were obtained for other flours (*33*), showing that flours from soft wheats absorb less water than those from harder wheats. The different water affinities and sorption capacities of each flour may play a relevant role in the different *P*/*L* ratios, maximum dough pressures, and viscoelastic behaviors of doughs.

Characterization of Freshly Prepared Unheated Doughs. *Rheological Behavior*. The characterization of the rheological behavior of the two selected doughs comprised the study of the (a) effect of strain, the linear viscoelastic behavior; (b) effect of oscillatory frequency, mechanical spectra; and (c) flow under constant applied stress. The stress sweep experiments showed that the linear viscoelastic region for the doughs is very short, in accordance with previous papers (*3*, *7*, *34*), with a strain threshold of around $0.2-0.4 \times 10^{-3}$ (**Figure 1**).

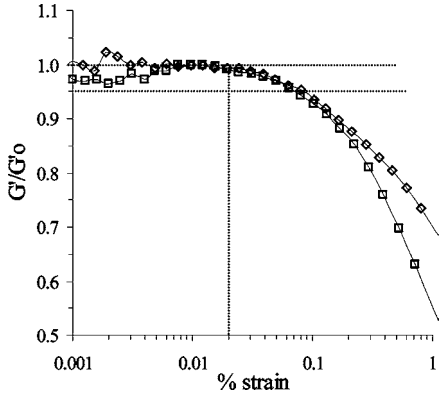

Figure 1. Reduced storage modulus (G'/G'_0) as a function of strain (20 °C, 0.5 Hz) for undeveloped doughs: (□) AMA doughs; (◇) SOR doughs. G'_0 denotes the G' at the beginning of the stress sweep experiment, after meaningful results were obtained.

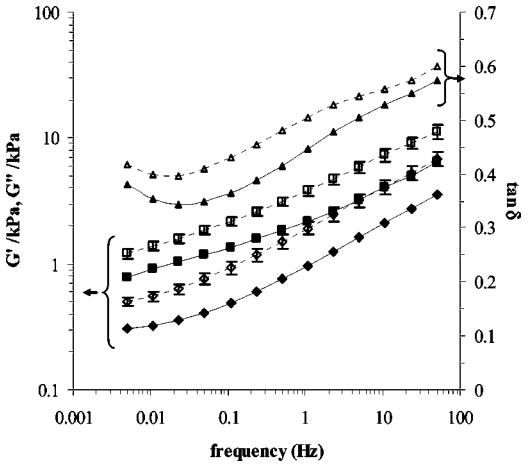

Figure 2. Viscoelastic moduli [G' (■, □) and G'' (◆, ◇)] and loss tangent (tan δ ▲, △) as a function of angular frequency (20 °C, 0.02% strain) for AMA (solid symbols) and SOR (open symbols) doughs. Error bars illustrate the magnitude of standard deviations for triplicate measurements.

The effect of frequency on the viscoelastic behavior of undeveloped doughs is shown in **Figure 2**. Qualitatively similar viscoelastic profiles were obtained for both flour doughs. The mechanical spectra exhibit the typical profile of structured systems, prevailing the solid-like character, but also show a relatively high dependence of the viscoelastic moduli upon oscillatory frequency, meaning that the overall chain mobility within the network is still relatively high. The main differences observed between the two dough samples were the higher moduli observed for SOR dough (harder variety) and the lower loss tangent (tan $\delta = G''/G'$) exhibited by the AMA dough. Also, for AMA dough, the minimum in tan δ was slightly shifted toward higher frequencies and, consequently, the viscoelastic response seems to have been shifted toward shorter times. This indicates that despite the higher rigidity, in the 0.001−100 s time scale, indicated by the higher storage moduli for the SOR dough network, the structural links involved in the transient network formed by the AMA dough are more permanent over the time scale considered, allowing a lower degree of structural

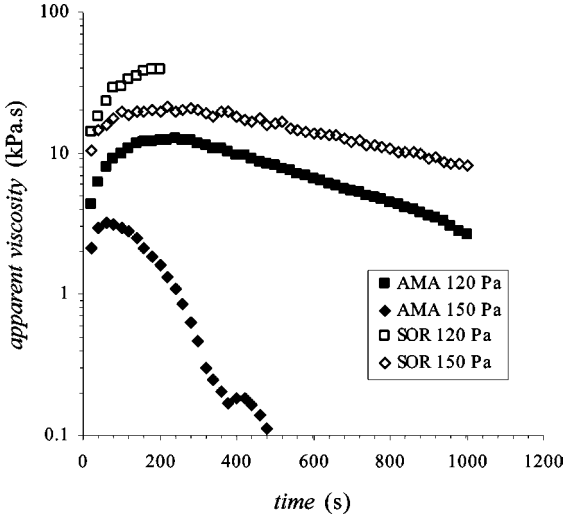

Figure 3. Apparent viscosity plotted against time from peak hold step tests performed at two constant shear stresses, 120 (■, □) and 150 Pa (◆, ◇), for undeveloped doughs: AMA doughs (solid symbols); SOR doughs (open symbols).

rearrangements and less dissipative processes to occur in the softer variety AMA than in SOR dough.

The shear flow behavior of the doughs was investigated by applying a constant stress to the samples. Shear viscometry imposes high strain levels to the sample, and this kind of test may be useful to distinguish between doughs from flours of different varietal origins. In fact, it was previously reported that small strain rheology is unable to differentiate between functionally different flours (*8*), and little relationship to the end-use performance of the flour may be expected (*35*). It is generally accepted that the steady-state shear flow is difficult if not impossible to be achieved for doughs owing to the small range of weak rubber-like behavior exhibited by those systems. Indeed, during the viscometry tests at constant applied stress performed here, the doughs never reached a steady state. Both doughs exhibited an initial "hardening" with shear viscosity increasing with time of applied stress, reaching a maximum, followed by a shear-thinning response corresponding to the partial breakdown of the dough network structure (**Figure 3**). Similar general behavior under simple shearing flow was previously reported (*7*, *36*) for doughs tested under constant applied shear rate. SOR dough showed higher shear viscosities and maximum peak viscosities than AMA dough. For applied stresses lower than 150 Pa, the SOR dough stopped flowing after some time, and no flow was detected during the remaining test time (total = 30 min), contrarily to what was observed for the AMA dough. Dough has been described as an intermediate network between rubber elasticity and plastic flow (*37*). One may conceive, therefore, that the rubber elastic character will be more pronounced in the case of the cv. SOR dough.

^{13}C and ^{1}H MAS NMR Spectroscopy. The present study aims at using MAS NMR methods to obtain information on component molecular dynamics and arrangement, in the dough system, and to attempt a complementary molecular level view to be added to the rheological results. The main method used was ^{1}H MAS spectroscopy, in tandem with measurements of proton T_1 (spin−lattice) and T_2 (spin−spin) relaxation times for specific peaks observed in the MAS spectrum. In some cases, ^{13}C cross-polarization and magic angle spinning (CP/MAS) and single-pulse excitation (SPE) were also employed to register information about the most rigid and most mobile populations of the system, respectively.

Figure 4A shows the effect of hydration up to 50% water in the ^{13}C CP/MAS spectra of SOR flour, similar observations having been registered for AMA flour. The most intense signals arise from starch and show a clear resolution enhancement upon hydration. This effect has been noted previously on starch samples and results from an increase in molecular organization due to the plasticizing of the system and consequent narrowing of the preferred conformations range. The signals noted at higher and lower fields arise, respectively, from gluten aliphatic groups (overlapping with a weak contribution from residual lipids) and gluten aromatic and carbonyl carbons. Interestingly, gluten signals are almost completely absent in the ^{13}C CP/MAS spectrum of flour dough (top spectrum). This indicates that hydration of the protein occurred efficiently, leading to a marked mobility enhancement in the sub-millisecond time scale and, hence, to noneffective cross polarization (CP). This is consistent with the observation of very weak gluten peaks in the ^{13}C SPE spectra (not shown). The slightly lower signal-to-noise ratio for starch peaks in the dough spectrum (top) should reflect the lesser amounts of sample in the rotor (for the doughs) as well as a possible effect of starch mobilization by hydration.

^{1}H MAS NMR enables faster spectra recording and additional information to be obtained, for instance, regarding water. The effect of hydration was identical for both varieties and, as expected, consisted of a marked resolution increase (**Figure 4B**), resulting from a general mobility increase affecting all components. The ^{1}H spectrum of the dough (top spectrum) seems to accommodate a weak underlying broad component across the spectrum, but no broader components were observed. This means that all dough components seem to have been mobilized enough to give signals observable in the 0−10 ppm range. Peak narrowing occurs due to both decrease of anisotropic linebroadening effects (such as magnetic susceptibility and chemical shift anisotropy) and lengthening of proton spin−spin (T_2) relaxation times. Despite the significant degree of overlap across the proton MAS spectrum, some peaks arise mainly from only one component and may thus be taken as indicators for the behavior of that component: 3.8 ppm, starch; 4.8 ppm, water; 6.9−7.3 ppm, protein side chains; 8.2 ppm (weak), protein backbone. The peaks at 0.8 and 2.2 ppm arise from methyl and methylene protons, respectively, and should have contributions from both protein and lipids.

Proton relaxation times may be useful for the understanding of the dynamics of each component and interaction between components. Proton spin−lattice relaxation times, T_1, generally depend on the number (or density) of rapid molecular motions (of the order of hundreds of MHz) but, in the solid or dough states, this parameter is primarily determined by the sharing of magnetization through spin diffusion. The result is that intimately mixed components will share a common average T_1 value and, therefore, some information on component proximity and interaction may, in principle, be obtained. Proton spin−spin relaxation times, T_2, reflect general molecular mobility in the system (with important contribution from rapid motions of the order of hundreds of MHz or sub-microsecond time scale), with shorter values indicating slower dynamics (higher molecular hindrance). In this work, these parameters were recorded for each component of the dough for which an indicator peak was identified in the spectrum.

Proton T_1 values were shown to decrease from tens of seconds, in the dry state, to about 1 s (**Table 3**) in the dough, thus reflecting the increase in the density of rapid motions as a result of hydration. Because absolute T_1 values are sensitive to small variations (few percent) in the dough hydration level, only

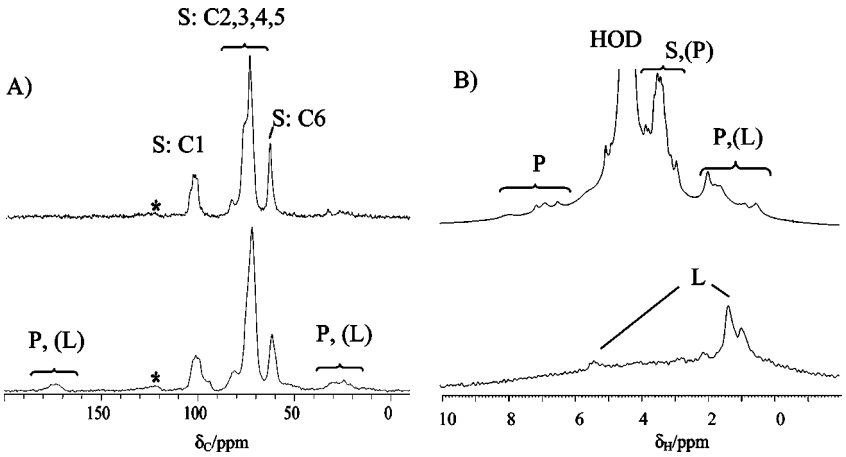

Figure 4. (**A**) 100 MHz ^{13}C CP/MAS spectra (500 scans and 4 kHz spinning rate) and (**B**) 400 MHz ^{1}H MAS spectra (32 scans and 5 kHz spinning rate) of dry (bottom) and hydrated (50%) (top) SOR wheat flour. *, spinning sidebands (rotation artifacts); P, protein; S, starch; L, residual lipids; indications in parentheses represent residual contributions.

Table 3. ^{1}H T_1 Relaxation Times (Seconds) for AMA and SOR Doughs: Unheated, Heated at 80 °C and Cooled to Room Temperature[a]

		AMA dough			SOR dough		
δ/ppm	assignment	20 °C (I)	80 °C	20 °C (II)	20 °C (I)	80 °C	20 °C (II)
0.8	protein/lipids–CH_3	1.01 ± 0.04	1.03 ± 0.05	1.18 ± 0.05	0.95 ± 0.04	1.23 ± 0.04	1.26 ± 0.04
2.2	protein/lipids–CH_2	1.00 ± 0.002	0.83 ± 0.03	1.22 ± 0.05	0.88 ± 0.04	0.81 ± 0.04	1.27 ± 0.03
3.8	starch H2,3,4,5,6 (+ small protein overlap)	0.88 ± 0.04	1.07 ± 0.02	1.24 ± 0.05	0.87 ± 0.04	1.25 ± 0.01	1.32 ± 0.02
4.8	HOD	0.99 ± 0.03	(2.02 ± 0.37)	1.28 ± 0.05	1.11 ± 0.020	1.34 ± 0.01	1.40 ± 0.01
5.5	starch H1 (+ possible contribution from water)	nd	nd	nd	1.04 ± 0.020	1.21 ± 0.01	1.46 ± 0.02
7.3	protein side chain NHs and aromatics	(0.95 ± 0.10)	(1.55 ± 0.23)	1.34 ± 0.07	1.01 ± 0.030	1.27 ± 0.03	1.51 ± 0.06

[a] nd, peaks for which T_1 could not be determined, mostly due to broadening of the peaks. Uncertainty intervals represent the accuracy of the fitting to the experimental relaxation curve; values with uncertainties >10% are indicated in parentheses.

Table 4. ^{1}H T_2 Relaxation Times for AMA and SOR Doughs: Unheated, Heated at 80 °C and Cooled to Room Temperature[a]

		20 °C (I)			80 °C			20 °C (II)		
δ/ppm	assignment	T_{2A}/ms	T_{2B}/ms	% T_{2B}	T_{2A}/ms	T_{2B}/ms	% T_{2B}	T_{2A}/ms	T_{2B}/ms	% T_{2B}
					AMA Dough					
0.8	protein/lipid–CH_3	(0.014 ± 0.003)	0.42 ± 0.057	46	nd			0.037 ± 0.004		
2.2	protein/lipid–CH_2	0.022 ± 0.01	0.25 ± 0.08	56	<0.01			<0.01		
3.8	starch H2,3,4,5,6[b]	(0.14 ± 0.03)	12.3 ± 0.5	56	<0.01			≪0.01	< 0.01	41
4.8	HOD	0.013 ± 0.001			<0.01			<0.01		
5.5	starch H1	nd	nd		nd			nd		
7.3	protein[c]	0.032 ± 0.005	(0.59 ± 0.14)	25	nd			<0.01		
					SOR Dough					
0.8	protein/lipid–CH_3		(1.9 ± 0.8)	100	(0.022 ± 0.006)	7.8 ± 0.6	47	0.046 ± 0.002	10.8 ± 0.3	64
2.2	protein/lipids–CH_2	(0.14 ± 0.06)			(0.023 ± 0.005)	9.5 ± 0.2	75	0.039 ± 0.005	0.77 ± 0.10	38
3.8	starch H2,3,4,5,6[b]	(0.05 ± 0.01)	7.1 ± 0.8	47	(0.036 ± 0.008)	5.7 ± 0.2	78	(0.064 ± 0.013)	6.1 ± 0.9	36
4.8	HOD		4.0 ± 0.8	100		2.2 ± 0.2	100		(3.8 ± 0.7)	100
5.5	starch H1	(0.62 ± 0.23)				5.8 ± 1.0	100	(0.81 ± 0.19)		
7.3	protein[c]		(2.9 ± 0.7)	100	(0.12 ± 0.13)	(12.5 ± 0.4)	65	(0.21 ± 0.09)		

[a] nd, peaks for which T_2 could not be determined due to peak broadening. [b] Peak has small overlap with protein resonances. [c] Peak relates to protein side chain NHs and aromatics. Uncertainty intervals represent the accuracy of the fitting to the experimental relaxation curve; values with uncertainties >15% are indicated in parentheses. Generally, T_2 values <0.01 ms may not be quantified with enough certainty.

relative differences within the same sample will be discussed. In this way, it is noted that for AMA dough, starch shows a slightly shorter value (0.88 s) compared to other components (about 1.0 s), which may indicate that starch granules are in a domain different from that of protein and water. The latter two components share the same T_1 value, which means that they are well mixed together, confirming the expected preferential hydration of the protein in freshly prepared dough. An identical behavior is noted for SOR dough. The relaxation behavior for peaks at 0.8 and 2.2 ppm is difficult to interpret in terms of single components, due to the overlapped nature of those signals.

Proton T_2 values show biexponential behavior for many peaks in the ^{1}H MAS spectrum of the doughs (**Table 4**), reflecting their dynamic heterogeneity and indicating the existence of two dynamic populations for some components: a more rigid fast-relaxing population (A) and a more mobile slow-relaxing one (B). The calculation of T_{2A} is often affected by higher uncertainty due to the more limited number of points available to define the earlier part of the relaxation curve. For AMA dough, this dynamic heterogeneity applies to all components (starch, protein, lipid), with the exception of water, which is characterized by a single very short T_2. This indicates that, in

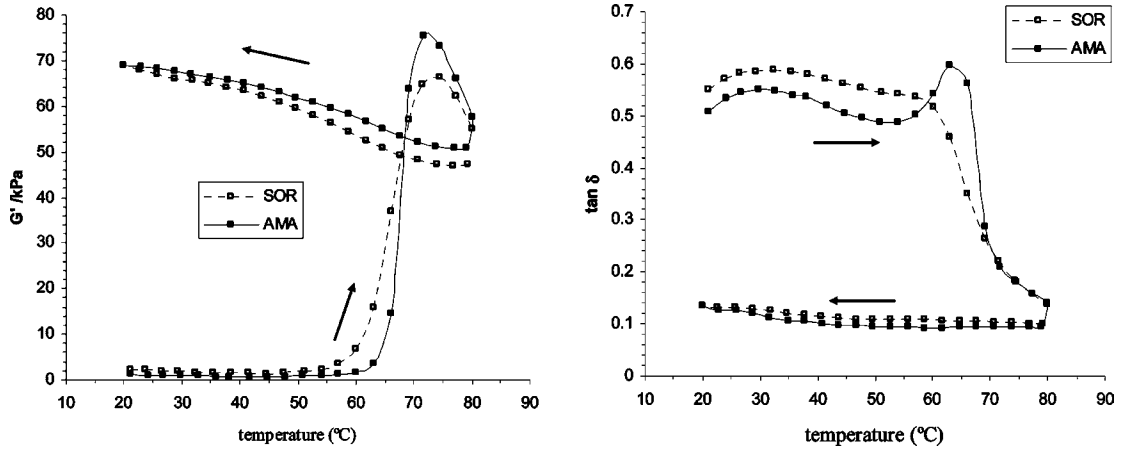

Figure 5. Storage modulus (G') and loss tangent (tan δ) as a function of temperature (2 °C/min, 0.5 Hz) for defatted undeveloped doughs: (■) AMA doughs; (□) SOR doughs.

this dough, water is strongly bound in the protein matrix, and this is confirmed by the T_2 of the 7 ppm peak (protein), which is of the same order of magnitude as that of the water and for which the more rigid population predominates (75% of population A). Interestingly, for SOR dough, both water and protein are significantly more mobile than in AMA. Regarding starch, the opposite effect is noted because the two populations A and B are rather more hindered in SOR than in AMA. It is possible that populations A and B of starch may, respectively, relate to inner (more rigid) and surface (more mobile) chains in the granules, the latter being more mobilized by hydration.

It is interesting to recall that SOR dough requires more water than AMA to achieve the technologically desired dough consistency. NMR shows that the water present in SOR dough does mix with the protein but does not form a rigid protein/water network as is the case in AMA dough. It may be suggested that the formation of this more rigid network as in AMA may be necessary, together with the contribution of dough mechanical mixing, for the correct dough consistency to be achieved. In addition, it is interesting to note that the higher rigidity observed at the supramolecular level, indicated by the higher storage moduli of SOR dough compared to AMA (**Figure 2**), does not seem to reflect directly the higher general molecular mobility of the protein/water matrix, as viewed by NMR. This is probably due to the fact that storage moduli reflect mostly slower main-chain motions (in the 0.001−100 s scale), whereas T_2 has a strong contribution from rapid motions (microsecond and submicrosecond), which may arise mostly from side-chain and short polymer segments. The lower degree of structural rearrangement indicated by the loss tangent (**Figure 2**) in AMA dough seems to be in agreement with the relatively lower molecular mobility registered by NMR. This would mean that such structural rearrangements involve, preferably, side chains and short polymer segments, rather than main-chain backbone.

Effect of Heating−Cooling on Freshly Prepared Doughs. *Viscoelastic Behavior.* **Figure 5** shows the typical viscoelastic behavior observed during heating and cooling of the doughs. During the initial part of the heating step, the storage modulus decreases slightly, reflecting the softening of the dough. However, no significant rheological changes occur until a certain critical temperature is reached. At this temperature an abrupt increase in storage modulus (G') can be observed, reaching a peak at 72−75 °C, related to the gelatinization of the starch fraction. This critical temperature is higher for AMA dough, which can be attributed to the higher starch damage level in SOR flour, an indirect consequence of its higher grain hardness

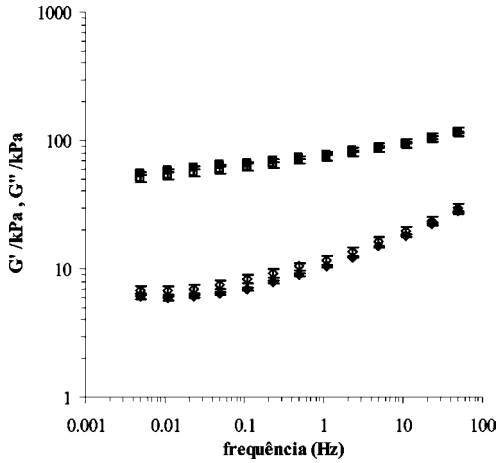

Figure 6. Viscoelastic moduli [G' (■, □) and G''(◆, ◇)] as a function of angular frequency (20 °C, 0.02% strain) for AMA (solid symbols) and SOR (open symbols) doughs. Error bars illustrate the magnitude of standard deviations for triplicate measurements.

(*29*). In fact, a high negative correlation was shown to exist between starch damage and the onset temperature of gelatinization, as measured by viscosimetric or calorimetric methods (*38*, *39*). The peak modulus and G' are higher for AMA dough during most of the cooling step, indicating a higher resistance to deformation caused by gelatinization of starch.

In the vicinity of the onset temperature, the loss peak observed for tan δ as a function of temperature (**Figure 5B**) is also more pronounced for AMA dough and may be related to the different water absorption capabilities of both cultivars. Similar relaxation peaks were previously reported for starch dispersions alone (*29*) and have been related to local energy-dissipative relaxation processes occurring due to the ingress of solvent into the amorphous regions of the starch granules.

After thermal treatment (20−80−20 °C), dough viscoelasticity is mainly controlled by the network of gelatinized starch granules, and only minor differences in the final viscoelastic behavior were observed for the different doughs (**Figure 6**).

^{13}C and ^{1}H MAS NMR Spectroscopy. For NMR studies, the process of heating and cooling (20−80−20 °C) of the doughs was carried out directly in the spectrometer on the same sample, and the results reproducibility was assessed qualitatively by duplicate measurements. The ^{13}C CP/MAS spectra recorded before and after the heating−cooling cycle (not shown) showed only a slight increase in resolution and loss of signal for the starch peaks. The resolution increase should arise mainly from

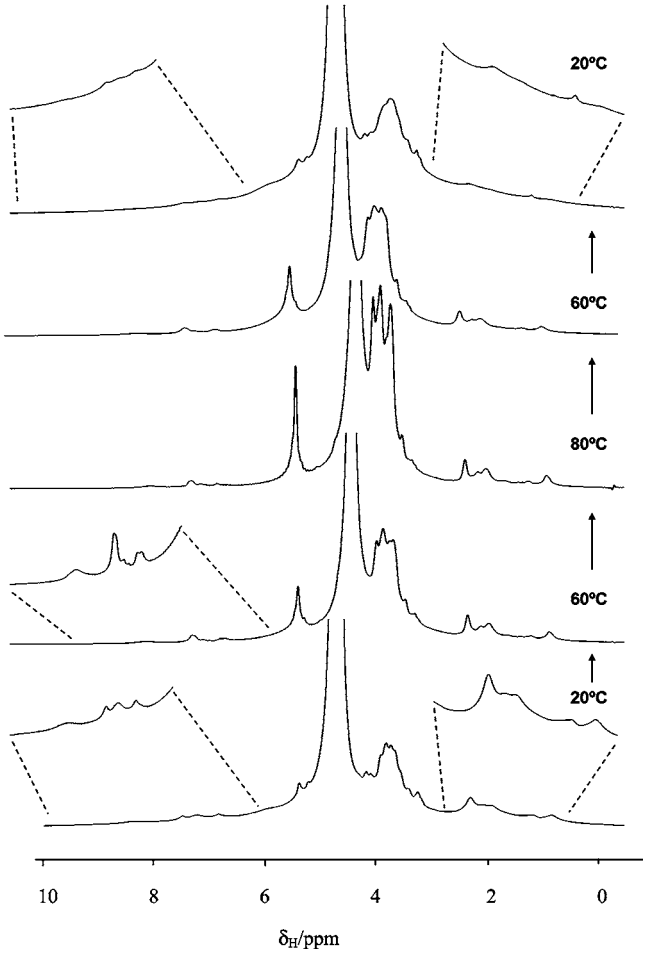

Figure 7. Series of ^{1}H MAS spectra recorded for SOR dough (with 50% D_2O) during heating and cooling, at 5 kHz spinning rate and with 32 scans at each temperature. The insets show expansions of smaller peaks.

decreased anisotropy, possibly due to starch retrogradation and, hence, increase in crystallinity. The loss of signal indicates that molecular mobility is also slightly increased, thus leading to less efficient cross-polarization. The small magnitude of these differences is consistent with no significant differences being observed in the ^{13}C SPE spectra recorded before and after sample thermal treatment (not shown).

Figure 7 shows the sequence of ^{1}H MAS spectra recorded for SOR dough during the heating−cooling cycle. The corresponding sequence of spectra for AMA was found to be very similar to that of SOR and is, therefore, not shown. With increasing temperature, the resolution increases in all regions of the spectrum; this could be due to not only an increase in mobility and consequent lengthening of T_2 relaxation times but also a decrease of the anisotropic effects in the sample, and these aspects will be discussed below. Upon cooling, the resulting spectrum is similar to the initial one, with the exception of the protein peaks, which have broadened significantly and practically disappear. This is consistent with the protein becoming considerably more hindered than initially, thus reflecting the occurrence of protein denaturation. For AMA dough, this broadening of the protein peaks was hinted at slightly earlier in the thermal cycle, compared to SOR (data not shown). It is, however, worth noting that the point at which protein and starch thermal processes start to occur, and hence leading to broadening of the ^{1}H MAS spectrum, is exquisitely dependent on the exact temperature/time evolution during the heating−cooling cycle. As a result, slight differences have been noted in the evolution of the ^{1}H MAS spectra, even for replica samples of the same variety.

Table 3 shows that most AMA T_1 values either do not change or increase only slightly with temperature. Upon cooling, T_1 values for water and protein (viewed through the 4.8 and 7.3 ppm peaks, respectively) tend to decrease again, approaching the initial values, whereas starch (3.5 ppm) shows an irreversible increase in T_1. In the final sample, all T_1 values are around 1.2−1.3 s, which indicates that all components are better mixed than in the initial sample. For SOR dough, the T_1 values increase steadily for all components throughout the heating−cooling process. The resulting system seems to be slightly less uniform, compared to AMA, because starch and protein differ slightly in T_1, with water showing an intermediate value, which may result from the fact that water is now distributed by both components and, hence, showing an intermediate average T_1. The T_1 behavior with heating−cooling suggests that water, which is initially hydrating the protein preferentially (water and protein share the same T_1 value, distinct from that of starch), redistributes between both protein and starch, after the heating−cooling cycle. Furthermore, component mixing seems to be slightly more homogeneous, at the molecular level, in AMA dough compared to SOR, because some T_1 variability is noted for the latter (**Table 3**).

The two varieties exhibit very different T_2 behaviors, even taking into account their different initial T_2 characteristics, which expressed higher mobility of the protein/water matrix and less mobility for starch for SOR dough, compared to AMA. At 80 °C, all components of AMA dough show the absence of the more mobile population (with T_{2B}) and all components are characterized by T_2 values shorter than 0.01 ms. Protein peaks at lower field become too broadened to enable any T_2 calculation attempt. This indicates that a rigid matrix has already formed in this dough at 80 °C and that it involves not only protein (most probably denatured) but also starch, which should have undergone gelatinization and may be held in a tangled network. Gelatinized starch may be characterized by less anisotropy than ungelatinized starch, thus explaining the resolved nature of the starch peaks at 80 °C (similarly to that seen for SOR in **Figure 7**), despite the hindrance caused by tangling with the protein matrix. Upon cooling, the system does not change significantly with the exception of starch, 60% of which sees its mobility further hindered, translated by a new fast relaxing component. Component B comprises 40% of the observed signal and has a T_2 qualitatively comparable to that observed at 80 °C. This suggests that about 40% of the starch remains uncrystallized, in a similar state as at 80 °C. It is possible that the rigid major component corresponds to crystallized starch, probably playing a determinant effect on the increase in G', characteristic of the cooling behavior of starch (**Figure 5**), and in the viscoelastic behavior noted (**Figure 6**).

For *SOR*, the main difference resides in the presence of the slower relaxing component, with T_{2B}, at 80 °C for all peaks, whereas only about 30% of protein and starch are becoming more rigid. Because the presence of a higher content of damaged starch was noted for SOR flour (**Table 1**), it is possible that the higher mobility of SOR starch results from the higher ease of water migration into the starch granules, also reflected by the lower gelatinization temperature in this dough (**Figure 5**). Interestingly, water and protein also remain very mobile, which suggests that denaturation is probably retarded in this dough. It should be noted, however, that water at 80 °C is slightly more hindered than initially, as in AMA, possibly due to its

redistribution between starch and protein. Cooling does result in a rigid (denatured) protein matrix, with protein T_2 decreasing by almost 1 order of magnitude. The fast-relaxing component is reinforced for starch and, similarly to AMA, about 60% of the starch seems to crystallize but remains characterized by a relatively higher mobility than in AMA. As seen previously, molecular rapid mobility (as viewed by T_2) may not be directly reflected by G', which may simply result from the slow backbone rearrangement in the formation of a crystalline starch network upon cooling. A final note regards the behavior of water, which also remains much more mobile in SOR than in AMA dough, after heating−cooling.

Conclusions. The undeveloped doughs of two wheat flours with different technological performance behaviors were hereby characterized by fundamental small-deformation oscillatory rheology and shear viscometry (supramolecular level) and by ^{13}C and ^{1}H MASNMR) spectroscopy (molecular level). For the harder variety, SOR, storage moduli were found to be higher compared to the softer variety, AMA, indicating higher rigidity in the 0.001−100 s range. For the hydrated unheated dough, storage moduli should reflect mainly the characteristics of the protein/water matrix, rather than of starch. However, proton NMR relaxation times indicated higher molecular mobility (mainly in the microsecond and sub-microsecond range) for the protein/water matrix in SOR. It is suggested that the slower molecular motions viewed by the storage moduli correspond to backbone long-segment motions, whereas the faster molecular motions viewed by T_2 relaxation times correspond to side-chain and/or short-segment motions. The higher side-chain/short-segment mobility observed for the harder SOR variety may be at the basis of the higher degree of structural rearrangement indicated by the loss tangent.

The rheological effects of heating−cooling reflected mainly starch behavior, whereas the corresponding ^{1}H MAS spectra and T_1 and T_2 relaxation times gave additional information on the degree of component mixing and molecular mobility. The softer variety AMA dough forms a more rigid lattice that, at higher temperature, accommodates all three components and suffers increased hindrance, whereas all components remain generally very mobile in the hard variety. Despite these differences, in both varieties ca. 60% of starch crystallizes, which may explain their similar rheological behaviors upon cooling.

The tandem use of rheology and NMR enables a molecular level perspective to be added to dough evaluation, with the advantage of probing a complementary dynamic range (faster motions) and the behavior of nonstarch components.

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