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Application of Electron Spin Resonance Spectroscopy and Spin Probes To Investigate the Effect of Ingredients on Changes in Wheat Dough during Heating

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The change in microviscosity of the aqueous and lipid phases of wheat flour dough, during heating and subsequent cooling, has been measured using novel spin probes based on the isoindolin-yloxyl structure. The spin probes, water and/or lipid soluble, were used with combinations of dough ingredients: diacetyl tartaric acid ester of monoglycerides (DATEM), salt, yeast, and sodium ascorbate. The lipid soluble probe showed that DATEM does not produce a homogeneous phase with endogenous lipids but is found in a separate, less mobile phase. Also, the lipids were shown not to be involved in the baking process, although DATEM may be incorporated into the gelled starch matrix. The water soluble probe enabled starch gelatinization to be investigated in detail and showed that gelatinization produces a reduction of dielectric constant. The technique is appropriate for the detailed examination of the behavior of different ingredients during baking and also potentially to examine interactions between ingredients and flour components in dough.

KEYWORDS: Electron spin resonance; spin probe; wheat flour; starch; gelatinization; baking

INTRODUCTION

It is a common practice to add particular ingredients to bread flour to improve the handling qualities of the dough and the overall quality and shelf life of bread. Ingredients used modify the interactions between dough components, including the gluten network and starch granules, interactions important in determining dough rheology and ultimately bread making quality. Salt, the simplest ingredient used, gives a significant effect on dough rheology at 2 wt % of flour and shows some cultivar dependence (*1*−*4*). The salt-mediated effects are thought to occur through interaction with the gluten network, and FT-IR has shown that NaCl can promote the formation of intermolecular β-sheet structures, thereby rendering the proteins more rigid and encouraging glutenin polymer formation (*5*). Ascorbate, the other major ingredient generally included in dough formulations, is an oxido-reductant, considered initially to be converted enzymatically to the reducing agent, dehydroascorbate. Dehydroascorbate can oxidize reduced glutathione in flour and thereby prevent the formation of mixed glutathione-protein disulfides with the gluten proteins. By limiting disulfide interchange with glutathione, the gluten network should be strengthened, with the quality of finished bread improved (*6*).

A second group of improvers widely used in bread making are emulsifiers, particularly the anionic surfactants diacetyl tartaric acid ester of monoglycerides (DATEM) and sodium stearoly lactylate (SSL). There are indications that each can interact with the surface of the starch granules (*7*) and also modify starch gelatinization temperature (*8*). DATEM may also interact with the gluten protein network, an interaction hypothesized to be mediated by hydrophobic interactions (*9*). Emulsifiers may also play a role in determining bubble size distribution (*10*). The implication is that DATEM either influences the interfacial properties of the liquid films that are proposed to line the bubble walls and thus retards bubble coalescence or contributes to a breakup of bubbles during mixing. Many questions remain unanswered regarding the mechanisms underlying the role of ingredients in bread making, particularly those mediated by emulsifiers. To answer these questions, suitable probes are required to monitor the complexity of events occurring during dough development in situ.

Electron spin resonance spectroscopy (ESR) has been used to study phase behavior during heating of dough (*11*) and has much potential to offer new insights into how food ingredients may affect phase behavior. Spin probes are stable free radicals that do not interact chemically with components of the system being studied (*12*). They can be chosen to be soluble in either the lipid or the aqueous phase or to partition between phases. The ESR spectra reflect the rotational motion that depends on the probe size and the microviscosity of the medium. Preliminary work (*11*) has shown the potential of some novel spin probes, based on the isoindolin-yloxyl structure, to monitor the microviscosities of the aqueous and lipid phase during the heating of dough. In an extension of the work, a more detailed study of

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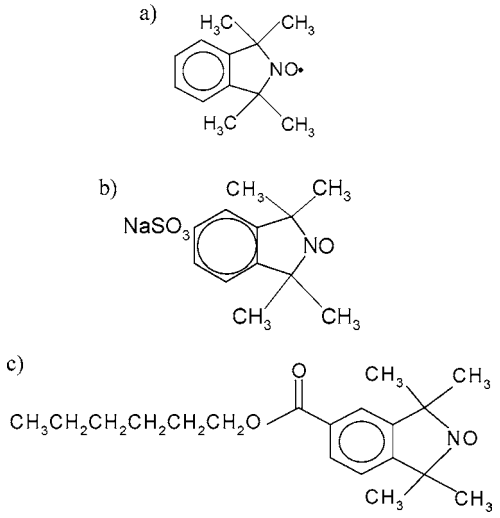

Figure 1. Free radical chemical structures: (**a**) 1,1,3,3-tetramethylisoindolin-2-yloxyl (TMIO), mainly lipid soluble but also water soluble (*17*, *18*); (**b**) the sodium salt of 1,1,3,3-tetramethylisoindolin-2-yloxyl-5-sulfonate (NaTMIOS), water soluble (*19*); (**c**) the *n*-hexyl ester of 5-carboxy-1,1,3,3-tetramethylisoindolin-2-yloxyl (HCTMIO), lipid soluble (*20*).

the gelatinization of starches has shown that ESR can distinguish two starch phases in the aqueous phase of heated dough (*13*).

In the current study, the objective was to use a computer-controlled minimixer to prepare dough (~5 g samples with defined ingredient recipes) under standardized mixing conditions, with mixing profiles recorded for subsequent analysis (*14*). The method was designed to mimic the mixing action of the Chorleywood bread making process (*15*, *16*).

MATERIALS AND METHODS

Triticum aestivum cv Hereward (Advanta Seeds, UK Ltd., Dorking, U.K.) was milled to 72% extraction using a Bühler mill at RHM Technology, High Wycombe, U.K. This was the source of flour used for all of the experiments described. Liquid DATEM esters (Loders Croklaan) were obtained from RHM Technology, High Wycombe, U.K., and all chemicals used were Analar grade and obtained through Sigma, U.K. Allinsons dried yeast was obtained locally and activated 30 min prior to use (38 mg yeast/mL in a 20 mg/mL sucrose solution at 30 °C).

The novel free radicals used as spin probes (**Figure 1**) are 1,1,3,3-tetramethylisoindolin-2-yloxyl (TMIO) (*17*, *18*), the sodium salt of 1,1,3,3-tetramethylisoindolin-2-yloxyl-5-sulfonate (NaTMIOS) (*19*), and the *n*-hexyl ester of 5-carboxy-1,1,3,3-tetramethylisoindolin-2-yloxyl (HCTMIO) (*20*). Partitioning experiments using *n*-octanol/water showed that the solubility of TMIO in water is <0.1%. The spin probe NaTMIOS is insoluble in nonpolar solvents whereas HCTMIO is insoluble in water. Preliminary experiments to study starch gelatinization have shown that these probes are more suitable for use in dough than are the commercially available probes such as 2,2,5,5-tetramethyl pyrrolidin-1-oxyl and 2,2,6,6-tetramethyl-piperidin-4-ol-1-oxyl (TEMPOL) (*13*).

Sample Preparation. Batches of experimental dough (5 g sample size) were prepared using a dough minimixer (*14*). Standard mixer settings were as follows: premix 15 s, set point 1.5 W, speed set point 250 rpm, and energy input 200 J (40 J/g dough). During mixing, speed (rpm), torque (mN m^{-1}), power (W), energy (J), and run time (s) were automatically displayed and recorded for off-line processing (**Figure 2**).

Dough samples were prepared using 100 parts flour plus 62 parts of water/100 parts flour, containing ~1 mM of either TMIO or NaTMIOS and including combinations of the following ingredients: DATEM (0.2 parts), activated dried yeast (~2 parts), sodium ascorbate (0.02 parts), and sodium chloride (2 parts). Since it is insoluble in water, the spin probe HCTMIO was introduced into the dough through either (i) coating the mixer paddles from a solution of probe in dichloromethane (in this case a premix of 30 s was used) or (ii) dissolved in DATEM with dough prepared under standard conditions. A sample of the dough was transferred to the barrel of a 5 mL syringe and ~40 mg extruded into a 30 mm ptfe. capillary (3 mm o.d. and 1.5 mm i.d). To minimize loss of water, the capillary was sealed at each end. After proof at 30 °C for 30 min, the dough sample was placed in a 5 mm o.d. NMR tube and introduced into the ESR resonance cavity. A T-type thermocouple was placed alongside the capillary and the temperature measured with a Comark 5000 digital thermometer.

ESR Instrumentation. A Varian E4 X-band ESR spectrometer was used in conjunction with a JEOL NM-PVT controller and digital set unit to provide sample temperature control. Spectra were obtained at temperature intervals of ~7 °C intervals from ~25 °C to ~95 °C and recorded digitally (1000 points) with a personal computer fitted with a Real Time Devices ADA2000 digitizer and EPRW software (*21*).

Data Treatment. *Mixing.* Profiles from each dough mix were obtained in triplicate, with data combined to provide a mean mixing profile. The mixing profile from each recipe was split into three component parts: premix (0−15 s), mixing (torque development), and mixed (torque plateau) (see **Figure 4a**). Regression analysis of data

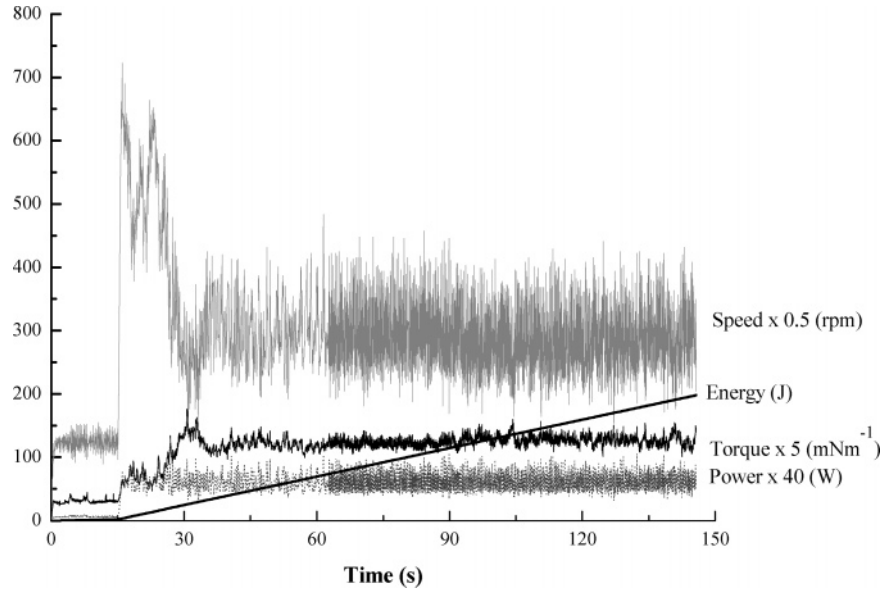

Figure 2. Dough mixing profile for a 5 g flour + water + salt + ascorbate + yeast dough recipe prepared under the set conditions (see Materials and Methods), to illustrate the change in rotor speed and torque development during dough preparation at set power input and energy level.

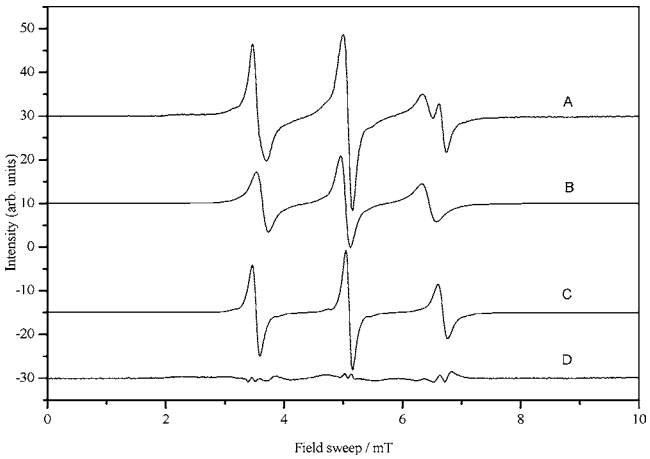

Figure 3. X-band ESR spectra at 24 °C of TMIO in dough prepared from flour + water: (A) experimental, deconvoluted spectra; (B) lipid phase; (C) water phase; (D) residual.

covered by the tangent, fitted visually, to each part of the mixing profile was used to derive the initial torque during premixing, rate of torque development, and final torque developed. Mixing time was taken as the point of intersection between the mixing time tangent and mixing plateau tangent, less 15 s for the premixing.

ESR. Since almost all of the ESR spectra obtained comprised at least two overlapping spectra, deconvolution is essential. Deconvolution was done for the two most intense spectra in an observed spectrum since it is not feasible to deconvolute more than two spectra in an experimental spectrum. Deconvolution of two overlapping nitroxide spectra was accomplished off-line using the EWVIOGTN iterative software (Scientific Software Services) (*21*). The fitting program calculates (i) nitrogen-14 hyperfine coupling constants, a^N, (ii) line positions, (iii) fractions of each component, (iv) Gaussian line widths, and (v) Lorentzian line widths. An example is shown in **Figure 3**. Deconvolution is assisted by the fact that a^N values are affected by the polarity of the medium, (range ~1.42 mT for a nonpolar medium to ~1.56 mT for a polar medium). From the Lorentzian line widths, the rotational correlation tine, τ(B), of a TMIO-type spin probe can be calculated *(19)* from the equation

$$\tau(\mathbf{B}) = 5.75(\pm 0.3)\{[W(-1) - W(+1)]/\mathbf{B}_0\} \times 10^{-7}\ \mathrm{s}$$

where $W(-1)$ and $W(+1)$ are, respectively, the low- and high-field Lorentzian line widths of the nitrogen-14 manifolds and $\mathbf{B}_0$ is the magnetic field at the center of the spectrum. Assuming that the Stokes−Einstein model is applicable and that the spin probe is a rigid sphere of radius r, $\tau(\mathbf{B})$ can be used to calculate the microviscosity of the medium in which the spin probe is dissolved:

$$\tau(\mathbf{B}) = 4\pi r^3 \eta / 3kT$$

where k is the Boltzmann constant and T is the absolute temperature. $\tau(\mathbf{B})$ has been used here as an indirect measure of microviscosity.

RESULTS AND DISCUSSION

Dough Mixing. Dough mixing can be characterized by physical measurements based on mixing torque or power consumption rather than chemical changes during dough development. Torque associated with dough mixing is a measure of how dough rheology varies with rate of work input, measured as resistance during standardized mixing conditions. Using the minimixer allowed changes in torque to be related to modifications in dough recipe. From the series of torque mixing profiles (**Figure 4**), it was apparent that the ingredients investigated had a marked effect on dough rheology. In the absence of any added ingredients (flour + water) torque developed rapidly on mixing, and mixing was effectively complete within 15 s. Addition of salt (**Figure 4c**) increased the time-to-plateau to around 60 s compared to around 30 s for simple flour−water dough, consistent with the observed increased mixing time effects of NaCl addition using the 2 g mixograph (*1*) Addition of salt and ascorbate (**Figure 4f**), extended mixing time to over 90 s. This observation is also consistent with known effects of ascorbate on mixing time, as determined by the 2 g mixograph (*22*). DATEM had little effect on the torque time-to-plateau (**Figure 4d**) in contrast to the strengthening effect observed previously (*22*), but this may be related to incorporating DATEM at half the level reported previously (*22*). Although torque development time varied according to ingredients present, the plateau values attained for each mix remained relatively constant. The absence of any apparent decrease in torque indicated overmixing of samples had not occurred. Similarly, since effect of ingredients on dough mixing was consistent with previous studies using different mixer types, the samples used for ESR measurement were considered typical of fully developed doughs.

Table 1. Mixing Profile Parameters for Different Dough Ingredient Recipes[a]

ingredient	mixing gradient (mN/m/s)	ΔTorque (mN/m)	time to plateau (s)
flour/water	1.02	18	17
flour/water/salt	0.35	19	60
flour/water/DATEM	0.50	15	25
flour/water/salt/DATEM	0.15	17	67
flour/water/salt/Ascorbate	0.24	15	65
flour/water/salt/DATEM/ascorbate	0.20	25	102
flour/water/salt/DATEM/ascorbate/ yeast	0.19	24	104

[a] The mixing gradient is the regression coefficient of torque developed between the end of the premix and the mixed dough. ΔTorque represents the difference in torque developed during mixing, i.e., $\Delta\mathrm{Torque} = \mathrm{Torque}_{\mathrm{plateau}} - \mathrm{Torque}_{\mathrm{premix}}$. Time to plateau = time (×) intersect of mixing gradient regression equation and plateau regression equation.

Regression analysis of the experimental mixing curves—as premix, mixing slope, and plateau (**Figure 4a**)—is summarized in **Table 1**. Thus, on introducing the different ingredients into the dough, development of torque was reduced relative to the flour−water mix. For salt and DATEM, there was some apparent cumulative reduction in torque development. On adding ascorbate, torque development remained relatively constant but the change during mixing (ΔTorque: **Table 1**) was higher, ~24 mM/m. DATEM and ascorbate in combination had the major influence on dough development. Yeast had no apparent effect on ΔTorque, and for all other combinations of ingredients tested, ΔTorque was around 15−19 mN/m.

Effect of Temperature on Spin Probes. The majority of free radicals having an unpaired electron centered appreciably on a nitrogen-14 atom have a hyperfine interaction temperature coefficient, $\mathrm{d}a^N/\mathrm{d}T$, of $+2.2 \times 10^{-4}$ mT K^{-1} (*23*). Preliminary experiments showed NaTMIOS in water had a coefficient of $+2.1(\pm 0.1) \times 10^{-4}$ mT K^{-1}, and this value was taken to be applicable to the other TMIO-based spin probes used.

The spin probes used are thermally very stable, within the chosen temperature range, but preliminary experiments suggested that probe concentration decreased with heating. Since there is the potential for the spin probes to react with some constituent in the flour and/or the ingredients used, the effect of temperature on total spin probe concentration was determined using a sample tube containing ~1 mM NaTMIOS in water placed alongside a sample tube containing dough without a spin probe. Results showed the apparent decrease in probe concen-

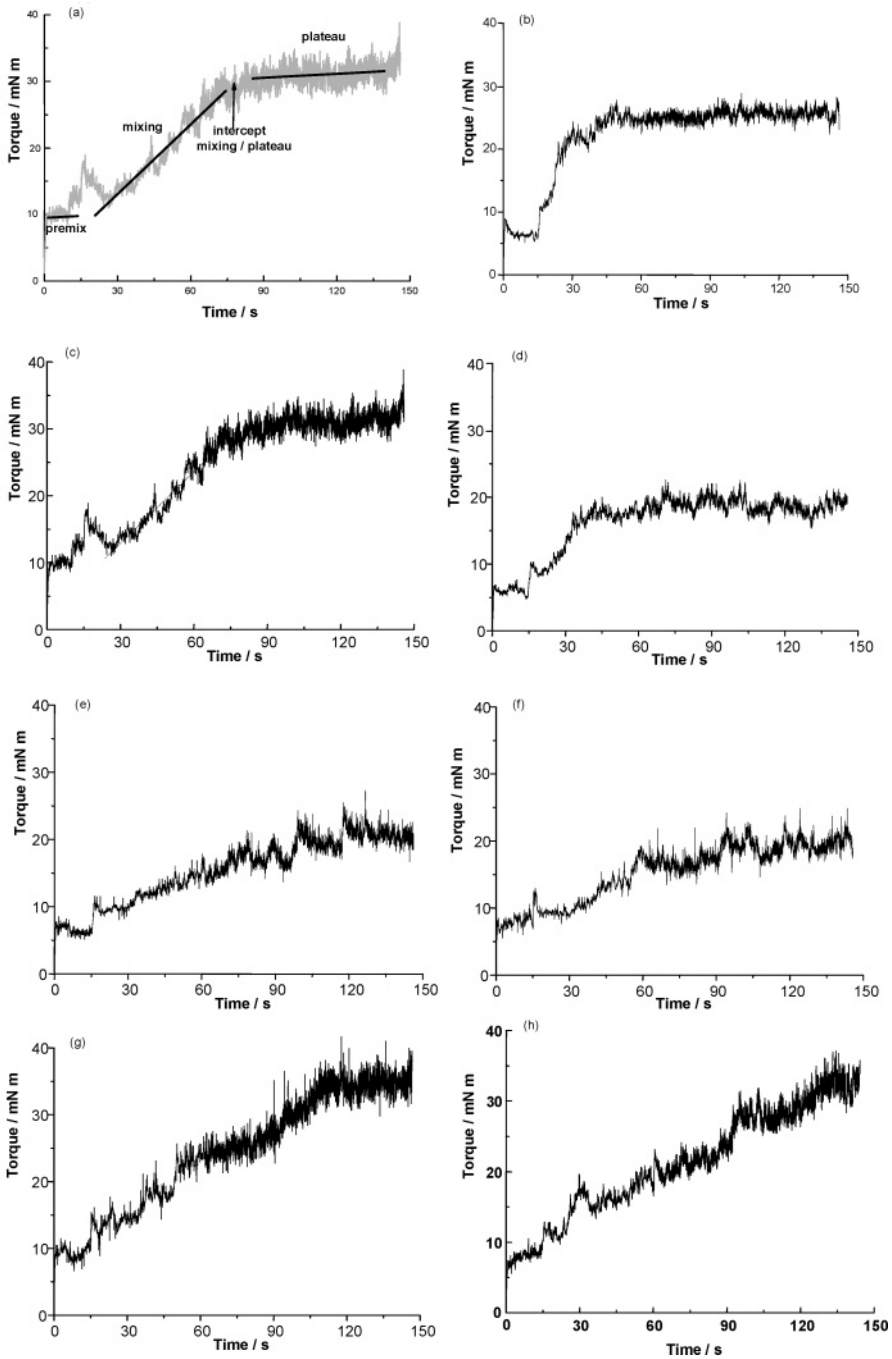

Figure 4. Mixing profiles (mean of three individual mixes for each recipe) for 5 g batches of dough prepared under standard mixing conditions for dough recipes with the test ingredients: (**a**) model to demonstrate the derivation of the mixing gradient and plateau (**Table 1**); (**b**) flour + water mix; (**c**) flour + water + salt mix; (**d**) flour + water + DATEM mix; (**e**) flour + water + salt + DATEM mix; (**f**) flour + water + salt + ascorbate; (**g**) flour + water + salt + DATEM + ascorbate; (**h**) flour + water + salt + DATEM + ascorbate + yeast.

tration was reversible, and the magnitude of the effect was in accord with the Curie Law, namely that $c_1/c_2 \sim T_2/T_1$. Thus, changes in dough properties have no effect on probe concentration, but there is a need for a correction in apparent concentration, c, of free radical at different temperatures. The correction may be needed to comply with (i) the Curie Law and (ii) an irreversible change in the quality factor, Q, of the spectrometer resonant cavity.

Figure 5 shows a typical set of results obtained by heating and cooling dough in the presence of each of the three spin probes. Although TMIO is much more soluble in lipid than in water, the preponderance of water results in a roughly equal partitioning of probe between the aqueous and lipid phases. Each phase is observed as a distinct population of TMIO in the dough. By contrast, the NaTMIOS is taken to partition only into the aqueous phase and HCTMIO only into the lipid phase. Selective use of the three probes provided information on the behavior of the aqueous and lipid phases present in each experimental dough.

TMIO in Dough. The experimental spectra could be deconvoluted since the nitrogen hyperfine splittings, a^N values of ~1.56 mT and ~1.42 mT, could be assigned to the aqueous and lipid phases, respectively. A τ(**B**) value of ~80 ps at room temperature was recorded, corresponding to the lipid phase, which reduced to ~40 ps at 90 °C. (**Figure 5a**). The process was essentially reversible. In contrast, TMIO in the aqueous

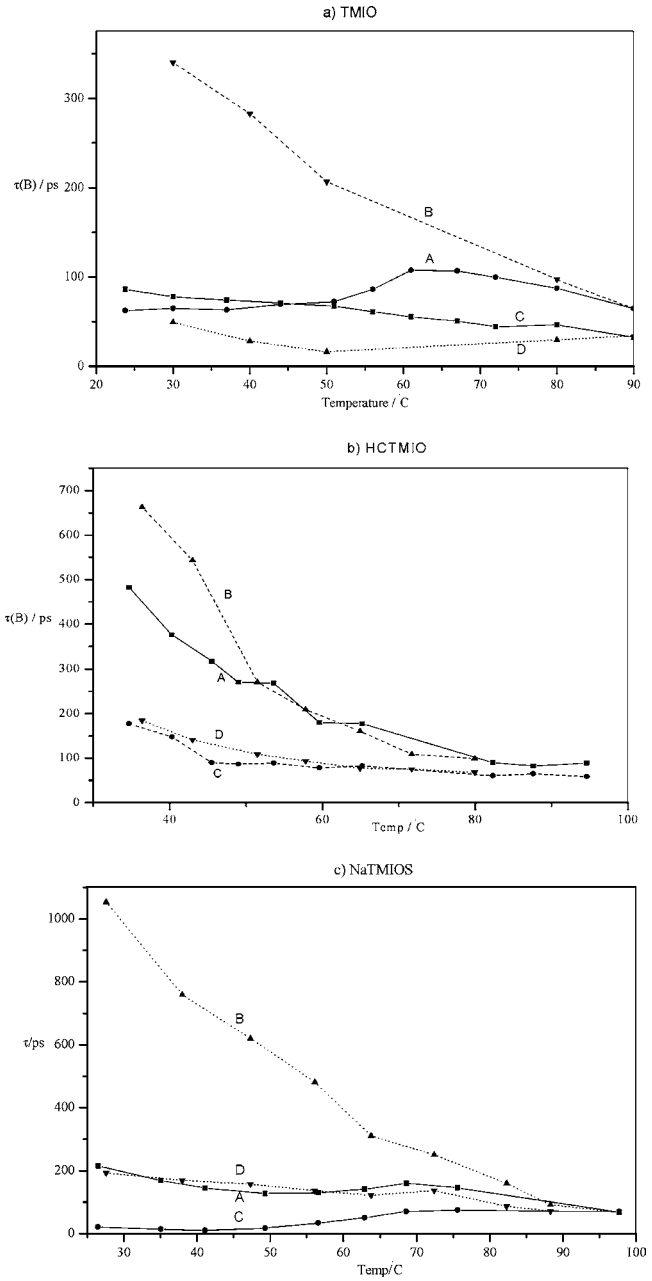

Figure 5. Effect of heating followed by cooling on the rotational correlation time, τ(**B**), of the three spin probes in dough. (**a**) TMIO in flour–water dough: A and B are, respectively, the aqueous phase on heating and cooling with $a^N \sim 1.56$ mT; C and D are, respectively, the lipid phase on heating and cooling with $a^N \sim 1.42$ mT. (**b**) HCTMIO in flour–water–DATEM dough: A and B are, respectively, the heating and cooling lipid phase at $a^N \sim 1.45$ mT; C and D are, respectively, the heating and cooling lipid phase at $a^N \sim 1.41$ mT. (**c**) NaTMIOS in flour–water–DATEM dough: A and B are, respectively, heating and cooling for the aqueous phase having $a^N \sim 1.55$ mT; C and D are, respectively, heating and cooling for the aqueous phase having $a^N \sim 1.57$ mT.

phase showed a sharp rise in τ(**B**) from ~60 ps at 24 °C to ~100 ps at ~55 °C. This corresponds to the expected gelatinization temperature range for starch. As temperature was increased further then τ(**B**) decreased. The process was not reversible, and on cooling τ(**B**) increased steadily to ~350 ps at 30 °C, consistent with the aqueous TMIO being located in a relatively rigid starch−protein matrix after baking. The predominance of starch in the dough might lead to the probe signal from the starch phase swamping any signal from protein

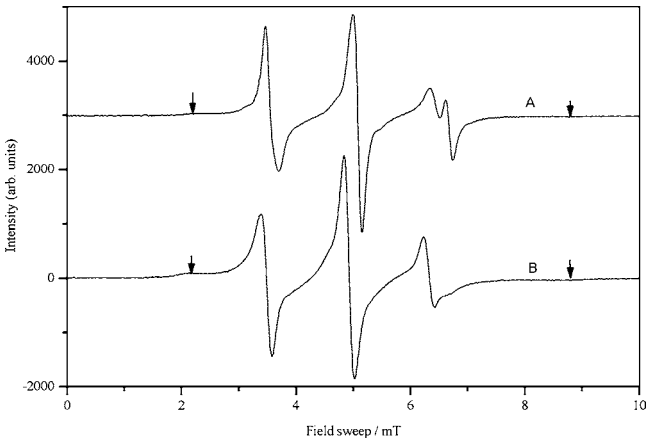

Figure 6. X-band ESR spectra at 24 °C of TMIO in dough prepared from flour + water: A, before heating; B, after heating to 90 °C. The arrows show how the amount of the third (less mobile) phase has increased after heating.

(gluten), so whether there is a gluten signal associated with the generation of the more viscous phase on cooling remains unknown.

The EWVOIGTN simulation program can only deconvolute two overlapping nitroxide spectra, but it may be seen from **Figure 6** that heating produced an increase in the amount of a third much-less mobile phase. It is unclear whether this immobile phase is related to starch, protein, or starch−protein complexes, but after one cycle of heating and cooling all changes are complete (*13*) and subsequent heating and cooling cycles give no further apparent change in the third less mobile phase.

Nitrogen-14 hyperfine coupling constants derived from the deconvoluted spectra show that the smaller values (attributable to the lipid phase) increased reversibly, with a temperature coefficient of $+6 \times 10^{-4}$ mT K^{-1}, somewhat larger than observed for simple spin probe solutions. However, the larger values of a^N associated with the aqueous phase behave abnormally in that they *decrease* with an increase in temperature and decrease even further upon cooling. This would suggest that gelatinization of starch causes a decrease in the dielectric constant of the aqueous environment. The addition of DATEM or DATEM plus salt to the dough mix did not affect the observed rotational correlation times, τ(**B**), for TMIO, consistent with the observed torque developed in doughs using these ingredients.

HCTMIO in Dough. When HCTMIO dissolved in DATEM was added to the flour−water dough mix, two phases were observed in the dough (**Figure 5b**), suggesting the presence of free lipid ($a^N \sim 1.45$ mT) and a bound lipid ($a^N \sim 1.41$ mT). However, no phase change was observed with change in temperature, as noted for starch gelatinization, and the process was reversible. The most likely explanation, that there is a temperature-related change in the microviscosity of the lipids, was checked from the linear relationship ($R = 0.95$) between ln τ(**B**) and the reciprocal of the absolute temperature. A value of 22 ± 3 kJ mol^{-1} was calculated for the microviscosity enthalpy, similar to that of 21.1 ± 0.6 kJ mol^{-1} determined for chloroform-extracted lipid from flour (cv Hereward) (*19*). This suggests that no lipid is bound to other dough constituents such as gluten and is consistent with confocal studies of dough, where lipid has been observed as discrete droplets (*24*).

Furthermore, with HCTMIO dissolved directly in DATEM and dispersed in water, τ(**B**) decreased from 1220 ps at 21 °C to 140 ps at 80 °C. The dependence of τ(**B**) on temperature

was exponential and gave an enthalpy of 31.8 ± 1.0 kJ mol^{-1}. This indicated that the lower value of 22 kJ mol^{-1} observed in dough was attributable to the endogenous lipid in the flour. Similarly, when HCTMIO was coated directly onto the mixer paddles and DATEM excluded from the dough mix, only one lipid phase was observed in the dough, corresponding to $a^N \sim$ 1.41 mT (**Figure 5b**). These data support the conclusion that DATEM and endogenous lipid do not form a homogeneous solution in dough. The phase having a τ(**B**) value of ~190 ps at room temperature is attributable to endogenous lipid whereas the larger value arises from DATEM. Since τ(**B**) values of more than ~400 ps cannot be measured with accuracy using the procedures to calculate τ(**B**) employed in this work, no firm conclusion can be drawn from the low-temperature divergence of the upper lines, A and B, in **Figure 5b**. However, it can be speculated that the divergence and apparent magnitude of τ(**B**) indicates that the DATEM may be incorporated into the protein−starch gel structure formed on heating above 60 °C (*7*).

NaTMIOS in Dough. The limited phase behavior changes in dough lipid favored the use the lipid insoluble spin probe, NaTMIOS, to investigate the effects of other ingredients. As shown in **Figure 5c** for dough containing DATEM, two phases were detected having very similar a^N values: ~1.55 mT and ~1.57 mT. Both phases were affected dramatically by heating above 60 °C. Taking account of the temperature change, proportion of probe associated with each phase, and the expected ~1:3 ratio of amylose:amylopectin in wheat starch (*25*), then the phase with $a^N \sim$ 1.55 mT probably corresponds to amylose and the phase with $a^N \sim$ 1.57 mT to amylopectin. Whether interactions exist between the different starch components and protein remains unclear, but previous work (*13*) has shown a close similarity between the behavior of isolated starches and the aqueous phase of wheat dough. This would suggest that the ESR signal from any protein−starch complexes is readily swamped by the presence of the high proportion of starch in the dough.

Analysis of the ESR spectra from dough containing ascorbate (**Figure 7b**,**c**), showed that the putative amylopectin phase, having $a^N \sim$ 1.55 mT, was apparently affected by the ascorbate, but the putative amylose phase, having $a^N \sim$ 1.57 mT, was unmodified. Notably, with ascorbate present the microviscosity during the cooling process did not increase substantially until the temperature was below 55 °C, with the microviscosity recorded being around half the value recorded in the absence of ascorbate. Since ascorbate has no known effect on starch gelatinization at the concentration used, it is more likely that its redox properties are responsible for the effects observed, e.g., in controlling disulfide bond formation in gluten (*26*, *27*).

Allied to this, a fluctuation in probe concentration was noted over the heating cycle as an apparent 3-fold increase in concentration. The implication is that the probe can be chemically reduced, by dehydroascorbate, during dough preparation but oxidized on heating. Initially, equimolar proportions of the probe and ascorbate were present. From the results, it is apparent that the probe was modified but not destroyed by the presence of ascorbate. Substituting an amide (nonradical) derivative of TMIO for NaTMIOS to check whether free radicals could be generated from the amide during the heating process gave no detectable signal, supporting the idea that when a TMIO-type spin probe is present it can be reduced to a nonradical hydroxylamine during the heating/cooling cycle and then oxidized back to a nitroxide.

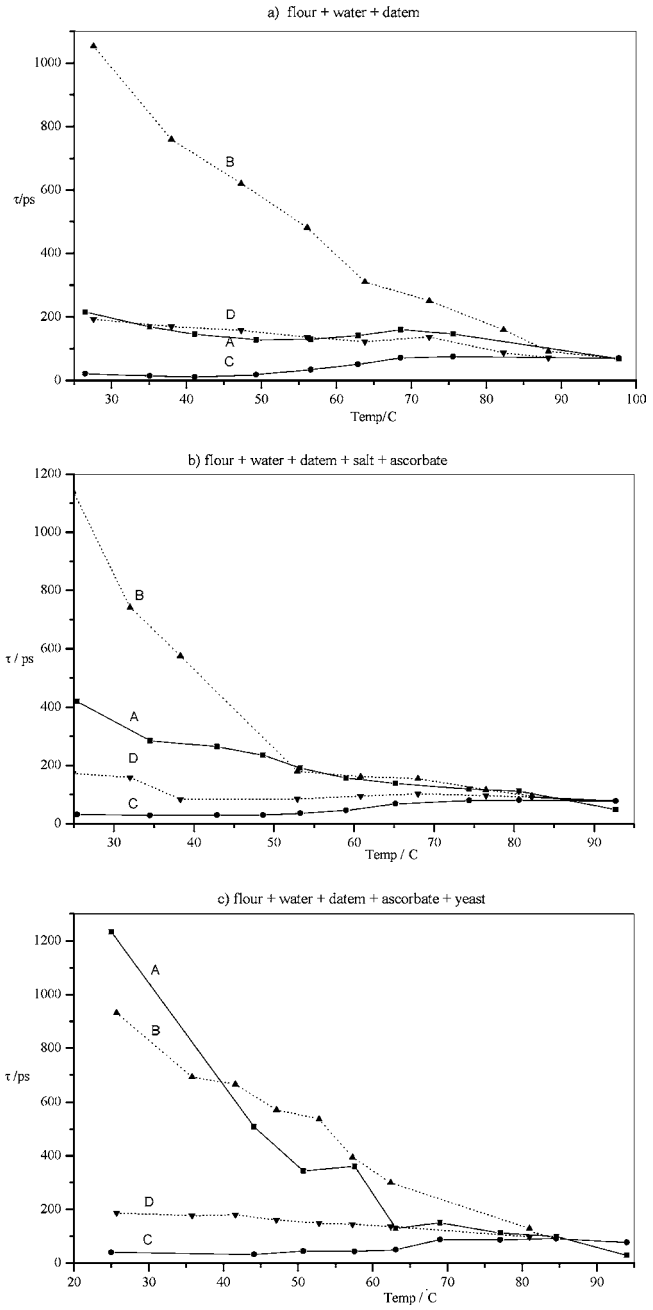

Figure 7. Effect of sodium ascorbate and the presence of yeast on the rotational correlation time, τ(**B**), of NaTMIOS. (**a**) Flour–water–DATEM dough: A and B are, respectively, heating and cooling for the phase having $a^N \sim$ 1.55 mT ('amylopectin'); C and D are, respectively, heating and cooling for the phase having $a^N \sim$ 1.57 mT ('amylose'). (**b**) Flour–water–DATEM–salt–sodium ascorbate dough: A and B are, respectively, heating and cooling for the phase having $a^N \sim$ 1.55 mT ('amylopectin'); C and D are, respectively, heating and cooling for the phase having a^N ~1.57 mT ('amylose'). (**c**) Flour–water–DATEM–sodium ascorbate–yeast dough: A and B are, respectively, heating and cooling for the phase having $a^N \sim$ 1.55 mT ('amylopectin'); C and D are, respectively, heating and cooling for the phase having $a^N \sim$ 1.57 mT ('amylose').

Including yeast along with all the other ingredients had a remarkable effect on the less mobile phase, characterized by $a^N \sim$ 1.55 mT; the more mobile phase, characterized by $a^N \sim$ 1.57 mT, behaved as previously (**Figure 7c**). The less mobile phase had a τ(**B**) value of over 1 ns even before heating, and the heating and subsequent cooling process appeared reversible.

The probe concentration apparently increased, as previously, about 3-fold over the heating cycle. A repeat experiment, using dough mixed with yeast and all ingredients except ascorbate, confirmed that ascorbate was responsible for the noted reduction followed by oxidation of the spin probe during the heating cycle, and yeast was responsible for the increased microviscosity of the amylose phase after mixing the dough (data not shown).

Conclusions. Using a standardized mixing regimen to prepare wheat flour doughs it has been demonstrated that useful and detailed information can be obtained from the application of the spin probes and ESR techniques to complex processes such as bread making. For example, compartmentalization of ingredients such as DATEM and endogenous lipid can be demonstrated, with neither apparently being modified in the dough, while ascorbate demonstrates oxidation and reduction properties in the dough. The information obtained can be correlated with differences in dough torque developed through mixing and subsequent changes expected during baking. The work could be usefully extended by more detailed examination of interactions between flour components and added dough ingredients, such as possible protein−starch−lipid interactions in dough, the oxido-reductant behavior of ascorbate, and the influence of gluten on bread baking by using ESR spectroscopy coupled with spin-labeled glutens.

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LITERATURE CITED

(1) Butow, B. J.; Gras, P. W.; Haraszi, R.; Bekes, F. Effect of different salts on mixing and extension parameters on a diverse group of wheat cultivars using a 2g-mixograph and extensigraph methods. *Cereal Chem.* **2002**, *79*, 826−833.

(2) Wehrle, K.; Grau, H.; Arendt, E. K. Effects of lactic acid, acetic acid and table salt on fundamental rheological properties of wheat dough. *Cereal Chem.* **1999**, *74*, 739−744.

(3) Preston, K. R. Effects of neutral salts on the lyotropic series on the physical dough properties of a Canadian red spring wheat flour. *Cereal Chem.* **1989**, *66*, 144−148.

(4) Salovaara, H. Effects of partial sodium chloride replacement by other salts on wheat dough rheology and breadmaking. *Cereal Chem.* **1982**, *59*, 422−426.

(5) Wellner, N.; Bianchini, D.; Mills, E. N. C.; Belton, P. S. Effect of selected Hofmeister anions on the secondary structure and dynamics of wheat prolamins in gluten. *Cereal Chem.* **2003**, *80*, 596−600.

(6) Grosch, W.; Weiser, H. Redox reactions in wheat dough affected by ascorbic acid. *J. Cereal Sci.* **1999**, *29*, 1−16.

(7) Collar, C.; Armero, E.; Martinez, J. Lipid binding of formula bread doughs—Relationships with dough and bread technological performance. *Z. Lebensm.-Unters. Forsch. A* **1998**, *207*, 110−121

(8) Russell, P. L. A kinetic study of bread staling by differential scanning calorimetry and compressibility measurements. The effect of added monglycerides. *J. Cereal Sci*. **1983**, *1*, 297−303.

(9) Knightly, W. H. Surfactants in baked foods: current practice and future trends. *Cereal Foods World* **1988**, *33*, 405−412.

(10) Campbell, G. M.; Herrero-Sanchez, R.; Payo-Rodriguez, R.; Merchan, M. L. Measurement of dynamic dough density and effect of surfactants and flour type on aeration during mixing and gas retention during proofing. *Cereal Chem.* **2001**, *78*, 272−277.

(11) Gillies, D. G.; Sutcliffe, L. H.; Wu, X.; Belton, P. S. ESR studies of the dynamics of water in and lipid in dough. *J. Agric. Food Chem.* **1999**, *47*, 4520−4524.

(12) Sutcliffe, L. H. The design of spin probes for electron magnetic resonance spectroscopy and imaging. *Phys. Med. Biol.* **1998**, *43*, 1987−1993.

(13) Robertson, J. A.; Sutcliffe, L. H. ESR investigation of starch gelatinisation using novel spin probes. *Magn. Reson. Chem.* **2005**, *43*, 457−462.

(14) Georget, D. M. R.; Gillies, D. G.; Mills, E. N. C.; Robertson, J. A.; Alva, J.; Sutcliffe, L. H. A Computer-controlled Mini-mixer to mimic Chorleywood Baking Process Dough Mixing. Submitted.

(15) Chamberlain, N. The Chorleywood Bread Process—International prospects. *Cereal Foods World*. **1984**, *29*, 656−658.

(16) Kelp, K. Bread Industry and Processes. In *Wheat: Chemistry and Technology*; Pomerantz, Y., Ed.; AACC Inc.: St Paul, MN, 1988; Vol. II, pp 371−406.

(17) Bolton, R.; Gillies, D. G.; Sutcliffe, L. H.; Wu, X. An EPR and NMR study of some tetramethylisoindolin-2-yloxyl free radicals. *J. Chem. Soc., Perkin Trans.* **1993**, *2*, 2049−2052.

(18) Bolton, R.; Sutcliffe, L. H.; Wu, X. Synthesis of isotopically labelled nitroxyl radicals for use as spin probes. *J. Labelled Compd. Radiopharm.* **1994**, *34*, 663−668.

(19) Belton, P. S.; Sutcliffe, L. H.; Gillies, D. G.; Wu, X.; Smirnov, A. I. A new water-soluble and lipid-insoluble spin probe: application to the study of aqueous sucrose solutions. *Magn. Reson. Chem*. **1999**, *37*, 36−42.

(20) Bottle, S. E.; Gillies, D. G.; Hughes, D. L.; Micallef, A. S.; Smirnov, A. I.; Sutcliffe, L. H. Synthesis, single-crystal X-ray structure and W-band (95 GHz) EPR spectroscopy of a new anionic isoindoline aminoxyl: synthesis and characterisation of some derivatives. *J. Chem. Soc., Perkin* **2000**, *2*, 1285−1291.

(21) Scientific Software Services, Bloomington, IL.

(22) Aamodt, A.; Magnus, E. M.; Faergestad, E. M. Effect of flour quality, ascorbic acid, and DATEM on dough rheological parameters and hearth loaves characteristics. *J. Food Sci*. **2003**, *68*, 2201−2210.

(23) Fairhurst, S. A.; Stewart, N. J.; Sutcliffe, L. H. ESR investigation of the dimerization of some dithiazolidinyl radicals. *Magn. Reson. Chem.* **1987**, *25*, 60−64.

(24) Dubreil, L.; Biswas, S. C.; Marion, D. Localization of Puroindoline-a and lipids in bread dough using confocal scanning laser microscopy. *J. Agric. Food Chem.* **2002**, *50*, 6078−6085.

(25) Parker R.; Ring, S. G. Aspects of the physical chemistry of starch. *J. Cereal Sci.* **2001**, *34*, 1−17.

(26) Every, D.; Griffin, W. B.; Wilson, P. E. Ascorbate Oxidase, Protein disulfide isomerase, Ascorbic acid, Dehydroascorbic acid and protein levels in developing wheat kernels and their relationship to protein disulfide bond formation. *Cereal Chem.* **2003**, *80*, 35−39.

(27) Koehler, P. Effect of ascorbic acid in dough: reaction of oxidised glutathione with reactive thiol groups of wheat glutelin. *J. Agric. Food Chem.* **2003**, *51*, 4954−4959.

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