Selected Spin Probes for the Electron Spin Resonance Study of the Dynamics of Water and Lipids in Doughs

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New water-soluble and lipid-soluble spin probes suitable for the ESR investigation of the physical states and interactions of components of dough have been developed. This paper reports some preliminary findings on the suitability of these probes for this type of investigation. Rotational correlation times have been measured for the spin probes in water, dough, oil, and a starch/water mixture. An increase in rotational correlation time of the spin probe corresponds to an increase in microviscosity of the medium. Changes observed in correlation times of the water-soluble spin probes in doughs and in water/starch mixtures clearly correspond to a gelatinization process when the mixtures are heated above 60 °C. These irreversible changes, clearly important in the baking process, were monitored by following the change of mobility of a spin probe in doughs over a wide temperature range. The similarity of the results from the two sets of experiments suggests that the phenomenon of the increase of correlation times with temperature in doughs is attributable to the starch component. The lipid-soluble spin probe was found to be located preferentially in the lipid phase of the dough.

Keywords: *ESR; spin probes; doughs; lipids; dynamics*

INTRODUCTION

Electron spin resonance (ESR) spectroscopy is a highly sensitive technique that can detect species with unpaired electrons and obtain information on the molecular surroundings of the species. These species can be used as spectroscopic probes having application to a wide variety of chemical and physical problems (Sutcliffe, 1998). The probes used in this work are nitroxyl free radicals the ESR spectra of which are characterized by three equally spaced lines having 1:1:1 intensities arising from hyperfine interaction of the nitrogen-14 nucleus with the unpaired electron. Because the interactions of the spin probe vary with the polarity of the solvent, the hydrophobic and hydrophilic nature of the environment surrounding the radical can be identified. In addition, asymmetric line broadening of the spectra can be used to measure the rotational correlation times of the probe. Compared with other spectroscopic techniques, ESR has the advantages of specificity, high sensitivity, and less interference. By using a spin label or a spin probe technique, a specific part of a complex system can be studied and, in the case of foods, physical and chemical changes of components can be monitored. An essential part of the application of the technique to food systems is the design and synthesis of suitable ESR spin probes (Sutcliffe, 1998; Bottle et al., 1999). Generally, free radical spin probes should be thermally and chemically stable and capable of being chemically modified by the attachment of specific groups. Additionally, the appropriate solubility of a spin probe in water or lipid is also important, and sometimes spin probes are required to have a molecular structure very similar to that of the compounds being studied (Chung et al., 1994; Britton et al., 1997). In this work we have used three members of a novel class of stable nitroxyl radicals that we have developed (Bolton et al., 1993, 1994) and for which we have obtained detailed magnetic resonance spectroscopic data (Bolton et al., 1993).

Some ESR studies on food systems have been reported in the literature, and much useful information has been obtained, for example, (i) structural changes in the viscosity of gel water in potato starch granules (Windle, 1985), in the hydration of whey protein−wheat starch systems (Schanen et al., 1990a,b), and in starch and protein modification during extrusion processing of corn starch and sunflower protein blends (Sotillo et al., 1994); and (ii) interactions of food components such as lipid−protein interaction in gluten (Nishiyama et al., 1981) and binding of spin probes to gelatinized starches (Nolan et al., 1986a,b; Schanen et al., 1990b; Sotillo et al., 1994). There have been ESR investigations of the lipid binding to starch and wheat gluten using stearic acid spin probes (Pearce et al., 1987a,b; Johnson et al., 1990a). Differences were found between waxy corn starches and amylose-containing starches. Tempo spin probes have been used in studies of water mobility in starch and gluten systems (Johnson et al., 1987b; Tsoubeli et al., 1995; Pearce et al., 1988). In the starch/

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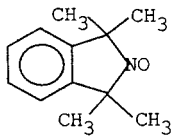
water mixtures, it was shown that both sugar type and concentration influenced the temperature range over which starch transformations take place.

Unlike some of these studies, the probes used in this work can be regarded as chemically inert and do not interact with dough components. This paper reports the results of some preliminary investigations designed to verify that several types of spin probes are useful for the study of microviscosity changes occurring in doughs brought about by heating. The probes were chosen to allow lipid and starch phases of the doughs to be studied separately or simultaneously.

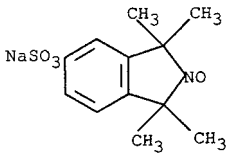
EXPERIMENTAL PROCEDURES

Materials. The flours used to make doughs were either Hereward, 1992 harvest, hard endosperm, HMW 7+9, 3+12 (milled on July 14, 1994, with a Quadrumat Senior mill, U.K.), or Hereward obtained from Advanta Seeds, harvested in 1997 (milled on May 5, 1998): both flours were stored at 4 °C. Lipid extraction of 1 g of 1992 Hereward flour was carried out at room temperature with 10 cm^3 of $CHCl_3$ with stirring for 20 min.

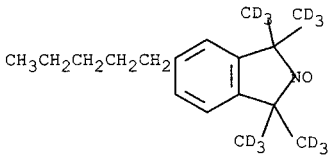
Three nitroxyl radicals were used as spin probes in this work, namely, 1,1,3,3-tetraisoindolin-2-yloxyl (TMIO) (Bolton et al, 1993)



sodium 1,1,3,3-tetraisoindolin-2-yloxyl-5-sulfonate (NaTMIOS) (Belton et al., 1998)



5-*n*-pentyl-1,1,3,3-tetrakis(trideuteriomethyl)isoindolin-2-yloxyl (PTMIOD) (Gillies et al., 1994)



Deuteration serves to give a useful reduction in the ESR spectral line widths: unfortunately, deuterated TMIO and NaTMIOS were not available for this work; nevertheless, the radicals have smaller linewidths than commercially available undeuterated nitroxide spin probes, such as Tempo. We have reported previously (Bolton et al., 1993; Gillies et al., 1994; Belton et al., 1998) the general synthetic procedures and ESR properties for the probes. Partition experiments using *n*-octanol/water showed that the solubilities in the water phase are TMIO <0.1%, NaTMIOS >99.9%, and PTMIOD <0.01%.

Sample Preparation. *(a)* Doughs were prepared by hand mixing 1 g of dry Hereward wheat flour with 0.75 g of water for 4 min.

(b) The dough containing the water-soluble radical NaTMIOS was prepared by hand mixing 1 g of flour and 0.75 g of 10 mM NaTMIOS aqueous solution for ~4 min.

(c) The dough containing the lipid-soluble radical PTMIOD was prepared by hand mixing 1 g of flour, 0.75 g of water, and, typically, 0.3 mg of PTMIOD for 4 min.

(d) The dough containing TMIO was prepared by first dissolving 2 mg of the radical in 1 cm^3 of dichloromethane and hand mixing the solution with 1 g of the 1998 flour. The solvent was removed under vacuum, and then 0.75 g of water was used to make the dough with hand mixing.

ESR Spectroscopy. A Varian E-4 spectrometer fitted with a V-4540 variable temperature controller and a JEOL JES-REIX spectrometer fitted with a DVT2 variable temperature controller were used to carry out ESR measurements. Both spectrometers operate at X-band frequencies of ~9.4 GHz. The dough and other samples were sucked into capillary tubes (1.3 mm i.d.) that were then sealed and put into 4 mm o.d. ESR sample tubes. The following ESR spectrometer parameters were used: modulation amplitude, 0.01 or 0.02 mT; microwave power, 1 mW.

Treatment of Data. Microviscosities can be evaluated by measuring the rotational correlation times of the spin probes. Nitroxyl radicals undergoing rapid rotational motion in an isotropic medium have their spectral anisotropy completely averaged and give rise to a three-line ESR spectrum. The widths of the lines can be accounted for by the motional modulation of the *g* factor and the hyperfine interaction tensors. The rate of tumbling can be determined, provided that the anisotropic *g* and *A* tensors are known and the rotational correlation time is <~1 ns. Two rotational correlation times, $\tau_c(B)$ and $\tau_c(C)$, can be calculated using eqs 1 and 2 (Fairhurst, 1983; Marsh, 1989) given below. The rotational correlation time $\tau_c(B)$ corresponds to averaging of the three anisotropic hyperfine interactions and the three anisotropic *g* values, whereas $\tau_c(C)$ reflects only the averaging of the hyperfine interactions. Ideally, both have the same value, but in general $\tau_c(B)$ is more reliable. Plots of ln $\tau_c(B)$ or ln $\tau_c(C)$ against the reciprocal of the absolute temperature should produce straight lines, the slopes of which provide the activation enthalpy. If two parallel lines are obtained, then the radical can be regarded as undergoing isotropic motion.

$$\tau_c(B) = 15B/(4b\Delta B_0)\ \text{(s)} \quad (1)$$

$$\tau_c(C) = 8C/(28020000b^2)\ \text{(s)} \quad (2)$$

where

$$\Delta = 2\pi\beta_e[g_{zz} - (g_{xx} + g_{yy})/2]/h\ (\text{mT}^{-1}\ \text{s}^{-1})$$

$$b = 4\pi[A_{zz} - (A_{xx} + A_{yy})/2]/3\ \text{(mT)}$$

$$B = 0.866W(+1)[1 - (h(+1)/h(-1))^{1/2}]\ \text{(mT)}$$

$$C = 0.866W(0)[(h(0)/h(+1))^{1/2} + (h(0)/h(-1))^{1/2} - 2]\ \text{(mT)}$$

where B_0 is the magnetic field at the center of the spectrum (mT).

The parameters $h(+1)$, $h(0)$, $h(-1)$, $W(+1)$, and $W(0)$ are line heights (mm) and peak-to-peak linewidths (mT), respectively. The relevant anisotropic ESR parameters for the TMIO series of radicals for *g* and *A* have been found to be (Bolton, 1993) $g_{xx} = 2.00820$, $g_{yy} = 2.00523$, $g_{zz} = 2.00147$; $A_{xx} = 0.500$ (mT), $A_{yy} = 0.439$ (mT), and $A_{zz} = 3.382$ (mT). For slower spin probe rotations, a simulation program devised by Schneider and Freed (1989) can be used to estimate rotational correlation times.

For the experiments with TMIO as a spin probe in dough, two superimposed ESR spectra are observed originating from the water and lipid phases. Spectral parameters from each of the spectra were obtained using the EWVOIGTN computer program supplied by Scientific Software Services of Illinois, Normal, IL. This program enables linewidths to be measured with precision from two overlapping nitroxyl spectra.

RESULTS

NaTMIOS Spin Probe. ESR spectra of the water-soluble probe NaTMIOS in dough were recorded over

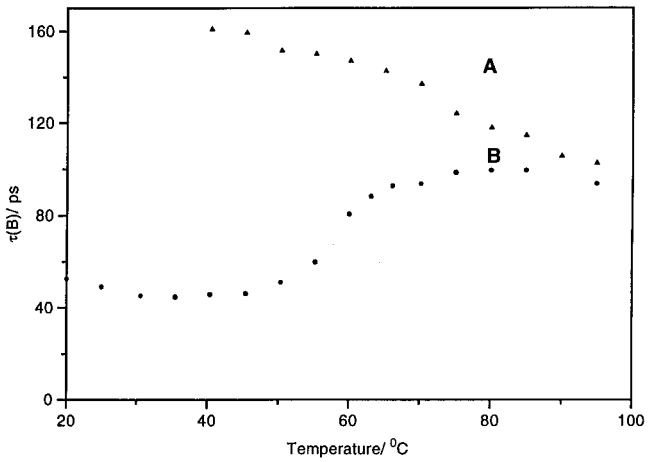


Figure 1. Effect of temperature on $\tau_c(B)$ of the radical NaTMIOS in (A) dough after heating and (B) dough.

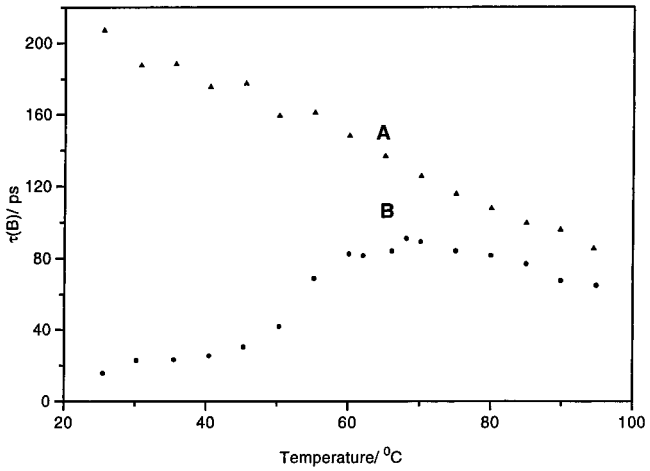


Figure 2. Effect of temperature on $\tau_c(B)$ of the radical NaTMIOS in (A) starch/water mixture after heating and (B) starch/water mixture.

the temperature range −10 °C to 95 °C at 5 °C intervals. Because NaTMIOS is soluble only in polar environments, it will be located solely in the aqueous phase in the dough. As mentioned above, the nitrogen hyperfine interaction constant of a spin probe varies with the polarity of the solvent. The nitrogen-14 isotropic hyperfine splitting constants for NaTMIOS in both water and dough are similar at room temperature, being 1.569 and 1.563 mT, respectively. This indicates that the polarities of the molecules surrounding NaTMIOS are similar for both media. Above 0 °C, the ESR spectra correspond to rapid motion; hence, the rotational correlation times of the probe can be calculated from eqs 1 and 2. At −8 °C, the ESR signal is very broad and corresponds to a solid-state ("powder") spectrum. Plots of $\tau_c(B)$ against temperature for temperatures above 20 °C are shown in Figure 1: those for the dough (Figure 1B) have a complex dependence that can be considered sigmoidal. As the temperature of the dough is increased, the values of $\tau_c(B)$ deviate increasingly from linearity above 35 °C, increase to a maximum at ∼75 °C, and then decrease again. After the dough was heated to 95 °C, it was cooled to room temperature and the rotational correlation times of NaTMIOS were measured again over a similar temperature range: the latter procedure enabled the irreversibility of the baking process to be checked. It may be seen that the rotational correlation times (Figure 1A) have an approximately linear dependence on temperature (it is actually a first-order exponential dependence). For the data in Figure 1A, the logarithm of the correlation time should be linearly dependent on the reciprocal of the absolute temperature, and from this relationship the enthalpy of activation can be calculated. It was found that for the data in Figure 1A, $\Delta H^* = 8.3 \pm 0.7$ kJ mol^{-1}. This is a surprisingly low value because $\Delta H^* = 18.6 \pm 0.5$ kJ mol^{-1} for NaTMIOS in pure water (Belton et al., 1998), a value that is confirmed by measurement of the enthalpy of activation ($\Delta H^* = 17.23 \pm 0.05$ kJ mol^{-1}) obtained from the temperature dependence of the viscosity of water (Berstad et al., 1988).

The rotational correlation times for NaTMIOS in a 1:1 mixture of wheat starch and water were measured in the temperature range 20−95 °C, and the results are shown in Figure 2. It may be seen that at 20 °C, the $\tau(B)$ is ∼16 ps for both pure water and the starch solution. Thus, the increase in $\tau(B)$ to 53 ps for the probe in dough must be caused by the presence of protein. Otherwise, the results are similar to those for the dough; that is, the temperature dependence of the rotational correlation time is sigmoidal, there being a sharp rise in the rotational correlation time in the range 45−70 °C (Figure 2B). After cooling to room temperature, the mixture of wheat starch and water became a gel. The rotational correlation times for the spin probe in the gel were measured in the same temperature range: the results are presented in Figure 2A. The data from Figure 2A give an enthalpy of activation of 11.3 ± 0.5 kJ mol^{-1}, which again is much lower than the value found for NaTMIOS in pure water.

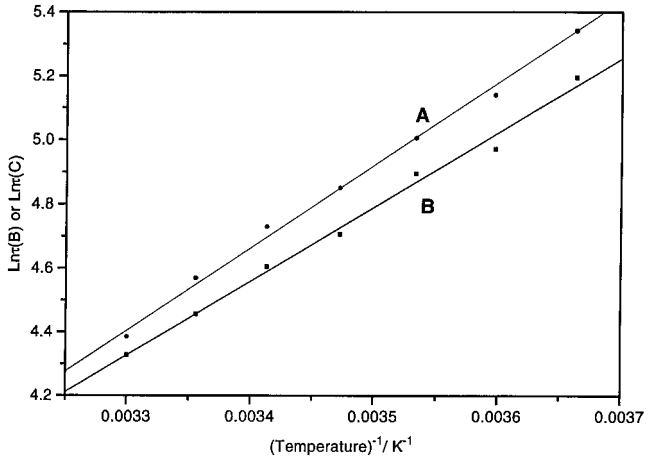


Figure 3. Plots of (A) ln $\tau_c(B)$ and (B) ln $\tau_c(C)$ versus $1/T$ for PTMIOD in degassed oil extracted from flour.

PTMIOD Spin Probe. The rotational correlation times of PTMIOD in degassed oil extracted from the flour were determined for the temperature range 0−30 °C: the activation energy plots for $\tau_c(B)$ and $\tau_c(C)$ are linear and almost parallel (Figure 3), so the rotation of the probe must be isotropic in this temperature range. The activation enthalpies for $\tau_c(B)$ and for $\tau_c(C)$ are 21.1 ± 0.6 and 19.3 ± 0.7 kJ mol^{-1}, respectively. At 20 °C, $\tau_c(B)$ is 113 ps for the oil, but there is an increase to ∼30 ns for PTMIOD in dough, presumably due to the presence of starch and protein. At 20 °C, the nitrogen-14 hyperfine coupling constant was found to be 1.459 mT, typical of a nonpolar environment. The oil freezes at −80 °C, and the frozen solution of the radical exhibits a typical ESR powder spectrum. The lines of the ESR powder spectrum of PTMIOD in dough are much broader than those observed for the oil.

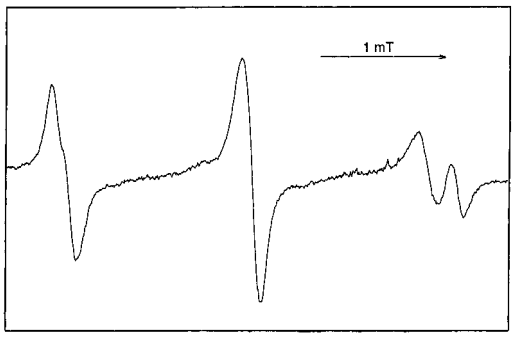


Figure 4. X-band ESR spectrum at 21.5 °C of TMIO in dough before heating.

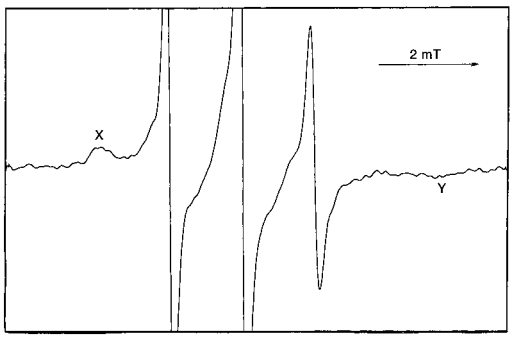


Figure 5. X-band ESR spectrum at 22.1 °C of TMIO in dough after heating to 90 °C. The central three sharp lines arise from TMIO in the lipid phase; the features marked X and Y originate from the outer lines of the powder spectrum of TMIO in the water phase.

TMIO Spin Probe. Although the experiments with PTMIOD in dough indicated that lipids are not involved in the gelatinization process, it was thought to be worthwhile to use TMIO as a spin probe as it would be located in both the lipid and starch phases and thus provide a simultaneous measure of the change of mobility during heating. Figure 4 shows that the two phases can be distinguished by the difference of the nitrogen hyperfine splitting constants for the two polarities and by the small difference in g factors for the two media. Note that the spectral lines are relatively sharp, even for the lipid phase, indicating that the oxygen concentration of the dough is low. Upon heating the dough, the ESR lines from TMIO in the water phase broaden while those in the lipid sharpen, thus confirming the findings reported above. After the dough had been heated to 90 °C and then allowed to cool, the spectrum shown in Figure 5 was obtained. Now it is clear that the TMIO in the water phase is characteristic of a powder spectrum, whereas the TMIO in the lipid ($a^N = 1.44$ mT) still has considerable mobility. It is difficult to estimate the value of $\tau_c(B)$ for the lipid phase because the main part of the powder spectrum overlays the three sharp lines (see Figure 5). However, when the dough was being heated in stages to 90 °C, $\tau_c(B)$ for the lipid could be estimated but $\tau_c(C)$ could not because of exact overlap of the central line with that from TMIO in water. From these measurements, the enthalpy of activation associated with $\tau_c(B)$ was found to be 19.0 ± 0.6 kJ mol^{-1} in the temperature range 22−72 °C: this value is close to those of 21.1 ± 0.6 and 19.3 ± 0.7 kJ mol^{-1} found, respectively, for $\tau_c(B)$ and $\tau_c(C)$ values for the isolated oil, using PTMIOD as a spin probe.

DISCUSSION

Although the motion of NaTMIOS in dough is fairly rapid, it is at least a factor of 3 times slower than in bulk water. The effect must arise from interactions of the water with the biopolymer matrix. Because viscosities of normal liquids decrease with temperature, it is usual for rotational correlation times to decrease with increase of temperature, but here we find the reverse is the case for dough and starch (Figures 1B and 2B). The most likely explanation is that starch gelation has occurred, causing release of amylose and amylopectin previously stored compactly in granules. The release of these polymers generates a more homogeneous biopolymer network with which the water may interact more readily, thus increasing the microviscosity. Figures 1A and 2A show a normal temperature dependence of the correlation times in the new more viscous medium formed after heating to 95 °C, as would be expected because the release of the biopolymers is irreversible. The enthalpies of activation found from the data in Figures 1A and 2A are also similar; hence, we may conclude that the behavior of the rotational correlation time with temperature observed for the dough originates from the starch component.

Lipids comprise 1.2−4% of the flour by weight and can be separated into two major types, free and bound. Free lipid decreases drastically at the first stage of dough mixing, which means that there has been an increase in lipid interactions with gluten and starch (Marsh, 1985; Chung et al., 1986). The ESR spectra of PTMIOD in dough show that there is less mobility in this than in the oil extracted from it. The spin probe in the dough is likely to reside in the lipid phase where there are ordered aggregates. The latter may be either micelles or layers formed on the framework of the water-insoluble proteins. In either case, PTMIOD molecules should stay in the hydrocarbon-rich region of the lipid phase. If the PTMIOD is in the lipid micelles, which are dispersed in water, the ESR spectra will be affected by gelatinization. No such effect has been observed in our study. Therefore, we believe that the radical stays in the lipid attached to the protein surface, thus restricting the probe's mobility. By contrast, the experiments with the TMIO probe in flour show that this probe is soluble only in the free lipid.

CONCLUSIONS

It has been shown that, by using spin probes of appropriate solubilities, the microviscosities of different phases in doughs can be monitored during the baking process. We propose to extend these preliminary measurements to well-characterized and different types of flour and to their constituent lipids and glutens.

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LITERATURE CITED

Belton, P. B.; 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.* **1998**, *37*, 36−42.

Berstad, D. A.; Knapstad, B.; Lamvik, M.; Skølsvik, P. A.; Tørklep, K.; Oye, H. A. Accurate measurements of the viscosity of water. *Physica* **1988**, *A151*, 246−280.

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**, 2049−2052.

Bolton, R.; Sutcliffe, L. H.; Wu, X. Synthesis of isotopically-labelled nitroxyl radicals for use as spin probes. *J. Labelled Compd. Radiopharm.* **1994**, *34* (7), 663−668.

Bottle, S. E.; Gillies, D. G.; Micallef, A. S.; Reid, D. A.; Sutcliffe, L. H. ESR measurements of some new spin probes in *n*-octanol/water. *Magn. Reson. Chem.* **1999**, in press.

Britton, M. M.; Fawthrop, S. A.; Gillies, D. G.; Sutcliffe, L. H.; Wu, X.; Smirnov, A. I. Matched spin probes for the study of the overall motion of lubricants. *Magn. Reson. Chem.* **1997**, *35*, 493−501.

Chung, O. K. Lipid-protein interaction in wheat flour, dough gluten and protein fractions. *Cereal Foods World* **1986**, *31*, 242−256.

Chung, Y-L.; Gillies, D. G.; Sutcliffe, L. H. Application of matched spin probes to the investigation of the overall motion of model lubricant molecules. *J. Chem. Soc., Faraday Trans.* **1994**, *90*, 2547−2550.

Fairhurst, S. A.; Pilkington, R. S.; Sutcliffe, L. H. Rotational correlation times and radii of dithiazol-2-yl and dithiazolidin-2-free radicals. *J. Chem. Soc., Faraday Trans. 2* **1983**, *79*, 439−452.

Gillies, D. G.; Sutcliffe, L. H.; Wu, X. NMR determination of EPR hyperfine coupling constants of some 5-(*n*-alkyl)-1,1,3,3-tetrakis(trideuteriomethyl)isoindolin-2-yloxyls. *J. Chem. Soc., Faraday Trans.* **1994**, *90*, 2345−2349.

Gillies, D. G.; Sutcliffe, L. H.; Wu, X.; Belton, P. S. Molecular motion of a water-soluble nitroxyl radical in gelatin gels. *Food Chem.* **1996**, *55*, 349−352.

Johnson, J. M.; Davis, E. A.; Gordon, J. Lipid binding of modified corn starches studied by electron spin resonance. *Cereal Chem.* **1990a**, *67*, 236−240.

Johnson, J. M.; Davis, E. A.; Gordon, J. Interactions of starch and sugar water measured by electron spin resonance and differential scanning calorimetry. *Cereal Chem.* **1990b**, *67*, 286−291.

Marsh, D. ESR spin label studies of lipid-protein interaction. In *Progress in Protein-Lipid Interactions*; Watts, A., de Pont, J. J. H. M., Eds.; Elsevier: Amsterdam, The Netherlands, 1985; Vol. 1, pp 143−172.

Marsh, D. Experimental methods in spin-label spectral analysis. In *Spin Labeling. Theory and Applications*; Berliner, L. J., Reuben, J., Eds.; Academic Press: New York, 1989; pp 255−304.

Nishiyama, J.; Kuninori, T.; Matsumoto, H. ESR studies on lipid-protein interaction in gluten. *Agric. Biol. Chem.* **1981**, *45*, 1953−1958.

Nolan, N. L.; Faubion, J. M.; Hoseney, R. C. An electron spin resonance study of native and gelatinized starch systems. *Cereal Chem.* **1986a**, *63*, 287−291.

Nolan, N. L.; Hoseney, R. C.; Faubion, J. M. The effect of fatty acid spin labels upon starch. *Cereal Chem.* **1986b**, *63*, 291−294.

Pearce, L. E.; Davis, E. A.; Gordon, J.; Miller, W. G. Stearic acid-starch interactions by electron spin resonance. *Cereal Chem.* **1987a**, *64*, 154−157.

Pearce, L. E.; Davis, E. A.; Gordon, J.; Miller, W. G. An electron spin resonance study of stearic acid interactions in model wheat starch and gluten systems. *Food Microstruct.* **1987b**, *6*, 121−126.

Pearce, L. E.; Davis, E. A.; Gordon, J.; Miller, W. G. Electron spin studies of isolated gluten systems. *Cereal Chem.* **1988**, *65*, 55−58.

Schanen, P. A.; Pearce, L. E.; Davis, E. A.; Gordon, J. Hydration of whey protein-wheat starch systems as measured by electron spin resonance. *Cereal Chem.* **1990a**, *67*, 124−128.

Schanen, P. A.; Pearce, L. E.; Davis, E. A.; Gordon, J. Lipid binding in whey protein-wheat starch systems as measured by electron spin resonance. *Cereal Chem.* **1990b**, *67*, 317−322.

Schneider, D. J.; Freed, J. H. Calculating slow motional magnetic spectra: a user's guide. In *Spin Labeling. Theory and Applications*; Berliner, L. J., Reuben, J., Eds.; Academic Press: New York, 1989; pp 1−76.

Sotillo, E.; Hettiarachchy, N. S.; Meinhardt, S. W. Changes in starch and protein on extrusion of corn starch and sunflower protein blends. *J. Food Sci.* **1994**, *59*, 436−440.

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

Tsoubeli, M. N.; Davis, E. A.; Gordon, J. Dielectric properties and water mobility for heated mixtures of starch, milk protein and water. *Cereal Chem.* **1995**, *72*, 64−69.

Windle, J. J. An ESR spin probe study of potato starch gelatinization. *Starch/Staerke* **1985**, *37*, 121−125.

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