THE EFFECTS OF DOUGH FORMATION AND BAKING ON IRON ENRICHMENT OF BREAD AS STUDIED BY ELECTRON PARAMAGNETIC RESONANCE[1]

J. J. WINDLE, C. C. NIMMO, and ELLEN J.-L. LEW, Western Regional Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Berkeley, CA 94710

Cereal Chemistry 53(5): 671-677

ABSTRACT

The state of iron in dough and bread and the effects of the breadmaking process on reduced iron additive have been investigated by electron paramagnetic resonance (EPR) spectroscopy. EPR spectra are presented for the intact wheat kernel, and for flour, dough, and bread. The results of this study suggest that in unenriched dough and bread iron is present in both the ferrous and ferric states and that baking results in the oxidation of ferrous iron to ferric. When reduced iron has been added, dough formation and baking cause some of the reduced iron to be oxidized to the ferrous and ferric states, but most of the reduced iron remains unchanged throughout the breadmaking process.

Since 1941, enriched flour and bread have been required to contain iron at levels of 13 to 16.5 and 8.0 to 12.5 mg/lb, respectively. Recently, new levels have been proposed that increase these amounts to 40 and 25 mg, respectively. Various forms of iron have been used to meet these requirements, the most common being ferrous iron salts such as ferrous sulfate, and finely powdered metallic iron known as reduced iron. Very little information is available on the state of iron in bread (1,2), or on the reactions it undergoes during the breadmaking process. In an effort to provide a better understanding of the role of iron in enrichment and its effects on breadmaking, we have investigated the effects of dough formation and baking on the endogenous iron and the added reduced iron in dough and bread. The technique of electron paramagnetic resonance (EPR) spectroscopy was applied to this problem because it is a highly sensitive method capable of distinguishing different states of iron, and because the measurements can be made directly and nondestructively on the dough and bread specimens, thereby giving information for the iron *in situ*.

The EPR signals for ferric iron depend upon the complex formed. Salts, such as ferric chloride, give spectra different from ferric heme-type complexes which, in turn, are different from non-heme ferric complexes. Metallic iron, such as reduced iron, gives a spectrum quite different from the ferric iron spectra; thus, different states of iron can be identified from their EPR spectra. The principal limitations of the EPR method are that iron in the ferrous state cannot be detected directly and that, in its present state of development, the method is mainly a qualitative rather than a quantitative one.

MATERIALS AND METHODS

Samples of unenriched dough and bread, and dough and bread made from flour enriched with reduced iron (hydrogen reduction, Glidden-Durkee, food grade, 10–40 μ particle size) at a level of 100 mg/lb were lyophilized and ground.

[1]Portions of this paper were presented at the 59th Annual Meeting, Montreal, Canada, Oct. 1974.

Higher than normal levels of reduced iron were used in this study in an effort to reduce experimental errors and to better clarify and characterize the changes which occur.

Dough and bread specimens of each were mixed with deionized water on an Omnimixer while being continuously flushed with nitrogen gas. Excess reduced iron was removed from the enriched samples by stirring with Teflon-coated magnetic bars until no visible trace of metallic iron was observed adhering to the magnet. This was usually accomplished after three consecutive 10-min magnetic separation periods, and with an efficiency of from 80 to 90%. Some dough and bread slurries were then extracted and centrifuged three times with 0.01*M* acetic acid at pH 3.4. The resulting supernatants and residues were lyophilized and weighed. The acid treatment extracted 30% of the dough solids and 16% of the bread solids. The EPR measurements were made on a Varian E-3 EPR spectrometer equipped with a multipurpose EPR cavity and a variable temperature accessory. All measurements were made on 60-mg samples in standard 3-mm i.d. quartz tubes, at a temperature of −185° C, with a modulation amplitude of 10G and a field scan of 5000G. The EPR spectra are presented as the first derivative of the EPR absorption curve. Care was taken throughout the sample preparation to avoid iron contamination.

Changes in the ferric iron concentrations were estimated from changes in the amplitude of the Fe(III) signal at a *g*-factor of 4.3. Such estimates are reliable, provided the EPR line shape remains unchanged. Then the concentration, which is given by the area under the absorption curve, is proportional to the signal amplitude. This was the only type of ferric iron signal found in these studies and, thus, it is presumed to be proportional to the ferric iron content of the samples.

RESULTS

The EPR spectrum for intact wheat (3 kernels, total weight 60 mg) is shown in Fig. 1. The spectrum is composed of three signals: in the center, at a *g*-factor of 2.00, are superimposed the spectra from manganous ion, identified by its characteristic shape and sextet hyperfine pattern, and a sharp, single peak due to a free radical. The free radical signal results from an unidentified, photosensitive component located in the pericarp (3,4). The third signal is located on the low field side at a *g*-factor of 4.3 and is due to ferric iron. This signal is found in many biological materials and has been attributed to ferric iron in an environment of rhombic or lower symmetry (5).

Flour and shorts milled from the same variety of wheat give the EPR spectra shown in Fig. 2. The spectrometer gain setting for the lower spectrum was 1/5 that used for the flour, showing that much of the iron and manganese are removed by the refining process (3). The absence of the free radical signal in these fractions indicates that the free radical-containing component has also been removed by the milling process.

Dough and Bread

Lyophilized, proofed dough (Fig. 3) exhibits an EPR spectrum quite similar to that found in flour, indicating that the dough-forming process does not significantly alter the endogenous iron or manganese. The EPR signal for lyophilized bread (Fig. 3) shows that the manganese signal remains unchanged,

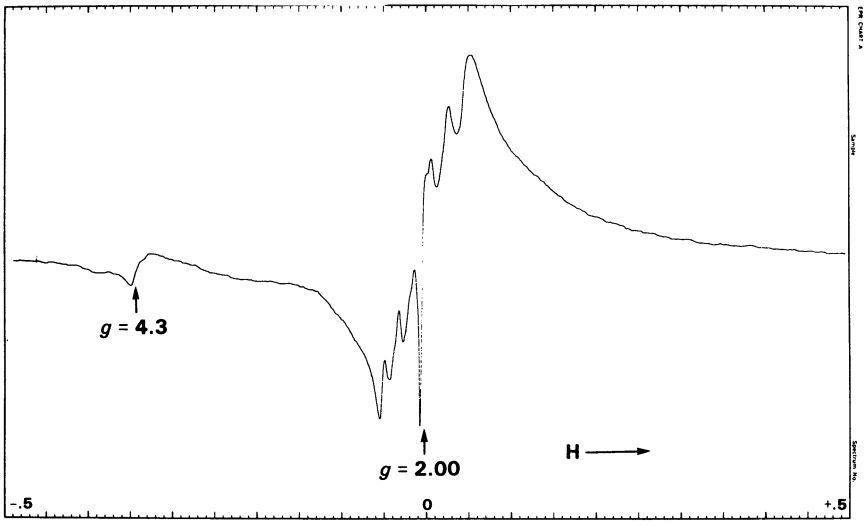

Fig. 1. EPR spectrum for three intact wheat kernels (total weight, 60 mg) at −185°C, showing manganese and free radical signals at a *g*-factor of 2.00 and ferric iron signal at a *g*-factor of 4.3. Total magnetic field scan of 5000G increasing from left to right.

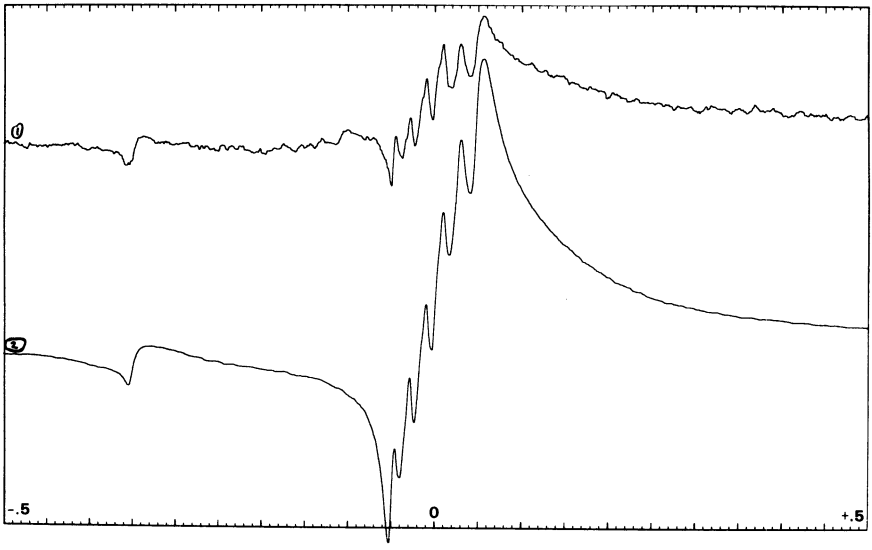

Fig. 2. EPR spectra for 60-mg samples of 1) flour and 2) shorts at −185°C. Spectrometer gain for flour spectrum is 5 times that for shorts.

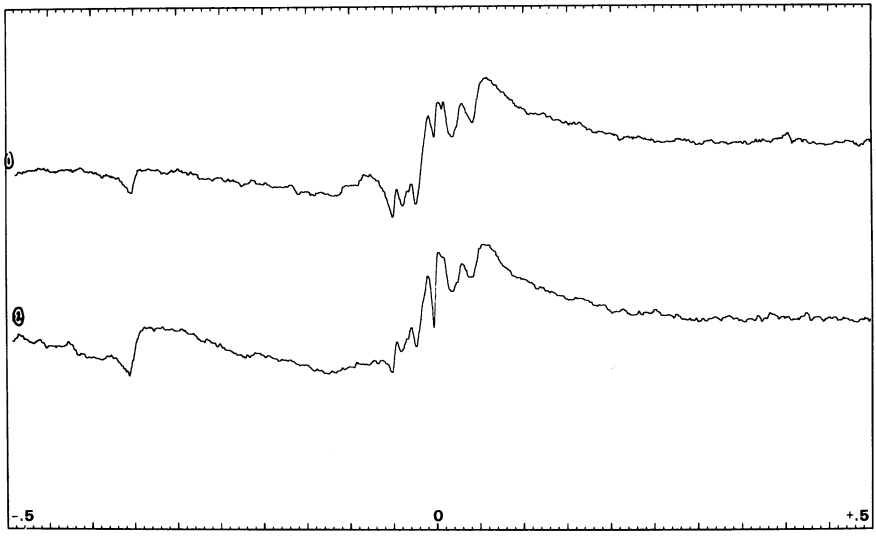

Fig. 3. EPR spectra of 60-mg samples of 1) lyophilized dough and 2) bread at −185°C.

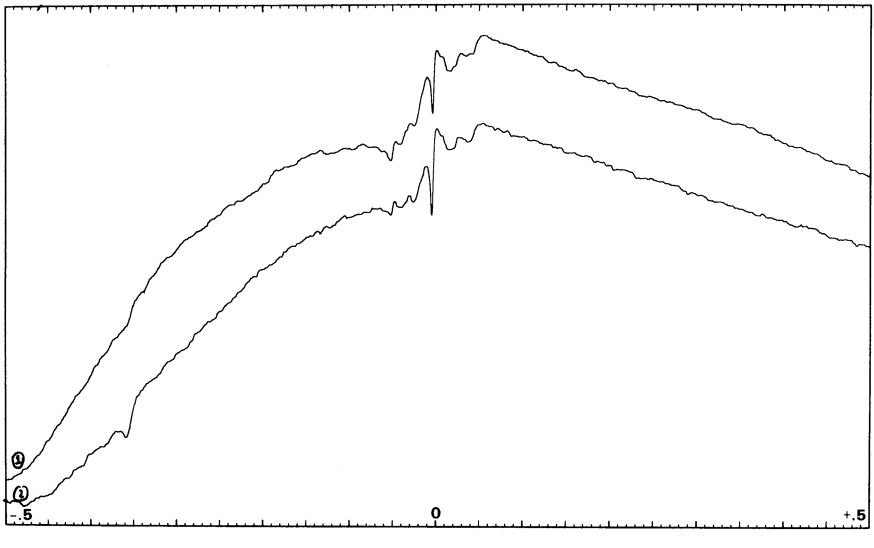

Fig. 4. EPR spectra for 60-mg samples of 1) lyophilized dough and 2) bread containing reduced iron at −185°C before extraction.

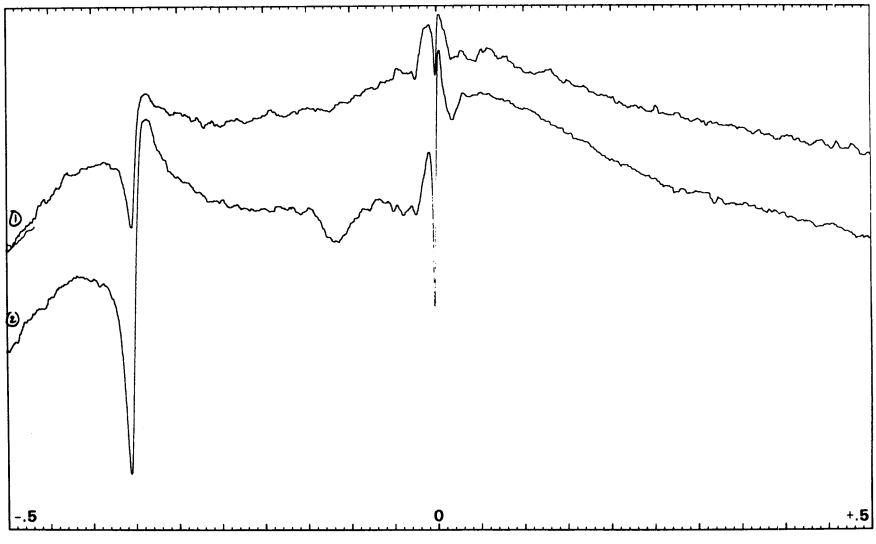

Fig. 5. EPR spectra for 60-mg samples of lyophilized residues (after magnetic and acidic extraction) of 1) enriched dough and 2) bread at −185° C.

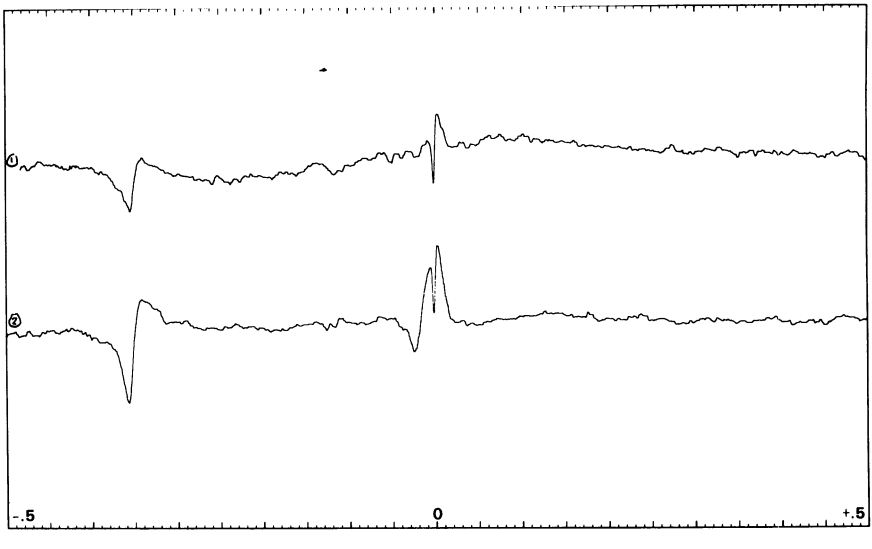

Fig. 6. EPR spectra for 60-mg samples of lyophilized residues of 1) dough and 2) bread without added reduced iron at −185° C.

but that the iron signal has increased by almost 60% and a free radical signal has appeared after baking.

Dough and Bread with Reduced Iron

The addition of reduced iron results in a strong, broad resonance, over 5000G in width, due to ferromagnetic resonance of the finely powdered metallic iron (5). This spectrum is superimposed upon the manganese, ferric iron, and free radical signals (Fig. 4).

The ferric iron signals are somewhat masked and distorted, but we estimate that they have increased about 35 and 20%, respectively, in dough and bread over the unenriched samples, and that in the enriched bread the signal is about 45% stronger than in the enriched dough. No change is observed in the manganese signals, but a free radical signal is now evident in the dough as well as in the bread.

Residues

The EPR spectra for the lyophilized residues from enriched dough and bread after magnetic and acetic acid extraction are shown in Fig. 5. When compared to unextracted dough and bread, a substantial increase in the ferric iron and free radical signals is found, with the greatest change occurring in the bread. The manganese signal has disappeared from both the bread and dough residues. Dough and bread without added reduced iron were extracted in the same way, and the results are shown in Fig. 6. Again, an increase in the ferric iron and free radical signals is observed, although not as great as with the enriched samples, and the manganese signal has disappeared. The EPR results for dough and bread are summarized in Table I.

Extracts

The supernatants from the three magnetic and acid extractions were combined and lyophilized in each case for the dough and the bread, and examined by EPR. The extracts of dough and bread give quite similar spectra; each exhibits a strong manganese signal and a very weak signal from ferric iron. These results show that the acid extraction removes the manganese from the dough and bread, but that little of the ferric iron is removed by this treatment.

TABLE I
Summary of EPR Results on Dough and Bread

Treatment		Fe^{3+} EPR Signal (Arbitrary Units)	
Reduced iron	Extraction[a]	Dough	Bread
No	No	7.6	12.5
No	Yes	12.0	23.5
Yes	No	11.0	15.0
Yes	Yes	30.5	81.0

[a]Amplitudes of non-extracted samples were multiplied by factors of 1.2 for bread and 1.4 for dough to correct for concentration effect resulting from extraction.

DISCUSSION

The finding that the ferric iron signal in bread is greater than in the corresponding dough can be understood if it is assumed that iron is present in wheat in a form not detectable by EPR, most probably in the ferrous state, and that during baking some of this iron is oxidized to the ferric form, thus giving an increased ferric EPR signal. The increase in the ferric signal after acid extraction also is attributed to the oxidation of ferrous iron to ferric during this treatment. The additional increase in the ferric signal in enriched dough and bread can result from the oxidation of some of the reduced iron during dough formation and baking, and this same mechanism accounts for the larger ferric signals in the residues from enriched dough and bread after the acid extraction. In the latter case, the ferric signal in the bread residue is considerably larger than in the corresponding dough, whereas before extraction, the bread signal is only slightly larger. To account for this difference, we suggest that during the baking process some of the reduced iron is converted to the intermediate ferrous oxidation state, which is undetectable by EPR until it is further oxidized to the ferric state during the acid extraction. The acid extraction removes all of the manganese from the dough and bread, but practically none of the ferric iron, showing that the iron complexes are not as readily extractable nor as easily broken by the acid treatment as is the manganese. The increase in the free radical signal, concomitant with the increase in the ferric content, may result from oxidation of the iron to the ferric state by means of one electron transfer from iron to oxygen or peroxide (7), which then may react with the the substrate by hydrogen abstraction to give an as yet unidentified free radical trapped in the matrix.

Literature Cited

1. LEICHTER, J., and JOSLYN, M. A. The state of iron in flour, dough and bread. Cereal Chem. 44:346 (1967).
2. HINTON, J. J. C., CARTER, J. E., and MORAN, T. The addition of iron to flour. I. The solubility and some related properties of iron powders including reduced iron. J. Food Technol. 2: 129 (1967).
3. WINDLE, J. J. ESR study of a new light sensitive free radical in the coats of wheat and other seeds. Photochem. and Photobiol. 15: 361 (1972).
4. WINDLE, J. J., and EVANS, J. J. Electron spin resonance study of stable free radicals in wheat. Cereal Chem. 48: 351 (1971).
5. BLUMBERG, W. E. The EPR of high spin Fe^{3+} in rhombic fields, p. 119. In: Magnetic resonance in biological systems, ed. by A. Ehrenberg, B. G. Malmström, and A. T. Vänngård. Pergamon: Oxford (1967).
6. BERSOHN, M., and BAIRD, J. C. An introduction to electron paramagnetic resonance, p. 148. W. A. Benjamin, Inc. New York (1966).
7. deGROOT, J. J. M. C., VELDINK, G. A., VLIEGENTHART, J. F. G., BOLDINGH, J., WEVER, R., and van GELDER, B. F. Demonstration by EPR spectroscopy of the functional role of iron in soybean lipoxygenase-1. Biochim. Biophys. Acta 377: 71 (1975).

[Received June 2, 1975. Accepted November 7, 1975]