Cereal Chemistry

Electron Spin Resonance Study of Stable Free Radicals in Wheat[1]

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ABSTRACT

Electron spin resonance (ESR) spectra have been obtained on single wheat kernels; on the bran, shorts, and flour from two varieties; and on gluten, starch, and water-solubles from flour. The wheat kernel exhibited two signals, one attributable to manganese and the other to a free radical. Each of the milling fractions also showed free radical spectra similar to the wheat kernel, with the signal from the bran and shorts being about ten to twenty times as intense as that from the break and reduction flours. When flour was separated into gluten, starch, and water-solubles, a different spectrum was obtained, with the largest free radical signal occurring in the gluten. Owing to lack of hyperfine structure, the identities of the free radicals could not be established. The ESR spectrum in the milling fractions showed manganese to be concentrated mainly in the bran and shorts fractions. Among the flour fractions, only the water-solubles gave a manganese spectrum that remained after dialysis. A comparison of the intensity of free radical and manganese signals in various milling fractions with their nitrogen, fiber, ash, and fat content indicated that these signals are concentrated in outer portions of the kernel.

Free radicals, as shown by electron spin resonance (ESR), are formed in flour and flour components by severe mechanical action (1), and indirect evidence suggests that free radicals are formed in wheat-flour dough during mixing (2). Free radicals are also known to be involved in chemical and enzymatic oxidative processes (3,4,5) and, therefore, may play a significant role in the formation and breakdown of dough and in the stability of wheat products. To better understand the occurrence and significance of free radicals in wheat and wheat products, we have undertaken this ESR study. We report results obtained with wheat kernels, mill fractions, and major flour constituents. In later papers we will discuss the

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effects of light, heat, and moisture on free radicals and the effect of these factors and free radicals on the stability and properties of wheat and wheat products.

MATERIALS AND METHODS

Two varieties of wheat, Red River 68 and Gaines, were tempered overnight to moisture levels of 15.5 and 13.5%, respectively, before milling in a C. W. Brabender Quadrumat Senior mill to yield break and reduction flours, shorts, and bran mill fractions. Samples were milled in a N_2 atmosphere by enclosing a C. W. Brabender Quadrumat Junior mill containing the wheat sample in a large polyethylene bag and purging with dry N_2 for about 20 min. before milling. Immediately after milling, flour and bran samples were packed in the ESR spectrometer tubes in the N_2 atmosphere and capped to exclude O_2.

For flour fractionation, 100 g. of Gaines break flour was added to 300 ml. of distilled water and stirred for 10 min. This slurry was centrifuged for 20 min. at 27,000 X g, yielding a supernatant or solubles fraction. The precipitate was fractionated by washing the starch from the gluten with 300 ml. distilled water. All three fractions were lyophilized to dryness before determining their ESR spectra. Moisture, fiber, fat, ash, and nitrogen were determined by standard AOAC methods (6).

The ESR spectra were obtained on a Varian E-3 spectrometer. The ESR spectrum is recorded as the first derivative of the absorption curve and thus appears as a curve which extends above and below the base line (see Fig. 1, B). The free radical concentration is proportional to the amplitude of this signal expressed in arbitrary units as the vertical distance between the two peaks. Samples were run in conventional 3-mm. ID quartz tubes. The milled samples were packed in the tubes and restricted to 5 mm. or less in height, and were centered in the resonant cavity. When samples were prepared in this way, the ESR signal was proportional to the sample weight, which enabled comparison on a unit weight basis.

RESULTS AND DISCUSSION

To obtain an ESR spectrum, the specimen must possess unpaired electrons. Except for molecules with an odd number of electrons and a few with triplet ground states, unpaired electrons are a characteristic of free radicals and certain transition metal ions which are paramagnetic, such as manganese. In general, it is possible to distinguish between free radicals and paramagnetic ions and to identify them from features of their ESR spectra such as the electron g-factor, line width, and hyperfine splittings.

For wheat, the ESR spectrum from a single Gaines wheat kernel showed signals from both sources (Fig. 1, A). The sharp, single line in the center of the spectrum, due to the free radical, is superimposed upon the much broader spectrum of manganese. The manganese spectrum is characterized by six hyperfine lines separated by 90 to 100 gauss centered at g = 2.00. The spectrum was quite similar to that observed for other manganese-containing biological materials (7). The free radical signal consists of a single, slightly asymmetric line, with a width of about 7.5 gauss and a g value of 2.00. A typical spectrum is shown in Fig. 1, B. The free radical concentration was estimated to be about 10^{13} spins by comparison with the Varian weak pitch standard. No resolvable hyperfine structure could be detected;
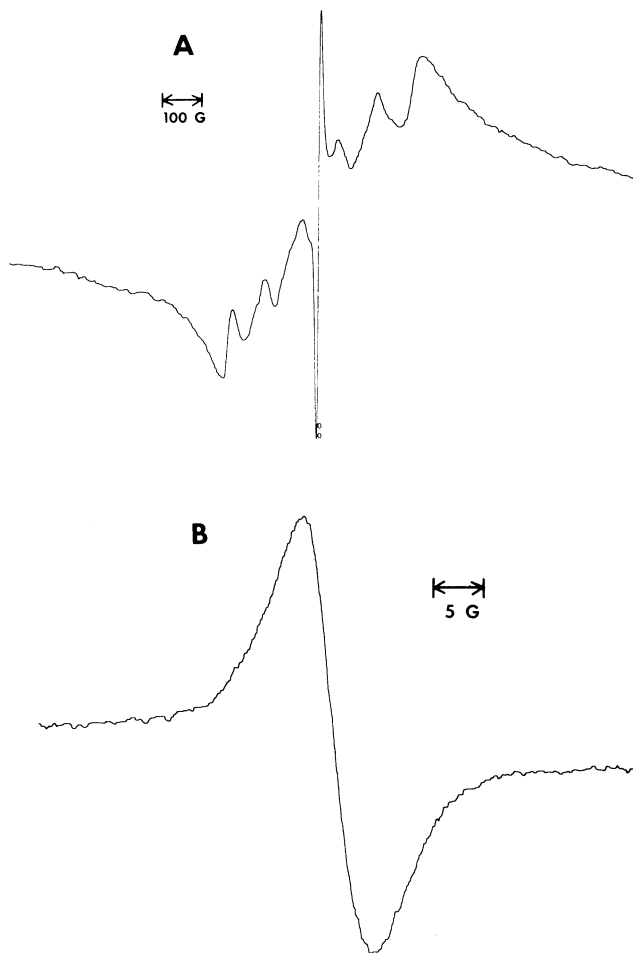
Fig. 1, A. ESR spectrum of a Gaines wheat kernel. A magnetic field scan of 2,000 gauss increasing from left to right and a modulation amplitude of 10 gauss were employed. The sharp peak in the center of the spectrum is due to the free radical and is superimposed on the much broader sextet spectrum characteristic of bound manganese.

Fig. 1, B. ESR spectrum of the free radical signal from the above spectrum. In this spectrum the magnetic field scan has been reduced to 100 gauss increasing from left to right and the modulation amplitude has been reduced to 2.5 gauss. This effectively excludes the manganese signal and expands the free radical signal to permit a more detailed examination of its spectrum. The phase difference between this signal and the above spectrum is instrumental and has no significance.

TABLE I.   ANALYTICAL RESULTS AND FREE RADICAL CONTENT OF MILL
FRACTIONS FROM GAINES AND RED RIVER 68 WHEAT

Wheat Type	N %	Fiber %	Fat %	Ash %	Moisture %	Relative Free Radical Content
Gaines						
Break flour	1.74	0.2	0.85	0.46	12.8	3.4
Reduction flour	1.91	0.4	1.37	0.57	12.4	6.0
Shorts	2.67	4.7	3.04	2.62	11.0	62.0
Bran	2.79	7.2	3.50	3.88	12.3	51.2
Red River 68						
Break flour	2.43	0.7	1.16	0.44	12.7	3.8
Reduction flour	2.30	0.5	1.24	0.52	9.9	6.1
Shorts	2.47	3.2	3.42	2.37	11.7	31.5
Bran	2.69	9.9	4.88	5.06	13.0	65.0

thus, identity of the free radical could not be determined from its spectrum. The same general spectrum was obtained among different wheat grains of the same or of different varieties, other cereal grains, and seeds from various assorted genera (8), differing only in the relative amounts of free radical and manganese[3].

After the wheats were freshly milled, the largest ESR signals were found in the bran and shorts fractions (Table I). On a weight basis, these fractions have signals ten to twenty times as intense as the flour fractions. The ESR spectra of the mill fractions exhibited the same line shape and width as found in the intact wheat kernel. The moisture content of the various mill fractions was also determined (Table I). Although there were slight variations in moisture levels, the moisture content could not account for the large differences in free radical content of the flour fractions vs. the shorts and bran fractions in both varieties of wheat.

Since it is known that free radicals can be induced in some materials by mechanical action such as abrasion and grinding and that such radicals can be affected by the presence or absence of air (1,9), we milled the wheat in an atmosphere of dry nitrogen. No significant change in free radical concentration or line shape was found for any of the milling fractions when compared with wheat milled under normal atmospheric conditions, indicating that the radicals are reasonably stable to air and ambient moisture. To determine whether the milling action induces free radicals, we compared the signals from a single wheat grain before and after grinding in a Wiley mill. No change in signal intensity or shape was observed. Since the milling action is designed to separate the endosperm from the bran and germ, the preponderance of free radicals in the bran and shorts indicates that they are located in the portions of the intact kernel contributing to these fractions. The occurrence of a small free radical signal in the flour fraction is believed to be due primarily to residual amounts of bran present. Table I also shows the analyses for nitrogen, fat, fiber, and ash content of the various milling fractions. These results further substantiate that the free radical is associated with outer portions of the kernel.

The manganese spectrum for the milling fractions was also found to be localized mainly in the bran and shorts with substantially less being found in the flour

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[3]In some species other paramagnetic signals are sometimes found, such as from iron.

fraction. Thus, in the intact wheat kernel, the manganese is also located mainly in the external portions of the kernel.

When the break and reduction flours were fractionated into gluten, starch, and water-solubles fractions and the lyophilized components examined, quite similar free radical signals were found in each fraction. These signals differed from signals found in the intact kernel and the milled-wheat fractions in that they were more asymmetric, being broader on the high field side and possessing a line width of 10 gauss as compared to 7.5 gauss for wheat.

The largest signal was found in the gluten; the water-solubles contained about one-third and the starch one-sixth the free radical content of the gluten. The origin of these signals is not known. They do not appear to be derived from the wheat signal, which would very likely not survive in aqueous solution, but it is possible that they resulted from the lyophilization procedure.

Among the flour fractions, only the solubles fraction gave a manganese spectrum. A portion of the solubles fraction was extensively dialyzed against distilled water and lyophilized, and the ESR spectrum determined. The manganese signal persisted and the ESR spectrum indicated that manganese was present in some form of complex or chelate (7).

These experiments have shown that the major metallic component of wheat detectable by ESR is manganese, which appears to be localized mainly in the bran, although there is some evidence of its presence in the flour fraction to a substantially lesser degree. In addition, wheat contains a naturally occurring, stable free radical easily detectable in a single kernel. The radicals are localized in the external (bran) portions of the kernel and are unaffected by milling. No appreciable free radical appears to be derived from endosperm tissue, as shown by the weak signal from flour.

If free radicals are involved in dough formation or breakdown, as suggested by some (2,10), they are probably induced after milling by some subsequent treatment or operation. The high concentration of free radicals in the bran and shorts, however, may play a significant role in the storage stability of these wheat mill fractions.

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