ELECTRON PARAMAGNETIC RESONANCE AND BAKING STUDIES ON GAMMA-IRRADIATED FLOUR[1]

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ABSTRACT

Irradiation of flour at its usual moisture level of 12–14% with up to 10^6 rads of Co^{60} gamma rays gave no observable electron paramagnetic resonance (EPR) spectrum. After reduction of the moisture content to 8 or 4%, irradiation resulted in EPR absorption indicating presence of radiation-induced free radicals. These radicals are destroyed rapidly in the presence of water vapor. With the irradiated flour kept in a sealed tube, the EPR spectrum fades with time. The fading was quite rapid for the first few days and then proceeded more slowly. For this particular flour, irradiation resulted in a moderate increase in loaf volume at fairly low radiation dosages, followed by a gradual decrease in loaf volume at higher dosages. It was also noted that the loaf volume tended to decrease as the elapsed time between irradiation and baking increased. This finding, when considered in conjunction with the fading of the EPR spectrum, indicated that the EPR-detectable free radicals in the irradiated flour did not cause a strengthening of the gluten to produce an increase in loaf volume, as these radicals disappeared on becoming stable compounds.

The literature concerning the effects of ionizing radiation on wheat and flour has been listed up to 1959 by Lai, Finney, and Milner (4). The contradictory findings among different workers of apparent improvement or of damage to breadmaking quality were pointed out. It was suggested that irradiation produced fermentable degradation products of starch which may cause moderate increases in loaf volume when bread was made with suboptimum amounts of sugar, malt, or bromate in the baking formula, and that no improvement would be observed if an optimum baking formula were used (4). Previous work in our laboratory (5,6) also indicated the breakdown of starch and gluten upon irradiation of flour with Co^{60} gamma rays. Similarities in behavior on irradiation of flours derived from different varieties of wheat of varying breadmaking quality suggested that the extent of such degradations may be largely dependent on total radiation dosage, without very much discrimination among flours of different baking quality (6). The changes in flour after irradiation very probably involve reactions of radiation-induced free radicals. The electron paramagnetic resonance (EPR) absorption is a powerful tool for the detection of free radicals, *in situ* (2,8,9). The present paper reports some observations on the EPR absorption of a flour that has been irradiated with Co^{60} gamma rays and on some baking studies with the irradiated flour.

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Materials and Methods

The flour used in all experiments was milled from Western Canadian hard red spring wheat obtained in 1960 from the mill of the Saskatchewan Wheat Pool. It was of "baker's grade" and it had not been subjected to improver treatments. Its crude protein and ash contents, on a 14% moisture basis, were 15.0 and 0.47%, respectively. Much of the flour irradiated was first dried to moisture levels of 8 and 4%. This was accomplished by prolonged evacuation of air-dried flour in a vacuum oven at room temperature. Sufficient amounts of flour at each of these moisture levels were prepared, thoroughly mixed, and kept in tightly covered cans. All subsequent experiments at a given moisture level were performed using the same composite stock of dried flour.

The irradiation was carried out in a Gamma Cell[2] containing 1,000 curies of Co^{60} which provided a dose rate of about 10^5 rads per hour. Two-kilogram batches of flour in tightly covered tin cans were irradiated. Immediately after irradiation, each batch was thoroughly mixed. Small samples were sealed in capillary tubing for EPR measurements. The bulk of the irradiated flour was kept in the tightly covered cans for subsequent baking studies.

EPR absorptions were determined at room temperature with an electron paramagnetic resonance spectrometer, model V-4500-10, supplied by Varian Associates. Baking tests were carried out using the following formula: 100 g. flour, 3 g. yeast, 5 g. sucrose, 1.75 g. sodium chloride, 3 g. shortening, 4 g. nonfat dry milk, 0.3 g. nondiastatic malt, 0.1 g. ammonium dihydrogen phosphate, and optimum absorption for baking.

Results and Discussion

EPR Studies on Flour without and with Prior Drying in Vacuum Oven. With the flour at its usual moisture content of 12–14%, samples irradiated with up to 10^6 rads showed no observable EPR spectrum[3]. On the other hand, when the flour was first dried in a vacuum oven at 90°C. overnight, irradiation gave rise to strong EPR absorption peaks indicating the presence of free radicals. The formation of the free radicals was induced by the irradiation, and not by drying, since drying alone did not cause any resonance absorption. Apparently, with flour containing the usual amount of moisture, the

[2] Supplied by the Atomic Energy of Canada Limited.

[3] Spectrometer sensitivities vary with the details of their construction, frequency, working temperature, and especially the nature of the sample (9). Under the present operating conditions, the minimum detectable free radical concentration for the dried flour is estimated to be 10^{15}–10^{16} radicals or about 10^{-8} moles per ml. of sample.

radicals formed during irradiation were destroyed by reaction with water before their presence could be detected. That water can destroy the radicals produced by irradiation was also demonstrated by the fact that when a sample of irradiated dry flour showing strong EPR absorption was placed overnight in a desiccator above a layer of water, the EPR spectrum completely disappeared. This effect of water on radiation-induced free radicals has been noted previously by O'Meara and Shaw (8) for a number of irradiated food constituents, though flour was not included in that study.

EPR Studies on Flour at 4 and 8% Moisture. In order to obtain irradiated flour for both EPR and baking studies, flours dried to 4 and 8% moisture were used. Irradiation of such samples resulted in EPR absorption. Figure 1 shows the derivative curves for the flour with 4% moisture irradiated with different dosages of gamma rays. As expected, higher dosages resulted in more intense peaks. Similar spectra but of lower intensity were observed with the flour at 8% moisture.

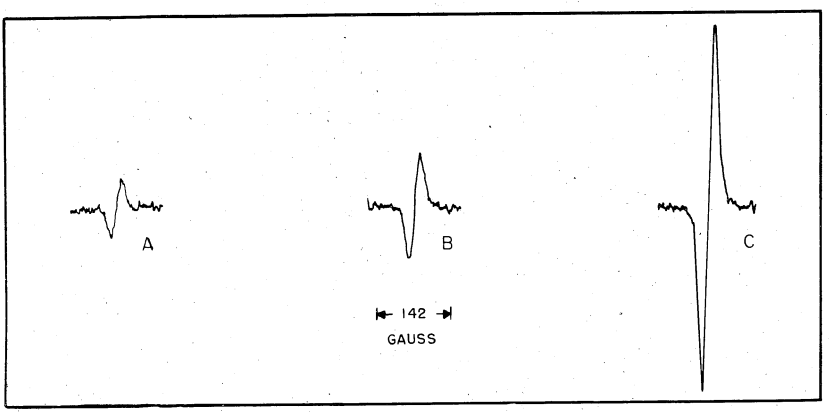

Fig. 1. Electron paramagnetic resonance spectra of irradiated flour which contained 4% moisture. A, B, and C correspond to radiation dosages of 0.25, 0.50, and 1.0 million rads, respectively.

Under conditions of higher resolution, the EPR spectrum for the 4% -moisture flour that had been irradiated with 2 million rads is shown in Fig. 2. The shape of this curve (Fig. 2) is quite similar to that reported for corn starch by O'Meara and Shaw (8). This is not surprising, since starch is the largest single component of flour. However, dried samples of starch and of gluten derived from the flour, as well

as samples of whole kernels of wheat, upon irradiation also resulted in EPR absorption, indicating that starch is not the only component of flour in which free radicals are induced by ionizing radiation. Actually, the asymmetric nature of the derivative curve (Fig. 2) would suggest the presence in the irradiated flour of several different radicals (1,8). The shape of Fig. 2 also indicates that the integrated absorption spectrum of the irradiated flour would have an overlapping doublet

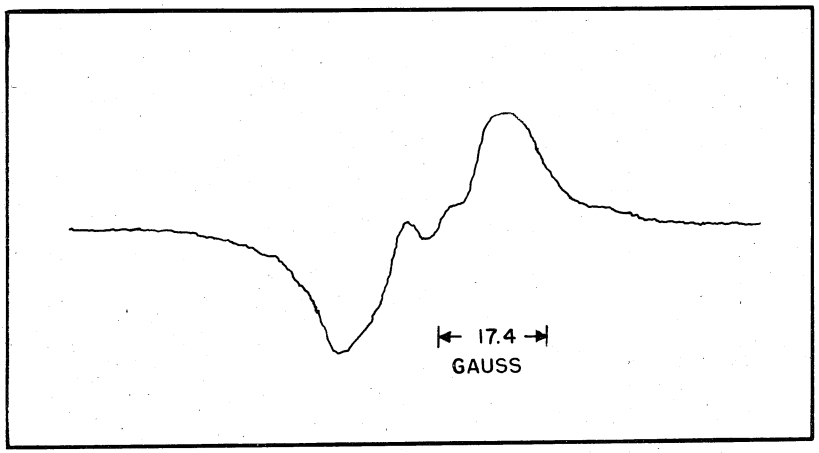

Fig. 2. Electron paramagnetic resonance spectrum under high resolution for flour with 4% moisture irradiated with 2.0 million rads.

structure. Doublet structures have been reported for starch (1) and for a number of proteins and peptides (3), and have been interpreted as indicating that a dominant feature must be the coupling of the free electron to only one proton (1). However, the detailed structures of the free radicals in the irradiated flour still remain unknown.

Fading of EPR Absorption with Time. The intensity of EPR absorption of the irradiated flour, kept in sealed capillary tubing, was found to decrease with time. The fading was quite rapid for the first few days and then proceeded more slowly. A typical behavior is given by Fig. 3 for the flour with 4% moisture irradiated with 2 million rads. While the total radical concentration may be estimated by finding the area of the integrated absorption band and comparing this area to the area of the integrated absorption band of a known standard solution of free radicals such as diphenylpicrylhydrazyl, the process

is a rather tedious one. In Fig. 3, peak heights of the observed derivative curves recorded at various times after irradiation are used as an index, but not a direct measure, of the radicals present in the sample. The disappearance of the EPR spectrum is, of course, associated

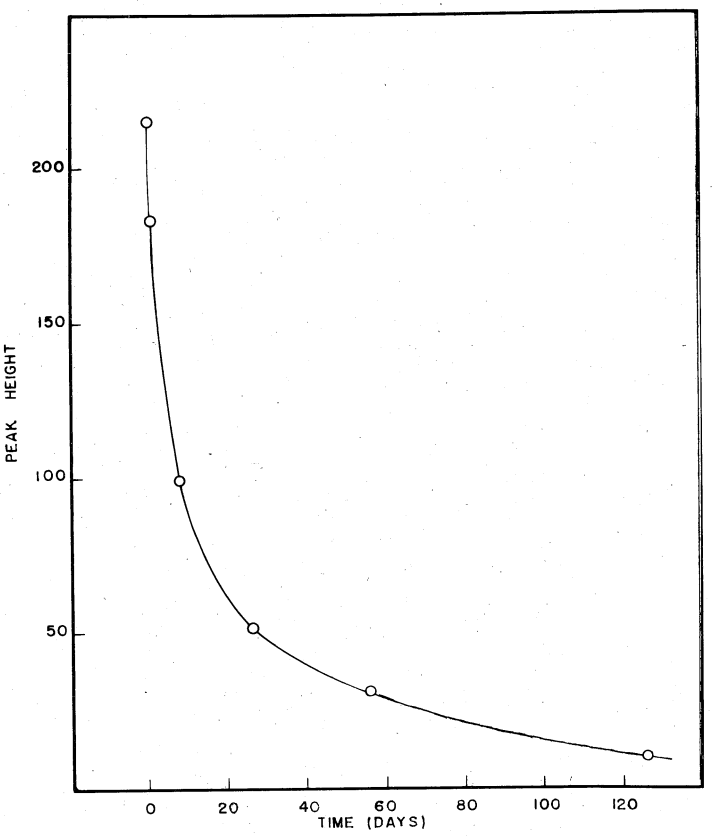

Fig. 3. Fading of electron paramagnetic resonance spectrum of flour with 4% moisture irradiated with 2.0 million rads. Peak height is expressed in arbitrary units.

with changes of the free radicals to stable compounds. A suggestion has been made by Maes (7) that there is a possibility of radiation-induced polymerization which may strengthen the gluten and thus account for the increase in loaf volume observed after irradiation of some flours such as weak European flours. If radiation-induced poly-

merization does occur, one possible route for the polymerization may be the union between two radiation-induced free radicals. It is, therefore, of interest to investigate the relationship, if any, between the disappearance of free radicals, as indicated by the fading of the EPR spectrum, and the baking performance of the irradiated flour.

Baking Studies on Unirradiated Flours. Before baking studies were made on irradiated flours containing 4, 8, and 14% moisture, the effects of drying on loaf volume of bread made from unirradiated flour were investigated. The averaged results are given in Table I, showing that drying to 4% moisture resulted in a highly significant decrease in loaf volume, whereas drying to 8% moisture did not cause any statistically significant difference from the control at 14% moisture.

TABLE I

EFFECTS OF DRYING ON LOAF VOLUME OF BREAD FROM UNIRRADIATED FLOUR

Moisture, %	14 (control)	8	4
Mean loaf volume[a], ml.	907.5	901.8	854.8**

[a]Mean value of sixteen loaves baked in replicates of four on four different dates.
**Significantly different from the control at the 1% level. The least significant difference (LSD) is 19.0 ml.

Baking Studies on Irradiated Flours. The effects of radiation dosage on loaf volume for the flours containing 4, 8, and 14% moisture were also studied. The mean values for loaf volume of bread from the flour with 4% moisture irradiated with 11 different dosages of gamma rays are given in Table II. Statistical analysis of the data showed highly significant differences among the different radiation treatments. For this particular flour using the baking formula given earlier in this paper, there was a moderate increase in mean loaf volume up to a

TABLE II

EFFECTS OF RADIATION DOSAGE ON LOAF VOLUME OF BREAD FROM IRRADIATED FLOUR CONTAINING 4% MOISTURE

DOSAGE	MEAN LOAF VOLUME[a]	DOSAGE	MEAN LOAF VOLUME[a]
10^3 rads	*ml*	*10^3 rads*	*ml*
0	854.8	150	877.2
25	873.6	200	867.2
50	877.6	250	867.0
75	883.0	500	847.6
100	875.2	750	830.4
125	873.2	1000	792.8
	LSD** = 7.0 ml.		

[a]Mean value of sixteen loaves baked in replicates of four on four different dates.
**Least significant difference. Level of significance at 1%.

maximum at the treatment with 75,000 rads. Beyond this maximum, irradiation with higher dosages resulted in a gradual decrease in mean loaf volume. In the studies with flours containing 8 and 14% moisture, lesser numbers of treatments at different dosages were carried out; but a similar trend of a moderate increase to a maximum loaf volume followed by a gradual decrease at higher dosages was observed.

The above results indicate that the flour used in these experiments responded to moderate doses of radiation with moderate increases in loaf volume. If this response were the result of polymerization through union of radiation-induced free radicals, the fading of the EPR spectrum of the irradiated flour might also be associated with polymerization and strengthening of the gluten. In such an event, for flours that show EPR absorption, namely, flours containing 4 and 8% moisture, loaf volume might be expected to increase with increasing length of time that elapsed between irradiation and baking, because the EPR spectrum of an irradiated flour fades with time. To investigate this possibility, baking was carried out with the irradiated flours at various times extending up to 4 weeks after irradiation. First of all, it was noted that with unirradiated flours at 4, 8, and 14% moisture, within each moisture level, there were no statistically significant differences in mean loaf volumes of bread baked at different times over a 4-week period. With the irradiated flours, highly significant differences among mean loaf volumes of bread baked at different times after irradiation were observed. The results are tabulated in Table III. A common trend was noted for the flours with moisture

TABLE III

MEAN LOAF VOLUMES[a] OF BREAD FROM IRRADIATED FLOUR BAKED AT DIFFERENT TIMES AFTER IRRADIATION

MOISTURE CONTENT OF FLOUR	TIME OF BAKING, DAYS AFTER IRRADIATION				LSD**
	1	2	7	28	
%	*ml*	*ml*	*ml*	*ml*	*ml*
14	936.0	924.5	897.5	910.5	12.9
8	946.4	937.5	902.8	892.9	10.5
4	863.3	851.6	862.8	846.8	10.9

[a]Mean values of 16, 36, and 44 loaves, respectively, for flour with 14, 8, and 4% moisture. Each mean value is the average of all loaves from flour that had been irradiated with different dosages of gamma-rays.
** Least significant difference. Level of significance at 1%.

contents of 4, 8, and 14%. In all cases, the mean loaf volumes tend to decrease with increasing length of time that elapsed between irradi-

ation and baking. It might be pointed out that in Table III, each value of mean loaf volume is the over-all average of all loaves derived from flour that had received different doses of radiation. For example, at 4% moisture, each mean loaf volume is the over-all average of 44 loaves baked in replicates of 4 for 11 different radiation dosages ranging from 25,000 to 1,000,000 rads. Since statistically, the average of all loaves from 11 radiation doses or 11 treatments can indicate a trend as well as, or even better than the average from only one treatment, the over-all averages are given in Table III. As an illustration of the baking behavior of flour that had received a given dose of radiation, the variation of loaf volume with baking time for dosages of 50,000 and 1,000,000 rads are given in Table IV for the flour with 4% moisture.

TABLE IV

MEAN LOAF VOLUMES[a] AT DIFFERENT TIMES OF BAKING FOR FLOUR WITH 4% MOISTURE IRRADIATED AT TWO DOSE LEVELS

DOSAGE	TIME OF BAKING, DAYS AFTER IRRADIATION			
	1	2	7	28
10^3 rads	*ml*	*ml*	*ml*	*ml*
50	888	886	880	842
1000	807	798	798	756

[a] Mean of four loaves.

Data in Tables III and IV indicate that irradiation appears to cause changes in the flour which resulted in a fairly rapid deterioration of its baking quality with age. This trend of decreasing loaf volume with age cannot be associated with fading of the EPR spectrum, since irradiation of the flour containing 14% moisture also showed this trend, although it did not show observable EPR absorption. Moreover, whatever may be the mechanism responsible for the disappearance of the free radicals as indicated by the fading of the EPR spectra, it may be concluded that for the particular flour used in the present studies, the EPR-detectable free radicals, produced in the irradiation of the flour with its moisture at the 4 and 8% levels, did not cause a strengthening of the gluten to give an increase in loaf volume, as these radicals disappeared on becoming stable compounds.

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