KINETIC STUDIES WITH ELECTRON PARAMAGNETIC RESONANCE ABSORPTION. ON THE DISAPPEARANCE OF RADICALS TRAPPED IN GAMMA-IRRADIATED FLOUR[1]

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ABSTRACT

Radical concentrations in flour after irradiation with ^{60}Co gamma rays were determined by means of electron paramagnetic resonance. The disappearance of such radicals follow second-order kinetics, the rate of disappearance in flour with 3.0% moisture being greater than that in the corresponding oven-dried flour. Possible mechanisms of reaction of the radiation-produced radicals are discussed. Consideration of such reaction mechanisms in conjunction with baking behaviors suggests that the predominant effects of irradiation would be degradation, with little or no polymerization.

In a previous study in this laboratory (1), the electron paramagnetic resonance (EPR) absorption spectrum was utilized to detect the presence of free radicals trapped in flour after irradiation with ^{60}Co gamma rays. EPR absorption was observed after irradiation of flour with reduced moisture content. The intensity of the absorption peak

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decreased with time, and moisture apparently could enhance the rate of this disappearance of the trapped radicals. The present paper reports the kinetics of the disappearance of EPR-detectable radicals in some irradiated flours. In addition, mixograms, farinograms, and baking data obtained from a flour before and after irradiation are compared.

Materials and Methods

The Flours. Three grades of flour, milled from Western Canadian hard red spring wheat and designated as patent, baker's, and clear grades, were obtained from the mill of the Saskatchewan Wheat Pool. Their crude protein and ash contents, on 14% moisture basis, are given below.

Grade	*Protein*	*Ash*
	%	%
Patent	13.0	0.32
Baker's	14.9	0.40
Clear	18.9	0.77

For the kinetic studies, the three flours were oven-dried (vacuum oven at 60°C. for 48 hr.), or the moisture level was reduced to 3.0% by evacuation in a vacuum oven at room temperature for 1 week. For studies with the mixograph and farinograph and for baking, only the baker's grade, either oven-dried or as-received (13.6% moisture), was used.

Experimental Procedures. The irradiation, the taking of the EPR spectra, and the baking tests were carried out as outlined previously (1). Mixograms were drawn with a Swanson-Working recording dough mixer utilizing 35 g. of flour; farinograms were obtained[3] using a Brabender Farinograph with a 300-g. bowl.

The Kinetic Studies. Each grade of flour, either oven-dried or at 3.0% moisture, was irradiated with 10^6 rads of ^{60}Co gamma rays. Samples were then sealed in small glass tubes and the EPR spectra taken at different times after the irradiation. Estimates of radical concentrations from these spectra provided data for the kinetic calculations. Such estimates of the absolute quantities of "spins" were obtained through indirect comparison of the spectra of the samples and the spectra of freshly prepared solutions of alpha,alpha-diphenyl-beta-picrylhydryl (DPPH), of known concentration, in benzene. Because the DPPH solutions are unstable, indirect comparisons were made through the use of gamma-irradiated barium salt of dichloroacetic acid, which gave an EPR spectrum stable with respect to time (2).

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[3] By courtesy of J. R. Reynolds of the Saskatchewan Wheat Pool.

Results

Radical Concentrations. The EPR spectrometer draws the spectrum as the first derivative curve exemplified by Fig. 1, A. Integration of the first derivative gives the corresponding absorption spectrum (Fig. 1, B). Now, integrating the absorption spectrum will give its area, which is directly proportional to the concentration of radicals. Thus each spectrum obtained was integrated twice successively to provide the area of the absorption curve.

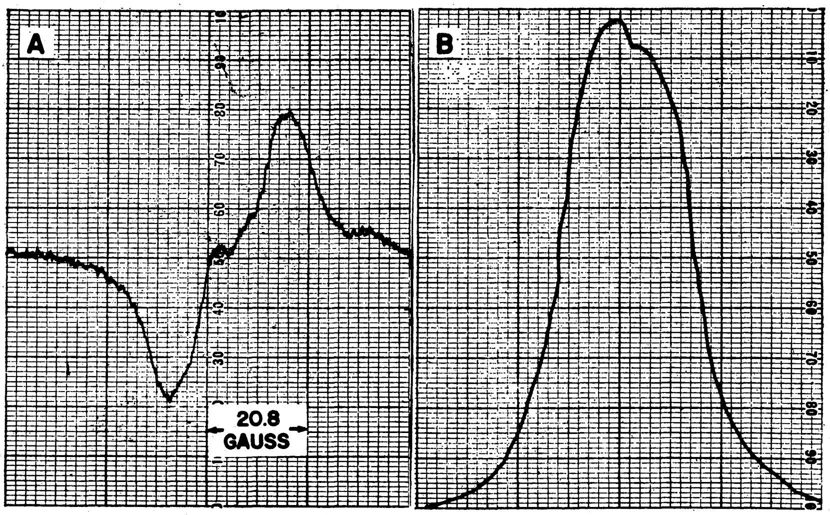

Fig. 1. Typical EPR spectrum of gamma-irradiated flour. A is the first derivative curve as drawn by the spectrometer. B is the absorption curve from integration of A.

For quantitative comparisons, all spectra were obtained under identical operating conditions. Under these conditions, a reference standard of gamma-irradiated barium dichloroacetate gave a spectrum with a peak height of 50 units. A freshly prepared 1.4×10^{-3} molar solution of DPPH showed a spectrum with an integrated area of 6,577 sq. units. Figure 1 is the spectrum of the oven-dried clear flour taken immediately after irradiation. Its integrated area is 5,812 sq. units, which would correspond to a radical concentration of:

$$5812 \times (1.4 \times 10^{-3} \times 6.02 \times 10^{23} \times 10^{-3})/6{,}573 = 7.45 \times 10^{17} \text{ spins/ml.}$$

While the spectrum of the DPPH solution will change with time, the spectrum of the gamma-irradiated barium dichloroacetate will remain unaltered or show only slight variations due to instrumental fluctuations. For example, the same clear flour at 20 days after irradiation gave a spectrum with an integrated area of 1,769 sq. units. At that time, the barium dichloroacetate reference standard showed a peak height of 51 units. Thus the radical concentration of this flour at 20 days after irradiation would be:

$$[1769 \times (1.4 \times 10^{-3} \times 6.02 \times 10^{23} \times 10^{-3})/6{,}577] \times 50/51 = 2.22 \times 10^{17} \text{ spins/ml.}$$

Radical concentrations, in spins per ml., calculated in the above manner for the three flours either oven-dried or at 3.0% moisture, at various times after irradiation, are given in Tables I and II.

TABLE I

RADICAL CONCENTRATIONS IN OVEN-DRIED FLOURS AFTER IRRADIATION WITH 10^6 RADS

DAYS AFTER IRRADIATION	(SPINS/ML.) × 10^{-17}		
	Patent	Baker's	Clear
0	6.87	8.14	7.45
1	5.19	6.81	5.63
2	4.58	5.04	4.46
3	4.35	4.59	4.61
5	3.65	3.81	3.42
7	3.32	3.64	3.72
10	3.70	3.17	3.25
14	2.44	2.37	2.63
20	1.84	2.09	2.22
27	1.79	1.25	1.66
34	1.23	0.97	1.56
41	0.94	0.85	1.24
47	0.99	0.78	1.07
55	0.92	0.62	1.03

TABLE II

RADICAL CONCENTRATIONS IN FLOURS WITH 3.0% MOISTURE AFTER IRRADIATION WITH 10^6 RADS

DAYS AFTER IRRADIATION	(SPINS/ML.) × 10^{-17}		
	Patent[a]	Baker's	Clear
0	5.60	4.77	5.67
2	3.35	3.55	4.35
4	2.94	2.73	4.11
7	2.32	2.76	3.03
11	1.37	1.38	2.15
17	0.92	0.95	1.37
24	0.51	0.60	0.98
32	0.51	0.39	0.63
39	0.51	0.30	0.48

[a] Since radical concentrations are calculated from doubly integrated areas of the EPR signals, the errors in the concentrations corresponding to very small areas would be relatively high. Such errors may contribute to the apparent leveling in radical concentration for this flour at 24–39 days after irradiation.

Kinetics of the Disappearance of Radicals Trapped in Irradiated Flour. Preliminary graphical trials indicate that the experimental results will be most compatible with second-order kinetics. With the usual symbols of chemical kinetics, one can write

$$dx/dt = k_2 (a - x)^2,$$

and on integration,

$$1/(a - x) = k_2t + C,$$

where a is the initial concentration and (a — x) is the concentration at time t. A plot of 1/(a — x) vs. t would give a straight line with the slope equaling the specific rate constant k_2.

Using the data of Tables I and II, straight-line plots of the reciprocals of radical concentration against time after irradiation were worked out by the method of least squares, with the aid of a digital computer[4]. These are shown in Figs. 2, 3, and 4. The slopes of these lines, also calculated by the computer, corresponded to k_2 in units of ml. $spin^{-1}$ day^{-1}. Appropriate conversion factors were applied so that the second-order specific rate constants may be expressed in the more conventional units of l. $mole^{-1}$ $sec.^{-1}$. For example, the slope of the plot of 1/(a — x) vs. t for the oven-dried patent flour is

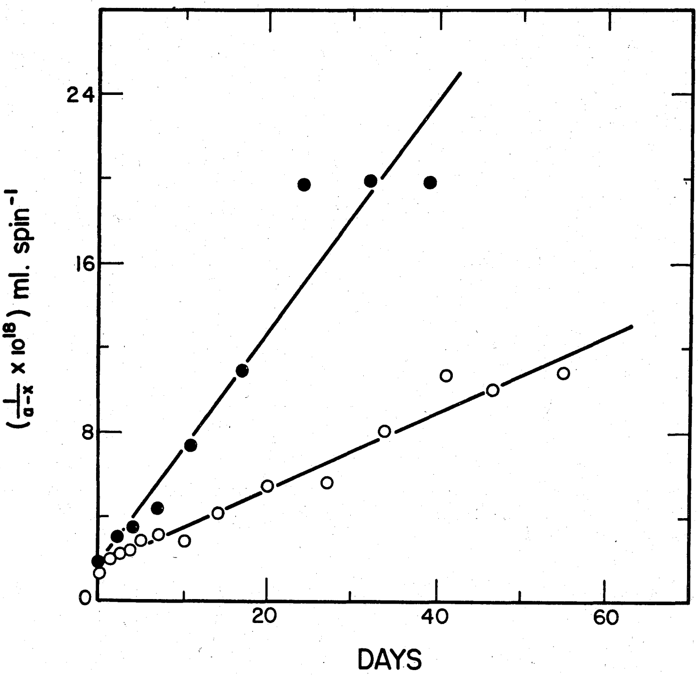

Fig. 2. Second-order kinetic plots for the disappearance of radicals trapped in patent flour after irradiation with 10^6 rads. Open circles, oven-dried flour; closed circles, flour with 3.0% moisture.

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[4] Model L.G.P.-30, supplied by General Precision (Canada) Ltd.

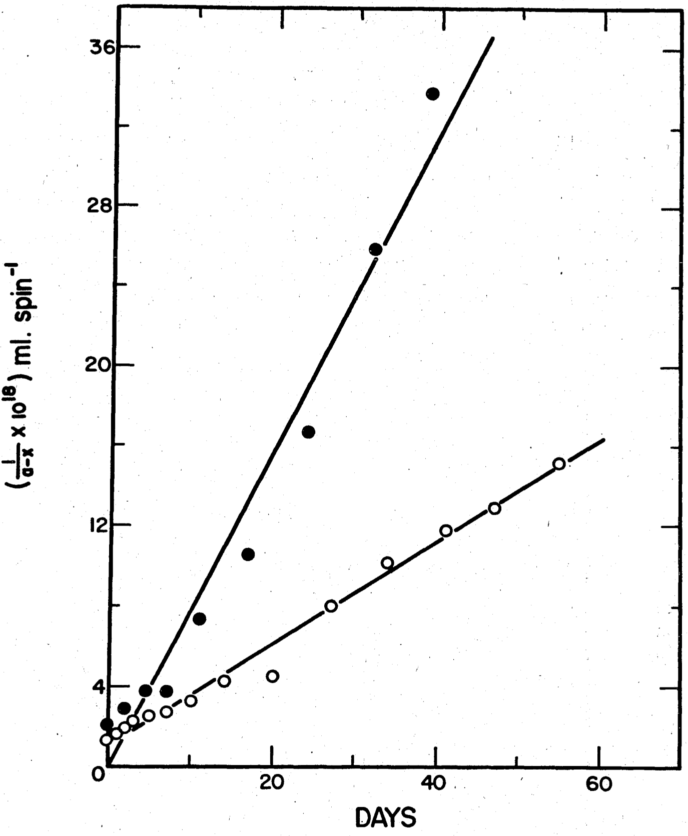

Fig. 3. Second-order kinetic plots for the disappearance of radicals trapped in baker's flour after irradiation with 10^6 rads. Open circles, oven-dried flour; closed circles, flour with 3.0% moisture.

1.81×10^{-19} ml. spin^{-1} day^{-1} which, after multiplication by the appropriate conversion factors, gives a k_2 of 1.26×10^{-3} l. mole^{-1} sec.$^{-1}$. Values of k_2 for the three grades of flour, either oven-dried or at 3.0% moisture, are summarized in Table III.

TABLE III

SECOND-ORDER SPECIFIC RATE CONSTANTS FOR THE DISAPPEARANCE OF TRAPPED RADICALS IN FLOURS IRRADIATED WITH 10^6 RADS

GRADE	$k_2 \times 10^3$, l.mole^{-1} sec.$^{-1}$	
	Oven-Dried	3.0% Moisture
Patent	1.26	3.74
Baker's	1.76	5.60
Clear	1.06	3.37

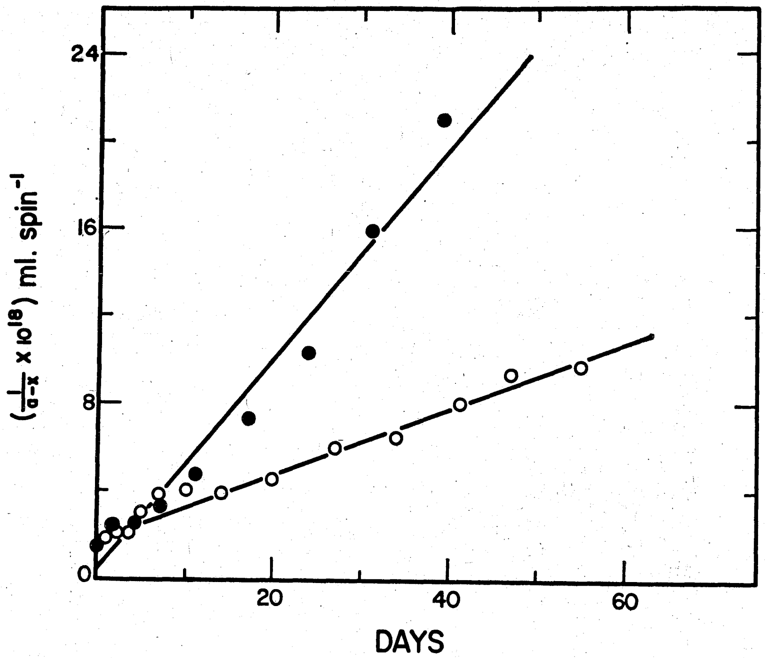

Fig. 4. Second-order kinetic plots for the disappearance of radicals trapped in clear flour after irradiation with 10^6 rads. Open circles, oven-dried flour; closed circles, flour with 3.0% moisture.

Farinograms, Mixograms, and Baking. Samples of the baker's flour, as received without drying, were irradiated with 0.5 and 1.0 million rads. The effects of such irradiation on the farinograms and mixograms are shown in Fig. 5.

In the previous work (1), it was noted that the loaf volumes of bread from irradiated flour, when baked at different times after

TABLE IV
MEAN LOAF VOLUMES[a] OF BREAD FROM IRRADIATED BAKER'S FLOUR AT DIFFERENT TIMES AFTER IRRADIATION

MOISTURE OF FLOUR	DOSAGE	TIME OF BAKING, DAYS AFTER IRRADIATION		
		1	7	28
	megarads	*ml.*	*ml.*	*ml.*
As-received	0	890	880	885
	0.5	850	815	830
	1.0	610	605	605
Oven-dried	0	765	770	760
	0.5[b]	630(3.30)	580(3.09)	550(0.81)
	1.0[b]	490(6.25)	420(3.91)	380(1.35)

[a] Mean of four loaves.

[b] Values in parentheses are concentrations of radicals remaining in the irradiated flour at the time of baking in (spins/ml.) × 10^{-17}.

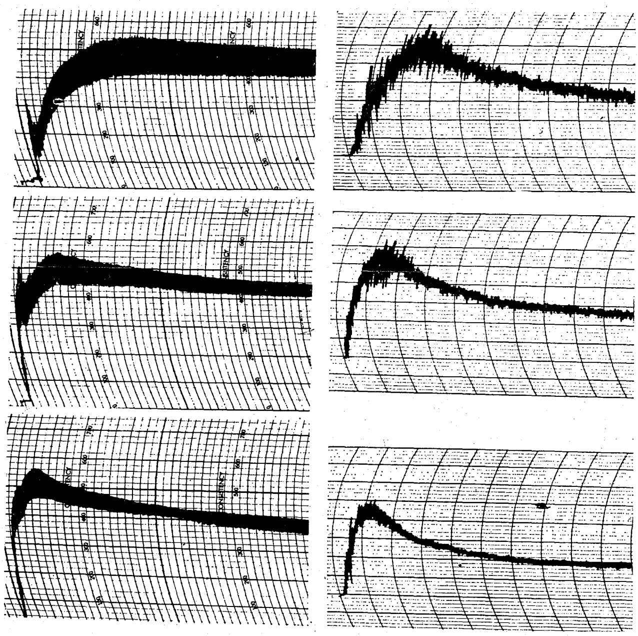

Fig. 5. Farinograms and mixograms of baker's flour before and after irradiation. Radiation dosages from top row to bottom row were 0, 0.5, and 1.0 million rads, respectively.

irradiation, tended to decrease as the elapsed time between irradiation and baking is increased. A similar trend is found in the present study with baker's flour (Table IV). For comparison, mixograms were run for some of the samples just before baking. These are shown in Fig. 6.

Discussion

The results shown in Tables I, II, and III verify in a quantitative way the earlier qualitative finding that moisture will enhance the rate of disappearance of the EPR-detectable radicals in gamma-irradiated flour. The second-order specific rate constant for the disappearance of trapped radicals in a flour with 3.0% moisture is about three times as great as the k_2 for the corresponding oven-dried flour (Table III). The influence of 0.5 and 1.0 million rads of ^{60}Co gamma rays on the farinogram and mixogram (Fig. 5) also corroborates earlier observations in this laboratory (3) on the deleterious effects of such dosages of radiation on the baking quality of similar flours.

The finding that the disappearance of trapped radicals followed second-order kinetics for both oven-dried flour and flour with 3.0%

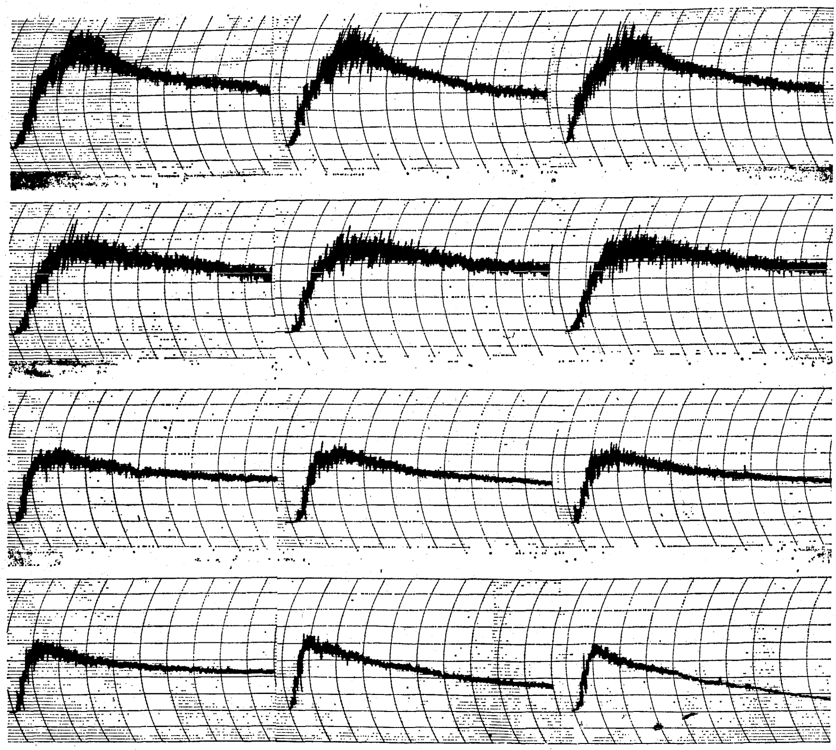

Fig. 6. Mixograms of baker's flour. Top to bottom rows correspond, respectively, to nonirradiated as-received flour, nonirradiated oven-dried flour, oven-dried flours irradiated with 0.5 million rads, and oven-dried flour irradiated with 1.0 million rads. Columns from left to right show curves taken at different times corresponding, respectively, to 1, 7, and 28 days after irradiation.

moisture is of considerable interest. One might suggest that this kinetic order arises from radical recombination as the equation:

$$R\cdot + R\cdot \rightarrow R - R \qquad (1)$$

However, this appears to be unlikely in the case of flour containing 3.0% moisture. The densities of the tightly packed flours used for the EPR measurements were about 0.7 g. per ml. With a water content of 3.0%, the water concentration in such flours would be approximately 10^{-3} moles/ml. Most of the radical concentrations summarized in Tables I and II are within the range of 10^{17} to 10^{18} spins/ml., which is of the order of 10^{-5} moles per ml. After irradiation of flour with 3.0% moisture, water concentration would, therefore, be about 100 times as large as radical concentration. Reaction between radical and water would be more likely than reaction between two radicals as in equation 1. That the disappearance of the gross amounts of radicals in flour containing moisture still follows second-order kinetics may be visualized as proceeding through the following steps:

$$R\cdot + H_2O \rightarrow RH + OH\cdot \tag{2}$$

$$R\cdot + \cdot OH \rightarrow ROH \tag{3}$$

The net result could be depicted by the equation:

$$2R\cdot + H_2O \rightarrow RH + ROH \tag{4}$$

In the case of oven-dried flour, radical recombination as depicted by equation 1 may be possible. However, since the gamma-induced radicals in the flour are present among a great excess of flour constituents from which hydrogen can be abstracted, the fate of the radicals may follow a route analogous to equations 2 and 3, rather than 1.

$$R\cdot + HA \rightarrow RH + A\cdot \tag{5}$$

$$R\cdot + A\cdot \rightarrow RA \tag{6}$$

where HA is a flour constituent capable of losing hydrogen. It is also possible that more than one flour constituent may be involved.

$$R\cdot + HA \rightarrow RH + A\cdot \tag{7}$$

$$A\cdot + HB \rightarrow HA + B\cdot \tag{8}$$

$$R\cdot + B\cdot \rightarrow RB \tag{9}$$

In this way, the rate of disappearance of the radicals would also be of second order. The fact that radical disappearance is faster in moist than in dry flour could be explained on the basis of greater mobility for the hydroxyl radicals and the water molecules. Water can destroy radicals trapped in various parts of flour more efficiently, while involvement of less mobile constituents, HA, HB, etc., which could be carbohydrates and proteins, would lead to slower destruction of the trapped radicals.

Table IV shows the results of the studies on possible changes in baking performance when bread was made with irradiated flour kept for various lengths of time after the irradiation. Over the 4-week period covered in these experiments, the nonirradiated flour did not give any significant changes in loaf volumes. After irradiation, the trend, most pronounced with the oven-dried flour, is that the loaf volume tends to decrease as the elapsed time between irradiation and baking increases. This observation is in agreement with previous results (1). From Fig. 6, it can also be noted that while drying as well as irradiation will cause marked differences in dough-mixing characteristics, the changes in baking performance with time resulted in only very minor, if at all noticeable, effects on the mixograms.

The contradictory findings among different workers of apparent improvement or of damage to breadmaking quality caused by ionizing radiation have been pointed out (4). Maes (5) has suggested the possibility that radiation-induced polymerization, which could strengthen the flour, may be responsible for improvements observed after irradiation. The results of the present work, however, suggest that when flour is irradiated at its usual moisture level of about 12 to 14%, the predominant fate of any free radical fragments resulting from the irradiation will not be polymerization, but more likely will be reaction with water via processes such as equations 2 and 3. The net effect of such reactions will be breakdown of flour constituents, which would be responsible for deterioration in baking quality as well as enhancement in fermentability of starchy components (3).

If polymerization were to occur during irradiation, the most favorable circumstances for it would be during irradiation of dried flour. After irradiation of oven-dried flour, both the concentration of trapped radicals and baking quality decrease with increasing time (Table IV). How the changes in radical concentration and baking quality may be related is not completely clear; but it can be stated that the reactions which the trapped radicals would undergo did not cause a net strengthening of the flour. From such considerations, it is concluded that in the cases where radiation-induced improvements in breadmaking quality were observed, an explanation, such as that suggested by Lai, Finney, and Milner (4), involving increased gas production due to radiation-produced fermentable degradation products, is more reasonable than an explanation involving polymerization.

Acknowledgment

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