

## STUDIES WITH RADIOACTIVE TRACERS

### V. The Reduction of $S^{35}$ -Labeled Persulfate to Sulfate in Flour and Dough Systems<sup>1</sup>

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#### ABSTRACT

$S^{35}$ -labeled persulfate is reduced completely to sulfate by water-flour slurries in about 60 minutes or by water-flour doughs in 15 to 30 minutes. Gluten slurries can also reduce some persulfate to sulfate, but other flour constituents may also take part in reactions with persulfate. Simple extraction of slurry or dough with water alone appears capable of extracting more sulfate than persulfate. A more efficient extraction resulted in the recovery of greater amounts of persulfate. Apparently, persulfate is more strongly adsorbed. In sharp contrast to the behavior of bromate, defatting of the flour by extraction with petroleum ether or with water-saturated *n*-butanol showed hardly any significant influence on the rapid decomposition of persulfate by doughs prepared from ordinary or defatted flour.

In previous work in this laboratory with radioactive tracers, information obtained from studies with  $Br^{82}$ -labeled bromate in flour and dough systems have been reported (6,7,8). An extension of these studies included investigations using  $S^{35}$ -labeled persulfate. Although persulfate, as measured by a fluorometric method (1), has been found to decompose rapidly in flour doughs, a more detailed examination of persulfate decomposition in flour and dough systems by means of the sensitive tracer technique is of interest. This paper reports results obtained on the conversion of  $S^{35}$ -labeled persulfate to sulfate in flour or gluten slurries and in doughs made from ordinary or defatted flour.

#### Materials and Methods

*$S^{35}$ -Labeled Potassium Persulfate.*<sup>2</sup> Potassium persulfate- $S^{35}$  was prepared from potassium sulfate- $S^{35}$  by electrolytic oxidation (4,10). The electrolytic cell was a 20-ml. test tube into which the electrodes were placed. The electrodes consisted of two smooth platinum wires, 12 mm. long and 0.4 mm in diameter, sealed onto a piece of Pyrex tubing. During electrolysis, the anode extended straight downward nearly to the bottom of the cell while the cathode was looped around the Pyrex tubing above the anode near the surface of the electrolyte (4). Immediately before an electrolysis, the electrodes were carefully heated until glowing to ensure the efficiency of a smooth anode (5).

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<sup>2</sup>This compound was prepared by R. Tkachuk.

A solution of 300 mg. reagent grade potassium sulfate and 1.5 mg. potassium ferrocyanide in 3.75 ml. of 1N sulfuric acid containing 5 mc.  $S^{35}$  was placed in the cell. The inclusion of the ferrocyanide has been reported to give better yields (5). The electrodes were then immersed into the electrolyte and electrolysis was carried out for 15 hours with a current of 200 to 210 milliamperes at a potential of 6.5 to 7.0 volts. The temperature of the electrolyte was kept at 12 to 17°C. during the electrolysis by cooling the cell in an ice bath.

After the electrolytic oxidation, the resulting mixture was cooled to just above its freezing point. The crystalline product was filtered off and washed with ice-cold water. To this product, 1.00 g. of ordinary potassium persulfate and 200 mg. of ordinary potassium sulfate were added; the latter served as hold-back carrier to aid the purification. The mixture was rapidly recrystallized twice from water at 90°C. The dried product weighed 1.076 g. The radiochemical yield, based on the  $S^{35}$  activity recovered in the product, was 72%. The radiochemical purity of the product was found to be greater than 99.5% persulfate.

*Flour and Freeze-Dried Gluten.* The flour used in all experiments was an untreated, commercially milled, patent flour. Its protein and ash contents, on 14% moisture basis, were 12.4% ( $N \times 5.7$ ) and 0.35%, respectively. The freeze-dried gluten was prepared from this flour according to the method of Lusena (9).

*Methods for Water Slurries of Flour or Freeze-Dried Gluten.* Ten grams of flour (at 14% moisture) and 100 ml. of distilled water, containing  $S^{35}$ -labeled potassium persulfate amounting to 100, 200, 300, 400, or 1000 p.p.m. based on the weight of flour, were stirred in a Waring Blendor at high speed for 5, 15, 30, or 60 minutes. The resulting mixture was centrifuged and the clear supernatant liquid, to be called the simple aqueous extract, was used for determinations of total recovery of  $S^{35}$  and of conversion of persulfate to sulfate.

The recoveries of  $S^{35}$  in the simple aqueous extracts described above were relatively low. In another set of experiments with water-flour slurries, the extraction procedure was modified so as to give greater  $S^{35}$  recoveries. The method adopted was essentially that of Auerbach and co-workers (1), with the exception that zinc chloride was used in place of zinc sulfate. One gram of flour and 34 ml. of distilled water containing 100, 200, 300, 400, or 1000 p.p.m. potassium persulfate- $S^{35}$  based on the weight of flour were shaken for 5 minutes by a mechanical shaker. Four milliliters of 0.3N zinc chloride, 12 ml. of 0.1N sodium hydroxide, and 0.5 g. of Celite filter aid were then added and the mixture shaken for an additional 15-minute period.

After centrifugation, the supernatant liquid, to be called the zinc hydroxide-clarified extract, was used for activity assays.

In the studies with freeze-dried gluten slurries, 1.0 g. of the gluten and 34 ml. of distilled water containing 100, 200, 300, 400, or 1,000 p.p.m. labeled postassium persulfate based on the weight of gluten were shaken for 5 minutes. The resulting mixture was worked up to give the zinc hydroxide-clarified extract exactly as described for the water-flour slurries.

*Methods for Doughs.* Nonfermenting doughs were prepared from 100 g. (at 14% moisture) of ordinary flour, flour defatted by extraction with petroleum ether, b.p. 40 to 60°C., or flour defatted by extraction with water-saturated *n*-butanol (11), 60 ml. of distilled water, and 100, 200, 300, 400, or 1,000 p.p.m. S<sup>35</sup>-labeled potassium persulfate. The fat removed by extraction with petroleum ether and with water-saturated *n*-butanol were, respectively, 0.69 and 1.47% of the weight of flour. Each of these doughs was mixed for 3 minutes in a Hobart mixer and then placed in a cabinet maintained at 30°C. Suitable subsamples were removed at 0, 15, and 30 minutes after mixing and worked up to give either simple aqueous extracts or zinc hydroxide-clarified extracts of the dough.

Simple aqueous extracts were obtained from doughs from ordinary and petroleum ether-extracted flours by dispersing a 20-g. subsample in 100 ml. of distilled water in a Waring Blendor operated at high speed for 5 minutes. Zinc hydroxide-clarified extracts were obtained from doughs from ordinary, petroleum ether-extracted, and water-saturated *n*-butanol-extracted flours. In each extraction, a 2.0-g. subsample of dough was dispersed in 68 ml. of distilled water, 8 ml. of 0.3*N* zinc chloride, 24 ml. of 0.1*N* sodium hydroxide and 1.0 g. of Celite in a Waring Blendor operated at high speed for 5 minutes.

*Determinations of Sulfate and Persulfate.* Aqueous extracts from flour slurries, gluten slurries, or doughs were assayed for sulfate and persulfate by the removal of the sulfate as insoluble barium sulfate (3,12). Suitable quantities of inactive sulfate as carrier and inactive persulfate as hold-back carrier were added before the precipitation of the barium sulfate. Since aqueous extracts from these experiments may contain both sulfate-S<sup>35</sup> and persulfate-S<sup>35</sup>, the accuracy of the actual experimental procedure was tested by trial assays with known mixtures of sulfate-S<sup>35</sup> and persulfate-S<sup>35</sup>. For example, with a mixture of 20% sulfate-S<sup>35</sup> and 80% persulfate-S<sup>35</sup>, after removal of barium sulfate, the activity remaining in solution amounted to 79.5 and 80.2% for duplicate trials.

Corrections for radioactive decay in the counting of all S<sup>35</sup> samples

were made in the following way: Immediately before and after the counting of any given sample, a sample containing a fixed amount of  $S^{35}$ , to be called the standard, was also counted. The  $S^{35}$  activity of the standard used in all the present experiments was 1,000 c.p.m. at an arbitrarily fixed zero time. If the activity of a given sample were  $n$  c.p.m. and the mean activity of the standard counted before and after the counting of the sample were, for example, 900 c.p.m., then the activity of the sample corrected to zero time would be

$$\frac{1000}{900} \times n \text{ c.p.m.}$$

### Results and Discussion

As an illustration of the actual experimental results and calculations, a description is given for the determination of  $S^{35}$  recovery and sulfate content in one of the duplicate experiments with the simple aqueous extract from the flour slurry which was stirred in the Waring Blendor for 5 minutes with 100 p.p.m. labeled potassium persulfate. To 40 ml. of this extract, 1.0 ml. of a solution of ordinary potassium persulfate (27 mg. or 0.1 mmole per ml.) and 1.0 ml. of a solution of ordinary potassium sulfate (27 mg. or 0.15 mmole per ml.) were added as carriers. Two-milliliter aliquots were then dried in aluminum pans and counted. The mean activity, corrected to zero time, was 1,940 c.p.m.

The total volume of aqueous extract, including the water from the 14% moisture in the flour, was 101.4 ml. Hence the total recovery of  $S^{35}$  in the simple aqueous extract was  $(101.4/2) \times (42/40) \times 1,940 = 103,300$  c.p.m.

To determine the total  $S^{35}$  activity originally added to the flour slurry, a solution was prepared by adding a known weight of potassium persulfate- $S^{35}$  to an aqueous extract from nonradioactive slurry obtained exactly in the same way as in the experiments with slurries containing labeled persulfate. The counting of dried aliquots of such a known solution gave 202,500 c.p.m. as the activity, corrected to zero time, for the 1.0 mg. of potassium persulfate- $S^{35}$  used in this 100-p.p.m. experiment.

Therefore, the recovery of  $S^{35}$  in this sample aqueous extract was  $(103,300/202,500) \times 100 = 51\%$ .

For the estimation of the sulfate content in this aqueous extract, 20 ml. of the 42 ml. of extract plus carriers were treated with 1.0 ml. of a solution of barium chloride (40 mg. or 0.19 mmole per ml.). The precipitated barium sulfate was removed by centrifuging and 2.0-ml. aliquots of the supernatant liquid dried and counted. The

mean activity, corrected to zero time, was 790 c.p.m.

Therefore, the total  $S^{35}$  activity remaining after removal of sulfate was  $790 \times (21/20) \times (101.4/2) \times (42/40) = 44,100$  c.p.m.

Since the total  $S^{35}$  recovered in the extract was 103,300 c.p.m., the sulfate content was  $(103,300 - 44,100/103,300) \times 100 = 57\%$  of the recovered  $S^{35}$ .

In an analogous manner, the  $S^{35}$  recoveries and sulfate contents in all the extracts from the slurries and doughs were determined. The mean results from duplicate experiments are tabulated in Tables I to V.

*Discussion of Results from Studies with Slurries.* For water-flour slurries, conversion of persulfate to sulfate was apparently complete after a stirring time of about 60 minutes (Table I). The recoveries of  $S^{35}$  in the simple aqueous extracts of flour slurries ranged between 51 and 68% of the persulfate initially added (Table I). Some 30 to 50% of the initially added persulfate was thus lost from the aqueous phase,

TABLE I  
RECOVERY OF  $S^{35}$  AND CONVERSION OF PERSULFATE TO SULFATE IN SIMPLE  
AQUEOUS EXTRACTS FROM WATER-FLOUR SLURRIES

INITIAL PERSULFATE (I.P.) LEVEL	STIRRING TIME (MINUTES)							
	5		15		30		60	
	$S^{35}$ Recovery in Extract	Sulfate Content of Recovered $S^{35}$	$S^{35}$ Recovery in Extract	Sulfate Content of Recovered $S^{35}$	$S^{35}$ Recovery in Extract	Sulfate Content of Recovered $S^{35}$	$S^{35}$ Recovery in Extract	Sulfate Content of Recovered $S^{35}$
ppm	%I.P.	%	%I.P.	%	%I.P.	%	%I.P.	%
100	52	57	64	82	64	97	57	100
200	53	56	61	77	60	97	62	100
300	54	50	58	75	60	98	61	100
400	56	51	59	77	56	97	58	100
1000	60	37	68	75	66	94	65	99

TABLE II  
RECOVERY OF  $S^{35}$  AND CONVERSION OF PERSULFATE TO SULFATE IN ZINC HYDROXIDE-  
CLARIFIED EXTRACTS FROM WATER SLURRIES OF FLOUR AND OF FREEZE-DRIED GLUTEN<sup>a</sup>

INITIAL PERSULFATE (I.P.) LEVEL	$S^{35}$ RECOVERY IN EXTRACT		SULFATE CONTENT OF RECOVERED $S^{35}$	
	Flour Slurry	Gluten Slurry	Flour Slurry	Gluten Slurry
ppm	%I.P.	%I.P.	%	%
100	96	76	37	78
200	100	87	36	73
300	97	71	33	74
400	98	78	38	74
1000	93	77	26	71

<sup>a</sup> The slurries were worked up after 1.0 g. of flour or 1.0 g. of freeze-dried gluten was shaken in 34 ml. of distilled water for 5 minutes.

probably through adsorption by the flour. There is, however, no apparent relationship between the degrees of  $S^{35}$  recovery and experimental variables such as initial persulfate level or length of stirring time. When the extraction of the flour slurry was effected by the method involving clarification with zinc hydroxide, recovery of all  $S^{35}$  in the aqueous phase was nearly complete (Table II).

It may be of interest to note that the sulfate contents in the zinc hydroxide-clarified extracts of flour slurries were some 10 to 20% less than the sulfate contents in the corresponding simple aqueous extracts of flour slurries that had been stirred for only 5 minutes (compare Tables I and II). This finding appears to indicate that simple extraction with water alone may remove more sulfate than persulfate, or in other words, persulfate may be more strongly adsorbed than sulfate. Another factor which probably contributed to the lower sulfate contents in the zinc hydroxide-clarified extracts may be regarded as a concentration effect. It will be recalled that in obtaining a simple aqueous extract of flour slurry, a total of 100 ml. of water was used on 10 g. of flour, while in obtaining the zinc hydroxide-clarified extract, the final volume was 50 ml. for 1.0 g. of flour. For a given initial persulfate level, the persulfate concentration in the aqueous phase is, therefore, higher under the conditions where the simple aqueous extract was obtained. Such a higher concentration of a reactant may lead to faster reaction, resulting in a greater amount of conversion of persulfate to sulfate in the simple aqueous extract.

Slurries of freeze-dried gluten also reduced some persulfate to sulfate. The extents of conversion of persulfate to sulfate by 5 minutes of shaking of water slurries of 1.0-g. samples of gluten were in the neighborhood of 75% (Table II). Under similar conditions, the conversions of persulfate to sulfate by water slurries of 1.0-g. samples of flour were approximately 35% (Table II). Since only about 0.15 g. of freeze-dried gluten was obtainable from 1.0 g. of flour, the gluten in 1.0 g. of flour could probably account for  $0.15 \times 75 = 11\%$  of the conversion of persulfate to sulfate, which is considerably less than the actual 35% conversion effected by 1.0 g. of flour under these conditions. Such considerations may be regarded as indicating that components of flour other than gluten also undergo oxidation-reduction reactions with persulfate. A similar conclusion was arrived at from measurements of the degrees of reduction of  $Br^{82}$ -labeled bromate to bromide by water slurries of flour and of freeze-dried gluten (8). Another explanation for the difference between gluten and flour in conversion of persulfate to sulfate may also be considered. If some de-

naturation had occurred during the preparation of the freeze-dried gluten, then it is possible that the isolate gluten would not react with as much persulfate as an equivalent amount of gluten in the original flour.

*Discussion of Results from Studies with Doughs.* In the studies with doughs, simple extraction of 20-g. subsamples with 100 ml. of water resulted in recoveries in the extract of only about 35 to 45% of the  $S^{35}$  initially introduced as persulfate. The  $S^{35}$  present in such simple aqueous extracts was practically all in the form of sulfate, even when the water extraction was carried out immediately after the dough was formed by 3 minutes of mixing. Similar results were observed when the dough was prepared from either ordinary or petroleum ether-extracted flour.

Extraction of 2.0-g. subsamples of dough by the more efficient method involving zinc hydroxide clarification resulted in higher  $S^{35}$  recoveries of the order of 70 to 80%. For doughs extracted immediately after mixing, some unchanged persulfate was present in the zinc hydroxide-clarified extracts, as the sulfate contents of such extracts were in the neighborhood of 80% of the recovered  $S^{35}$ . That the nonsulfate portion of the recovered  $S^{35}$  was indeed unchanged persulfate was indicated by the observation that such residual  $S^{35}$ -containing material could again be completely converted to sulfate by heating with dilute hydrochloric acid. The hydrochloric acid presumably was oxidized by the unchanged persulfate. If the doughs were allowed to rest for various lengths of time before extraction, conversion of persulfate to sulfate was practically complete when the rest time was 15 to 30 minutes. Essentially similar results were observed for doughs prepared from ordinary flour, petroleum ether-extracted flour, or water-saturated *n*-butanol-extracted flour (Tables III, IV, V).

Comparing the conversions of persulfate to sulfate in doughs and in flour slurries, it is clear that such conversions are faster in the doughs. A factor, though not necessarily the only factor, that contributes to the differences in rates of persulfate decomposition in slurries and doughs may be the effects of reactant concentration. Since the volume of water used in making up a dough is much less than the volume used for a slurry, one may regard the concentration of reactants in doughs as higher than in slurries. Thus, in a dough the reactions are faster, and consequently there is a more rapid conversion of persulfate to sulfate.

The rapid and complete reduction of persulfate to sulfate by water-flour doughs is quite different from the behavior of bromate (6).

TABLE III  
RECOVERY OF S<sup>35</sup> AND CONVERSION OF PERSULFATE TO SULFATE IN ZINC  
HYDROXIDE-CLARIFIED EXTRACTS OF DOUGHS FROM ORDINARY FLOUR

INITIAL PERSULFATE (I.P.) LEVEL	REST TIME AFTER MIXING OF DOUGH (MINUTES)					
	0		15		30	
	S <sup>35</sup> Recovery in Extract	Sulfate Content of Recovered S <sup>35</sup>	S <sup>35</sup> Recovery in Extract	Sulfate Content of Recovered S <sup>35</sup>	S <sup>35</sup> Recovery in Extract	Sulfate Content of Recovered S <sup>35</sup>
<i>ppm</i>	<i>%I.P.</i>	<i>%</i>	<i>%I.P.</i>	<i>%</i>	<i>%I.P.</i>	<i>%</i>
100	72	83	74	97	83	100
200	82	78	74	96	81	100
300	77	81	76	97	81	99
400	73	81	81	98	84	100
1000	76	71	77	90	79	97

TABLE IV

RECOVERY OF S<sup>35</sup> AND CONVERSION OF PERSULFATE TO SULFATE IN ZINC HYDROXIDE-  
CLARIFIED EXTRACTS OF DOUGHS FROM PETROLEUM ETHER-EXTRACTED FLOUR

INITIAL PERSULFATE (I.P.) LEVEL	REST TIME AFTER MIXING OF DOUGH (MINUTES)					
	0		15		30	
	S <sup>35</sup> Recovery in Extract	Sulfate Content of Recovered S <sup>35</sup>	S <sup>35</sup> Recovery in Extract	Sulfate Content of Recovered S <sup>35</sup>	S <sup>35</sup> Recovery in Extract	Sulfate Content of Recovered S <sup>35</sup>
<i>ppm</i>	<i>%I.P.</i>	<i>%</i>	<i>%I.P.</i>	<i>%</i>	<i>%I.P.</i>	<i>%</i>
100	71	87	73	97	80	100
200	67	80	73	97	63	100
300	75	81	74	97	72	99
400	79	75	76	93	72	98
1000	72	68	73	88	79	96

TABLE V

RECOVERY OF S<sup>35</sup> AND CONVERSION OF PERSULFATE TO SULFATE IN ZINC HYDROXIDE-  
CLARIFIED EXTRACTS OF DOUGHS FROM WATER-SATURATED *n*-BUTANOL-EXTRACTED FLOUR

INITIAL PERSULFATE (I.P.) LEVEL	REST TIME AFTER MIXING OF DOUGH (MINUTES)					
	0		15		30	
	S <sup>35</sup> Recovery in Extract	Sulfate Content of Recovered S <sup>35</sup>	S <sup>35</sup> Recovery in Extract	Sulfate Content of Recovered S <sup>35</sup>	S <sup>35</sup> Recovery in Extract	Sulfate Content of Recovered S <sup>35</sup>
<i>ppm</i>	<i>%I.P.</i>	<i>%</i>	<i>%I.P.</i>	<i>%</i>	<i>%I.P.</i>	<i>%</i>
100	81	85	81	93	80	98
200	74	83	80	99	75	99
300	80	82	82	96	82	99
400	80	83	83	95	77	99
1000	76	69	82	91	76	97

The fact that persulfate is a more powerful oxidizing agent than bromate may at least be partly responsible for the different behavior of persulfate and bromate in flour slurries and doughs.

From the studies with doughs made from flour defatted by extraction with petroleum ether or with water-saturated *n*-butanol, the results obtained show hardly any significant differences in the rapid decomposition of persulfate by doughs from ordinary or defatted flours (Tables III to V). Such results are again in sharp contrast to the behavior of bromate. Lee and Tkachuk (7) have noted a marked decrease in the degree of conversion of Br<sup>82</sup>-labeled bromate to bromide when the dough was prepared from petroleum ether-extracted flour. Similarly, decreases in the rates of disappearance of bromate, measured by amperometric titration, were reported by Cunningham and Hlynka (2) for doughs prepared from flours defatted by extraction with petroleum ether or water-saturated *n*-butanol. Thus flour lipids appear to be involved, either directly or indirectly, in the oxidation-reduction reactions of bromate in dough systems. With persulfate, on the other hand, the flour lipids do not appear to exert any appreciable effect on its rapid decomposition by the dough.

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