

The Decomposition
Of
S³⁵-Labeled Potassium Persulfate By
Flour Doughs

A Thesis

Submitted to the Faculty of Graduate Studies
in Partial Fulfilment of the Requirements
for the Degree of
Master of Science

in the Department of Chemistry
University of Saskatchewan

by



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Saskatoon, Saskatchewan

March, 1959.

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Acknowledgment

The author wishes to express his gratitude to Dr. C.C. Lee for his assistance throughout the course of the experimental work and his advice in the preparation of this thesis.

I also wish to thank Mr. R. Tkachuk for his preparation of the radioactive persulfate used throughout the work, to Mr. R.G. Teed for his technical assistance and to the Saskatchewan Research Council for the award of a Province of Saskatchewan Graduate Research Scholarship for 1957-58.

-- Table of Contents --

	page
1. Introduction.....	1
11. Review of applications of radioactive isotopes to studies in Cereal Chemistry.....	7
111. Experimental methods and results	
A. Preparation of S ³⁵ -labeled potassium persulfate.....	10
B. Determination of sulfate and persulfate.....	11
C. Conversion of persulfate to sulfate by flour slurries.....	11
Table 1 - Recovery of S ³⁵ and conversion of persulfate to sulfate in simple aqueous extracts from water-flour slurries.....	14
Table 2 - Recovery of S ³⁵ and conversion of persulfate to sulfate in zinc hydroxide clarified aqueous extracts from water-flour slurries.....	16
D. Conversion of persulfate to sulfate by water-gluten slurries.	17
Table 3 - Recovery of S ³⁵ and conversion of persulfate to sulfate in zinc hydroxide clarified aqueous extracts from water-gluten slurries.....	19
Table 4 - Recovery of S ³⁵ and conversion of persulfate to sulfate in zinc hydroxide extracts from water slurries of petroleum ether extracted gluten.....	20
E. Conversion of persulfate to sulfate by water-flour doughs.....	18
Table 5 - Recovery of S ³⁵ and conversion of persulfate to sulfate in simple aqueous extracts of water-flour doughs from ordinary flour.....	23
Table 6 - Recovery of S ³⁵ and conversion of persulfate to sulfate in simple aqueous extracts of water-flour doughs from petroleum ether extracted flour.....	24
Table 7 - Recovery of S ³⁵ and conversion of persulfate to sulfate in zinc hydroxide clarified aqueous extracts of water-flour doughs from ordinary flour.....	27
Table 8 - Recovery of S ³⁵ and conversion of persulfate to sulfate in zinc hydroxide clarified aqueous extracts of water-flour doughs from petroleum ether extracted flour.....	28
Table 9 - Recovery of S ³⁵ and conversion of persulfate to sulfate in zinc hydroxide clarified aqueous extracts of water-flour doughs from water-saturated n-butyl alcohol extracted flour.....	30

(Table of contents 2.....cont'd)

Page

F. Conversion of persulfate to sulfate by extracted "fat" of flour.....	29
G. Determination of unchanged persulfate in extracts.....	31
1V. Discussion of results.....	33
Figure 1 - Mean % conversion of persulfate to sulfate in simple aqueous extract from water-flour slurries vs. length of time slurry was stirred in Waring Blendor.....	34
Figure 2 - Mean sulfate content in zinc hydroxide clarified aqueous extracts of doughs vs. reaction time after mixing.....	40
V. Bibliography.....	43

1. Introduction

The botanical classification Triticum vulgare, or common wheat, includes many varieties of wheat used for bread making. Hard red spring wheat grown in Western Canada is a bread wheat used for the manufacture of high quality flour.

On the basis of the percentage of dark, hard and vitreous kernels, hard red spring wheat may be graded into three subclasses: dark northern spring, northern spring and red spring. Other factors considered in the final determination of the numerical grade are damaged grain, the presence of foreign matter, mustiness, excessive water, heating grain, or any factor that may render the grain of distinctly low quality. In general, when well matured, the kernels are hard and flinty, rich in protein and possess the qualities that permit the baking of large loaves of bread from a given quantity of flour.

Canadian wheat is described as "strong" wheat of high "quality". These loose terms are used to describe collectively the properties of the wheat. Strength in wheat may be regarded as the factor or factors which will normally produce large well-piled loaves, provided that the gas production is sufficient. In practice this can often be judged from the behaviour and the properties of the fermenting doughs. A number of mechanical dough testing instruments have made possible the analysis of "strength" in terms as such physical properties as elasticity, extensibility and consistency.

The government of Canada has provided steps at maintaining high quality of our wheat. The Canada Grain Act stipulates that no new variety can be authorized unless its quality is equal to or better than **Marquis**. This quality evaluation has been entrusted to an associate committee of the National Research Council.

Various experimental data are considered collectively in evaluating quality. Some of the factors considered in determining wheat quality are bushel weight, flour yield, wheat protein, flour protein, flour color, flour pigment, baking absorption, loaf volume, crumb texture and color.

One of the most reliable criteria on which quality evaluation is made is the baking test. Experimental loaves of bread actually baked under controlled conditions are scored on loaf volume and shape, crumb color and crust color. The baking test is generally carried out with and without an added "flour improver" such as potassium bromate. The ability of the flour to respond to improvers is also considered in the overall quality evaluation.

In the baking industry, it was known for a long time that ageing of the flour improves the character of the resulting bread. Similar effects are obtained by the addition of minute quantities of oxidizing agents or flour improvers. Some of the commonly used improvers (1) are potassium and ammonium persulfates, potassium bromate and potassium iodate. Persulfates are popular in Europe, and at one time most of the flour in England was treated with persulfate. Bromate is especially liked in Canada and the U.S.A. and it is used as a normal dough ingredient in the standard baking test.

Certain chemical agents show a bleaching action as well as improving action. Nitrogen trichloride, sold under the name Agene, has a marked strengthening effect upon the gluten. The bleaching and maturing of flour with chlorine dioxide is capable of giving results comparable with those obtained by the use of nitrogen trichloride (1).

The nature of the improver action is still obscure, though bromate improvement was observed over forty years ago (2). At first, improvers

were thought to be nutrients for the yeast, thus stimulating gas production. It is now generally accepted that improvers have no significant influence upon gas production but function by improving gas retention (1). How this comes about has not been solved. There are two theories which are most widely studied.

Jorgensen (3,4,5) suggested that improvers exert their effect by suppressing indirectly the action of proteolytic enzymes of flour. His theory is that oxidizing agents inactivate the proteinases of the wheat flour. When these proteinases are inactivated, the breakdown of the protein of the dough diminishes. As a result, the gas retaining capacity of the dough membrane increases. A number of important arguments supporting the theory are as follows:

1. The activity of many proteinases from tissues or cells is strongly reduced by oxidizing agents.

2. Potassium chlorate which does not improve the baking strength of wheat flour, does not depress the activity of proteinases.

3. It can be shown that the activity of the proteinases of wheat malt and wheat germ is reduced by potassium bromate.

4. Ascorbic acid, known to be able to reduce the activity of plant proteinases, has been found to be a good improver of the baking strength of wheat flour.

Though the proteolytic activity in flour has been found to be very low (6), Jorgensen claimed that in the presence of a suitable activator, glutathione for instance, the effects of the powerful but latent proteolytic enzymes would become important.

Support for the proteolytic theory was advanced by Balls and Hale (7). It was pointed out that proteinases usually produced first a

coagulation of the protein; later the coagulated material is broken down and perhaps ultimately dissolved. Applying this to the flour gluten, in the first phase of proteinase action, the gluten would probably become more tenacious; in the second phase, it would be broken down to a thinner, more nearly liquid material. It is to be expected, therefore, that a small amount of proteinase in flour would be beneficial. A large amount of proteinase would, on the contrary, be most harmful. The behaviour of flours with application of improvers was postulated to be due to a diminution of the proteolytic activity, brought about by the oxidation of the activator of the flour proteinase.

A strictly chemical hypothesis was presented by B. Sullivan and coworkers (8,9,10). These workers suggested that the effect of improvers is attributable to a direct oxidation of the sulfur linkages of the flour proteins. They pointed out that the deleterious effect of wheat germ on the baking quality of flour was due to glutathione. The fact that it was glutathione in germ, rather than the proteolytic enzyme itself, which is the cause of the injurious action of the germ, was shown by boiling the water extract for a short time. The heated extract still had an injurious effect on the dough quality. They concluded by postulating two main theories. Both indicate that changes in the sulfur linkages of the gluten proteins are responsible for many of the effects observed. The postulates are as follows:

1. There are present in gluten certain -S-H groups bound to other protein linkages or -S-S- groups capable of reduction to -S-H. During fermentation, some of these sulfhydryl groups can be oxidized by oxygen from the air or from oxidizing agents such as bromate, giving rise to disulfide cross-linkages between protein chains, thus improving the gas

retention power of the gluten.

2. If it were assumed that the gluten strands are coiled fibrils, it is conceivable that a main or side chain containing R-S-S-R may be acted on by oxygen and water (or agents capable of supplying oxygen) to form compounds where the sulfur linkage would be altered or broken. If a scission of the molecule should take place, it would result in shorter strands and less extensibility, as observed when an excess of an improver is used. Similarly certain reducing agents might attack the sulfur linkage not causing cleavage but only the uncoiling of the long fibrils producing greater softness and extensibility but less elasticity.

Considering all evidence, Freilich and Frey (11) concluded that the whole picture of flour improvers could not be justified by use of one theory. They suggested that three different factors, namely, direct action on the gluten, inhibition of proteolytic action and oxidation of reducing substances may all be involved in the effects of oxidation in dough.

In some of the more recent studies on the improver problem, one line of attack has been the determination of the rate of reduction or decomposition of the improvers by flours and doughs. Thus the decomposition of bromate in fermenting and nonfermenting doughs has been followed by Cunningham and Anderson (12) by means of amperometric titration, while Lee, Tkachuk and Finlayson (13) have measured the conversion of bromate to bromide in fermenting and nonfermenting doughs and in baked bread by use of radioactive bromine -82 as tracer. Detailed studies on persulfate, however, are still lacking. A fluorometric method for the determination of persulfate in flour and dough has been reported (14). It was claimed to be sensitive for the quantitative determination of as little as 5 to 10

parts ammonium persulfate per million parts of flour or dough. The method involves the re-oxidation of leuco-fluorescein to fluorescein through the action of persulfate ion. Under controlled conditions, the intensity of the developed fluorescence bears a reproducible relationship to the persulfate content of the sample.

Since persulfates are used only in the order of 200 p.p.m., the measurement by ordinary analytical means of such minute amounts of improver in relatively large quantities of flour is, at best, rather difficult. The application of radioactive tracers, with its high sensitivity and the ease of identification of the labeled compounds by the carrier technique, provides an excellent tool for determining the fate of flour improvers in dough systems. Conn and coworkers (15), for example, have used I^{131} -labeled potassium iodate to investigate the decomposition of iodate during the baking of bread, and have shown the major decomposition product to be iodide. In the present work, the degrees of reduction of S^{35} -labeled persulfate to sulfate are determined for water slurries of flour and of gluten, and for nonfermenting doughs prepared from ordinary and defatted flours.

11. Review of Applications of Radioactive Isotopes to Studies in Cereal Chemistry

The earliest work done in this field was by Spinks and Tollefson (16), who described a convenient and rapid radioactive tracer method of determining adsorption of ruthenium ions by flour. The adsorption of Ru^{+++} by four wheat flour mill streams increased with decreasing grade of flour, and they suggested that it was possible that the method may be of value as a quick way for estimating baking quality on a small sample of flour.

Conn and coworkers (15) used potassium iodate containing radioactive iodine, I^{131} , to determine the fate of iodate during the baking of bread. Bread samples baked from dough containing radioactive potassium iodate were extracted with ammoniacal methanol to recover iodate and iodide. Carriers were added to facilitate the separation of these salts, and to minimize the extent of the side reactions of these components during the extraction process. Less than 7.5 % of the original 3.5-4 p.p.m. iodate was found in the baked bread, the major decomposition product being iodide.

The determination of sulfhydryl groups in flour by means of o-iodosobenzoate and radioactive iodine, carried out by Nordin and Spencer (17), was also one of the earlier applications of tracers. The original method for the determination of sulfhydryl groups in certain proteins by means of o-iodosobenzoate had to be modified when used for flour due to the interference of starch. This modification consisted of determining the excess iodosobenzoate by the addition of sodium iodide containing radioactive iodide. The free iodine liberated was adsorbed by the starch and the unreacted sodium iodide was then determined in the supernatant by a dip counter. By this method, results were obtained which indicated that the sulfhydryl content ranged from 1.2×10^{-6} equivalents per gram for a patent flour, 1.6×10^{-6} for a break flour to 2.3×10^{-6} for the red dog portion.

Burtle and Sullivan (18) used C^{14} -labeled carbon dioxide to test the permeability of fermenting sponges to carbon dioxide. Results showed that the carbon dioxide retained above fermenting dough in cabinet fermentation was easily capable of diffusing back into the sponge even against the developing internal gas pressure of the mass.

A recent application of tracers to Cereal Chemistry was by Lee, Tkachuk, and Finlayson (13), who studied the conversion of Br^{82} -labeled bromate to bromide in nonfermenting and fermenting doughs and in baked bread. Conversions of the labeled bromate to bromide were investigated for initial bromate concentrations of 5, 10, 15, 20 and 30 p.p.m. Reduction of part of the bromate to bromide was found in all cases, the rates of their conversion ranging from 0.08 to 0.36 p.p.m. per hour for the nonfermenting doughs and 0.25 to 1.05 p.p.m. per hour for the fermenting doughs. The amounts of bromate converted to bromide were greater in the bread than in the corresponding fermenting doughs when these doughs were ready for baking, indicating reductions of further quantities of the bromate during baking. While these observations do not constitute proof of any mechanism of improver action, the findings show unequivocally that some oxidation-reduction reactions have taken place and would, therefore, be consistent with mechanisms involving oxidation-reduction reactions.

Lee (19) also showed the effects of Co^{60} gamma rays on the baking quality and maltose value of irradiated flour. Loaf volumes of bread baked from two grades of flour (patent and baker's) were decreased by irradiation of the flours with Co^{60} gamma rays at dosages of 0.25, 0.5 and 1.0 million roentgens. Recoveries of crude gluten from all the irradiated samples were also decreased. A marked adverse bromate effect as well as an increase in the water soluble non-protein nitrogen were found for the

flours that were treated with 10^6 r. A regular increase in maltose value and gassing power with increasing dosages of radiation was observed for both grades of flour, and this was attributed to fragmentation of the starch, thus enhancing its ability to undergo hydrolysis.

Lee and Tkachuk (20) also used Br^{82} -labeled potassium bromate to study the presence of unchanged bromate in bread. Experimental loaves of bread were baked from a commercial, intermediate grade flour treated with 5, 10, 15, 20, 25, or 30 p.p.m. bromate. Aqueous extracts of the bread accounted for 53-60 % of Br^{82} activity as bromide, while 33 to 42 % was bromate. Contrary to the earlier belief that all bromate used as improver would have decomposed after baking these results indicate that some unchanged bromate is still present in the finished bread.

The most recent studies with radioactive tracers by Lee and Tkachuk (21), show the effects of defatting and of benzoyl peroxide on the decomposition of Br^{82} -labeled bromate by water-flour doughs. The decomposition of Br^{82} -labeled bromate to bromide by water-flour doughs was studied using commercially milled patent, intermediate and low grade flours. Doughs containing 15 p.p.m. labeled bromate were prepared from each grade of the original flours, defatted flours, original flours treated with benzoyl peroxide and defatted flours treated with benzoyl peroxide. Measurements of the bromide and bromate contents in aqueous extracts of various doughs showed that the lower flour grade, the greater the decomposition of bromate to bromide. Defatting as well as treatment with benzoyl peroxide markedly decreased the amounts of bromate reduced to bromide for all the flours studied. The results led to the conclusion that bromate, a nonspecific oxidizing agent, may take part in a number of oxidation-reduction reactions with various oxidizable constituents of dough.

111. Experimental Methods and Results¹

A. Preparation of S³⁵-labeled potassium persulfate²

The S³⁵ was purchased as carrier free sulfate from the Atomic Energy of Canada Limited, Chalk River, Ontario. Its radiochemical purity was established by following its half-life, which gave the accepted value of 87 days³. The potassium persulfate-S³⁵ was prepared by electrolytic oxidation (22,23,24) from potassium sulfate containing approximately 5 mc. S³⁵. The electrolytic cell was an ordinary 20 ml. test-tube into which the electrodes were placed: The electrodes consisted of smooth platinum wires projecting out of a "Pyrex"-glass tube, and were 12 mm. long, and 0.4 mm. in diameter. The cathode was so arranged that during electrolysis it looped around the glass tubing, above the anode which projected downwards. A current of 200 ma. at 6.5 volts during electrolysis supplies the high current density of approximately 1.25 amperes /cm² which is required in this preparation. Just previous to a preparation, the electrodes were carefully heated until glowing.

A solution of 300 mg. reagent grade potassium sulfate and 1.5 mg. potassium ferrocyanide in 3.75 ml. 1.0N sulfuric acid was placed in a 20 ml. test-tube. The two electrodes were then immersed into the electrolyte and electrolysis was carried out for 15 hours, with a current of approximately 200-210 ma. at a potential of 6.5-7.0 volts. The temperature of the electrolyte was kept at 12-17°C during the electrolysis. When electrolytic oxidation was complete, the reaction mixture was well cooled to just above its freezing point. The crystalline product was filtered off and washed with cold water. To this product, 1.0 g. of inactive potassium persulfate and 200 mg. potassium sulfate were added. The mixture was then twice, rapidly recrystallized from water at 90°C. The dried product weighed 1.076 g.

1. All experiments were carried out at room temperature with the exception that when the doughs were held at rest in the fermentation cabinet at 30°C.

2. Kindly carried out by Mr. R. Tkachuk.

3. Half-life of S³⁵ is 87.1 days.

equivalent to a yield of 72.5 %. By the carrier technique, radioactivity measurement after removal of barium sulfate showed that this material contained 99.6% persulfate.

B. Determination of Sulfate and Persulfate¹

Aqueous extracts of flour, gluten or dough initially treated with potassium persulfate-S³⁵ were assayed for sulfate and persulfate by the removal of the sulfate as insoluble barium sulfate. (25,26) Suitable quantities of inactive sulfate as carrier and inactive persulfate as hold-back carrier were added before the precipitation of the barium sulfate. The accuracy of the actual experimental procedure was ascertained by trial assays on known mixtures of sulfate-S³⁵ and persulfate-S³⁵. For example, with a mixture of 20 % sulfate-S³⁵ and 80 % persulfate-S³⁵, after removal of barium sulfate, the activity remaining in solution amounted to 79.5 % and 80.2 % for duplicate trials. More detailed illustrations of actual experimental data will be given in subsequent sections.

C. Conversion of Persulfate to Sulfate by Flour Slurries

The flour used in the studies as slurries or as doughs was a commercial patent grade flour milled from Western Canadian hard red spring wheat by the Flour Mill Division, Saskatchewan Wheat Pool. It has not been treated with improvers at the mill. Its protein and ash contents, on a 14 % moisture basis, were 12.4 % (N x 5.7) and 0.35 %, respectively.

Ten grams of flour (at 14 % moisture) and 100 ml. of distilled water containing S³⁵-labeled potassium persulfate at initial levels of 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 determination of total recovery of S³⁵ and of conversion of persulfate to sulfate.

Total recovery of S³⁵-labeled compounds from each sample was measured

1. All radioactivity measurements were made by means of an end-window Geiger Counter. Samples from any given set of experiments were of the same geometry and thickness, consequently, no self absorption corrections were required. All samples were counted for a sufficient length of time to insure that statistical errors were within ± 2 %.

by drying and counting an aliquot of the aqueous extract. Immediately before and after the counting of each sample, a sample containing a fixed amount of S^{35} , to be designated the standard, was also counted. This "sandwiching" technique, corrects for the radioactive decay and counter efficiency variations with time. The standard used in all the counting has a S^{35} activity of 50 registers¹ /min. at an arbitrarily fixed zero time. If the activity of a given sample were y reg. /min. and the average value for the activity of the standard counted before and after the counting of the sample were, for example, 45 reg. /min., then the corrected activity of the sample would be $y \times \frac{50}{45}$ reg. /min.

As an illustration of the actual experimental data, take, for example, the flour slurry containing 100 p.p.m. labeled potassium persulfate which was stirred in the Waring Blendor for 5 minutes. The simple aqueous extract was obtained from treatment of 10 g. of flour with 1.0 mg. of potassium persulfate- S^{35} and 100 ml. of water. To 40 ml. of this extract, 1 ml. of solution of ordinary potassium persulfate (27 mg. or 0.1 m. mole per ml.) and 1 ml. of a solution of potassium sulfate (27 mg. or 0.15 m. mole per ml.) were added as carriers. Two-ml. aliquots were then dried in aluminum pans and counted. The mean corrected activity was 1.94 reg. /min.

The total volume of aqueous extract, including the water from the 14% moisture in the flour, is 101.4 ml. Hence the total recovery of S^{35} in the simple aqueous extract was:

$$\frac{101.4}{2} \times \frac{42}{40} \times 1.94 = 103.3 \text{ reg. /min.}$$

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 in the same

¹One register equals 64 disintegrations counted.

manner as in the experiments with slurries containing labeled persulfate. The counting of dried aliquots of such a known solution gave corrected activity of 4.05 reg. /min. per 0.02 mg. $K_2S^{35}_2O_8$.

Hence for 1.0 mg. $K_2S^{35}_2O_8$ used in the 100 p.p.m. experiment, total S^{35} activity originally added to the slurry was $\frac{1.0}{0.02} \times 4.05 = 202.5$ reg. /min.

Therefore the recovery of S^{35} in the aqueous extract =

$$\frac{103.3}{202.5} \times 100 = 51 \%$$

For the estimation of sulfate content in the aqueous extract, 20 ml. of the 42 ml. of extract plus carrier was treated with 1.0 ml. of a solution of barium chloride (40 mg. or 0.19 m. mole per ml.). The precipitated barium sulfate was removed by centrifuging and 2.0-ml. aliquots of the supernatant dried and counted. The mean corrected activity was 0.79 reg. /min.

Therefore the total S^{35} activity remaining after removal of sulfate =

$$0.79 \times \frac{21}{20} \times \frac{101.4}{2} \times \frac{42}{40} = 44.1 \text{ reg. /min.}$$

Since the total S^{35} recovered in the extract was 103.3 reg. /min., the sulfate content =

$$\frac{103.3 - 44.1}{103.3} \times 100 = 57 \% \text{ of the } S^{35} \text{ in the aqueous extract.}$$

The results of this set of experiments are summarized in table 1.

In another set of experiments with water-flour slurries, the method was modified so as to give greater recoveries of S^{35} in the aqueous extract. The extraction procedure adopted was essentially that of Auerbach and coworkers (14). One gram of flour and 34.0 ml. of distilled water containing 100,200,300,400 or 1000 p.p.m. S^{35} -labeled potassium persulfate, based on the weight of the flour, were shaken for 5 minutes on a Fisher Gyrosolver.

Table 1: Recovery of S35 and Conversion of Persulfate to Sulfate in Simple Aqueous Extracts from Water-Flour Slurries

Stirring Time (min.)	5		15		30		60	
	S35 recovery in extract (% of S35 added)	Sulfate Content (% of S35 in extract)	S35 recovery in extract (% of S35 added)	Sulfate Content (% of S35 in extract)	S35 recovery in extract (% of S35 added)	Sulfate Content (% of S35 in extract)	S35 recovery in extract (% of S35 added)	Sulfate Content (% of S35 in extract)
100	51	57	62	85	60	98	56	100
	54	56	66	79	68	96	58	100
200	53	54	59	78	60	96	63	100
	53	58	62	75	59	97	60	100
300	54	48	58	75	62	97	61	100
	53	52	57	75	58	98	61	99
400	57	56	57	76	56	97	59	100
	55	47	60	77	56	97	56	100
1000	61	39	70	72	71	95	65	99
	59	35	65	78	61	93	64	99

To aid the removal of colloidal materials, 4.0 ml. of 0.3 N zinc chloride, 12.0 ml. of 0.1 N sodium hydroxide and 0.5 g. of Celite filter aid were added and the mixture shaken for an additional 15 minutes. After centrifuging, the supernatant liquid, to be called the zinc hydroxide clarified aqueous extract, was used for sulfate assays.

To illustrate with the actual experimental data, take, for example, the flour slurry originally containing 100 p.p.m. labeled potassium persulfate. The total volume of the aqueous extract, allowing for the 0.14 g. of moisture from the 1 g. of flour, was 50.14 ml.

To 40 ml. of the extract were added 1.0 ml. of ordinary $K_2S_2O_8$ and 1.0 ml. of K_2SO_4 as carriers. One-ml. aliquots were dried and counted, giving a mean corrected activity of 2.20 reg. /min.

Therefore the total S^{35} recovered in the zinc hydroxide clarified aqueous extract =

$$2.20 \times 50.14 \times \frac{42}{40} = 115.6 \text{ reg. /min.}$$

The corrected activity of the $K_2S^{35}_2O_8$ used was found to be 2.42 reg. /min. per 0.002 mg.

The 1 g. flour sample treated with 100 p.p.m. labeled potassium persulfate contained the equivalent of 0.1 mg. $K_2S^{35}_2O_8$ or an original S^{35} activity of $\frac{0.1}{0.002} \times 2.42 = 121.0$ reg. /min.

Therefore the recovery of S^{35} in the zinc hydroxide clarified aqueous extract =

$$\frac{115.6}{121.0} \times 100 = 96 \%$$

As described before, 20 ml. of the 42 ml. of extract plus carriers was treated with 1.0 ml. of barium chloride followed by the removal of the barium sulfate by centrifuging. One-ml. aliquots of the supernatant were

Table 2: Recovery of S^{35} and conversion of persulfate to sulfate in zinc hydroxide clarified aqueous extracts from water-flour slurries.

Initial persulfate level (p.p.m.)	S^{35} recovery in extract (% of persulfate added)	Sulfate content (% of S^{35} in extract)
100	96	37
	96	36
200	100	35
	100	37
300	96	32
	97	33
400	99	35
	96	40
1000	92	27
	93	24

dried and counted, giving a mean corrected activity of 1.33 reg. /min.

Hence the total activity remaining after sulfate removal =

$$1.33 \times 50.14 \times \frac{42}{40} \times \frac{21}{20} = 73.3 \text{ reg. /min.}$$

As the total S^{35} recovered in the extract was equivalent to 115.6 reg. /min., the sulfate content =

$$\frac{115.6 - 73.3}{115.6} \times 100 = 37\% \text{ of the } S^{35} \text{ in the extract.}$$

The results obtained from zinc hydroxide clarified aqueous extracts of water-flour slurries are given in table 2.

D. Conversion of Persulfate to Sulfate by Water-Gluten Slurries

Samples of freeze-dried and spray-dried gluten were obtained from Dr. W.B. McConnell of the Prairie Regional Laboratory, National Research Council.

The freeze-drying procedure used by Dr. McConnell was that of Lusena (27). Briefly, it consisted of:

1. Wash the gluten out of the flour.
2. Disperse the washed gluten in 0.005 N acetic acid at pH 5.0-5.2 in a Waring Blendor for 5 minutes.
3. Centrifuge for 10 minutes to remove any starch.
4. Reprecipitate the gluten by treatment with calcium hydroxide to bring pH to 6.5-6.8.
5. Redisperse in 0.005 N acetic acid in 0.005 N acetic acid. Centrifuge the dispersion and the supernatant is freeze-dried.

The spray-dried material was derived from an ammonium hydroxide dispersion of gluten. A thick slurry consisting of 1 part of flour and 4 parts 0.16 N ammonium hydroxide was thoroughly mixed in a high speed homogenizing mixer. The starch was centrifuged off and the extract containing all water soluble

materials as well as gluten was spray-dried.

In the experiments with S^{35} -labeled persulfate, 1.0 g. of freeze-dried or spray-dried gluten and 100, 200, 300, 400 or 1000 p.p.m. potassium persulfate in 34 ml. of distilled water were shaken for 5 minutes. This was followed by the addition of 4.0 ml. of 0.3 N zinc chloride, 12.0 ml. of 0.1 N sodium hydroxide and 0.5 g. of Celite and an additional 15 minutes of shaking. Centrifugation gave zinc hydroxide clarified aqueous extracts which were assayed for sulfate content as described for the similar experiment with water-flour slurries. The results are tabulated in table 3.

Samples of the freeze-dried and spray-dried glutens were extracted with petroleum ether, b.p. 40-60°C, for 17 hours in Soxhlet extractors. Conversion of S^{35} -labeled persulfate to sulfate by water slurries of these extracted glutens was determined as in the studies with the non-extracted glutens. The results are given in table 4.

E. Conversion of Persulfate to Sulfate by Water-Flour Doughs

Nonfermenting doughs were prepared from 100 g. of flour (14 % moisture basis) and 60 ml. of distilled water containing 100, 200, 300 or 400 p.p.m. S^{35} -labeled potassium persulfate. The doughs were mixed for 3 minutes in a Hobert mixer and then placed in a fermentation cabinet maintained at 30°C. Twenty-gram subsamples of the dough were removed at 0, 15, 30, 60 and 120 minutes after mixing. Each subsample was extracted by 100 ml. of water in a Waring Blendor operated at high speed for 5 minutes. The resulting dispersion was centrifuged to give a simple aqueous extract of the dough. The S^{35} recoveries and the sulfate contents in these extracts were determined in the usual manner. As an illustration of the actual experimental data, take, for example, the water-flour dough containing 100 p.p.m. S^{35} -labeled potassium persulfate which was worked up at 0 minutes after mixing.

Table 3: Recovery of S35 and conversion of persulfate to sulfate in zinc hydroxide clarified aqueous extracts from water-gluten slurries.

Initial persulfate level (p.p.m.)	Freeze-dried gluten		Spray-dried gluten	
	S35 recovery in extract (% S3520g added)	Sulfate content (% S35 in extract)	S35 recovery in extract (% S3520g added)	Sulfate content (% S35 in extract)
100	83	82	81	80
	69	74	86	75
200	85	71	72	75
	88	74	78	72
300	75	77	80	79
	66	71	81	71
400	77	76	84	73
	79	72	90	73
1000	76	71	78	71
	78	71	87	64

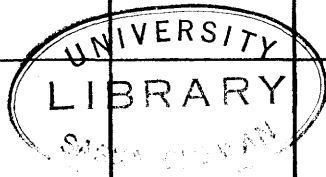


Table 4: Recovery of S35 and conversion of persulfate to sulfate in zinc hydroxide clarified aqueous extracts from water slurries of petroleum ether extracted gluten.

Initial persulfate level (p.p.m.)	Freeze-dried gluten		Spray-dried gluten	
	S35 recovery in extract (% S35 _{20g} added)	Sulfate content (% S35 in extract)	S35 recovery extract (% S35 _{20g} added)	Sulfate content (% S35 in extract)
100	77	80	66	66
	85	78	83	72
200	77	74	76	72
	84	79	82	66
300	76	76	74	68
	88	74	86	69
400	75	72	77	71
	93	73	76	72
1000	78	72	73	66
	78	65	84	69

The total volume of simple aqueous extract for the 20-g. subsample of dough equals 100 ml. plus the water derived from the dough. Since the dough was prepared from 86 g. of dry flour, 14 g. moisture and 60 ml. of added water, 20 g. of dough would contain $\frac{20}{160} \times 74 = 9.25$ ml. of water. The total volume of the extract would, therefore, be 109.25 ml.

To 40 ml. of this extract were added 1.0 ml. each of the $K_2S_2O_8$ and K_2SO_4 solutions as carriers. Two-ml. aliquots were dried and counted, giving a mean corrected activity of 2.18 reg. /min.

Therefore the S^{35} activity recovered from the 20-g. subsample of dough =

$$2.18 \times \frac{109.25}{2} \times \frac{42}{40} = 125 \text{ reg. /min.}$$

A solution was prepared by adding known weights of $K_2S_2^{35}O_8$ to an extract of nonradioactive dough. The counting of aliquots of this solution showed that the corrected activity of the $K_2S_2^{35}O_8$ used was 10.1 reg. /min. per 0.04 mg.

For a 20-g. subsample of the dough originally treated with 100 p.p.m. labeled persulfate, its total $K_2S_2^{35}O_8$ content was $\frac{20}{160} \times 10 = 1.25$ mg. Hence the total original $K_2S_2^{35}O_8$ activity in the 20-g. subsample of dough =

$$\frac{1.25}{0.04} \times 10.1 = 316 \text{ reg. / min.}$$

Therefore the recovery of S^{35} in the simple aqueous extract of the dough =

$$\frac{125}{316} \times 100 = 40 \%$$

As before, 20 ml. of the 42 ml. of extract plus carriers was treated with 1.0 ml. of barium chloride. After removal of the barium sulfate, 2-ml. aliquots were dried and counted giving a mean corrected activity of 0.035 reg. /min.

Therefore the S^{35} activity remaining after removal of sulfate =

$$0.035 \times \frac{21}{20} \times \frac{109.25}{2} \times \frac{42}{40} = 2.1 \text{ } \mu\text{g. /min.}$$

Hence the conversion to sulfate =

$$\frac{125 - 2.1}{125} \times 100 = 98 \% \text{ of the activity in the extract.}$$

Results from this set of experiments are summarized in table 5.

Nonfermenting doughs were also prepared from water and "defatted" flour. In one series of experiments, defatting was carried out by extraction with petroleum ether, b.p. 40-60°C, in Soxhlet extractors for 17 hours. The weight of petroleum ether soluble materials extracted averaged 0.69 % of the weight of flour. Batches of the extracted flour were thoroughly mixed and sieved to give a single stock supply to insure uniformity.

Doughs were prepared from 100 g. of such petroleum ether extracted flour and 60.0 ml. of distilled water containing 100, 200, 300 or 400 p.p.m. S³⁵-labeled potassium persulfate. Simple aqueous extracts were obtained from 20-g. subsamples of each dough at 0, 15, 30, 60 and 120 minutes after mixing as described previously. The S³⁵ recoveries and sulfate contents in these extracts were measured and the results are tabulated in table 6.

The reduction of persulfate to sulfate in nonfermenting doughs made from ordinary and petroleum ether extracted flours was also studied using the more efficient extraction method involving clarification with zinc hydroxide. As before, doughs were prepared from 100 g. of ordinary or petroleum ether extracted flour (at 14 % moisture basis) and 60 ml. of distilled water containing 100, 200, 300, 400 or 1000 p.p.m. S³⁵-labeled potassium persulfate. At various time intervals after mixing of the dough, a 2.0-g. subsample was taken and dispersed in 68 ml. of distilled water, with 1.0 g. of Celite, 24 ml. of 0.1 N sodium hydroxide and 8 ml. of 0.3 N zinc chloride in a Waring Blendor operated at high speed for 5 minutes.

Table 5: Recovery of S³⁵ and conversion of persulfate to sulfate in simple aqueous extracts of water-flour doughs from ordinary flour.

Initial S ³⁵ 208 level (ppm)	Rest time after mixing of dough (min.)												
	0		15		30		60		120				
	S ³⁵ Rec. in the extract (% of S ³⁵ 208 added)	S ³⁵ 0 ₄ content (% of S ³⁵ in extract)	S ³⁵ Rec. in the extract (% of S ³⁵ 208 added)	S ³⁵ 0 ₄ content (% of S ³⁵ in extract)	S ³⁵ Rec. in the extract (% of S ³⁵ 208 added)	S ³⁵ 0 ₄ content (% of S ³⁵ in extract)	S ³⁵ Rec. in the extract (% of S ³⁵ 208 added)	S ³⁵ 0 ₄ content (% of S ³⁵ in extract)	S ³⁵ Rec. in the extract (% of S ³⁵ 208 added)	S ³⁵ 0 ₄ content (% of S ³⁵ in extract)	S ³⁵ Rec. in the extract (% of S ³⁵ 208 added)	S ³⁵ 0 ₄ content (% of S ³⁵ in extract)	
100	40	98	41	99	38	99	37	98	37	98	37	98	
	35	99	40	99	40	99	41	99	39	99	39	99	
200	38	99	36	100	34	100	33	99	32	99	32	99	
	39	98	38	99	36	98	38	98	37	98	37	98	
300	39	99	48	99	38	99	38	98	37	99	37	99	
	38	99	33	99	34	99	34	98	35	99	35	99	
400	37	98	37	100	35	100	36	99	34	100	34	100	
	40	99	39	99	37	99	36	99	33	99	33	99	

Table 6: Recovery of S35 and conversion of persulfate to sulfate in simple aqueous extracts of water-flour doughs from petroleum ether extracted flour.

Initial S35208 level (ppm)	Rest time after mixing of dough (min.)									
	0		15		30		60		120	
	S35 Rec. in the extract (% of S35208 added)	S3504 content (% of S35 in extract)	S35 Rec. in the extract (% of S35208 added)	S3504 content (% of S35 in extract)	S35 Rec. in the extract (% of S35208 added)	S3504 content (% of S35 in extract)	S35 Rec. in the extract (% of S35208 added)	S3504 content (% of S35 in extract)	S35 Rec. in the extract (% of S35208 added)	S3504 content (% of S35 in extract)
100	48	98	45	98	44	100	43	99	43	100
	46	99	42	98	44	98	42	99	41	98
200	46	98	45	99	43	98	41	98	42	99
	44	100	45	100	41	100	39	100	40	100
300	44	99	43	100	40	99	39	100	40	100
	46	99	43	100	43	99	41	99	41	100
400	44	99	41	100	40	99	41	100	40	99
	43	98	45	99	42	99	41	100	42	99

The mixture was centrifuged to give zinc hydroxide clarified aqueous extracts. The S^{35} recovery and the sulfate content in the aqueous extract were determined in the usual manner. An illustration of the actual experimental data is given below. Take, for example, the water-flour dough from ordinary flour, ^{originally} containing 100 p.p.m. labeled potassium persulfate which was worked up at 0 minutes after mixing.

Since a 2.0-g. subsample of dough would contain $\frac{2}{160} \times 74 = 0.92$ ml.

of water, the total volume of extract equals 100.92 ml.

As before, to 40 ml. of the extract were added 1.0 ml. each of the K_2SO_4 and $K_2S_2O_8$ solutions as carriers. Two-ml. aliquots were dried and counted to give a mean corrected activity of 2.40 reg. /min.

Therefore total activity recovered in the zinc hydroxide clarified extract of 2 g. of dough =

$$2.40 \times \frac{100.92}{2} \times \frac{42}{40} = 127 \text{ reg. /min.}$$

A solution was prepared by adding a known weight of $K_2S^{35}_2O_8$ to a zinc hydroxide clarified aqueous extract of nonradioactive dough. Counting of dried aliquots of this solution showed that the corrected activity of the $K_2S^{35}_2O_8$ used was 26.3 reg. /min. per 0.02 mg.

In the 2.0-g. subsample of dough originally treated with 100 p.p.m. labeled persulfate, the $K_2S^{35}_2O_8$ content was $\frac{2}{160} \times 10 = 0.125$ mg.

Hence the originally $K_2S^{35}_2O_8$ activity in the 2 g. of dough =

$$\frac{0.125}{0.02} \times 26.3 = 164 \text{ reg. /min.}$$

The recovery of S^{35} , therefore, in the zinc hydroxide clarified extract =

$$\frac{127}{164} \times 100 = 78 \%$$

Again, 20 ml. of the 42 ml. extract plus carriers was treated with 1 ml. of barium chloride as described previously. Two-ml. aliquots of the supernatant were dried and counted, giving a mean corrected activity of 0.40 reg. /min.

Hence the S^{35} activity remaining after sulfate removal =

$$0.40 \times \frac{21}{20} \times \frac{100.92}{2} \times \frac{42}{40} = 22.2 \text{ reg. /min.}$$

Therefore conversion to sulfate =

$$\frac{127 - 22.2}{127} = 83 \% \text{ of the activity in the extract.}$$

The results on S^{35} recoveries and sulfate contents for zinc hydroxide clarified aqueous extracts of doughs made from ordinary and petroleum ether extracted flours are given in table 7 and 8.

Nonfermenting doughs were also prepared from water and flour extracted with water-saturated n-butyl alcohol. The procedure used in the extraction was that of Mecham and Mohammed, (28) which has been reported as capable of removing all the "lipids" of flour. Each gram of flour was mixed with 10 ml. of water-saturated n-butyl alcohol and allowed to remain overnight with occasional agitation. Centrifugation aided the removal of the alcohol and several washings with water-saturated ether provided an alcohol free product. The weight of the water-saturated n-butyl alcohol soluble materials extracted averaged 1.47 % of the weight of the flour. Batches of this extracted flour were also thoroughly mixed and sieved to give a single stock supply to insure uniformity.

Doughs were prepared from 50 g. (at 14 % moisture) of such water-saturated n-butyl alcohol extracted flour and 30 ml. of distilled water containing 100, 200, 300, 400 or 1000 p.p.m. S^{35} -labeled potassium persulfate. At various time intervals after mixing of the dough, a 2-g.

Table 7: Recovery of S35 and conversion of persulfate to sulfate in zinc hydroxide clarified aqueous extracts of water-flour doughs from ordinary flour.

Initial S35208 level (ppm)	Rest time after mixing of dough (min.)											
	0		15		30		60		120			
	S35 Rec. in the extract (% of S35208 added)	S3504 content (% of S35 in extract)	S35 Rec. in the extract (% of S35208 added)	S3504 content (% of S35 in extract)	S35 Rec. in the extract (% of S35208 added)	S3504 content (% of S35 in extract)	S35 Rec. in the extract (% of S35208 added)	S3504 content (% of S35 in extract)	S35 Rec. in the extract (% of S35208 added)	S3504 content (% of S35 in extract)		
100	78	83	78	100	89	100	72	98	77	98	98	
	65	83	69	93	77	100	74	98	84	98	98	
200	82	76	65	94	76	100	76	100	76	100	99	
	82	79	83	97	86	99	82	100	75	100	100	
300	76	81	74	96	85	99	79	100	76	100	100	
	77	80	77	98	77	99	66	99	79	100	100	
400	74	80	79	98	88	100	75	100	71	100	100	
	72	82	83	97	79	100	76	100	86	99	99	
1000	79	71	77	90	73	97	71	98	84	99	99	
	73	70	77	89	84	97	81	98	85	100	100	

Table 8: Recovery of S35 and conversion of persulfate to sulfate in zinc hydroxide clarified aqueous extracts of water-flour doughs from petroleum ether extracted flour.

Initial S35208 level (ppm)	Rest time after mixing of dough (min.)									
	0		15		30		60		120	
	S35 Rec. in the extract (% of S35208 added)	S3504 content (% of S35 in extract)	S35 Rec. in the extract (% of S35208 added)	S3504 content (% of S35 in extract)	S35 Rec. in the extract (% of S35208 added)	S3504 content (% of S35 in extract)	S35 Rec. in the extract (% of S35208 added)	S3504 content (% of S35 in extract)	S35 Rec. in the extract (% of S35208 added)	S3504 content (% of S35 in extract)
100	70	85	71	97	79	100	83	100	71	100
	71	88	74	97	80	100	84	100	78	100
200	64	79	69	97	62	99	68	100	79	99
	69	81	77	97	63	100	71	100	71	100
300	72	78	71	96	78	99	72	100	73	100
	78	83	76	97	65	99	71	99	69	100
400	84	78	74	94	76	98	68	99	71	100
	74	72	77	92	68	98	78	99	81	100
1000	72	66	69	88	75	97	75	99	77	100
	72	70	77	88	82	95	79	99	79	99

subsample was taken and dispersed in 68 ml. of distilled water, 1 g. Celite, 24 ml. of 0.1 N sodium hydroxide and 8 ml. of 0.3 N zinc chloride in a Waring Blendor operated at high speed for 5 minutes. Centrifugation gave zinc hydroxide clarified aqueous extracts and S^{35} recovery and sulfate content in the aqueous extract were determined in the usual manner. The results are summarized in table 9.

F. Conversion of Persulfate to Sulfate by the Extracted "Fat" of Flour

The "fat" was obtained by Soxhlet extraction with petroleum ether or by extraction with water-saturated n-butyl alcohol as described previously.

The petroleum ether extract from 60 g. of flour was evaporated to remove all the solvent, the last stage of the evaporation being done in a vacuum oven at 40°C for 30 min. The residue was dissolved in 120-ml. purified dioxane (29). Ten-ml. aliquots of the dioxane solution, each containing the equivalent of fats from 5 g. of flour, and 100, 200, 300, 400 or 1000 p.p.m. S^{35} -potassium persulfate (based on flour weight) in 30 ml. of water were shaken on a Fisher gyrosolver for 5 min. A duplicate run, with a 2 hour reaction time, was also done. Sulfate- S^{35} and persulfate- S^{35} determined by carrier technique showed essentially no conversion of the persulfate to sulfate.

Similar results were obtained when the fat was extracted by water-saturated n-butyl alcohol. A volume of the alcohol extract equivalent to 20 g. of flour was vacuum distilled to near dryness. The fat and remaining alcohol were dissolved in several ml. of chloroform and filtered through a medium porosity sintered glass filter and then evaporated. Complete removal of all solvents was insured by placing the fat in a vacuum oven at 50°C for 30 min. The fat was dissolved in 40 ml. of purified dioxane and 10-ml. aliquots and 100 or 1000 p.p.m. S^{35} -labeled potassium persulfate (based on

Table 9: Recovery of S35 and conversion of persulfate to sulfate in zinc hydroxide clarified aqueous extracts of water-flour doughs from water-saturated n-butyl alcohol extracted flour.

Initial S35208 level (ppm)	Rest time after mixing the dough (min.)											
	0		15		30		60		120			
	S35 Rec. in the extract (% of S35208 added)	S3504 content (% of S35 in extract)	S35 Rec. in the extract (% of S35208 added)	S3504 content (% of S35 in extract)	S35 Rec. in the extract (% of S35208 added)	S3504 content (% of S35 in extract)	S35 Rec. in the extract (% of S35208 added)	S3504 content (% of S35 in extract)	S35 Rec. in the extract (% of S35208 added)	S3504 content (% of S35 in extract)		
100	84	86	81	95	81	99	74	99	83	99	99	
	78	84	81	90	79	96	84	98	79	98	98	
200	73	85	79	98	71	99	84	100	86	99	99	
	74	80	81	99	79	99	74	99	91	99	99	
300	85	82	84	96	82	99	91	99	91	99	99	
	75	82	79	96	81	99	81	99	85	97	97	
400	80	81	83	93	80	99	83	98	83	99	99	
	79	84	92	96	74	98	74	98	73	99	99	
1000	74	69	80	91	80	96	81	93	78	97	97	
	78	69	83	90	72	97	83	99	85	98	98	

flour weight) in 30 ml. of water were shaken on a Fisher gyrosolver for 5 minutes. The absence of conversion of persulfate to sulfate in these solutions, determined in the usual manner, was again observed.

As an illustration of the actual data, first of all, a blank experiment was carried out by shaking for 5 minutes 10 ml. of pure dioxane and 30 ml. of water containing 0.5 mg. of labeled persulfate. After addition of 1 ml. each of the persulfate and sulfate solutions as carriers, 1-ml. aliquots were dried and counted, showing a mean corrected activity of 7.82 reg. /min.

To 20 ml. of this solution, 1 ml. of barium chloride was added and the precipitated barium sulfate centrifuged off. The supernatant showed a mean corrected activity of 7.86 reg. /min. per ml. after the dilution factor of 21/20 was taken into account. Therefore, there was no conversion of persulfate to sulfate in the blank experiment.

When 10 ml. of dioxane solution of the extracted fat and 30 ml. of water containing various levels of S^{35} -labeled persulfate were worked up exactly as described for the blank experiment, results also showed the absence of conversion of persulfate to sulfate.

G. Determination of Unchanged Persulfate in Extracts

In the experiments where the conversion of persulfate to sulfate is not complete, the exact nature of the S^{35} -containing material remaining after removal of the barium sulfate has not been determined, though it was presumed to be unchanged persulfate.

To verify this presumption, zinc hydroxide clarified aqueous extracts for ordinary flour slurries and doughs originally containing 1000 p.p.m. S^{35} -labeled potassium persulfate were obtained as before. By carrier technique, as previously described, the activity of the unconverted persulfate could be determined. To a 10-ml. aliquot, of the

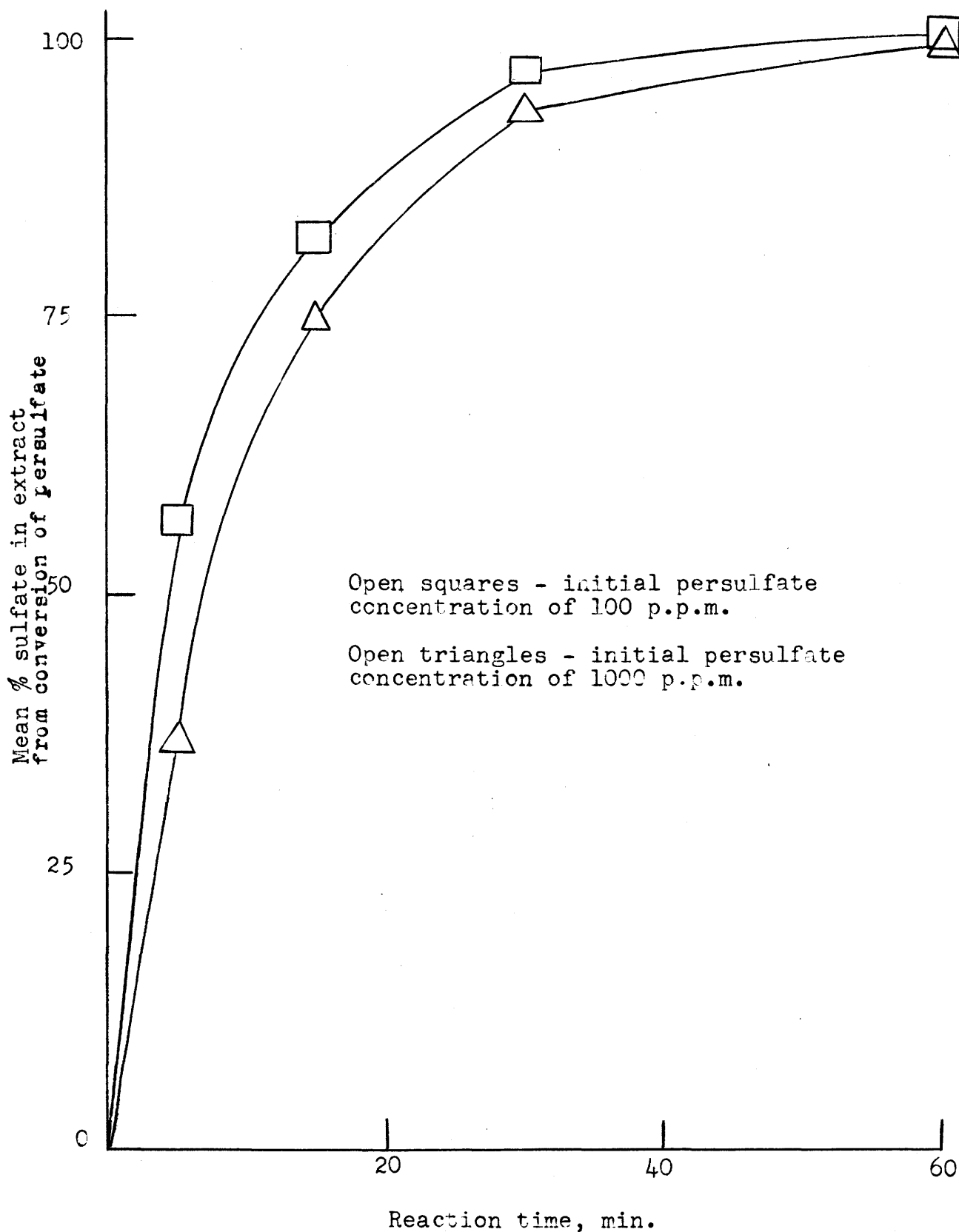
extract after sulfate removal, 1.0 ml. of dilute HCl was added and the solution was heated on a boiling water bath for 30 min. The second precipitate of barium sulfate was centrifuged off and aliquots were again dried and counted. Essentially no activity was observed, signifying complete conversion of the residual S^{35} activity to sulfate, hence the residual S^{35} was quite probably in the form of persulfate.

IV. Discussion of Results

The results from the experiments described in the previous section, as summarized in tables 1 to 9, clearly show that persulfate is readily reduced to sulfate by flour slurries or doughs. Although it is generally expected that oxidative improvers such as persulfate would take part in oxidation-reduction reactions with constituents of flour and dough, the present results unequivocally demonstrated the reduction of persulfate to sulfate. Since in many instances, the persulfate was completely reduced to sulfate, the improver effects of persulfate could involve oxidation-reduction reactions. Mechanisms of improver action involving oxidation-reduction reactions either directly or indirectly would, therefore, be consistent with the present results.

For water-flour slurries, apparently complete conversion of persulfate to sulfate occurred after a stirring time of about 60 minutes (table 1, page 14). The mean percentage conversion of persulfate to sulfate in the simple aqueous extracts from water-flour slurries plotted as a function of the length of time the slurry was stirred in a Waring Blendor is shown in figure 1 for initial persulfate levels of 100 and 1000 p.p.m. As one might expect, after a given time of stirring, the % sulfate in the extract from the slurry with the rather high initial persulfate level of 1000 p.p.m. is lower than the corresponding value for the extract of the slurry initially treated with 100 p.p.m. persulfate. Thus percentage-wise, the rate of conversion, expressed as the % of total persulfate reduced to sulfate in a given time, is lower the higher the total initial persulfate concentration. In absolute quantities, however, the reverse is true. For example, after 5 minutes of stirring, the mean sulfate contents in the simple aqueous extracts of slurries initially treated with 100 and 1000 p.p.m.

Figure 1: Mean % conversion of persulfate to sulfate in simple aqueous extract from water-flour slurries vs. length of time slurry was stirred in Waring Blendor.



persulfate were, respectively, 56.5 % and 37 % of the S^{35} recovered in the extracts. Since the S^{35} recovered in the extracts amounted to a mean of 52.5 % of 100 p.p.m. and 60 % of 1000 p.p.m., the actual rates of conversion for the initial dosages of 100 and 1000 p.p.m. were, respectively, 5.9 and 44 p.p.m. of persulfate converted to sulfate per minute during the first 5 minutes of stirring.

The recoveries of S^{35} in the simple aqueous extracts of flour slurries ranged between 51 and 71 % of the persulfate initially added (see table 1). Some 30 to 50 % of the initially added persulfate was thus lost from the aqueous phase, probably through adsorption by the flour. There is, however, no apparent relationship between the degree of recovery and experimental variables such as initial persulfate level or length of stirring time. When the extraction of the slurry was effected by the method involving clarification with zinc hydroxide, nearly complete recovery of all S^{35} in the aqueous phase was obtained (table 2, page 16). It is of interest to note that in the zinc hydroxide clarified extracts of slurries worked up after 5 minutes of stirring time, the sulfate contents were some 10 to 20 % less than the sulfate contents in the corresponding simple aqueous extracts. In tables 1 and 2, the data appear to indicate that simple extraction with water alone may remove more sulfate than persulfate, or in other words, persulfate may be more tightly adsorbed than sulfate. Why this is so is not apparent from the present studies. Another factor which likely contributed to the lower sulfate contents in the zinc hydroxide clarified extracts is a concentration effect. It may 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 g. of flour. With a given initial persulfate level, for example, 100 p.p.m. based on the weight of flour present, the persulfate concentration in the aqueous solution is obviously higher under the conditions where the simple aqueous extract was obtained. Such a higher concentration of a reactant may lead to a faster reaction, resulting in a greater conversion of persulfate to sulfate in the simple aqueous extract.

In the studies with slurries of freeze-dried and spray-dried glutes, conversion of persulfate to sulfate was also observed (table 3, page 19). There is no significant difference in the degrees of conversion for the two types of gluten. Petroleum ether extraction did not appreciably change the reducing power of freeze-dried gluten. However, the spray-dried gluten, after petroleum ether extraction, seemed to cause slightly less conversion of persulfate to sulfate (table 4, page 20). Since the two types of gluten were prepared in different ways (pages 17 - 18), their exact natures are expected to be different. Possibly, some minor components in the spray-dried gluten, which can react with persulfate, may have been removed by petroleum ether extraction.

The conversions of persulfate to sulfate by water slurries of 1-g. samples of gluten were in the neighborhood of 75 % after a stirring time of 5 minutes (table 3). Under similar conditions, the conversions of persulfate to sulfate by water slurries of 1-g. samples of flour were approximately 35 % (table 2). Since the protein content of the flour was 12.4 %, in 1 g. of flour, the weight of gluten would be about 0.12 g. This amount of gluten could probably convert $0.12 \times 75 = 9$ % of the persulfate to sulfate under the

conditions of the flour slurry experiments of table 2. Since the actual conversions by the flour slurries were in the neighborhood of 35 % considerably higher than 9 %, this comparison indicates that components of flour other than gluten also undergo oxidation-reduction reactions with persulfate.

In the studies with water-flour doughs, simple extraction of 20-g. subsamples of the dough with 100 ml. of water resulted in recoveries in the extracts of only about 35 to 45 % of the S^{35} initially added 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 done immediately after the dough was formed by 3 minutes of mixing of the flour and water containing persulfate- S^{35} . Similar results were observed when the dough was prepared either from ordinary or petroleum ether extracted flour (tables 5 and 6, pages 23 and 24).

Extraction of 2-g. subsamples of dough by the more efficient method involving clarification with zinc hydroxide resulted in higher S^{35} recoveries of the order of 70 to 80 %. When extractions were done immediately after mixing of the doughs, 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 S^{35} in the extract. If the doughs were allowed to rest for various lengths of time before extraction, practically complete conversion of persulfate to sulfate was found when the rest time was 15-30 minutes. Essentially similar results were observed for doughs prepared from ordinary flour, petroleum ether extracted flour or water-saturated n-butanol extracted flour (tables 7, 8, and 9, pages 27, 28 and 30).

From these studies with doughs, first of all, it may be noted that

recovery of S^{35} by extraction, even with the more efficient procedure, was considerably below 100 %. In the estimation of persulfate decomposition in doughs by ordinary chemical means, such as that reported by Auerbach and coworkers (14), it was assumed that all unchanged persulfate was extracted quantitatively from the dough. Such an assumption is obviously not justified.

As in the case with flour slurries, persulfate is apparently less readily extracted since the simple aqueous extracts of doughs contained only sulfate while the zinc hydroxide clarified extracts showed higher S^{35} recoveries as well as the presence of some unchanged persulfate in the extracts obtained from dough samples worked up immediately after mixing. Such results suggest that persulfate is more tightly adsorbed than sulfate. Although the fundamental reasons for such difference between sulfate and persulfate is not clear, it is of interest to refer to the suggestion made by Hoffer (30) that flour improvers must first be adsorbed before they can exert their improving action. It was postulated that in the natural state, flour proteins are globular in nature. During the mixing process, these proteins are unfolded, forming a three dimensional network of fibres holding the starch, etc. After mixing, the proteins tend to revert to the natural globular condition. However, if an improving agent is present, it is adsorbed on the protein chain and stabilizes the system by preventing the reversion of the chain from the extended state to the globular state by mechanical blocking. The present finding that persulfate appears to be more tightly adsorbed than sulfate in flour slurries and doughs is at least consistent with, though not a proof of the adsorption hypothesis of Hoffer.

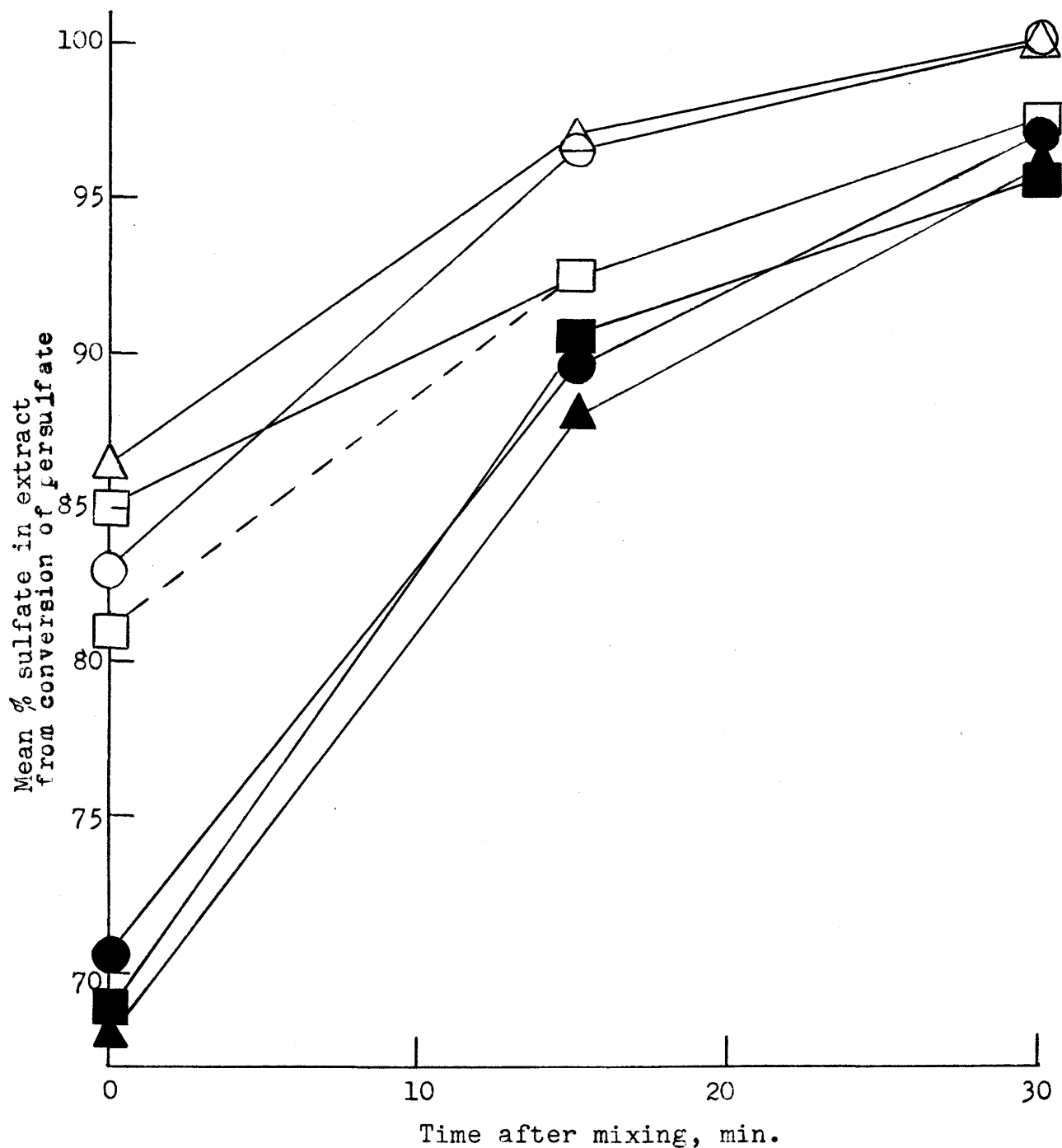
Data on the sulfate contents in the simple or zinc hydroxide

clarified extracts of doughs (tables 5 to 9) show the essentially complete decomposition of the persulfate to sulfate in 15 to 30 minutes after the dough was formed. Comparing with the results on the conversion of persulfate to sulfate by flour slurries (tables 1 and 2), it is clear that the rate of decomposition of persulfate to sulfate is faster in doughs than in slurries. This is to be expected even if one were to consider only 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, hence in a dough system, the reaction is 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, another common flour improver. Work with Br⁸²-labeled bromate has shown that some unchanged bromate is present even in the finished bread (20). Since persulfate is a more powerful oxidizing agent than bromate, its greater oxidizing power may be a contributing factor in the observation that persulfate is decomposed faster than bromate by oxidizable constituents of flour or dough.

As an attempt to shed some light on a possible involvement of flour lipids in the improver reactions, studies were made on the conversion of persulfate to sulfate by doughs prepared from flour "defatted" by extraction with petroleum ether or water-saturated n-butanol. The results obtained (tables 7, 8 and 9) show hardly any significant differences in the rapid decomposition of persulfate by doughs from ordinary or defatted flours. A graphically presentation of some of the results is shown in figure 2 where the mean sulfate contents in the

Figure 2: Mean sulfate content in zinc hydroxide clarified aqueous extracts of doughs vs. reaction time after mixing.



Circles - doughs from ordinary flour.
 Triangles - doughs from petroleum ether extracted flour.
 Squares - doughs from water saturated butanol extracted flour.
 Open symbols for initial persulfate level of 100 p.p.m.
 Solid symbols for initial persulfate level of 1000 p.p.m.
 Dotted line - rewashed flour with water-saturated ether.

zinc hydroxide clarified extracts are plotted against reaction time after mixing for the three types of doughs initially treated with 100 or 1000 p.p.m. potassium persulfate.

The absence of any significant effect on persulfate decomposition by defatting is again in sharp contrast to the behavior of bromate. Lee and Tkachuk (21) noted a marked decrease in the degree of conversion of bromate to bromide when the dough was prepared from petroleum ether extracted flour. For the same patent flour used in the present experiments, Lee and Tkachuk (21) found that with an initial potassium bromate- Br^{82} treatment of 15 p.p.m., the bromide content was about 21 % in the aqueous extract from an ordinary flour dough worked up about 4 hours after mixing. Under the same conditions for a dough prepared from petroleum ether extracted flour, the bromide content decreased to about 10 %. Similarly, decreases in the rates of disappearance of bromate, measured by amperometric titration, were reported by Cunningham and Hlynka (31) 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 significant influence on its rapid decomposition by the dough. This was further verified by observation that dioxane solutions of extracted lipids failed to show any reaction with aqueous solutions of persulfate.

In summary, from the present studies, the following conclusions may be drawn:

1. Persulfate is rapidly and completely reduced to sulfate by

water-flour slurries or water flour doughs, thus unequivocally showing that oxidation-reduction reactions take place. This does not constitute a proof of any mechanism of improver action, but the results are consistent with mechanisms involving oxidation-reduction reactions.

2. Gluten slurries can reduce persulfate to sulfate, but the extent of reduction by the gluten present in 1 g. of flour is not as high as the extent of reduction by 1 g. of flour, thus indicating that flour constituents other than gluten also take part in reactions with persulfate.

3. Persulfate is apparently more strongly adsorbed by the flour slurry or dough than sulfate as evidenced by the finding that simple extraction of slurry or dough with pure water showed that sulfate is more readily extracted than persulfate. This observation is consistent with the suggestion of Hoffer that adsorption plays an important role in the mechanism of improver action.

4. The rate of reduction of persulfate to sulfate is greater in the dough than in the slurry. This could be explained as a concentration effect. Since the concentrations of reactants may be regarded as higher in the dough than in the slurry, reactions would take place faster in the dough than in the slurry.

5. In sharp contrast to the behavior of bromate, defatting of the flour by extraction with petroleum ether or water-saturated n-butanol showed hardly any significant influence on the rapid decomposition of persulfate by doughs prepared from ordinary or defatted flours. This was further verified by the finding that dioxane solutions of the extracted flour lipids did not react with aqueous solutions of potassium persulfate.

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