

## COMPARATIVE STUDY ON REACTIONS OF IODATE, AZODICARBONAMIDE, AND ACETONE PEROXIDES IN SIMPLE CHEMICAL SYSTEMS AND IN DOUGH<sup>1</sup>

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### ABSTRACT

A comparative study has been made on reactions of iodate, azodicarbonamide (ADA), and acetone peroxides (AP) in simple chemical systems as well as in dough. AP has been found to be an effective oxidizing agent for glutathione and thiogel. A general chemical equation has been postulated for the reaction between AP and sulfhydryl (-SH) compounds, when the monomeric form of AP is assumed to be the reactant. From the rate studies in simple chemical systems and in dough, it is concluded that AP, iodate, and ADA are all fast -SH-oxidizing agents. Doughs treated with these agents show an improving effect, as indicated by the increase in resistance to extension and the decrease in extensibility in the extensigram. On an equivalent basis, ADA is more rapid than iodate or AP in oxidizing -SH groups of dough and in exerting the improving effect on dough under our experimental conditions. Unlike ADA and iodate, which have little or no bleaching effect, AP has both bleaching and maturing actions on dough.

Very recently two new flour improvers, azodicarbonamide (ADA) and acetone peroxides (AP), have been introduced by the Wallace & Tiernan Co. and Sterwin Chemicals, Inc., respectively; both have been approved by the Food and Drug Administration for inclusion with the Standards of Identity for flour as bleaching and maturing agents (2). The usefulness of ADA as a flour-maturing agent in improving dough properties and baking qualities has been reported by Joiner (7). The studies of Ferrari *et al.* (3) and Johnson *et al.* (6) have shown that AP is effective for flour maturing and bleaching. Our recent studies on the reactions of iodate, ADA, and AP in dough have indicated that all are fast -SH-oxidizing agents. The improving effect is closely associated with the sulfhydryl oxidation in dough (10-13).

The importance of and need for flour improvers with fast maturing action have been increased by the introduction of continuous dough

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processes such as the Baker Dō-Maker, the Amflow, and the Chorleywood Process. In view of the practical application of these improvers, a comparative study has been made on reactions of iodate, ADA, and AP in simple chemical systems as well as in dough. The reaction of these agents has also been followed with the extensigraph to determine the corresponding changes in rheological properties of the dough.

### Materials and Methods

*Reagents.* All chemicals used in this study were reagent grade. ADA and Maturox (a starch mixture containing 10% ADA) were kindly supplied by Wallace & Tiernan, Inc. A sample of AP blend (acetone peroxides blended with a starch carrier) was a gift from the Research Laboratory, J. R. Short Milling Co. The AP blend had 10% hydrogen peroxide equivalent of acetone peroxides or 0.59% equivalent of acetone peroxides, as determined by potassium permanganate titration. In this report, the peroxide strength of the AP blend is expressed as equivalent of AP calculated from the determination made on the original sample. Glutathione (GSH) and thiogel (lot No. TGA 6101) were purchased from Schwarz Bio-Research, Inc. Nitrogen used in this study was of commercial grade (at least 99.7% nitrogen) and was passed through two gas washing bottles containing vanadous sulfate solution to remove traces of oxygen from the gas (8). Distilled water was passed through a "Deeminizer" before use.

*Flour and Dough.* This study was made on an untreated straight-grade flour milled from a blend of Canadian hard red spring wheat. The protein ( $N \times 5.7$ ) and ash contents of the flour were 13.7 and 0.50% (on 14% moisture basis). Doughs were prepared from 100 g. of flour (14% moisture), improver, and sufficient salt solution to give an absorption of 55% and salt content of 1% (flour basis); they were mixed in the GRL mixer (5) under nitrogen. Precautions were also taken to minimize the oxidation of dough by air: flour or flour plus improver was purged with nitrogen under alternate vacuum and pressure in an airtight bowl before mixing; the solutions used for mixing were saturated with nitrogen.

*Extensigrams.* For extensigraph tests, the following treatments were used:

(a) Doughs were mixed in nitrogen for 2.5 min. They were given a reaction time of 10 min., rounded and shaped, and then stretched after a rest period of 20 min. After stretching, a dough sample was taken for analyses of -SH and pigment contents.

(b) Doughs were also given a 10-min. reaction time, rounded, shaped, and then stretched after resting for 45 and 135 min.

During the reaction time and rest period the doughs were kept in a cabinet maintained at 35°C. and 95% r.h.

*Analytical Methods.* A dough sample of about 20 g. was frozen in liquid nitrogen. It was then freeze-dried, ground in a micro Wiley mill (60-mesh) and stored at -40°C. for subsequent analytical uses. Sulfhydryl contents of thiogel, flour, or doughs were determined according to the modification of the method of Sokol, Mecham, and Pence (9) described previously (12).

For the titration of GSH, the titration solution contained 0.01% gelatin instead of 6M urea. The presence of gelatin, used as a maximum suppressor, was found to give more reproducible current readings.

The carotenoid pigment contents were measured by the procedure proposed by Binnington, Sibbitt, and Geddes (1), with the following modifications: 20 ml. of water-saturated n-butyl alcohol were introduced into a 50-ml. glass-stoppered Erlenmeyer flask containing 4.0 g. of sample. The flask was shaken thoroughly for about 1 min. and allowed to stand for 1 hr. After filtration through Whatman No. 1 paper, the absorbance of the extract was measured at 435.8  $m\mu$  in a 1-cm. cell with a Beckman Model DU spectrophotometer.

## Results and Discussion

*Reactions of Acetone Peroxides and Iodate in a Simple Chemical System.* Previous studies have shown that AP, methyl ethyl ketone peroxides (MEKP), iodate, and ADA, when added to doughs in increasing amounts, cause progressive decreases in -SH content and increases in extensigram height (10,11,12).

To explore further the reaction mechanism and the rate at which these reagents react with -SH groups, studies were made using a simple chemical system. Such a system has been applied successfully toward the establishment of the ADA reaction mechanism (10). Since the -SH compounds GSH and thiogel used in this system are readily oxidizable by air (14-16), all operations and titrations in these experiments were done under a nitrogen atmosphere (by constantly flushing the system with nitrogen). A typical experiment with the simple chemical system is described below.

Various amounts (see Table I) of the AP blend were added to 20 ml. of GSH solution (total GSH content, 4.85  $\mu$ eq.) or thiogel solution (total -SH content, 2.97  $\mu$ eq.). The mixture was then stirred slowly with a magnetic stirrer for 2.5 min. to let the reaction take place. The residual GSH or -SH of thiogel was then determined by amperometric titration. The results, given in Table I, show that

as more AP is added, more GSH or thiogel is oxidized. This behavior is analogous to that of dough reported earlier where AP or MEKP, when added in increasing amounts, decreases the -SH content of the dough (12). All these findings demonstrate that AP oxidizes -SH groups of GSH, thiogel, and flour very effectively. However, the acetone peroxides in the blend consist principally of the monomeric and dimeric forms (3), and accordingly the stoichiometric relation between -SH groups oxidized and acetone peroxides consumed cannot be established. Under normal conditions, when acetone peroxides are prepared by oxidation of acetone with hydrogen peroxide, the monomeric form predominates in the mixture. If this form is assumed to be the reactant, the following chemical equation of the oxidation is most likely:

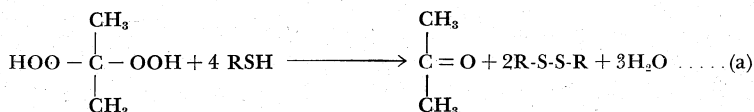


TABLE I  
REACTION OF ACETONE PEROXIDES WITH SULFHYDRYL COMPOUNDS

-SH COMPOUND	AP BLEND (Eq. AP) ADDED		RESIDUAL -SH CONTENT	-SH REACTED
	<i>g.</i>	<i>eq.</i>	<i>μeq.</i>	<i>μeq.</i>
GSH	0.005	( 3.0 × 10 <sup>-5</sup> )	4.04	0.81
	0.015	( 8.9 × 10 <sup>-5</sup> )	2.86	1.99
	0.025	(14.8 × 10 <sup>-5</sup> )	1.67	3.18
	0.035	(20.7 × 10 <sup>-5</sup> )	0.49	4.36
Thiogel	0.005	( 3.0 × 10 <sup>-5</sup> )	2.57	0.40
	0.015	( 8.9 × 10 <sup>-5</sup> )	1.92	1.05
	0.025	(14.8 × 10 <sup>-5</sup> )	1.26	1.71
	0.035	(20.7 × 10 <sup>-5</sup> )	0.77	2.20

Additional experiments were carried out to measure the oxidation rate of GSH or thiogel by AP or iodate in the simple chemical system.

The reaction system consisted of 5 ml. of GSH or thiogel solution (for -SH contents, see Table II) and 15 ml. of solution containing AP or iodate. The mixture was stirred with a magnetic stirrer and allowed to react for various periods of time. At the end of a given reaction time, the residual -SH content was determined. The results obtained from these experiments, together with those from the previous study on the ADA reaction (10) for comparison, are summarized in Table II.

The data (Table II) indicate that all of these agents can oxidize these -SH compounds readily. On an equivalent basis, iodate is more

TABLE II  
EFFECT OF REACTION TIME ON THE OXIDATION OF SULFHYDRYL COMPOUNDS BY  
VARIOUS IMPROVERS

-SH COMPOUND	REACTION TIME	-SH REACTED			
		AP Added:		Iodate Added: 5.6 $\mu$ eq.	ADA Added: 4.0 $\mu$ eq. for GSH; 3.3 $\mu$ eq. for Thiogel
		5.6 $\mu$ eq.	$2.1 \times 10^{-4}$ eq.		
	min.	$\mu$ eq.	$\mu$ eq.	$\mu$ eq.	$\mu$ eq.
GSH	1.0	0.13 <sup>a</sup>	4.36 <sup>b</sup>	3.87 <sup>c</sup>	3.64 <sup>d</sup>
	2.5	0.17	4.36	3.96	3.64
	5.0	0.20	4.37	4.05	3.63
	10.0	0.28	4.41	4.07	3.63
	20.0	0.37	4.41	4.10	...
Thiogel	1.0	0.04 <sup>e</sup>	1.67 <sup>e</sup>	3.30 <sup>f</sup>	3.08 <sup>g</sup>
	2.5	0.09	2.24	3.51	3.06
	5.0	0.19	2.76	3.72	3.07
	10.0	0.27	3.33	3.92	3.06
	20.0	0.29	3.93	3.99	3.07

<sup>a,b,c,d</sup> Original GSH content, respectively: 4.92, 4.85, 4.97, and 5.00  $\mu$ eq.

<sup>e,f,g</sup> Original total -SH content in thiogel solution, respectively: 4.91, 4.93, and 3.90  $\mu$ eq.

effective than AP as the oxidizing agent. Although the equivalent ratios of ADA to GSH or thiogel used in the previous study (10) are different from those of AP or iodate to GSH or thiogel, it is, however, quite evident that ADA is the most active of the three. GSH (a peptide) is oxidized more rapidly than thiogel (a protein) by these agents. The mole ratios of the reactants, GSH/ADA and thiogel -SH/ADA, were previously found to be 1.79 and 1.84 (10). If the equivalent figure of iodate is converted to its mole value, the results, presented in Table II, show that the mole ratios for GSH/iodate vary from 4.18 to 4.43 and for thiogel -SH/iodate from 3.54 to 4.28, as the reaction time is extended from 1 min. to 20 min. The ratio of -SH/iodate could then be assumed to be about 4 which agrees well with the value obtained from dough treated with iodate (13) or from the study of Hird *et al.* (4).

*Reaction Rates of AP and Iodate with Sulfhydryl Groups in Dough.* Further studies were made to determine the rates of reaction of iodate and AP with -SH groups in dough during prolonged resting and with prolonged mixing.

Doughs were prepared from 100 g. of flour and 0.0536 g. of the AP blend (3.2  $\mu$ eq. of AP per g. of flour) or iodate at the concentration of 0.8  $\mu$ eq. iodate per g. of flour. They were mixed for 2.5 min., transferred to plastic containers, and placed in a cabinet maintained at 30°C. and 95% r.h. There dough samples were rested for 0, 1, 2, 3, and 4 hr. At the end of the selected rest period, the sample was immediately frozen in liquid nitrogen, freeze-dried, and ground for -SH determinations.

In another series of experiments doughs were mixed for 2.5, 5, 10, or 20 min. After mixing for the desired time, the dough sample was frozen in liquid nitrogen and freeze-dried, and its -SH content was determined. It should be mentioned here that a part of the experiment on iodate in this study repeats our previous work (13). However, the repetition is necessary in order to obtain comparable data under the same experimental conditions. The results are presented in Table III, showing that AP and iodate are fast -SH-oxidizing agents. On an equivalent basis iodate is more effective than AP, for the extent of -SH losses in the treated doughs is about the same in spite of the fact that more AP is used than iodate under the identical condition.

TABLE III  
SULFHYDRYL OXIDATION IN DOUGHS TREATED WITH AP AND IODATE DURING  
PROLONGED RESTING AND PROLONGED MIXING

	TIME	-SH CONTENTS OF DOUGHS	
		AP-Treated	Iodate-Treated
		<i>hr.</i>	<i>hr.</i>
Prolonged resting	0	0.71	0.66
	1	0.57	0.53
	2	0.49	0.45
	3	0.46	0.44
	4	0.46	0.44
	<i>min.</i>		
Prolonged mixing	2.5	0.71	0.66
	5.0	0.61	0.57
	10.0	0.58	0.56
	20.0	0.59	0.59

*Extensigrams and Sulphydryl Contents of AP-, Iodate-, and ADA-Treated Doughs.* Three sets of dough samples were prepared according to the procedure mentioned in the "Materials and Methods" section: one set of samples was prepared from 100 g. of flour and 0.0268 g. of the AP blend (1.6  $\mu$ eq. of AP per g. of flour); the other two sets of samples were prepared from 100 g. of flour plus iodate or ADA (Maturox) at a concentration of 0.4  $\mu$ eq. of iodate or ADA per g. of flour. The extensigrams of the treated doughs and controls are presented in Figs. 1 to 4.

In comparison with the control, all treated doughs show an improving effect, as indicated by the increase in resistance to extension and decrease in extensibility. The treatment levels of improvers used in this experiment were selected to produce similar extensigrams and to give as significant losses of -SH content in treated doughs as possible. However, with 0.4  $\mu$ eq. of ADA per g. of flour, the dough ap-

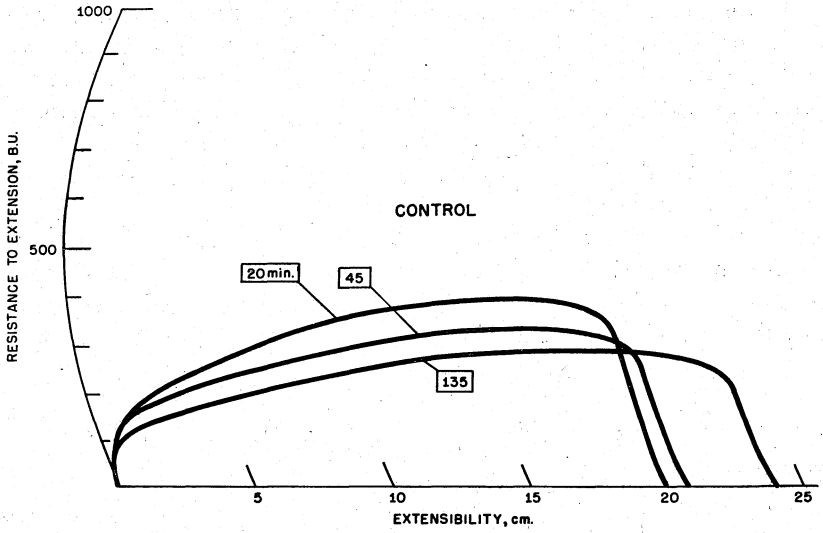


Fig. 1. Extensigrams of the control dough.

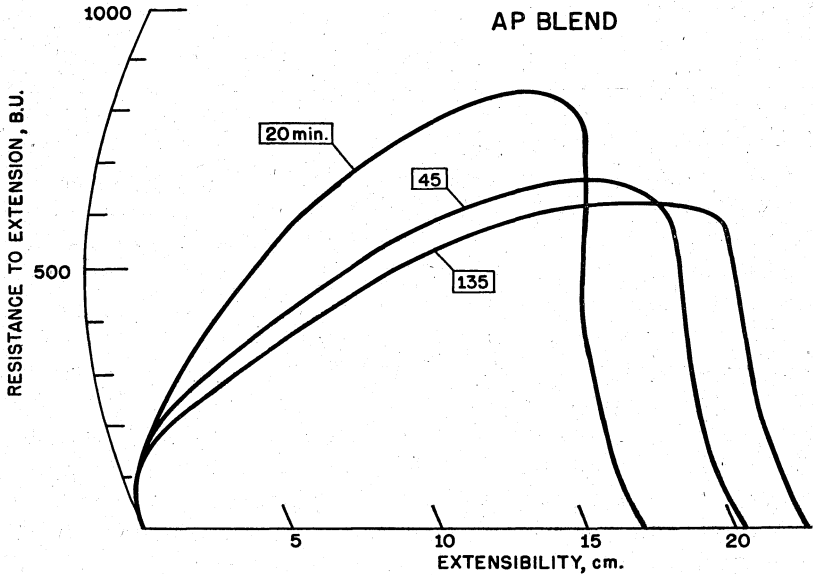


Fig. 2. Extensigrams of dough treated with AP blend.

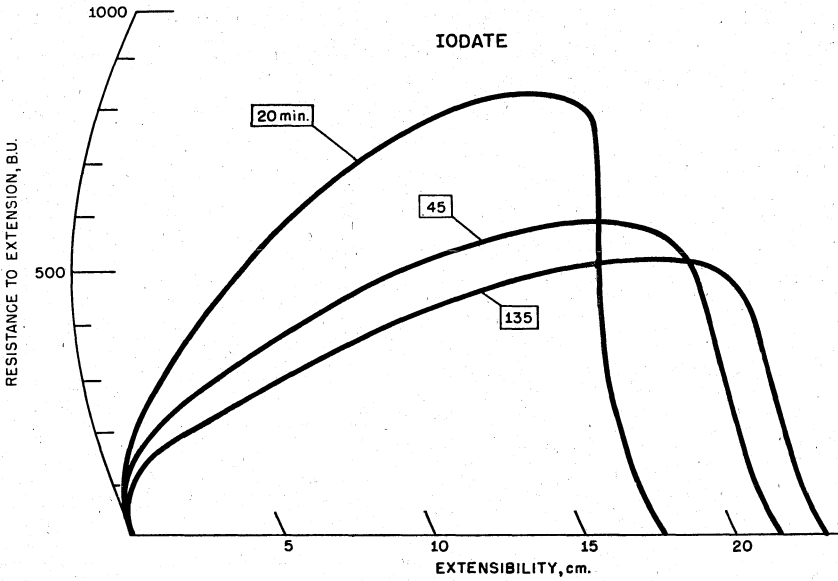


Fig. 3. Extensigrams of dough treated with iodate.

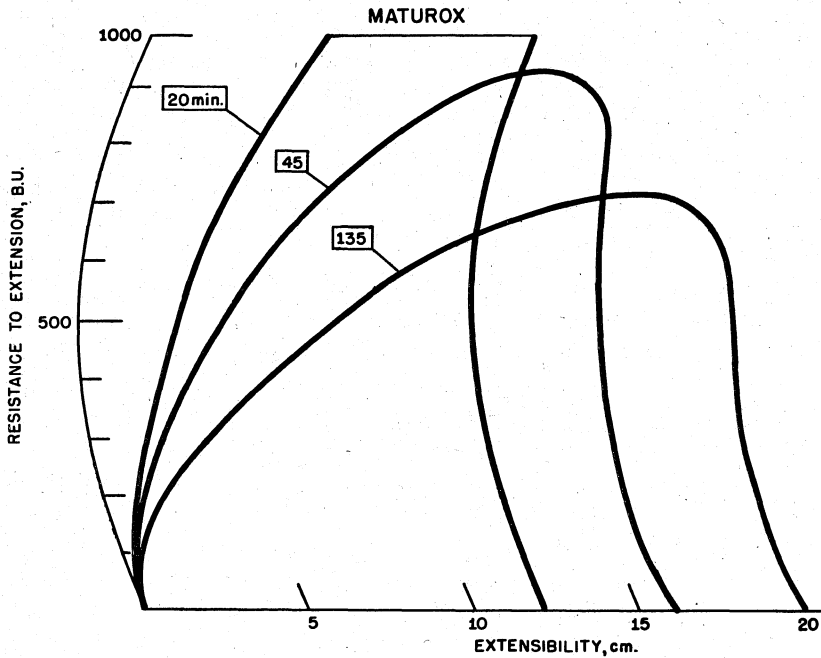


Fig. 4. Extensigrams of dough treated with Maturox.



peared to be overtreated, as reflected by its high resistance. The magnitude of the improving effect, of course, depends upon the kind and quantity of an improver used. On an equivalent basis, ADA is more rapid than iodate or AP in exerting the improving effect, as shown in Figs. 3 and 4.

Sulfhydryl contents of the doughs (with a 10-min. reaction time and a 20-min. rest period) after stretching were also determined. The results, given in Table IV, show that ADA is a faster -SH-oxidizing agent than iodate or AP for dough.

TABLE IV  
LOSSES OF -SH CONTENT IN DOUGHS TREATED WITH THE IMPROVERS

AGENT	AMOUNT ADDED <i>μeq./g. flour</i>	-SH CONTENT <i>μeq./g. dough</i>
None		0.94
Iodate	0.4	0.71
ADA	0.4	0.57
AP	1.6	0.71

*Bleaching Effect of AP on Flour Pigments.* Unlike ADA and iodate which have little or no bleaching effect (10), AP has both bleaching and maturing actions. To study the bleaching effect during dough processing, dough samples were prepared from flours treated with various amounts of the AP blend (see Table V) and immediately mixed in nitrogen for 2.5 min. The samples were then frozen in liquid nitrogen, immediately freeze-dried, and ground. The carotenoid pigment contents of these samples were determined. The results, given in the top half of Table V, show that AP bleaches the pigments in dough effectively.

The experiment was then extended to study the effect of storage on bleaching. Flour samples were treated with the same amounts of the AP blend in a tightly closed bottle and stored in a cabinet maintained at 30°C. for 3 weeks. Doughs were then prepared as above and the pigment contents of the doughs were again measured. The results, given in the bottom half of Table V, show that the bleaching effect of AP increases with storage, when flours have been treated with low amounts of AP. No additional bleaching was observed, however, for the dough containing 0.1072 g. of the blend per 100 g. of flour. The maximum bleaching effect appears to have been reached with this concentration even without storage.

No significant changes in -SH contents and in results of extensigraph tests are found for doughs prepared from the treated flours

TABLE V  
BLEACHING EFFECT OF AP ON CAROTENOID PIGMENT IN DOUGH  
BEFORE AND AFTER STORAGE OF FLOUR

STORAGE PERIOD	AP BLEND (Eq. AP) ADDED	CAROTENOID PIGMENT CONTENT
weeks	g. or eq./100 g.	p.p.m. (dry basis)
0	0.0	2.65
	0.0268 ( $1.6 \times 10^{-4}$ )	1.57
	0.0536 ( $3.2 \times 10^{-4}$ )	0.89
	0.1072 ( $6.3 \times 10^{-4}$ )	0.49
3	0.0	2.53
	0.0268 ( $1.6 \times 10^{-4}$ )	1.19
	0.0536 ( $3.2 \times 10^{-4}$ )	0.61
	0.1072 ( $6.3 \times 10^{-4}$ )	0.52

after storage for 3 weeks, as compared to those from the same flours without storage.

In general, the results obtained from this study on the bleaching effect of AP during dough processing are in agreement with the conclusion reached by Ferrari *et al.* (3) and Johnson *et al.* (6), that AP is an effective bleaching and maturing agent.

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