THE REACTION MECHANISM OF AZODICARBONAMIDE IN DOUGH[1]

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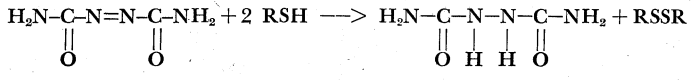
ABSTRACT

Azodicarbonamide (ADA) is a new flour-maturing agent. When mixed into doughs, it oxidized the sulfhydryl (–SH) groups and exerted an improving effect. The oxidation was rapid and almost complete during the mixing of dough for 2.5 min. Neither further mixing nor prolonged resting could give a significant and additional decrease in the –SH content. Because of its rapid reaction, ADA may possibly be used to replace iodate in a dough process where a faster maturing agent is required. ADA did not bleach flour pigments. Only slight variations in the pigment content were observed when doughs were treated with increasing amounts of ADA. From the reaction systems studied, the mole ratios of the reactants, GSH/ADA, thiogel –SH/ADA, and flour –SH/ADA, were found to be 1.79, 1.84, and 1.75, respectively; based on these ratios, the following chemical equation has been established to explain the maturing action of ADA:



Azodicarbonamide (ADA) is a new maturing agent introduced by Wallace and Tiernan, Inc. Its maturing action is of current interest to cereal chemists. Very recently Joiner has reported that ADA matures flour through oxidation. It does not react in the dry flour, but does react in the process of making dough. ADA-treated flours produce drier and more cohesive doughs than chlorine dioxide-treated flours. These drier doughs can tolerate higher absorption and are superior in machining properties. The bread made from ADA-treated flour is characterized by increased loaf volume and improved grain texture and outside appearance (7). In view of these interesting observations, this study has been undertaken to investigate the reaction mechanism of ADA in dough.

It is known that sulfhydryl groups (–SH) of flour proteins are involved in the reaction with various flour maturing or improving agents (3–5,8,9,13–16, and references therein). Our recent studies on the reactions of iodate, bromate (14), and acetone peroxides (Ketonox, a new flour bleaching and maturing agent introduced by the J. R. Short Milling Co.) (16), have clearly demonstrated that the improving effect of these agents can be attributed to their oxidizing action on –SH groups of flour. Although the maturing action of ADA

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has been established, no information appears to be available as to whether ADA exerts its action also through the oxidation of the –SH groups. If so, how fast does it react in dough and how effectively does it improve rheological properties of dough? Does it also oxidize flour pigments? Is there any stoichiometric relation between the reactants, ADA and glutathione (GSH), thiogel (thiolated gelatin), or –SH groups of flour? These problems have been investigated, and the results are reported in this paper.

Materials and Methods

Reagents. All chemicals used in this study were reagent grade. ADA and Maturox (a powdered premix containing 10% ADA) were kindly supplied by R. R. Joiner of Wallace and Tiernan, Inc., Belleville, New Jersey. GSH and thiogel (Lot No. TGA 6101) were purchased from Schwarz Bio-Research, Inc. Distilled water was passed through a "Deeminizer" before use. 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 nitrogen (10).

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 × 5.7) and ash contents of the flour were 13.7 and 0.50% (on 14% moisture basis). The –SH content was 1.07 μeq. per g. of flour. Doughs were prepared from 100 g. of flour (14% moisture) and sufficient salt solution to give an absorption of 55% and salt content of 1% (flour basis). They were mixed in the GRL mixer (6). Precautions were taken to minimize the oxidation of dough by air: flours or flours plus additives were purged with nitrogen under alternate vacuum and pressure in an air-tight bowl before mixing; the solutions used for mixing were saturated with nitrogen. Unless stated otherwise, all the mixings were done under nitrogen for 2.5 min.

Extensigrams. For extensigraph tests, doughs were given a reaction time of 10 min., rounded and shaped, and then stretched after a rest period of 20 min. During the reaction time and rest period the doughs were kept in a cabinet maintained at 30°C. and 95% r.h.

Analytical Methods. Unless otherwise indicated, a dough sample of approximately 20 g. was taken immediately after stretching. It was frozen in liquid nitrogen, freeze-dried, ground in a micro Wiley mill (60-mesh) and stored at −40°C. for subsequent analyses of –SH and carotene contents. –SH contents of thiogel, flour, or doughs were determined according to the modified method of Sokol, Mecham, and Pence (12) as described previously (16).

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

The carotene contents were measured by the procedure outlined by Binnington, Sibbitt, and Geddes (1) with the following modifications: Water-saturated n-butyl alcohol (20 ml.) was 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 in a 1-cm. cell with a Beckman Model DU spectrophotometer at 435.8 mμ.

Results and Discussion

Effect of Azodicarbonamide and Its Premix (Maturox) on Extensigram Height and –SH Content of Dough. The curves in Fig. 1 show that increasing amounts of Maturox, added in terms of ADA μmoles per g. of flour (14% moisture), cause progressive increases in extensigram height and decreases in extensibility of dough. This confirms the work of the Wallace and Tiernan Newark Laboratory that ADA is an effective flour-maturing agent (7). The extensigrams pre-

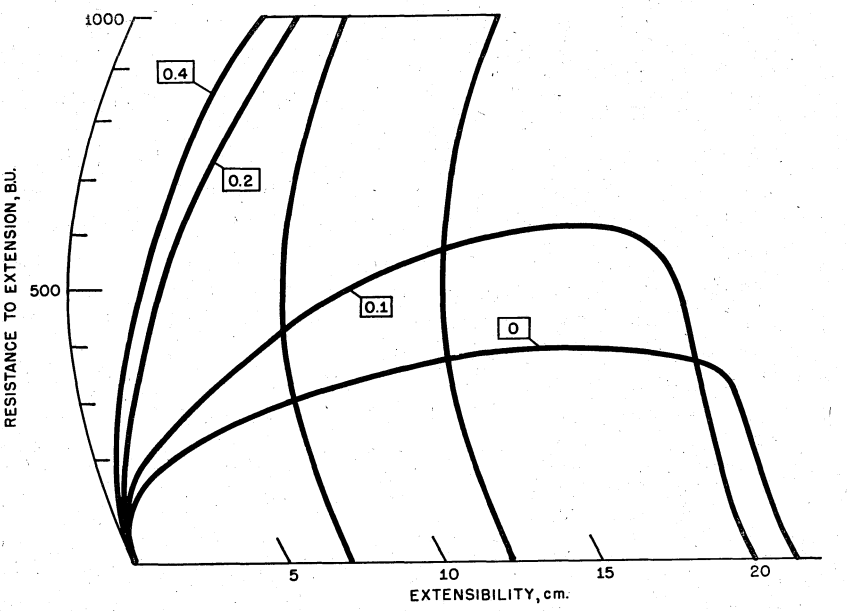


Fig. 1. Extensigrams for doughs treated with Maturox, expressed in terms of ADA μmoles per g. of flour.

pared from doughs with Maturox over 0.4 μmoles ADA per g. of flour are not presented in the figure because their extensigrams are almost identical to the one with Maturox of 0.4 μmoles ADA per g. In addition, these extensigrams in Fig. 1 show that when a dough is prepared from flour plus Maturox at or over 0.2 μmoles of ADA per g. of flour, its resistance to extension is raised beyond 1,000 B.U., while its extensibility is greatly reduced. This drastic change in dough properties probably indicates the overtreatment of flour with Maturox. The overtreatment with Maturox could result in some detrimental effects on final baking qualities, as observed by Sibbitt and Gilles (11).

To ascertain the chemical reaction of ADA with flour, –SH groups of these doughs were determined. The results, given in Fig. 2, show that a linear relation exists between the –SH loss and Maturox added up to 0.2 μmoles ADA per g. of flour. This relation demonstrates that ADA reacts with –SH groups of flour. The mole ratio of the reactants in dough, –SH groups oxidized/ADA added, is found to be 1.75. When larger amounts of Maturox were added, the linear relation, however, does not exist, mainly because the residual –SH groups become increasingly resistant to the oxidation. It appears likely that a portion of the total –SH content is masked in some protein structures. Once all of the accessible –SH groups have been oxidized, without unfolding of the structure, the remaining –SH groups become increasingly resistant to further oxidation by ADA.

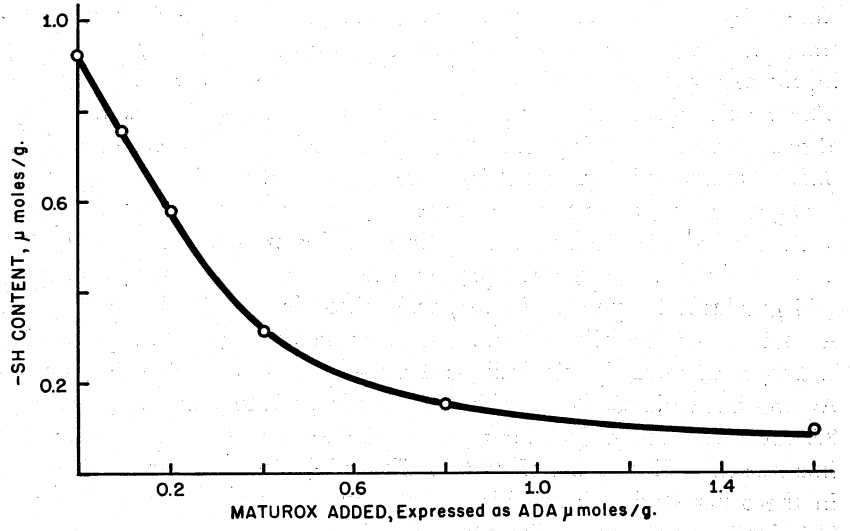


Fig. 2. Relation between added Maturox in terms of ADA and the sulfhydryl loss in dough.

Instead of Maturox, various concentrations of ADA solutions were added to flour before mixing. As shown in Table I, the extents of reduction in –SH content, increase in extensigram height, and decrease in extensibility of doughs treated with ADA solution are all essentially similar to those of doughs treated with Maturox containing an equal amount of ADA (Figs. 1 and 2). This means that ADA acts effectively as a maturing agent whether in the form of a premix or solution.

TABLE I

CHANGES IN EXTENSIGRAM HEIGHT AND EXTENSIBILITY AND LOSS OF –SH CONTENT IN DOUGHS TREATED WITH INCREASING AMOUNTS OF ADA

ADA Amount Added	Dough Properties		
	Extensigram		–SH Content
	Max. Height	Extensibility	
μmole/g. flour	*B.U.*	*cm.*	*μmole/g. dough*
0	395	21.4	0.93
0.1	590	20.8	0.69
0.2	over 1,000	13.2	0.58
0.4	over 1,000	7.4	0.32
0.8	over 1,000	7.2	0.17

Effect of Reaction Time and Mixing Period. Dough samples containing Maturox (0.4 μmoles ADA per g. of flour) were mixed for 2.5 min. and put into plastic containers. These containers were placed in a cabinet, maintained at 30°C. and 95% r.h., for a given length of time, referred to as the reaction time. At the end of the desired reaction time, 0, 1, 2, 3, or 4 hr., the samples were immediately frozen in liquid nitrogen and lyophilized. Their –SH contents were determined. The results, given in Table II, show that the reaction is fast and almost complete during the mixing period. The reaction time gives only a relatively insignificant increase in the –SH content.

Further experiments were made to determine whether the extent of reaction was limited by the physical barrier presented in the resting dough. Dough samples were prepared from flour containing Maturox (0.4 μmoles ADA per g. of flour) and mixed for 2.5, 5, 10, or 20 min. At the end of the mixing period, the sample was also frozen in liquid nitrogen at once, lyophilized, and its –SH content was determined. Again, the results show that the reaction proceeds to completion within 2.5 min. of mixing. Further mixing, on the contrary, causes a slight increase in –SH content of dough. This slight increase, as also shown in the other studies from this Laboratory, is apparently due to some "hidden" or "masked" –SH groups of flour proteins becoming accessible through continued mixing (2,14) .

TABLE II

LOSS OF –SH CONTENT IN DOUGHS TREATED WITH MATUROX DURING PROLONGED RESTING AND CONTINUED MIXING

	TIME	–SH CONTENT
	hr.	*μmole/g. dough*
Reaction time (prolonged resting)	0	0.32
	1	0.32
	2	0.33
	3	0.34
	4	0.34
	min.	
Mixing period (continuous mixing)	2.5	0.32
	5	0.33
	10	0.34
	20	0.37

Ineffectiveness of ADA as a Flour Bleaching Agent. To investigate the bleaching effect of ADA on flour pigments during dough processing, dough samples were prepared from flours treated with various amounts of Maturox (see Table III) and mixed in nitrogen for 2.5 min. The carotene contents of the freeze-dried doughs were determined. The results, given in the top half of Table III, show that ADA does not bleach carotenes in dough.

The experiment was then extended to study the effect of storage on the bleaching. Flour samples were treated with various amounts of Maturox in tightly closed bottles and stored in a cabinet maintained at 30°C. for 3 weeks. The carotene contents of the freeze-dried doughs, prepared from these flour samples after storage, were measured. The results, given in the bottom half of Table III, demonstrate that among these dough samples little evidence of bleaching was observed. This confirms that ADA does not bleach flour pigments. A similar conclusion was reported by Joiner (7).

TABLE III

INEFFECTIVENESS OF ADA IN BLEACHING OF PIGMENTS IN DOUGH

STORAGE PERIOD	MATUROX ADDED	CAROTENE CONTENT
weeks	*μmole ADA/g. flour*	*p.p.m. (dry basis)*
0	0.0	2.65
	0.1	2.58
	0.4	2.68
	0.8	2.83
3	0.0	2.53
	0.1	2.83
	0.4	2.32
	0.8	2.35

The Reaction Mechanism. Evidence presented has shown that ADA does not bleach the pigments, but does react with –SH groups of flour. Additional experiments were therefore made on simple chemical systems consisting of ADA and GSH or thiogel to elucidate the reaction mechanism. Since –SH compounds are readily oxidizable by air (17–19), all operations and titrations in these experiments were done under nitrogen atmosphere (by constantly flushing the system with nitrogen).

Various volumes of $1 \times 10^{-3}M$ GSH were added to a titration vessel containing 15 ml. of ADA solution (total ADA content, 2 μmoles). The mixture was then stirred slowly with a magnetic stirrer for 1 min. to let the reaction take place. When the amount of GSH added exceeded that required to react with the 2 μmoles of ADA in solution, the

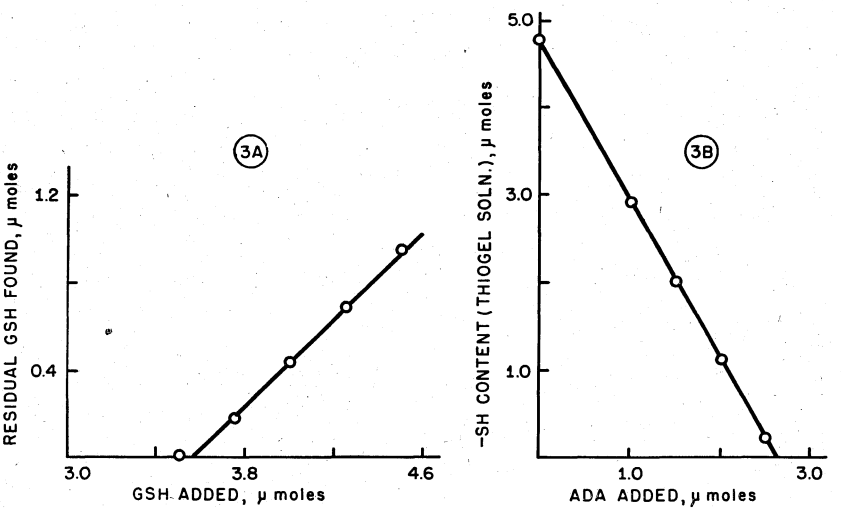
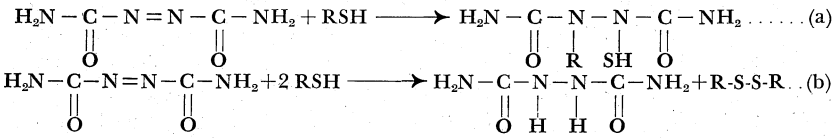


Fig. 3. A, reaction of ADA with GSH. B, reaction of ADA with thiogel.

residual GSH could then be determined by amperometric titration. Figure 3A shows that a linear response exists between further GSH added and residual GSH recovered. By extrapolating the line to the abscissa, the intercept is found to be 3.58 μmoles of GSH. Accordingly, this amount is required to react with 2 μmoles of ADA; the mole ratio of the reactants, GSH/ADA, is 1.79.

In an analogous manner, various volumes of $2 \times 10^{-4}M$ ADA were added to 5 ml. of 0.69% thiogel. The residual –SH groups were also determined. The results are plotted in Fig. 3B. From the figure, the mole ratio of the reactants for thiogel –SH/ADA is found to be 1.84.

The reaction of ADA and –SH compounds could take place according to either one of the following equations.



Theoretically the mole ratios should be 1 and 2 according to equations a and b. The average mole ratio with the simple chemical systems was experimentally found to be 1.82; with dough, it was 1.75. Thus, it is reasonable to assume that the reaction proceeds as indicated by equation b. In addition, Joiner has reported that the conversion of ADA to hydrazodicarbonamide (biurea) in reaction with flour is quantitative (7). The formation of biurea also supports the evidence that ADA oxidizes –SH groups of flour according to equation b. The reaction is therefore of the oxidation-reduction type (equation b) rather than the addition type (equation a).

The ADA reaction-rates were also determined for the following simple chemical systems: When 5 ml. of $1 \times 10^{-3}M$ GSH were added to 15 ml. of ADA solution (total ADA content, 2 μmoles), the mixture was allowed to react for 1, 2.5, 5, 10, or 20 min. by stirring with a magnetic stirrer under nitrogen. At the end of the reaction time, the residual GSH was determined.

TABLE IV

EFFECT OF REACTION TIME ON THE ADA REACTION WITH GSH OR THIOGEL

REACTION	REACTION TIME	RESIDUAL –SH	–SH REACTED	–SH/ADA
	min.	*μmoles*	*μmoles*	
ADA + GSH	1.0	1.36	3.64	1.82
	2.5	1.36	3.64	1.82
	5.0	1.37	3.63	1.82
	10.0	1.37	3.63	1.82
ADA + thiogel	1.0	0.82	3.08	1.85
	2.5	0.84	3.06	1.83
	5.0	0.83	3.07	1.84
	10.0	0.84	3.06	1.83
	20.0	0.83	3.07	1.84

Likewise, 5 ml. of thiogel (total –SH content, 3.903 μmoles) were added to 15 ml. of ADA solution (total ADA content, 1.669 μmoles). After the various reaction times, residual –SH contents were measured. The results, summarized in Table IV, show that the rate of oxidation of GSH, or thiogel –SH, by ADA is very rapid.

Iodate is a fast –SH oxidizing agent in dough. Our previous study demonstrated that the –SH content in dough decreased from 1.11 to 0.33 μeq. per g., when dough was treated with iodate at the concentration of 2.32 μeq. per g. of flour and mixed in nitrogen for 5 min. With further mixing the iodate reaction continued, but its rate decreased sharply (14). When dough was treated with ADA (0.8 μeq. per g. of flour) and mixed in nitrogen for 2.5 min., the –SH content dropped from 1.07 to 0.32 μeq. per g. The –SH content did not further decrease during continued mixing. It appears that on an equivalent basis ADA would oxidize more –SH groups in dough and also react faster than iodate. Thus, ADA may possibly be used to replace iodate in processes, such as the continuous breadmaking process, where a fast oxidizing agent is required.

Acknowledgment

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