THE IMPROVING MECHANISM OF ASCORBIC ACID[1]

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ABSTRACT

The improving action of ascorbic acid (AA) is due to the oxidation of –SH groups in dough by dehydroascorbic acid (DHA), an oxidation product of AA, as shown in this study. When flour treated with DHA, isoascorbic acid (IAA), or AA and mixed in nitrogen, only DHA-treated doughs show a significant decrease in –SH content. The –SH oxidation is closely associated with the improving effect of DHA on dough, proving that DHA has the typical action of a flour improver. DHA does not react with –SH groups in dough as fast as iodate or azodicarbonamide, but it reacts faster than bromate. The IAA-treated doughs, whether mixed in nitrogen, air, or oxygen, do not show any significant differences in –SH content from the controls. The inactivity of IAA reveals the specificity of the enzymatic system. AA is effective for the –SH oxidation in the presence of oxygen, indicating that AA should be oxidized to DHA first to exert its improving action. For a mixing process where the oxygen supply is limited, the use of bromate or air can accelerate the action of AA by oxidizing AA chemically rather than enzymatically. In addition, bromate can supplement DHA to oxidize –SH groups in dough to enhance the improving effect.

A series of studies on the chemical reactions of flour improvers such as bromate, iodate (1), acetone peroxides (2), and azodicarbonamide (ADA) (3) has been carried out in this Laboratory. Results of these studies all point out that the actions of these improvers are closely associated with the oxidation of sulfhydryl (–SH) groups in dough. These flour improvers are the ones most used in North America and in other parts of the world as well. In European countries ascorbic acid (AA) also has been widely used as a flour improver since about 29 years ago when its improving action was found by Jørgensen (4). AA differs from the other improvers in two respects: it is a vitamin; no possible objection could be raised against its use in the quantities employed. AA itself is a reducing substance but exerts its improving action as an oxidizing agent through an enzymatic system. AA is oxidized to dehydroascorbic acid (DHA) with AA oxidase in flour extracts (5,6) and in dough (7,8). It has been well established through the studies of Melville and Shattock (5), Sandstedt and Hites (6), and Maltha (9) that DHA is responsible for the improving action. Although there is evidence that DHA can be reduced to AA by DHA reductase in plant tissues (10) and in flour extract (6,9,11,12) with the addition of glutathione (GSH), the reaction between DHA and –SH

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groups of flour proteins has remained obscure. Sandstedt and Hites observed the disappearance of –SH groups in the flour extract with the addition of AA, as detected by the nitroprusside test (6). Since the test is quite unspecific for –SH groups (13) and the nitroprusside end point, as they have found, is indistinct in flour extract (6), their observation indicates only tentatively that some –SH groups of flour extract may be oxidized in the presence of AA. A few attempts were also made very recently by Kuninori and Matsumoto to estimate the changes in –SH content of flour extracts with the addition of DHA, but they failed to detect any –SH changes (12). Furthermore, all these studies (6,12) were done with flour extract, not with dough. In view of the fact that AA exerts its improving action on dough, it seems desirable to obtain direct evidence concerning the reaction of DHA with –SH groups in dough in order to explain the improving mechanism of AA.

This study has been undertaken to elucidate the improving mechanism of AA by investigating various effects of DHA, AA, and isoascorbic acid (IAA) on the oxidation of –SH groups in dough. It has also been extended to explore the use of bromate, iodate, ADA, or oxygen to oxidize AA chemically rather than enzymatically in order to accelerate the improving action of AA. Results of this study are reported in this paper.

Materials and Methods

Reagents. All chemicals used in this study were reagent grade. L(+)AA and DHA were purchased from Matheson Coleman & Bell Co. and Mann Research Laboratories, Inc., respectively. Nitrogen used 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 (14). Distilled water was passed through a Deeminizer before use.

Flour and Dough. An untreated straight-grade flour milled from a blend of Canadian hard red spring wheat was used throughout the study. The protein (N × 5.7) and ash contents of the flour were 14.0 and 0.48% (14% moisture basis), respectively. The –SH content was 1.20 μeq. per g. of flour. Doughs were prepared from 200 g. of flour (14% moisture) and sufficient salt solution containing AA or other reagent(s) to give an absorption of 59.6% and a salt content of 1% (flour basis). They were mixed in the GRL mixer (15). As a precaution to minimize the oxidation of dough by air, flour samples were stored in a desiccator filled with nitrogen overnight. AA and/or other reagent(s) was freshly prepared with oxygen-free salt solution before use. The flour sample was purged again with nitrogen under alternate vacuum and

pressure in an air-tight bowl before mixing. Unless otherwise stated, all the mixings were done under nitrogen for 2.5 min. When doughs were mixed in oxygen, oxygen was used instead of nitrogen in every step of the process.

Extensigrams. For extensigraph tests, a dough sample after mixing was divided into two pieces. They were given a reaction time of 5 min., rounded and shaped, and then stretched after a rest period of 20 min. for one dough-piece and of 80 min. for the other. 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 analytical use. Sulfhydryl contents of flour and doughs were determined according to the modification of the method of Sokol *et al.* (16) described previously (17). DHA was determined by the 2,4-dinitrophenylhydrazine method (18).

Results and Discussion

Effect of AA, IAA, and DHA on the –SH Oxidation and Rheological Properties of Doughs Mixed in Nitrogen, Air, or Oxygen. The results of –SH determinations, listed in Table I, lead to several conclusions. First, when these doughs are mixed in nitrogen and rested for 20 or 80 min., only the DHA-treated doughs show large decreases in –SH content. The decreases suggest that DHA oxidizes –SH groups in dough just as other flour improvers do. Second, AA is effective for the –SH oxidation in the presence of oxygen, indicating that AA should be oxidized to DHA first in order to exert the improving action. Third,

TABLE I

EFFECTS OF AA, IAA, AND DHA ON THE OXIDATION OF –SH GROUPS IN DOUGHS MIXED IN NITROGEN, AIR, OR OXYGEN

ADDITIVE	AMOUNT ADDED	REST PERIOD	–SH CONTENT		
			N_2	Air	O_2
	μmole/g. flour	*min.*	*μmole/g.*	*μmole/g.*	*μmole/g.*
O		20	1.15	1.05	0.91
IAA	0.1		1.14	1.05	0.91
AA	0.1		1.12	0.91	0.82
DHA	0.1		0.91	0.83	0.76
O		80	1.05	0.91	0.82
IAA	0.1		0.98	0.92	0.82
AA	0.1		0.94	0.82	0.70
DHA	0.1		0.81	0.70	0.67

IAA is inactive. The IAA-treated doughs, whether mixed in nitrogen, air, or oxygen, show very little change with respect to the –SH content in doughs as compared to the controls. The negligible change in –SH groups upon IAA treatment suggests the specificity of the enzymatic system.

Parallel with determinations of –SH groups in dough, extensigraph tests were also undertaken to measure the corresponding changes in rheological properties of these treated doughs. The extensigraph test, though empirical, provides us with a visual comparison of the improving effect. The extensigrams, given in Fig. 1, demonstrate the following points which agree well with the conclusions obtained from the –SH determinations. First, the extensigram height of the DHA-treated dough is higher than those of others. Second, the height of the AA-treated dough increases progressively with increasing concentrations of oxygen used for mixing. Third, there is no practical difference in extensigram height between the IAA-treated dough and the control. The extensigrams, given in Fig. 1, were made on the treated doughs rested for 20 min. Similar extensigrams with lower heights were also observed for the doughs rested for 80 min.

In short, the presented data and those of other workers (4–12) indicate that the improving mechanism of AA involves the oxidation of AA to DHA by AA oxidase and the subsequent reduction of DHA to AA by DHA reductase coupled with the oxidation of –SH groups in dough. As a result, DHA has the typical action of a flour improver.

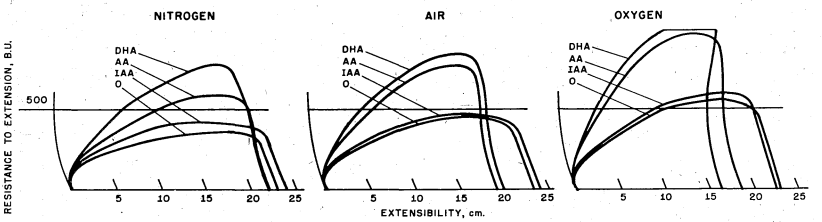


Fig. 1. Extensigrams for doughs treated with DHA, AA, or IAA and mixed in nitrogen, air, or oxygen.

Effects of Various Amounts of DHA. In view of the relation between the improving effect and the –SH oxidation, further experiments were made to ascertain the effect of various amounts of DHA on the –SH oxidation and dough properties.

The extensigrams, given in Fig. 2, show that increasing amounts of DHA cause progressive increases in extensigram height and decreases in extensibility of dough. The same conclusion can be drawn from the extensigram changes for the doughs rested for 80 min.

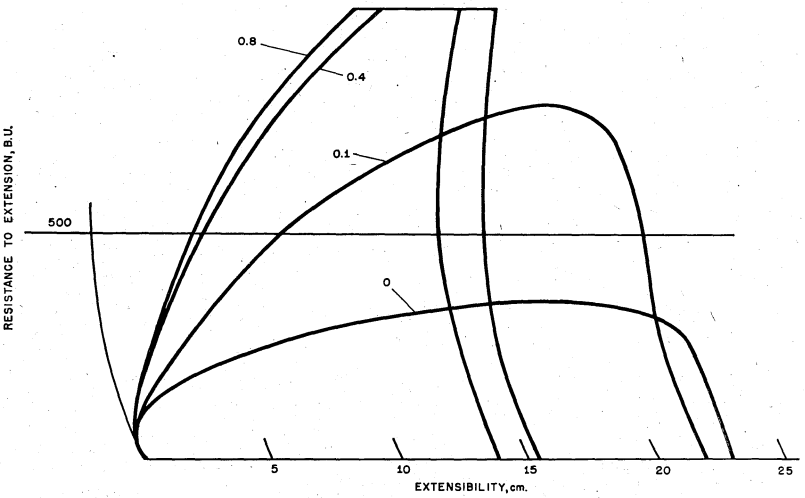
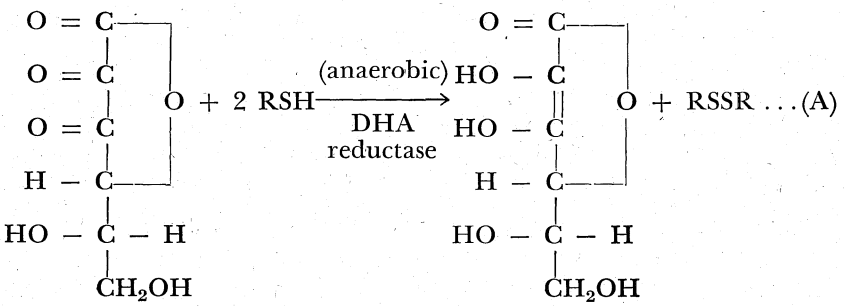


Fig. 2. Extensigrams for doughs treated with DHA, expressed in terms of μmole of DHA per g. of flour.

The relation between DHA added and –SH groups oxidized in these doughs is presented in Fig. 3. With the addition of 0.1 μmole of DHA per g. of flour, there are 0.15 to 0.24 μmole of –SH groups oxidized in dough (see also Table I). In other words, the mole ratio of the reactants in dough, –SH groups oxidized/DHA added, is approximately 2. This ratio is in agreement with that found by Kuninori and Matsumoto with flour extract in the presence of GSH (12). It seems that the oxidation of –SH groups by DHA takes place in dough according to equation A. However, at higher DHA levels, the mole ratio decreases sharply. The sharp decrease in the mole ratio indicates that –SH groups in dough probably are not as readily accessible to DHA and the reductase system as to other chemical improvers such as iodate (1), ADA (3), and acetone peroxides (2). Alternatively, DHA with the reductase system may not be as reactive as other improvers for the oxidation of –SH groups in dough. This finding may serve as a theo-



retical basis for explaining why flour can tolerate more AA than other improvers, as shown by baking results and discussed previously (19).

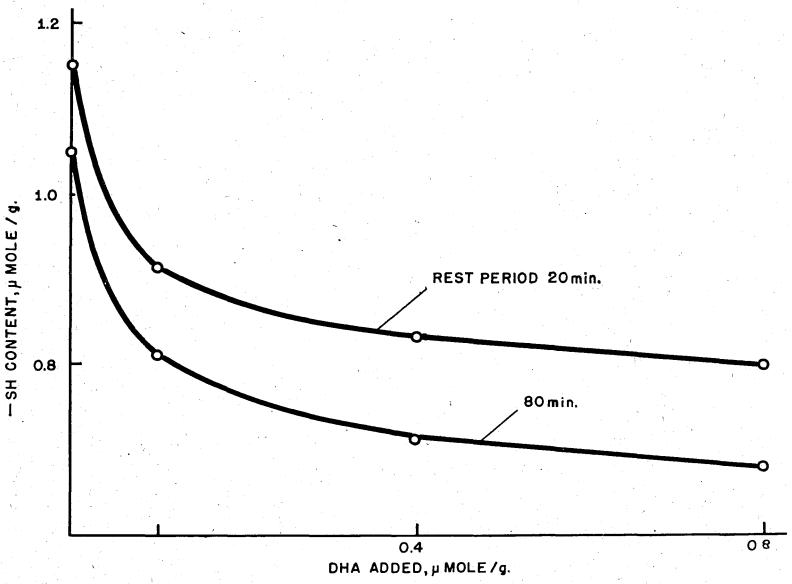


Fig. 3. Relation between DHA added and –SH groups oxidized in doughs rested for 20 and 80 min.

Effects of Reaction Time and Mixing Period. Experiments were then extended to evaluate the effect of physical barriers in dough on the DHA reaction by continuous mixing and prolonged resting. Dough samples containing 0.4 μmole of DHA per g. of flour were mixed for 2.5, 5, 10, or 20 min. Results, presented in Fig. 4, show that the continuous mixing of dough with DHA can increase the –SH oxidation. However, the increase is not so pronounced after mixing for 10 min.

In another series of experiments, doughs with 0.4 μmole of DHA per g. of flour were mixed for 2.5 min., transferred to plastic containers, placed in a cabinet maintained at 30°C. and 95% r.h., and rested for 0, 30, 60, 120, or 180 min. The curve in Fig. 4 shows that the –SH group is oxidized by DHA in dough quite rapidly during the first 60 min. Afterward the oxidation rate slows down rapidly.

When the rate of the DHA reaction is compared with the rates of reaction of bromate, iodate, and ADA with –SH groups in dough as reported previously (1,2,3) (also see the next section), it can be concluded that DHA does not react with –SH groups in dough as fast as iodate or ADA; but it reacts faster than bromate.

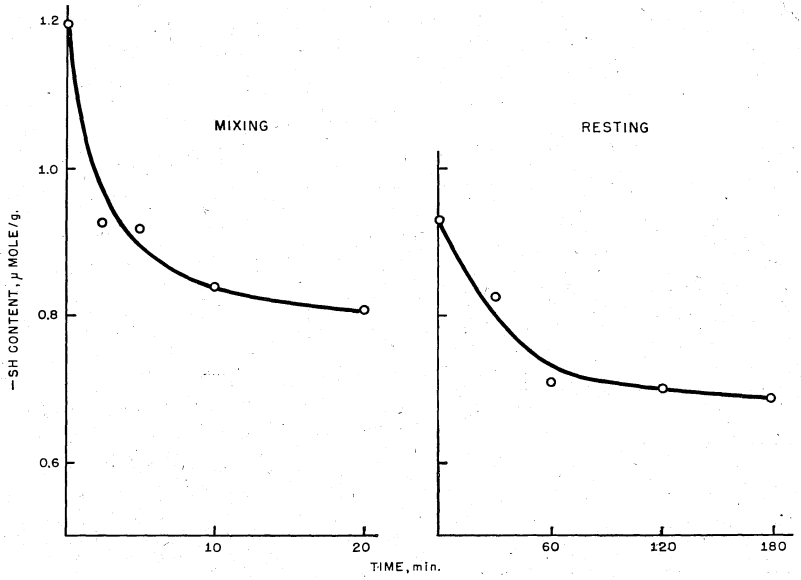
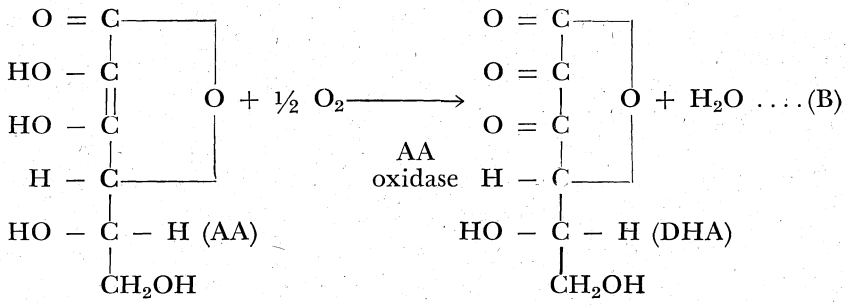


Fig. 4. Losses of –SH content in doughs treated with DHA during continuous mixing and prolonged resting.

Synergistic Action of AA and Bromate or Other Improvers. The effectiveness of DHA as an improver has been discussed in the previous sections; in practice it is, however, too expensive to use it directly. On the other hand, if AA is used, oxygen is required for its oxidation as indicated by equation B. In a continuous doughmaking process, or a batch process where the oxygen supply is limited, the use of ascorbic acid would be inefficient. In order to speed up the oxidation of AA to DHA, the use of bromate or other improvers would be advantageous to oxidize AA to DHA chemically rather than enzymatically. Bromate (or other improver) and DHA may also exert some supplementary action on the oxidation of –SH groups in dough. In addition, in the United Kingdom and several other countries where both AA and bro-



mate are permitted to be used as flour improvers, it is also profitable to replace part of the AA with bromate, for bromate is less expensive than AA.

As shown in Fig. 5, the extensigrams at the left were made from the doughs treated with bromate from 0.1 to 0.4 μmole per g. of flour; those at the right were from the doughs with the corresponding amounts of bromate plus 0.4 μmole of AA per g. of flour. For comparison, the extensigram of the dough with 0.4 μmole of AA is also included in Fig. 5; the dough contains 0.93 μmole of –SH group per g.

On the basis of these results, it is evident that the improving effect of ascorbic acid and bromate together is greater than that of bromate or ascorbic acid alone on dough.

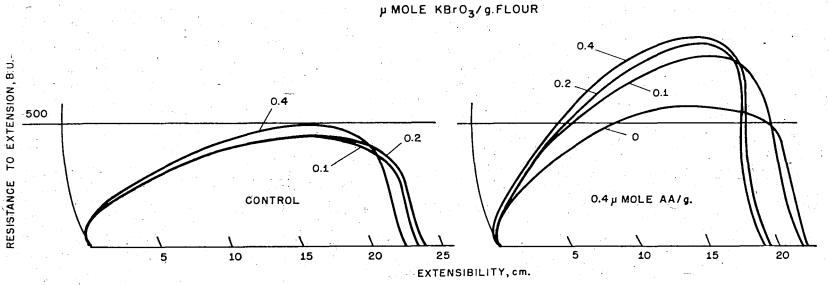


Fig. 5. Extensigrams for doughs treated with bromate and with bromate plus AA.

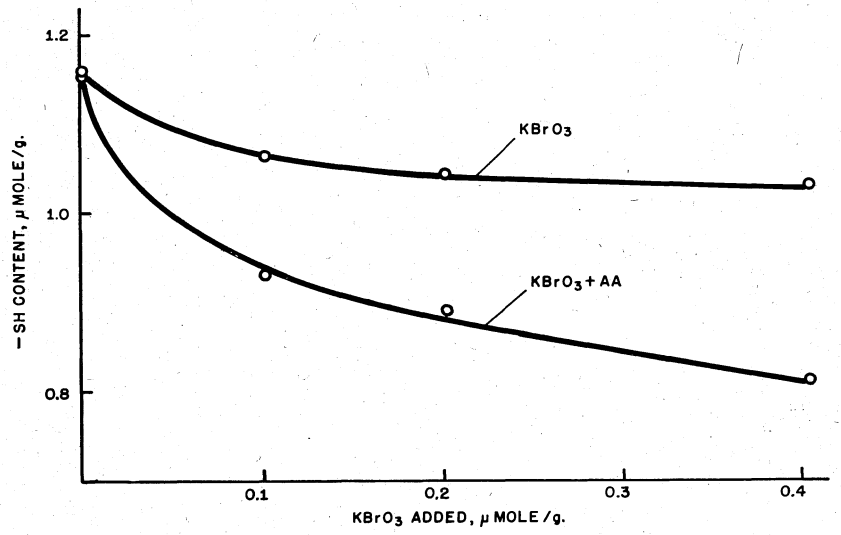


Fig. 6. Losses of –SH content in doughs treated with bromate and with bromate plus AA.

Corresponding with the changes in the extensigrams, more –SH groups in these doughs are oxidized by AA and bromate together than by bromate or AA alone, as shown in Fig. 6.

Further, an attempt was made to find out whether the extent of AA oxidized by bromate could be increased with increasing reaction times. The solution used for mixing was prepared by dissolving AA at the concentration of 0.4 μmole per g. of flour with oxygen-free bromate-salt solution (bromate at 0.2 μmole/g. flour; sodium chloride at 1% of flour) in a flask. Nitrogen was then flushed into the flask to remove any residual oxygen immediately before the flask was closed with a glass stopper. The flasks containing the solution were allowed to stand for 0, 0.5, 1, 2, or 19 hr. before mixing. The results show that the extensigram height of the dough increases from 830 (0 hr.) to 920 B.U. (19 hr.); its –SH content decreases from 0.89 to 0.82 μmole per g. The relatively small effect of the reaction time on the improving action or the –SH oxidation reveals that bromate does not oxidize AA in solution effectively under the experimental conditions. Then, the synergistic effect of bromate and AA could be due to the oxidation of AA by bromate in dough during mixing. This could also be due to the oxidation of –SH groups by bromate (1) in addition to the oxidation by DHA and the reductase system in dough. As a result, bromate can enhance the improving effect of AA.

The experiment was then expanded to use iodate or ADA instead of bromate to ascertain the effect of a combination of AA and iodate or ADA on dough. The results, listed in Table II, indicate that doughs treated with iodate or ADA, except that with 0.1 μmole ADA, show a

TABLE II

COMPARISON OF THE EFFECTS BETWEEN IODATE AND IODATE WITH AA, AND BETWEEN ADA AND ADA WITH AA ON THE –SH OXIDATION AND DOUGH PROPERTIES

ADDITIVE (AMOUNT ADDED)	DOUGH PROPERTIES	
	–SH Content	Extensigram Height
μmole/g. flour	*μmole/g. dough*	*B.U.*
Iodate (0.1)	0.68	810
Iodate (0.2)	0.53	1,000
Iodate (0.4)	0.31	over 1,000
Iodate (0.1) + AA (0.4)	0.86	710
Iodate (0.2) + AA (0.4)	0.73	974
Iodate (0.4) + AA (0.4)	0.50	990
ADA (0.1)	0.93	513
ADA (0.2)	0.69	890
ADA (0.4)	0.43	over 1,000
ADA (0.1) + AA (0.4)	0.84	670
ADA (0.2) + AA (0.4)	0.83	720
ADA (0.4) + AA (0.4)	0.80	930
AA (0.4)	0.93	608
O	1.15	350

greater loss in –SH content and a higher B.U. in extensigram height than corresponding doughs with iodate (or ADA) and AA together. As stated before, iodate or ADA can oxidize –SH groups faster than DHA to exert the improving effect. When iodate (or ADA) and AA are mixed together with dough, part of the iodate (or ADA) would be consumed for the oxidation of AA. The net effect of iodate and AA together would be less than that of iodate (or ADA) alone.

Oxidation of AA by Air. When bromate, iodate, and ADA are used to oxidize AA, only bromate together with AA accelerates the –SH oxidation and increases the improving effect, as shown and discussed in the previous section. An alternative would be to use air to oxidize AA before mixing. It is well known that AA is readily oxidizable by air especially in a neutral or alkaline solution. The first oxidation product is dehydroascorbic acid. Further studies were then made to oxidize AA directly by air.

Twenty milliliters of sodium acetate buffer (I = 0.05; pH 5.8) containing 0.8×10^{-4} mole of AA was poured into a flask. The flask was closed with a rubber stopper which had two small holes to let air into the flask. It was shaken by a Burrell shaker for 1 hr. The DHA content in the oxidized solution was found to be 0.32×10^{-4} mole. This solution was then saturated with nitrogen, and used for mixing under nitrogen.

The extensigrams, presented in Fig. 7, indicate that the oxidation

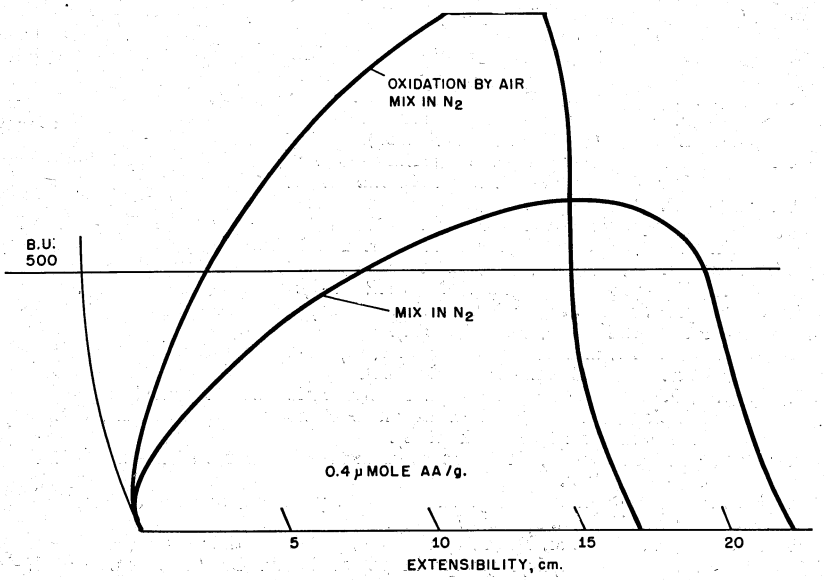


Fig. 7. Effect of the oxidation of AA by air on the extensigram.

of AA by air can enhance the improving effect significantly, as compared with the control. The improving effect obtained from the oxidation by air appears more pronounced than that from the oxidation by bromate. The –SH content was also decreased from 0.93 μmole per g. of the control dough to 0.81 μmole per g. of the dough treated with the oxidized AA solution.

So far as the oxidation of –SH groups and changes in dough properties were concerned, no significant variations were observed by using oxygen instead of air to oxidize the AA solution or by prolonging the oxidation period. Using a flour suspension or flour extract to provide AA oxidase for catalyzing the oxidation before mixing was also tried; but results showed that such a treatment was not as effective as the oxidation directly by air for exerting the improving action.

Acknowledgment

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