L-ASCORBIC ACID OXIDIZING SYSTEM IN DOUGH AND DOUGH IMPROVEMENT[1]

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ABSTRACT

The oxidation of L-ascorbic acid (L-AsA) in flour doughs was followed by a modification of Roe's method (Kedo *et al.*, [in Japanese] Eiyō to Shokuryō 13: 242–245; 1961; and Roe and Kuether, J. Biol. Chem. 147: 399–407; 1943) with metaphosphoric acid extract from dough. L-AsA incorporated in dough at 100 p.p.m. (flour basis) was oxidized 54, 67, and 81% in 5, 10, and 15 min. of mixing, respectively. However, 500 p.p.m. of sodium diethyldithiocarbamate (DDC) incorporated with L-AsA inhibited the reaction to the extent that only 24% was oxidized in 74 min. No further inhibition was found with increased DDC above this level. Rheological study of dough with extensigraph indicated a little but no definite inhibition by DDC to the improvement reaction of L-AsA. It is assumed the oxidation of L-AsA is not a limiting factor of dough improvement, from these data and those with ascorbic acid oxidase.

Jørgensen (8) reported that L-ascorbic acid (L-AsA) had an effect similar to that of an oxidizing agent in dough, although up to that time it had been regarded as a reducing agent.

Melville and Shattock (11) found that old lemon juice in which L-AsA is assumed to be present as dehydro-L-ascorbic acid (DHA), the oxidized form of L-AsA, still had an improving activity in dough, and that DHA was considerably more potent than L-AsA. They showed that flour contained ascorbic acid oxidase and that DHA was equivalent to bromate on a weight-for-weight basis. Feaster and Cathcart (5) obtained similar results, but D-ascorbic acid had no effect as a dough improver.

Cathcart and Edelmann (1) found that it was necessary to add one and one-half times as much L-AsA as bromate to get an equivalent effect on baking.

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There are many interesting reports dealing with dough-improving reaction of some L-AsA analogs, which may give an indication of the mechanism involved in the reaction.

Despite Elion's finding (4) that compounds having an enediol group act as inhibitors of papain and also as flour improvers, Sandstedt and Hites (16) could not detect as much improving effect with L-glucoascorbic and D-araboascorbic acid as with L-AsA, and showed that those compounds having an enediol group next to a carbonyl group were not always effective as improvers of dough. They found reversible oxidation of L-AsA in flour extract. Recently similar reaction was also discussed by Shimizu, Fukawa, and Ichiba (17), through the determination of DHA and L-AsA in the presence of glutathione. Experiments with five analogs, which possess a triketone group in their oxidized form, were carried out by Maltha (10). The effects of L-AsA on the rheological properties of dough were published by Shimizu, Nagao, and Ichiba (18).

These results indicate that the improving reaction of L-AsA involves at least two systems, namely, oxidation to DHA at the first stage and reduction of DHA by reducing materials or groups, as shown by Sandstedt and Hites (16).

Proskuryakov and Auerman (14) studied the L-AsA oxidizing system in flour suspension or flour extract using inhibitors such as potassium cyanide and sodium diethyldithiocarbamate (DDC) for oxidation, and estimated their effects on the rheological properties with the Chopin Alveograph. They found that potassium cyanide and DDC inhibited 35 and 45% of oxidation of L-AsA respectively in flour suspension for reaction times of 30 min. They also found that 400 p.p.m. of DDC added to dough could inhibit the effect of L-AsA, but incorporation of ascorbic acid oxidase in addition had no effect.

Since there is a higher concentration of the active group of gluten proteins, presumably involved in the reaction of L-AsA in dough, than in the aqueous extract and suspension, a better picture of the mechanism may be obtained by studying the reaction in dough. In this paper, the oxidation of L-AsA which was postulated as the first stage of dough improvement with L-AsA is studied in greater detail by analytical and rheological methods. The second stage will be reported in the second paper of this series.

Materials and Methods

Materials. The flour used in this study was unbleached, improver-free, straight-grade flour, commercially milled from a blend of Cana-

dian hard spring wheat, containing 13.2% crude protein and 0.43% ash, 14% moisture basis.

Sodium chloride, L-AsA, and DDC were analytical-grade commercial products purchased from Wako Pure Chemical Co.

Ascorbic acid oxidase was prepared from pumpkin rind by fractional precipitation with ammonium sulfate (6). One milliliter of this crude enzyme preparation catalyzed the oxidation of 0.96 mg. out of 1.01 mg. of L-AsA in 1.2 ml. of 2% metaphosphoric acid adjusted to pH 6.0 with sodium hydroxide during aeration for 10 min. at 30°C.

Preparation of Dough for Extraction and Determination of L-AsA. Doughs for determining the extent of oxidation of L-ASA were prepared by mixing 200 g. of flour and 65% distilled water (flour basis) containing L-AsA and DDC when these were added, in an open-bowl GRL mixer. Water was circulated through the bowl to maintain the temperature of the dough at 30°C. The time of mixing was varied for the different series of experiments. For the first, the dough was mixed for 7 min., allowed a reaction time of 30 min., mixed again for another 7 min., then allowed another 30-min. reaction time.

When the reagents, L-AsA or DDC, were added at different times, the volume of water was decreased by 10 ml. and this amount of water containing the reagent was added prior to the second mixing. In the third series of experiments, the mixing times were varied from 5 to 15 min. for the first mixing and 5 to 10 min. for the second.

Where reaction times were necessary, doughs in vessels were placed in a thermostat at 30°C. for a fixed time. Surface drying was minimized by covering the vessel with a moist cloth.

For the extraction of L-AsA, dough corresponding to 100 g. of flour was homogenized with 200 ml. of 5% metaphosphoric acid solution in a Waring Blendor for each 1 min., before and after a 1-min. rest period.

DHA in the extract was determined colorimetrically by a modification (9) of Roe's method (15), as the osazone with 2,4-dinitrophenylhydrazine at 530 mμ with a Hitachi EPU-2 spectrophotometer (Beckman type).

L-AsA was determined by the same method after oxidation with 2,6-dichlorophenolindophenol (13). The quantity of L-AsA plus DHA found was expressed as total ascorbic acid. Diketo-L-gulonic acid, which was an oxidized product of DHA and was determined as DHA by Roe's method, was neglected from this calculation because it was found to be negligible in our experiment.

Extensigraph Test. Doughs were prepared in a farinograph mixer with 300 g. of flour, 2% of sodium chloride, the reagents indicated,

and sufficient water to give 500 B.U. at maximum consistency. The mixing times were 1 min. and 3 min. before and after a 5-min. rest period, respectively. For the extensigraph test 150 g. of dough were taken as test pieces and stretched after 45 min. of resting on a dough holder. The second and third extensigrams were taken by remoulding the previously used sample and stretching after the same rest period.

Relaxation Test. Doughs were prepared by the same method as in the previous section. Relaxation curves were obtained by the method of Dempster, Hlynka, and Anderson (3) by plotting the load at 7 cm. extension on the kymograph against the rest period. All of these experiments were carried out at 30°C.

Results

Oxidation of L-AsA in Dough and the Effect of DDC. 1. Effect of DDC concentration. The extent of oxidation of DDC was determined 30 min. after the second mixing. The results are shown in Table I.

TABLE I

EFFECT OF DDC INCORPORATION ON THE OXIDATION OF L-ASA IN DOUGHS

SAMPLE No.	CONCENTRATION OF DDC [a]	L-ASA ADDED TO DOUGH [a]	DHA FOUND IN DOUGH [a]	L-ASA + DHA FOUND IN DOUGH [a]	PERCENT OXIDIZED [b]
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	%
1	0	115	88	95	93
2	250	112	58	91	63
3	500	108	25	103	24
4	1,000	112	25	99	25

[a] Flour basis (13.5% moisture).
[b] DHA/(DHA + L-AsA) × 100.

It was found that the oxidation of L-AsA, which proceeded up to 93%, was inhibited markedly with DDC. However, complete inhibition of oxidation could not be attained; the maximum occurred at

TABLE II

EFFECT OF DDC INCORPORATION AT DIFFERENT TIMES ON THE OXIDATION OF L-ASA

SAMPLE No.	COMPOUNDS ADDED [a]		L-ASA ADDED TO DOUGH [b]	DHA FOUND IN DOUGH [b]	L-ASA + DHA FOUND IN DOUGH [b]	PERCENT OXIDIZED [c]
	First Mixing	Second Mixing				
			p.p.m.	*p.p.m.*	*p.p.m.*	%
1	L-AsA	—	115	88	95	93
2	L-AsA	DDC	115	86	94	92
3	L-AsA, DDC	—	108	25	103	24
4	DDC	L-AsA	108	21	103	21

[a] L-AsA and DDC were 100 and 500 p.p.m. respectively (flour basis).
[b] See footnote a in Table I.
[c] See footnote b in Table I.

500 p.p.m. and further increase of DDC had no effect.

2. Effect of mixing order. The effect of DDC, incorporated into the dough before, after, and at the same time with L-AsA, on the oxidation of L-AsA is shown in Table II.

Comparison of samples 2 and 3 in Table II indicates that the inhibitory effect of DDC was instantaneous; the order in which it was incorporated into the dough was immaterial. From the results of samples 1 and 2 it appeared that the reaction of L-AsA was almost complete within the first 7 min. of mixing and 30-min. reaction time.

3. Effect of DDC added at various mixing times. The oxidation of L-AsA during the mixing process and the effect of DDC added at various mixing times are shown in Fig. 1.

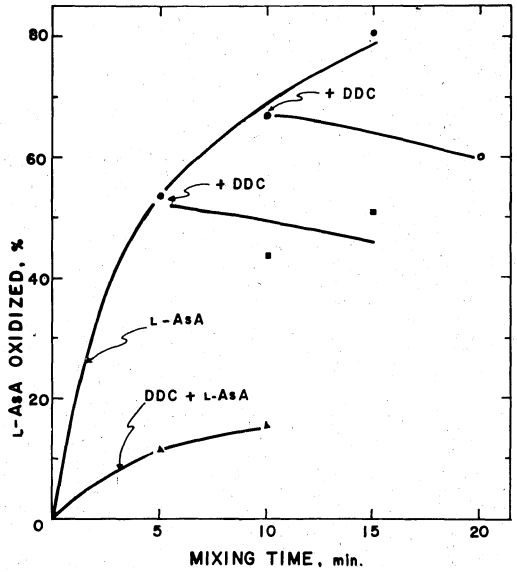

Fig. 1. Effect of DDC on the oxidation of L-AsA in dough during mixing. L-AsA, 100 p.p.m.; DDC, 500 p.p.m. (flour basis).

These data show that 50 and 80% of L-AsA were oxidized as a result of 5 and 15 min. of mixing, respectively. However, with DDC only 11% was oxidized after 5 min. of mixing.

Effects of L-AsA and DDC on the Rheological Properties of Dough. Two methods, the extensigraph test and the relaxation measurement, were used in this study.

1. Effects of L-AsA and DDC on extensigrams. Four kinds of sample doughs were prepared and tested with the extensigraph. Results are presented in Fig. 2.

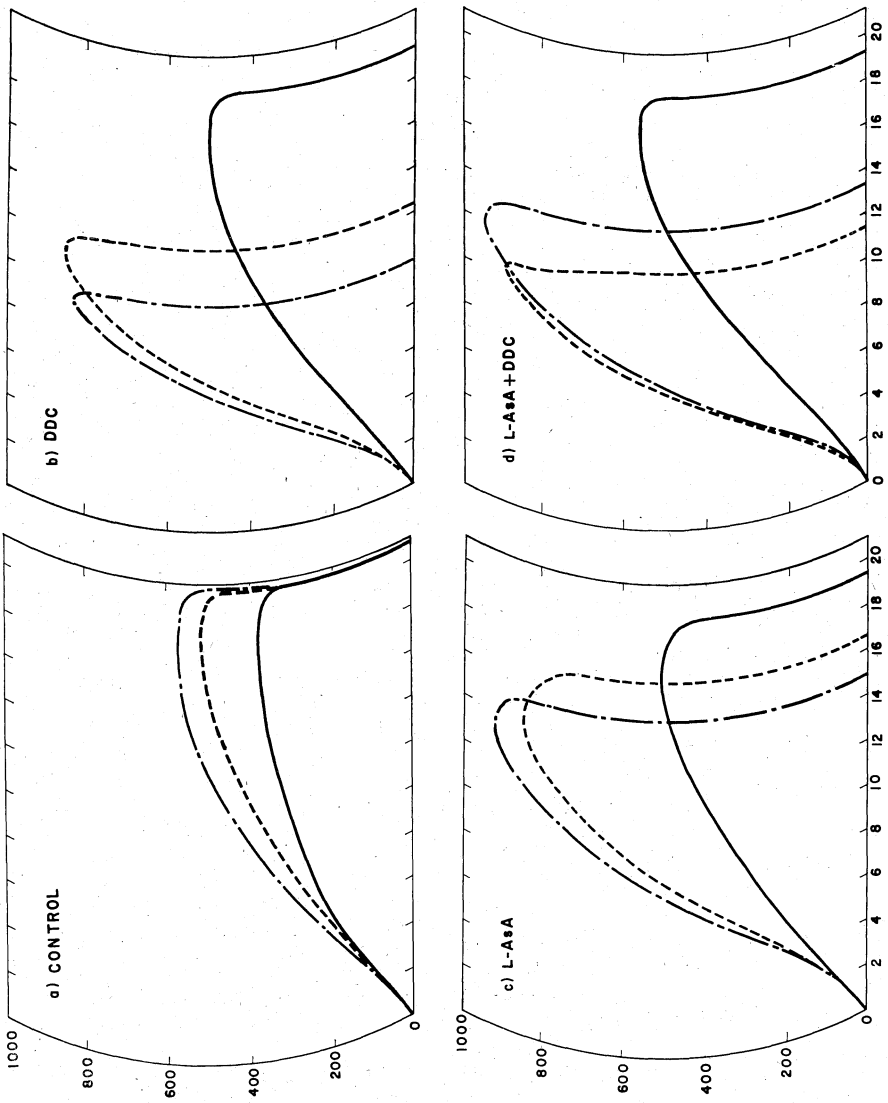

Fig. 2. Effects of DDC and L-AsA on extensigrams of dough. L-AsA, 20 p.p.m.; DDC, 500 p.p.m. (flour basis). Solid line, first stretch; dashed line, second stretch; dash-and-dot line, third stretch.

The effect of L-AsA was indicated by smaller extensibility and larger resistance of dough than in the control experiment; this showed a tightening of the dough. This effect, found in comparing Fig. 2(a) with Fig. 2(c), could not be found so clearly in comparing Fig. 2(b) with Fig. 2(d). These results showed that the effect of L-AsA was inhibited to some extent in the presence of DDC, though the large effect of DDC alone made it indefinite.

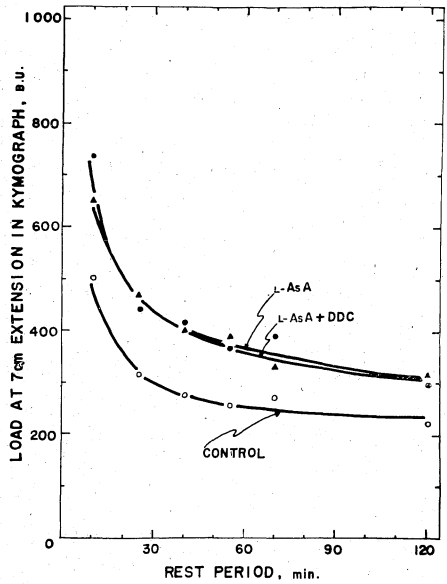

Fig. 3. Effects of DDC and L-AsA on relaxation curves at 0-hr. reaction time. L-AsA, 20 p.p.m.; DDC, 33 p.p.m. (flour basis).

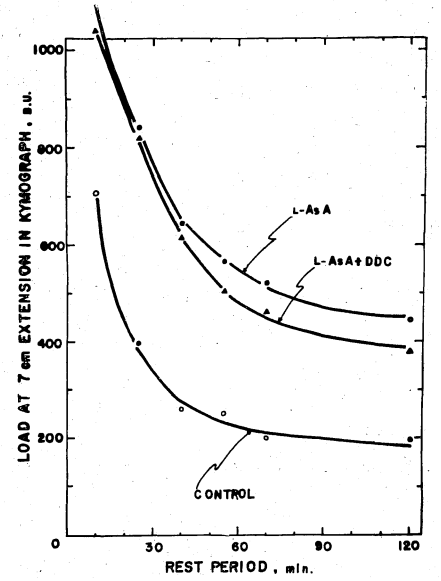

Fig. 4. Effects of DDC and L-AsA on relaxation curves at 3-hr. reaction time. L-AsA, 20 p.p.m.; DDC, 33 p.p.m. (flour basis).

2. Effects of L-AsA, DDC, and ascorbic acid oxidase on the relaxation of dough. Structural relaxation of dough according to the method of Dempster *et al.* (3) was determined with a lesser amount of DDC than in experiment 1. The results with 33 p.p.m. of DDC are shown in Fig. 3 and in Fig. 4 as a comparison of curves at 3-hr. reaction time. The improving effect of L-AsA was found to be decreased to a small extent by the addition of DDC. However, curves of L-AsA and L-AsA plus DDC were quite similar at zero-hour reaction time in Fig. 3, which indicated no inhibition of the effect of L-AsA by DDC, at the starting point, and also indicated that additional rheological effect of DDC over that of L-AsA could be neglected at this stage.

Relaxation curves of dough with or without crude ascorbic acid oxidase prepared from pumpkin rind are presented in Fig. 5. There was no difference between doughs with L-AsA plus ascorbic acid oxidase and L-AsA alone, nor between the control doughs with and without ascorbic acid oxidase.

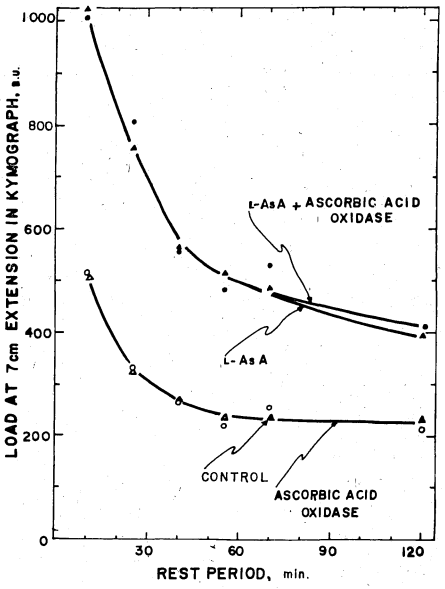

Fig. 5. Effects of ascorbic acid oxidase and L-AsA on relaxation curves at 3-hr. reaction time. L-AsA, 20 p.p.m. (flour basis); ascorbic acid oxidase, 7 ml. of crude preparation of the enzyme incorporated at the reduced absorption by 7 ml. for 300 g. of flour.

Discussion

The behavior of ascorbic acid in dough as well as in flour suspension should be followed when the mechanism of dough improvement

with ascorbic acid is studied. In the former case, there are two difficulties: 1) ascorbic acid and other reagents must be homogeneously incorporated into the dough; and 2) ascorbic acid must be extracted from dough with good recovery without further oxidation during the extracting procedure. The first difficulty seems to be eliminated with the GRL mixer; the data show high reproducibility and immediate reaction of DDC.

As for the second difficulty, the average recovery in these experiments is not as satisfactory as it might be. It is possible that L-AsA in the solution might be occluded into a nonhomogeneous precipitate upon the addition of metaphosphoric acid, and be eliminated from the determination. However, since it is the ratio of L-AsA to DHA that is to be considered on the reaction mechanism of L-AsA, the average recovery, as much as 86.8%, is regarded as available. Metaphosphoric acid was used to avoid further oxidation of L-AsA during extraction.

Thus the experimental basis, using dough as the reaction medium, was established.

Some of the results obtained by Proskuryakov and Auerman (14), who used wheat flour suspension and extract as the reaction medium, were confirmed in our experiments with dough.

In the present study, it may be noted that the concentration of L-AsA used is approximately the same as that used commercially in baking, and that the reaction of L-AsA was studied in dough which contains a higher concentration of the reactive groups of the protein than in aqueous flour suspension. Possibly the bound L-AsA oxidizing system shown by Merts (12) in corn root can be considered at the same time in this experiment which can not be estimated with flour extract. Thus, a more active oxidation is expected in dough than that in suspension as shown by Proskuryakov and Auerman (14). The fact that 80.6% of L-AsA was oxidized within 15 min. from the time of mixing confirms this view. This rate is comparable to the rate of oxidation of L-AsA by a crude preparation of ascorbic acid oxidase from pumpkin rind by the Fujita method (6).

However, the findings that DDC, which is a well-known chelating agent for heavy metals, inhibited this oxidation about 75%, and that no more oxidation of L-AsA could be found when it was incorporated during the reaction time of dough indicate the following two possible hypotheses:

1. The oxidation system in dough which contains heavy metal ions, such as cupric, may be inhibited through elimination with DDC. The total copper content in flour, determined by the colorimetric method

with DDC (2) after digestion with concentrated nitric acid, was found to be 1.79 p.p.m. by the present authors[3]. It is reasonable to assume that a part of this copper accelerates the oxidation of L-AsA as cupric ion or as a component of oxidase.

2. The reduction of DHA may proceed by means of sulfhydryl groups of protein, which were freed from heavy metals by chelation with DDC, and constitute a conjugated reduction system of DHA. This hypothesis is supported by the fact that sulfhydryl content and rheological improvement were known to be increased by the addition of ethylenediaminetetraacetic acid (EDTA) (7,19), though it is not a similar reagent to DDC.

However, no evidence can be given to explain the 20% of oxidation which could not be avoided even if DDC was incorporated in the dough before the addition of L-AsA as shown in Table II.

In the light of the results by the analytical and rheological methods used, the effect of L-AsA on the physical properties of dough may be attributed to the oxidizing action, possibly by DHA, which may be similar to the effect produced by bromate.

The fact that ascorbic acid oxidase had no effect, as shown in Fig. 5, can be explained on the basis that dough itself has a higher activity for the oxidation of L-AsA than is required for improving action.

The improving action of L-AsA may be represented by two reactions: oxidation of L-AsA, and reduction of DHA coupled with the oxidation of the reactive group. If the second reaction is the rate-limiting step, extremely rapid oxidation of step 1 is not necessary.

The rheological data are not sufficient to draw the conclusion that DDC inhibits the improving effect of L-AsA in dough, as the results showed the effect of DDC alone to be very large. Moreover, when only 33 p.p.m. of DDC were used (Fig. 4), only 15% of the oxidation was inhibited.

However, the conclusion that DDC inhibits the reaction of L-AsA to some extent is based on the assumption that the effect of L-AsA should be additive and that a little inhibition of oxidation of L-AsA would also affect dough properties. Further investigations will be necessary to confirm these points.

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[3] Unpublished data.

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