Ascorbic Acid as an Oxidant in Wheat Flour Dough. II. Rheological Effects[1]

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ABSTRACT

Rheological changes were measured by studying the spread of dough. L-Ascorbic acid (AA) has both an immediate and time-dependent rheological effect on dough and requires only a low concentration (15 ppm) to produce those effects. AA and yeast have a greater rheological effect than is obtained with a combination of AA and bromate or with AA alone. The oxidizing effect of yeast cannot be replaced by the use of AA. D-Isoascorbic acid was converted to dehydro-D-isoascorbic acid during mixing. Dehydro-D-isoascorbic acid did not have an oxidizing effect on dough, however, and did not overcome the reducing effect of thiol compounds such as was obtained with dehydro-L-ascorbic acid (DHAA). This result suggests that the oxidation effect of DHAA is an enzyme-mediated reaction. We hypothesized that the enzyme oxidizes a rheologically deleterious material in the water-soluble fraction of flour. We also hypothesized that DHAA or AA stops a time-dependent reaction in dough, which, without the oxidant present, causes dough to flow excessively. The time-dependent reaction is thought to be enzymatic.

The improving action of ascorbic acid (AA) in bread dough has been studied extensively (Cathcart and Edelmann 1944, Dahle and Murthy 1970, Johnston and Mauseth 1972, Kuninori and Matsumoto 1963, Meredith 1966, Tsen 1965). Jorgensen (1939) suggested that the improving effect of $KBrO_3$ and KIO_3 was due to their inhibitory effects on flour proteolytic enzymes and that AA functions as a flour improver because it inhibits the proteolytic activity in flour. Elion (1944) reported that compounds containing an enediol group adjacent to a carbonyl group not only inhibit proteinase but also act as chemical flour improvers. This conclusion does not agree with the results of Sandstedt and Hites (1945) and Cathcart and Edelmann (1944), who showed that D-isoascorbic acid (DIAA) was ineffective as a dough improver.

Sandstedt and Hites (1945), Kuninori and Matsumoto (1964), and Carter and Pace (1965) have all shown a rapid reaction of dehydro-L-ascorbic acid (DHAA) with glutathione (GSH) in flour-water extracts. Kuninori and Matsumoto (1964) reported that the reaction was specific for GSH and DHAA, that the molar ratio of GSH to DHAA was 2:1, and that the DHAA reductase was thermolabile. This suggests an enzymatic reduction system. Carter and Pace (1965) studied the distribution of the reductase in wheat and found high activity in the germ, but the largest amount of total activity was in the endosperm.

The purpose of this study was to use the recently developed spread test (Hoseney et al 1979) to characterize the effects of AA on the rheology of bread doughs.

MATERIALS AND METHODS

All chemicals used in the study were reagent grade. Compressed baker's yeast was used. L-AA was purchased from Sigma Chemical Co. and DIAA from Aldrich Chemical Co. DHAA was prepared by oxidation of AA with I_2.

The flour was a composite of many hard winter wheat varieties grown at many locations. It contained 12.2% protein (N × 5.7) and 0.42% ash.

Doughs (100.0 g) prepared for the spread test (Hoseney et al 1979) were mixed to optimum development and were fermented for 0, 30, 60, 105, 155, or 180 min at 30°C and 95% rh. (Fig. 1). Reported spread ratios are the average of two or more determinations. The standard deviations for determining the spread ratios were 0.112 for 0 min of fermentation and 0.043 for 180 min of fermentation.

Solutions of 0.5*N* HCl or 0.5*N* $NaHCO_3$ were used to adjust the pH of the doughs. After mixing, duplicate doughs were slurried in water and the pH measured.

Flour Fractionation and Reconstitution

One part of flour was suspended in 10 parts of distilled water and stirred continuously for 30 min. The suspension was centrifuged for 20 min at 1,000 × *g* (Fig. 2). The insoluble residue, gluten plus starch, was frozen, lyophilized, ground in a Moulinex grinder, and rehydrated in a proofing cabinet to about 14% moisture. The water-soluble fraction was either frozen or boiled and then frozen. Both water-soluble fractions were lyophilized and then reduced to a powder in a mortar. Mixing time and water absorption of each combination of reconstituted flour-water doughs were determined from mixograph data (Finney and Shogren 1972).

RESULTS AND DISCUSSION

Rheology of Dough: Effect of AA and DHAA

The spread test (Hoseney et al 1979) was used to measure the rheological change in dough as a result of oxidation. When a flour-water dough (control) was mixed to optimum, the spread ratio increased with fermentation time from 2.6 at zero time to 3.2 at 180 min of fermentation (Table I).

Inclusion of AA or DHAA in an optimum mixed dough caused both an immediate drop of the spread ratio (from 2.6 to 2.1 at zero fermentation time) and a time-dependent decrease in the spread ratio (from 2.1 at zero to 1.8 at 180 min of fermentation). The

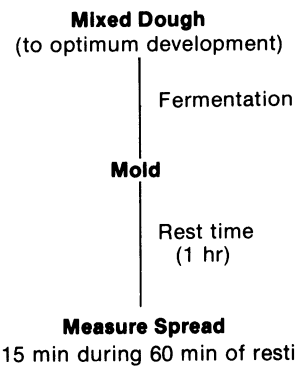

Fig. 1. Scheme for determining dough spread after 180-min fermentation. Dough is punched during fermentation after about 105 and 155 min. To obtain zero point, mold dough immediately after mixing, then start 1-hr rest. Measure spread with the formula: spread ratio = width of dough piece/height of dough piece.

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decrease in spread ratio is an indication of the oxidizing effects of AA and DHAA. When DHAA is added to a dough, hydrogen iodide, formed during oxidation of AA with I_2, is added along with the DHAA. Addition of hydrogen iodide alone had no effect on dough rheology.

The lower spread ratio obtained when both yeast and DHAA were included in the dough (Table I) shows that yeast has an oxidizing effect that cannot be entirely replaced with DHAA. Hoseney et al (1979) showed that the spread ratio of a full bread formula was 1.5 after 180-min fermentation. These results are similar to the value obtained here with a flour-water DHAA yeasted dough. This shows that the main rheological change in dough is the result of the oxidizing agent and the yeast.

Lower concentrations of AA or DHAA, from 50 ppm (0.28 μmole/g of flour) to as low as 12.5 ppm (0.07 μmole/g of flour) in flour-water doughs had an effect on the spread ratio (Table II). This indicates that only low concentrations of AA are required to change the rheology of dough.

Effect of DIAA

Sandstedt and Hites (1945) and Cathcart and Edelmann (1944) showed that DIAA was not effective as an oxidizing agent in bread making. When DIAA was included in flour-water doughs, the spread ratio was higher than the control at zero fermentation (3.2 vs 2.6) and stayed constant during fermentation. This shows that DIAA (or, more accurately, the dehydro-D-isoascorbic acid [DHIAA] produced during mixing) did not have an oxidizing effect on dough. This result is similar to that reported by others using bread making as the test system. No explanation is given for the higher spread ratio of the DIAA dough at zero fermentation time.

Effect of Potassium Bromate and Potassium Iodate

When $KBrO_3$ was included in flour-water dough mixed to optimum development, no rheological change was found at zero fermentation (Table III). However, the spread ratio decreased during fermentation. The data show that addition of $KBrO_3$ to flour-water doughs causes a slow and time-dependent rheological change. $KBrO_3$ did not decrease the spread ratio as much as AA or DHAA did. Meredith (1965) and Tsen (1965) concluded that a combination of $KBrO_3$ and AA caused more oxidative changes than did each reagent alone. Our data (Table III) are in agreement with those conclusions.

When KIO_3 was included in flour-water doughs mixed to optimum, it had an immedate rheological effect on dough (Table III). The rapid decrease in spread ratio due to the iodate addition is an indication of its effect as a rapid and strong oxidizing agent. Flour-water doughs containing a combination of KIO_3 and DHAA did not show any rheological effect over that of KIO_3 alone.

Effect of Cysteine and GSH

When cysteine or GSH was included in flour-water doughs mixed to optimum development, the spread ratio was significantly increased (Table IV). The major effect was evident at zero fermentation time. The changes in rheological properties during fermentation were similar to those of a flour-water dough. Thus, both cysteine and GSH have a direct chemical effect on the dough that increases the spread ratio and dough mobility, presumably as a result of the thiol-disulfide interchange reactions.

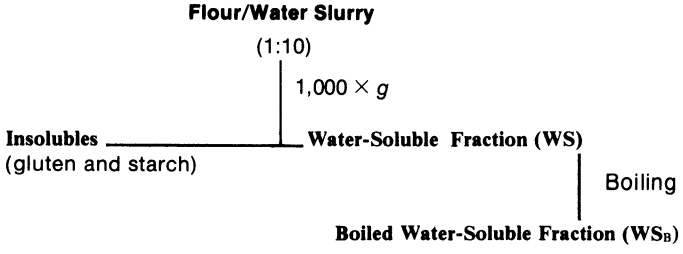

Fig. 2. Flour fractionation scheme.

Effect of Combination of Thiol Compounds and DHAA

When cysteine or GSH was included in flour-water doughs in combination with DHAA and the dough mixed to optimum development, the spread ratio was decreased at zero fermentation (Table IV) and declined further during fermentation. Thus the DHAA was effective in overcoming the effect of the thiol compounds. The data were somewhat surprising in view of the work of Kuninori and Matsumoto (1964) showing that GSH is effective as a hydrogen donor in the DHAA reductase system but that cysteine is not effective.

Of course, it is also possible that the DHAA oxidized the thiol groups without the help of an enzymatic system. To check that possibility, DIAA was studied. DIAA is converted to DHIAA during mixing (Elkassabany et al 1980). DHIAA would have the same oxidation potential as DHAA but has been shown to be ineffective in the DHAA reductase system (Kuninori and

TABLE I
Spread Ratio of Flour-Water Doughs Containing Ascorbic Acid[a] (AA), Dehydro-L-Ascorbic Acid[3](DHAA), and 2% Yeast

Treatment	Fermentation Time, min		
	0	60	180
Flour-H_2O	2.6	3.1	3.2
Flour-H_2O-AA	2.1	1.9	1.8
Flour-H_2O-DHAA	2.1	1.9	1.8
Flour-H_2O-yeast-DHAA	2.1	1.7	1.5

[a] 50 ppm.

TABLE II
Spread Ratio of Flour-Water Doughs Containing Various Concentrations of Dehydro-L-Ascorbic Acid (DHAA)

Treatment	Amount (ppm)	Spread Ratio
Control	...	2.6
DHAA	50	2.1
	25	2.1
	12.5	2.3
	10	2.4
	5	2.6

TABLE III
Spread Ratio of Flour-Water Doughs Containing Ascorbic Acid[a] (AA), $KBrO_3$,[b] or KIO_3,[b] and Certain Combinations

Treatment	Fermentation Time, min	
	0	180
Control	2.6	3.2
AA	2.1	1.8
AA and yeast	2.1	1.5
AA and $KBrO_3$	2.2	1.7
$KBrO_3$	2.7	2.1
KIO_3	1.9	1.9
AA and KIO_3	2.0	1.9

[a] 50 ppm.
[b] 30 ppm.

TABLE IV
Spread Ratio of Flour-Water Doughs Containing Combinations of Thiols[a] and Dehydro-L-Ascorbic Acid[b](DHAA)

Treatment	Fermentation Time, min	
	0	180
Control	2.6	3.2
Cysteine	4.1	5.1
Glutathione	5.2	5.6
Cysteine and DHAA	3.0	2.4
Glutathione and DHAA	3.0	2.3

[a] 30 ppm cysteine and 200 ppm glutathione.
[b] 50 ppm.

Matsumoto 1964). When DIAA was combined with cysteine in a flour-water dough mixed to optimum development, the spread ratio at zero fermentation was larger than the values obtained with DIAA or cysteine alone (Table V). These data indicate that DIAA (or the DHIAA produced during mixing) does not oxidize the thiol compounds. Therefore, the fact that DHAA oxidizes the thiols implies that both cysteine and GSH can be used as hydrogen donors for the DHAA reductase system.

Effect of pH

When flour-water doughs were mixed to optimum at different pHs, the mixing time was affected. Mixing time was increased at both extremes of pH. We also found that increasing or materially decreasing the pH of dough, compared with that of the control, had a stiffening effect on the dough (decreased spread) similar to that obtained when an oxidizing agent was added. Similar bell-shaped curves were obtained at 0 or 180-min fermentation, but much lower spread ratios were obtained at zero fermentation time (Fig. 3).

When DHAA or bromate was included in flour-water doughs mixed to optimum development at different pHs, the spread ratios obtained after 180-min fermentation were lower than those obtained with zero fermentation (Figs. 4 and 5). However, the shapes of the spread ratio curves at zero and 180-min fermentation for DHAA and bromate doughs were different.

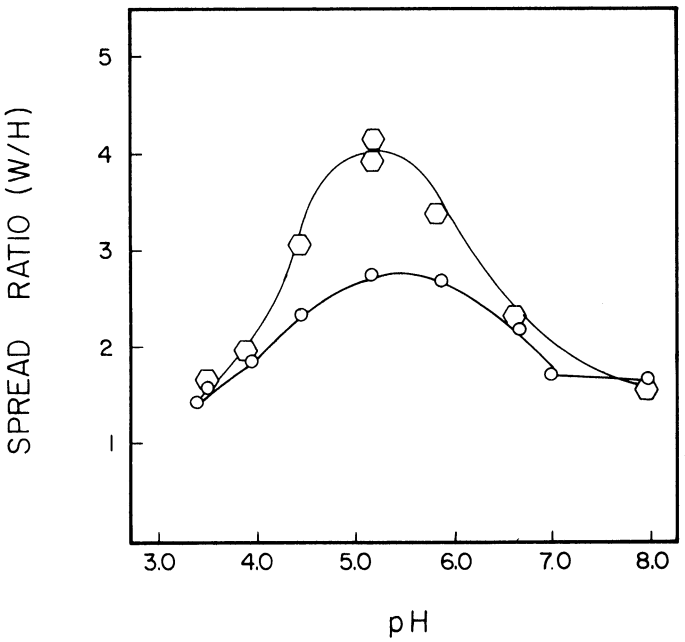

Fig. 3. Effect of pH on the spread ratio (width/height) of flour-water doughs mixed to optimum development: o = zero fermentation, ⬡ = 180-min fermentation.

TABLE V
Spread Ratio of Flour-Water Doughs containing Cysteine[a] and D-Isoascorbic Acid[b] (DIAA)

	Fermentation Time, min	
Treatment	**0**	**180**
Control	2.6	3.2
DIAA	3.2	3.3
Cysteine	4.1	5.1
Cysteine and DIAA	4.6	5.4

[a] 30 ppm.
[b] 50 ppm.

TABLE VI
Spread Ratio for Reconstituted Flour Mixed with Ascorbic Acid[a] (AA)

	Fermentation Time, min	
Treatment	0	180
Control	2.7	3.1
Gluten-starch	1.3	1.2
Gluten-starch-WS[b]	2.3	2.6
Gluten-starch-WS-AA	2.0	1.8
Gluten-starch-WS_B[c]	1.9	1.6
Gluten-starch-WS_B-AA	2.1	1.7

[a] 50 ppm.
[b] WS = Water-soluble fraction.
[c] WS_B = Boiled water-soluble fraction.

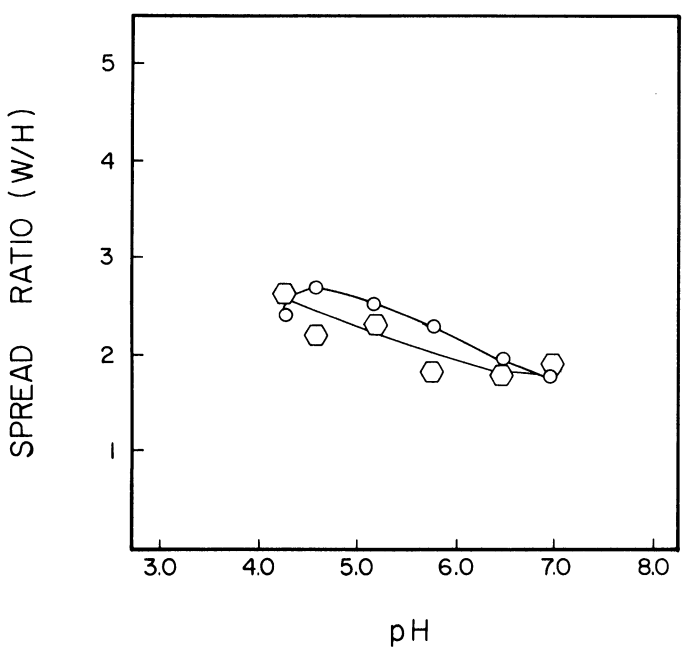

Fig. 4. Effect of pH on the spread ratio (width/height) of flour-water doughs mixed to optimum development in the presence of ascorbic acid: o = zero fermentation, ⬡ = 180-min fermetation.

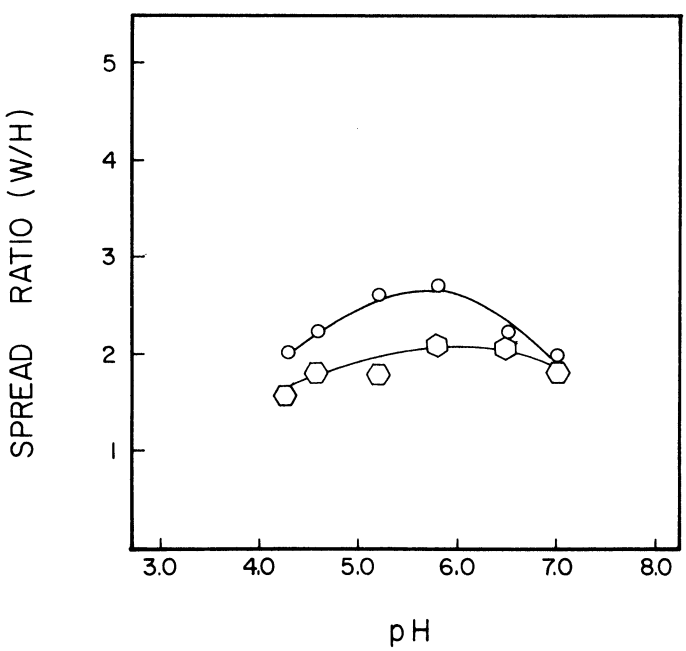

Fig. 5. Effect of pH on the spread ratio (width/height) of flour-water doughs mixed to optimum development in the presence of $KBrO_3$: o = zero fermentation, ⬡ = 180-min fermentation.

In flour-water doughs a maximum spread ratio was obtained at pH 5.5 (Fig. 3). Because the spread ratio increases with time and is pH-dependent, this effect could be enzymatic. When an oxidizing agent such as DHAA or bromate is added to dough at normal dosages, it stops the increase in spread ratio with time, possibly by inhibiting the enzyme reaction. In addition, DHAA (but not bromate) decreases the spread ratio at zero time (Figs. 4 and 5). With longer fermentation time (180 min), both DHAA and bromate decrease the spread ratio further.

Comparison of the spread ratios for doughs containing DHAA and bromate at different pHs (Figs. 4 and 5) shows that DHAA has only a minor effect on flour-water doughs at low pH. As pH increases, however, the improving effect of DHAA increases. Those data are in agreement with the findings of Kuninori and Matsumoto (1964) that pH 7 is optimum for DHAA reductase activity.

Effect of Flour Fractions

Flour was fractionated into gluten, starch, and water-soluble fractions, and the spread test (Hoseney et al 1979) was used to measure the rheological change in reconstituted flour dough (Table VI). When gluten, starch, and water-soluble fractions were reconstituted and a flour-water dough was mixed to optimum development, the spread ratio was lower than the control (unfractionated flour). However, the reconstituted dough showed a similar trend of increasing spread ratio during fermentation. Including AA in the reconstituted dough, brought both an immediate and a time-dependent decrease in the spread ratio. This is a similar trend to that obtained when AA was included in the unfractionated flour-water dough.

When gluten and starch were reconstituted without the water-soluble fraction and mixed into dough, the spread ratio was low at zero fermentation (1.3) and stayed constant throughout the fermentation time. Incorporation of AA into the gluten and starch system did not affect the spread ratio. Thus the factors responsible for the high spread ratio at zero time and the increase in spread during fermentation are found in the water-soluble fraction. Also, the oxidant has no effect on the gluten but stops the deleterious effect of water solubles.

When the water-soluble fraction was boiled to denature enzymes and used in a reconstituted dough, the spread ratio was low (1.9) at zero fermentation and decreased even more during 180-min fermentation. Addition of AA did not affect those values. Thus boiling the water-soluble fraction of flour has an oxidizing effect similar to that produced by AA.

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