Some Effects of Antioxidants in Dough Systems

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ABSTRACT

A number of antioxidants, including ascorbic acid, were tested in a continuous-doughmaking system. With varying magnitude, all effected a lower mixing requirement. Hydroquinone and *p*-benzoquinone caused a weakening of the farinograph curve. That this could be caused by SH-blocking action was suggested by the reactivity of *p*-benzoquinone to glutathione. Neither hydroquinone nor ascorbic acid weakened a synthetic dough system of starch, gliadin, and glutenin. No evidence was obtained for SS cleavage as a mechanism of the action of antioxidants in dough systems.

For a number of years, cereal chemists have investigated the behavior of bread-improver substances such as iodate, bromate, and ascorbic acid, with the goal of better elucidating the mechanism, or mechanisms, of improver action. The problem, a difficult and complex one, has yet to be resolved to the complete satisfaction of all working in this field. Evidence to date indicates that flour proteins are the principal targets of improver action. The changes induced in flour proteins by improver substances are believed to involve SH groups. One hypothesis is that new protein SS groups are formed by the oxidation of the SH groups and, by this additional SS cross-linking, increase the strength of the gluten and dough structure.

The improver action of ascorbic acid in conventional doughmaking systems was investigated fairly recently by C. C. Tsen (1). In these studies, he observed a loss of titratable sulfhydryl due to its reacting with the oxidized form of ascorbic acid, dehydroascorbic. Ascorbic acid oxidase was implicated. It was believed that, by this enzyme, ascorbic acid was oxidized to dehydroascorbic acid which, in turn, oxidized the SH groups. Isoascorbic acid failed to cause a loss of sulfhydryl, and this failure was attributed to the specificity of the enzyme for ascorbic acid.

More recently another type of action of ascorbic acid in dough systems has been revealed. In continuous-doughmaking equipment, it has been observed that ascorbic acid reduces the mixing requirement or power requirement in the dough developer. This is the basis of a patent held by Johnston and Mauseth (2). Published results of their work show that the reduced form of ascorbic acid (not dehydroascorbic) is required (3); dehydroascorbic acid has no effect. Isoascorbic acid was as effective as ascorbic acid. Similar results were obtained with continuous-system equipment in our laboratory.

The mechanism of the action of ascorbic acid in continuous systems appears to be entirely different from its action in conventional dough systems. Its action in the latter is explained as the effect of oxidation by dehydroascorbic acid; its action in continuous systems must involve the reducing action of the acid. How this reducing action can be rationalized on the basis of SH-SS phenomena is not immediately obvious. There is no reason to believe that ascorbic acid can chemically cleave a SS bond as can, for example, cysteine.

Very recently Zentner (4) published his study of the effect of ascorbic acid on gluten, in which he used the farinograph. His results indicated a weakening of the farinograph curve upon addition of the acid. The effect was obtained after prior

addition of sulfhydryl and urea to the gluten, suggesting that SH action was not involved. He was not able to find titratable sulfhydryl in the gluten, either before or after mixing it with ascorbic acid. He advanced the tentative hypothesis that ascorbic acid may change the water-binding properties of gluten.

Ascorbic acid is an antioxidant and its function as such in a cereal system is known. The use of ascorbic acid to preserve the yellow color of carotenoid pigments in durum products is the subject of a patent held by Schmalz and Risdal (5). Some years ago Irvine and Winkler (6) investigated the oxidative loss of durum pigments and lipoxidase action. Alpha-naphthol, a typical antioxidant, was found to preserve color, and this was attributed to its inhibitory action on the lipoxidase system.

The object of our investigation, then, was to explore the action of antioxidants in dough systems by comparing their effects in a continuous system and with the farinograph and to collect collateral data which might shed some light on the mechanism of their action.

MATERIALS AND METHODS

Baking Procedure

Continuous-mix data were obtained on a Wallace & Tiernan Do-Maker. For all studies, a patent flour of moderate strength (0.44% ash, 12.0% protein) derived from a blend of hard spring and hard winter wheats was used. Bromate (60 p.p.m.) and iodate (6 p.p.m.) were added in the form of a water solution. Ascorbic acid and the other antioxidants also were added as water solutions or water dispersions. The fat-soluble antioxidants first were dissolved in a minimum amount of ethanol and then added to water to form a dispersion just before being added to the flour in the mixer. Doughs were proofed to 1 in. over the pan and baked for 17 min. at 500°F.

Ascorbic and Isoascorbic Acid Measurement

After being taken from the extruder, 100-g. samples of the control and ascorbic acid-containing doughs were homogenized immediately with 400 ml. of 1% oxalic acid solution for 1 min. in a Waring Blendor. The slurries were centrifuged, and 150-ml. aliquots of the clear extracts were titrated with 0.025% 2,6-dichlorophenolindophenol solution. Suitable corrections were made for the amount of water in the original doughs. A standard solution of ascorbic acid (Mallinckrodt Chemical Works) containing 1 mg. per 5 ml. in 1% oxalic acid was used.

Sulfhydryl Reactivity

Reactivity to SH groups was measured by reacting each compound with glutathione in buffer at pH 8.0, after which the unreacted glutathione was measured colorimetrically by Ellman's procedure (7).

Synthetic Dough System

The compounds were added as water solutions (using part of the absorption water) to a synthetic dough system comprised of starch (32 g.), gliadin (4 g.), glutenin (11 g.), and water (33 ml.). The gliadin and glutenin were prepared according to the procedure previously published (8). The dry ingredients were

blended in the farinograph bowl, after which water was added and the farinograph was run in the usual way.

RESULTS AND DISCUSSION

When dough was taken from the extruder head of the Do-Maker and measured immediately for ascorbic acid and isoascorbic acid content, it was found that 88 and 86%, respectively, had been oxidized. Since the oxidizing agents, iodate and bromate, had been included in the dough mixture, most of the oxidation could be attributed to these agents.

Figure 1 shows the effect of ascorbic acid in reducing the mixing requirement of flour in continuous equipment. The laminations denote undermixing. This confirms the data of Mauseth and Johnston (3).

The first antioxidant tried was propyl gallate. As Fig. 2 shows, this compound definitely reduced the mixing requirement. An optimum was achieved at a level of 300 p.p.m. and 167 r.p.m. An effect similar to that shown in Fig. 2 was obtained with a group of water-soluble antioxidants. These included isoascorbic acid, hydroquinone, squaric acid (Aldrich Chemicals), and dihydroxymaleic acid, all used

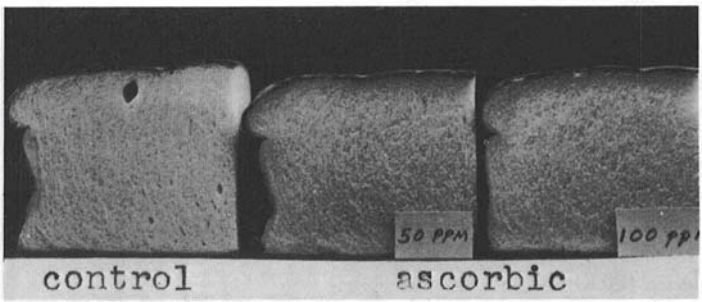

Fig. 1. Ability of ascorbic acid to lower the mixing requirement of flour in a continuous system; laminations denote undermixing. Developer speed was 167 r.p.m.

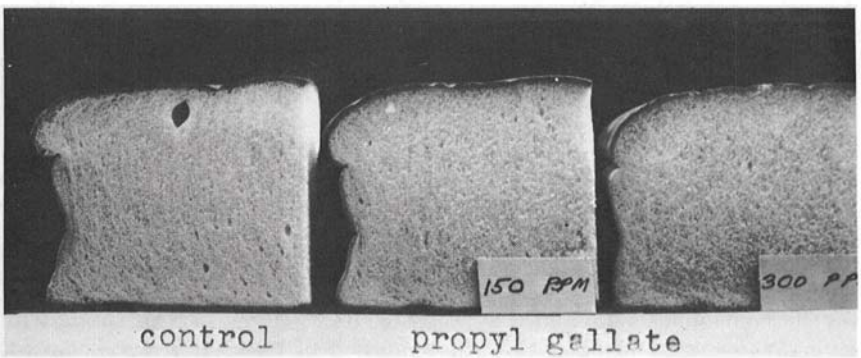

Fig. 2. Ability of propyl gallate to lower the mixing requirement of flour in a continuous system; developer speed was 167 r.p.m.

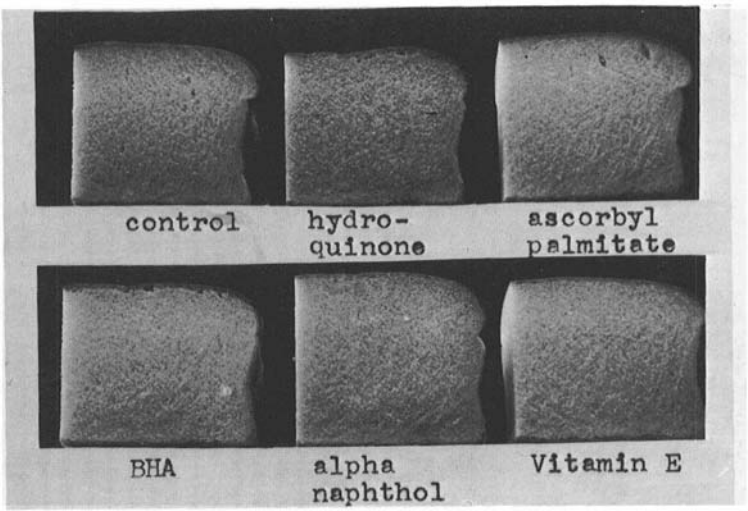

Fig. 3. Ability of fat-soluble antioxidants to lower the mixing requirement of flour in a continuous system, compared with hydroquinone. Hydroquinone and ascorbyl palmitate were at 150 and 300 p.p.m., respectively; BHA and alpha-naphthol were at 200 p.p.m. and vitamin E was at 250 p.p.m. Developer speed was 200 r.p.m.

at a level of 75 p.p.m. Except for hydroquinone, all have ene-diol groupings. Comparison with ascorbic acid indicated that all had the power to reduce the mixing requirement. It is noteworthy that isoascorbic acid, which was shown by the earlier work of Tsen (1) to be unaffected by the ascorbic oxidase enzyme system in flour, had a potency as great as that of ascorbic acid.

Figure 3 shows the effect of the fat-soluble antioxidants. All had the ability to reduce the mixing requirement. Hydroquinone and alpha-naphthol were at equimolar levels, but hydroquinone shows overmixing.

It is apparent that water-soluble antioxidants are effective at lower levels compared with fat-soluble antioxidants.

When linoleic acid was added to flour at a level of 5,000 p.p.m., with and without antioxidants, a slight reduction of the mixing requirement was observed when an antioxidant was present. As an example, Tenox 4 was blended into the linoleic acid at a level to give 100 p.p.m. of butylated hydroxy anisole and butylated hydroxy toluene in the flour.

The weakening effect of hydroquinone on the farinograph curve is seen in Fig. 4. Ascorbic acid did not have a comparable effect; this confirms the results of Ikezoe and Tipples (9). The oxidation product of hydroquinone, *p*-benzoquinone, had an effect similar to that of hydroquinone. To a lesser extent, the other phenolic antioxidants weakened the farinograph curves.

Since the air and oxidases present in flour could easily cause oxidation of hydroquinone to *p*-benzoquinone, the weakening effects observed might be attributable to SH-blocking action of the alpha, beta unsaturated carbonyl function of *p*-benzoquinone. The reactivity at pH 8 of glutathione to various agents when combined in equimolar proportions is seen in the table below. The blocking action

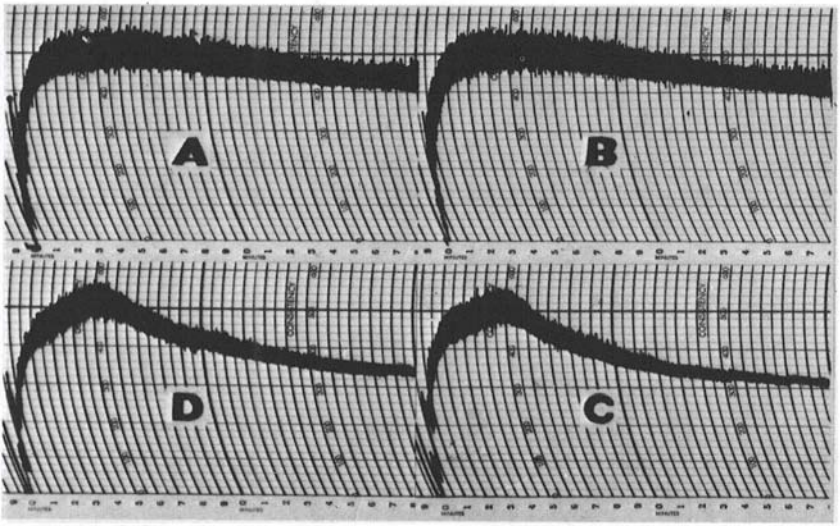

Fig. 4. Effect of ascorbic acid (B), hydroquinone (C), and *p*-benzoquinone (D) on the farinograph curve; all levels were 75 p.p.m. Control is A.

of quinones and their accompanying rheological effects have been noted by Narayanan and Hlynka (10).

Chemical Agent	*Glutathione Reacted* %
Ascorbic acid	none
Dehydroascorbic acid	none
Hydroquinone	none
p-Benzoquinone	93.4

A SS bond-cleaver such as dithiothreitol greatly weakens the farinograph curve of a flour. The weakening effect of this agent on a synthetic dough system comprised of starch, gliadin, and glutenin has been reported by Murthy and Dahle (8). When ascorbic acid and hydroquinone were tried under the same conditions, no weakening was observed, as is shown in Fig. 5, and thus no evidence of SS cleavage.

GENERAL DISCUSSION

From the data collected in this investigation, there is no evidence that ascorbic acid and other antioxidants cause scission of SS bonds. Thus, although cleavage of SS bonds might serve to explain the ability of cysteine to reduce mixing requirements in continuous-dough systems, another explanation is required for the compounds tested.

A logical inference is that oxidized lipid-protein interaction results in a change of the protein properties which require greater expenditure of energy for optimal dough development in continuous-dough systems and that this is prevented by

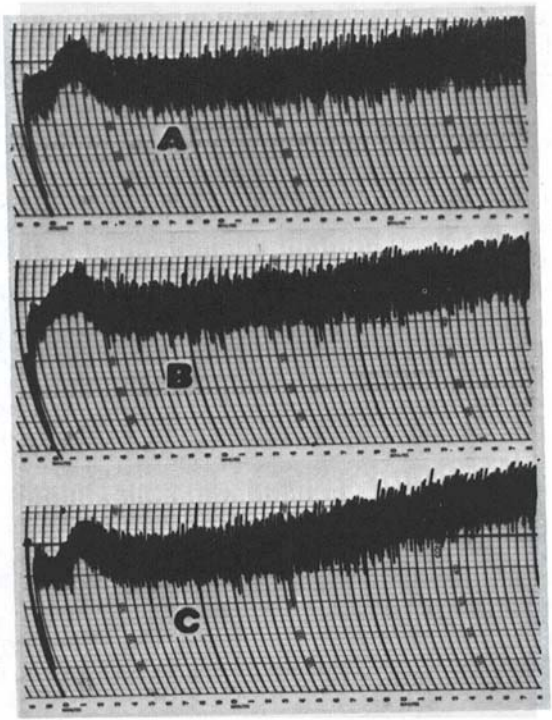

Fig. 5. Effect of ascorbic acid (B) and hydroquinone (C) on farinograph curve of synthetic dough system of starch, gliadin, and glutenin; all levels were 200 p.p.m. Control is A.

antioxidants which prevent lipid oxidation. More specifically, oxidized lipid-protein may well involve a type of coupled oxidation of lipid and protein, as is already known to occur between lipid and carotenoid pigments in durum flour systems. Moreover, there may be some preformed lipid-lipoxidase-oxygen complex present in flour, such as was postulated by Irvine and Winkler (6) to account for a small initial loss of carotenoid pigment in pasta processing, even in the absence of atmospheric oxygen.

The phenomena associated with lipid oxidation in flour systems have been the subject of numerous investigations. The work of Irvine and Winkler (6) has been mentioned. Published work of Dahle (11) and Matsuo et al. (12) has demonstrated the importance of free polyunsaturated fatty acids as preferred substrates.

The damaging effect of oxidized fatty acids on the rheological and baking properties of flour are reported in early studies of Sullivan et al. (13). Some extensive studies of lipid oxidation effects in conventional dough systems were reported 12 years ago by Smith, Andrews, and co-workers (14,15). They first determined the effect of atmospheric oxygen on dough properties by excluding it from dough systems. The most sensitive indicator of rheological change was the extensigraph. Doughs developed in nitrogen atmosphere were less resistant to extension. Further manipulation of lipid, enzyme, and substrate gave evidence that lipid oxidation was involved in the observed rheological changes. When they

attempted to correlate loss of flour protein sulfhydryl with rheological changes induced by lipid oxidation, they were unable to obtain satisfactory evidence.

Some more recent work by Bushuk and Hlynka (16) showed that less bromate was reduced in a dough when fat had been removed than when fat was present. The greatest reduction of bromate occurred in systems under nitrogen, with little difference between normal and defatted doughs. When they added the antioxidants propyl gallate and butylated hydroxy anisole, there was less bromate reduction, just as was observed when the flour was defatted. The authors interpreted these data as indicating a competition between bromate and oxygen for improver reaction; when there was no consumption of oxygen by lipid, more was available for improver reaction, resulting in less need for bromate. When they added various levels of cumene hydroperoxide, they were able to show competitive inhibition of the bromate reaction, which suggested to them that available SH groups could be oxidized by lipid hydroperoxides as well as by bromate.

The combined interaction of lipid, protein, and oxidants in a dough offers effects of great complexity which are difficult to resolve. However, sufficient evidence exists to show the importance of oxidized lipid in dough systems. Sulfhydryl groups certainly can be expected to be reactive; the phenolic residues of tyrosine also should be considered. Undoubtedly, there are many sites on flour proteins vulnerable to attack by oxidizing lipid.

Recent work by Daniels et al. (17) demonstrates the effect of air on lipid binding in mechanically developed doughs. A nitrogen atmosphere showed an increase in lipid binding at all rates of work input over doughs in an air atmosphere. All doughs contained 75 p.p.m. of ascorbic acid. It may be that the ascorbic acid and antioxidants in our system involved this same parameter of lipid binding.

A more explicit understanding of the effect of antioxidants in both continuous and conventional dough systems requires more research, some of which must be quite basic in nature. It is hoped that more knowledge will be accumulated on the subject.

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