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RHEOLOGICAL STUDIES OF THE ROLE OF LIPIDS IN DOUGH[1]

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ABSTRACT

Doughs from normal flour show only a modest response to mixing in atmospheric oxygen as indicated by structural relaxation data. Doughs from defatted flour show a very marked effect of air, indicating that the lipids have a protective action against the improver effect of oxygen. Flour extracted with petroleum ether and reconstituted with the lipids that were removed, regained the properties of normal flour. Reconstitution of extracted flour with free fatty acids showed both an initial improver effect of atmospheric oxygen and a subsequent protective effect similar to that of the lipid fraction. Oleic and linoleic acids showed a protective action, while palmitic did not. The unsaponifiable fraction of flour lipids, tocopherol, propyl gallate, nordihydroguaiaretic acid, butylated hydroxytoluene and butylated hydroxyanisole — all well-known antioxidants — had an improving effect when doughs were mixed in air. The improving agents, bromate and iodate, gave apparently a greater response to doughs from defatted flour on mixing in air, and this was due to the marked effect of oxygen on defatted flour.

Lipids normally present in flour play an important role in determining the properties of dough. In a recent paper Tsen and Hlynka (21) showed that: a) flour lipids readily formed peroxides when doughs were mixed in air; common antioxidants inhibited the reaction and lipoxidase enhanced it; b) organic peroxides and hydroperoxides had an improving effect on physical dough properties; and c) sulfhydryl-blocking reagents increased the extent of peroxidation. On the basis of these findings they suggested a hypothesis that flour lipids compete with sulfhydryl groups for available oxygen in dough. A review of the earlier literature was also given in that paper; accordingly no further review is intended here.

In addition to the studies based on essentially chemical techniques, we have also conducted parallel rheological studies on the role of

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lipids in dough. For this purpose the structural relaxation method was adopted (9–11,14). It has been known for a long time that oxygen incorporated into dough has a significant effect on the structural relaxation parameters. If it is assumed that the effect of oxygen is associated with the role of lipids, then the response of dough to the effect of oxygen provides a convenient means of studying the role of flour lipids.

The primary object of the experimental work was, firstly, to establish the general effect of lipid fraction in flour on the rheological properties of dough; secondly, to examine the effect of the principal lipid fractions; and thirdly, to supplement the data with experiments in which certain chemicals were added to obtain answers to specific questions. Some baking experiments were also done to supplement the rheological findings.

Materials

HRS

Flour used for this study was a straight-grade, improver-free, unbleached sample commercially milled from a blend of Canadian hard red spring wheat. The absorption as determined at a consistency of 500 farinograph units was 63%, protein content 13.5%, and ash 0.43% on a 14% moisture basis.

Defatted flour was prepared by extracting flour with petroleum ether (Skellysolve F 95) in a large Soxhlet extractor for 16 hours. The flour was then removed and spread out in a shallow dish to allow the solvents to evaporate. The defatted flour was kept under nitrogen in cold storage to prevent further oxidation. When sufficient quantities were collected they were blended in an atmosphere of nitrogen to make a uniform mixture of sample. The extracted lipids were recovered by careful evaporation and were used for reconstitution experiments.

Neutral fat which was used in the experiment was prepared as follows: Thirty grams of petroleum ether extract were dissolved in petroleum ether. The free fatty acids were washed out with dilute sodium carbonate solution and the "neutral" fat (free from free fatty acids) was washed several times with water to remove the alkali. The extract was reconstituted with defatted flour (3,000 g.) in an atmosphere of nitrogen.

Bound lipids were prepared by refluxing the defatted flour with aldehyde-free ethanol (95%) for about 30 minutes. After refluxing, the mixture was cooled slightly and centrifuged. The alcohol layer was poured into a separatory funnel and the flour remaining in the centrifuge tubes was washed once again with warm alcohol and then three times with petroleum ether. The combined alcohol washings were diluted with water and the lipids were extracted from the alcohol solution with petroleum ether by repeated washings. The combined petroleum ether phase thus collected was washed with water to free it from alcohol. It was then kept for some time to drain off the water, and the "bound" lipids thus collected were mixed with defatted flour on a 1% flour-weight basis.

Unsaponifiables were prepared by saponification of petroleum ether extract (30 g.) with alcoholic potash in the presence of pyrogallol as described by Tosic and Moore (20). After saponification the solution was cooled and diluted with water, and the unsaponifiables were extracted with peroxide-free ether. The extracts were washed with water, twice with dilute alkali, and then several times with water until the ether extract was free from alkali. The ether was evaporated under reduced pressure and the unsaponifiables were taken up in petroleum ether and mixed with defatted flour (3,000 g.).

Flour fatty acids were prepared by refluxing 30 g. of petroleum ether extract with alcoholic potash as described by Hilditch (13). After saponification was completed, most of the alcohol was removed by distillation under reduced pressure. The soaps were dissolved in water, and after extraction of the unsaponifiables with ether the soap portion was converted into their fatty acids by adding dilute sulfuric acid in the presence of nitrogen. When the acids were completely liberated they were extracted with petroleum ether in the presence of nitrogen. The extract was finally freed from mineral acids and mixed with 3,000 g. defatted flour.

Mineral oil (liquid petrolatum, U.S.P. — Robinson & Webber, Ltd., Winnipeg), palmitic acid (Research Laboratory, Eastman Kodak Co., Rochester, N.Y.), oleic acid (U.S.P. — J. T. Baker Chemical Co., Phillipsburg, N.J.), and 75% linoleic acid (Nutritional Biochemicals Corp., Cleveland, Ohio) were added to defatted flour on 1% flour-weight basis. Before addition to the flour the fatty acids were tested and found to be free from peroxides; other tests for chemical purity were not considered necessary at this stage.

Alpha tocopherol, butylated hydroxytoluene (BHT), nordihydroguaiaretic acid (NDGA), butylated hydroxyanisole (BHA), and propyl gallate (PG) were added to the flour on 0.005% flour-weight basis. BHT was oxidized for 5 hours with alkaline ferricyanide by the method of Cook *et al.* (5) and mixed with defatted flour.

All the fatty materials used in these experiments were dissolved in petroleum ether before being blended with flour. The ether was evaporated from flour before mixing by keeping the flour open to an atmosphere of nitrogen.

Potassium bromate and iodate used were reagent grade and they were added to the flour at concentration of 15 and 5 p.p.m. respectively.

Nitrogen used in these experiments was commercial tank nitrogen containing at least 99.7% nitrogen.

Method

Doughs were prepared from 200 g. flour (14% moisture) and sufficient water and salt solution to give an absorption of 59% and a salt content of 1% (flour basis). They were mixed in air or nitrogen for 2.5 minutes in a GRL mixer (15) under conditions to give dough at 30 ± 0.5°C. Flours to be mixed in nitrogen were conditioned in nitrogen overnight before use to desorb and displace atmospheric oxygen originally present in the flour. Changes in the properties of dough thus prepared were followed by the structural relaxation method using Brabender Extensigraph (9–11,14). Each structural relaxation curve obtained in this study was analyzed in the manner previously described (10,14) to obtain the relaxation constant, C. The changes which take place on the physical properties of dough with reaction time is shown by plotting relaxation constant, C, *vs.* reaction time. A large relaxation constant for a dough indicates a "tighter" dough which takes longer to relax than one with a smaller relaxation constant.

The basic AACC malt phosphate formula was employed for the baking tests. To determine the mixing tolerances of doughs (1) from normal and defatted flours and also flours containing antioxidants, duplicate loaves were baked from doughs mixed for 1.5, 3, 4.5, and 6 minutes in the GRL mixer (15).

Results

Effect of Defatting. Figure 1 emphasizes differences in the rheological behavior of doughs made from normal and defatted flours in various atmospheres. The changes in relaxation constants for doughs from normal flour are represented by solid lines and the results for defatted flour by dashed lines. An increase in the relaxation parameter, C, indicates an "improver" effect as reflected by the rheological properties of dough. In interpreting the changes in C with various treatments it is adequate for the present purpose to follow only the trends, whether "improver" effect has or has not been brought about.

A comparison of the curves obtained for relaxation constant, C, *vs.*

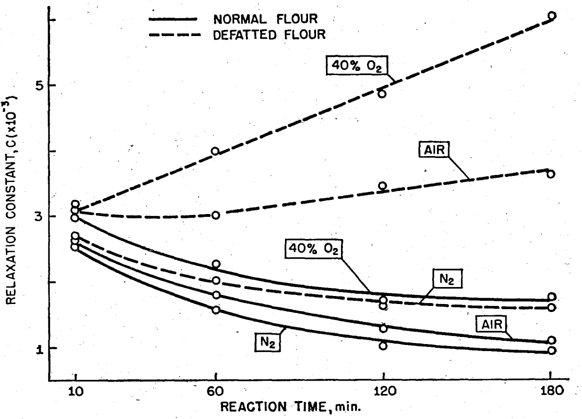

Fig. 1. Change in structural relaxation constant C with reaction time for doughs mixed from normal and defatted flours in various atmospheres.

reaction time for doughs mixed in air and nitrogen shows that doughs from normal flour give only a modest response to oxygen, whereas the effect of oxygen in doughs from defatted flour is very marked. For doughs mixed in a mixture of 40% oxygen and 60% nitrogen the curves for relaxation constant, C, *vs.* reaction time are a little more displaced upward than for doughs mixed in air, owing to a higher concentration of oxygen. This greater apparent effect of oxygen when doughs are prepared from defatted flour has also been observed by others (6,18). It appears, then, that the lipid fraction that is removed by defatting has a sort of protective action against the improver effect of oxygen in dough. The mechanism is likely a competition for oxygen between lipids extracted from flour, and flour proteins. If lipids are removed, more oxygen is directly available to react with flour proteins.

Effect of Reconstitution. Figure 2 shows the effect of reconstitution of some fractions of the extracted lipids to the defatted flour. When petroleum ether extract is added to the extracted flour on 1% flour-weight basis, the changes in relaxation constant, C, for doughs at various reaction times are very similar to those obtained for doughs from normal unextracted flour, both mixed in air. This indicates that the extraction and reconstitution procedures do not alter appreciably the flour properties. The protective action against oxygen is restored. The effect of the addition of "neutral" fat, on the basis of 1% petroleum ether extract, to the defatted flour on the relaxation constants for doughs at different reaction periods was similar to that obtained by addition of the crude petroleum ether extract. This shows that "neutral" fat in dough is also involved in the protective action against oxygen.

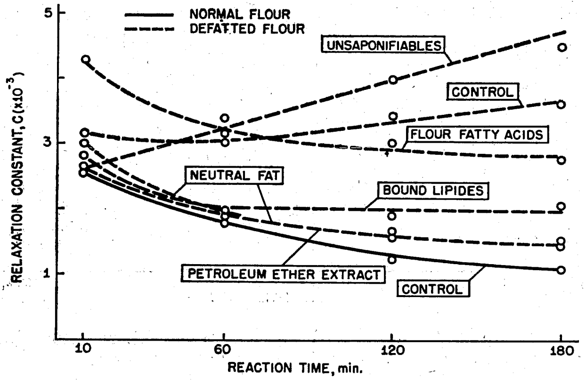

Fig. 2. Change in structural relaxation constant C with reaction time for doughs mixed from defatted flour in air after reconstitution with different lipid fractions of flour.

The extraction of flour lipids by petroleum ether under the experimental conditions described here gives only about 50% of the total flour lipids. The removal of the "bound" lipids was achieved by treatment of defatted flour with alcohol and subsequent extraction with petroleum ether. For doughs containing these "bound" lipids (added to defatted flour on 1% flour-weight basis), the graph for structural relaxation constant, C, *vs.* reaction time is somewhat similar to that for doughs with petroleum ether-extractable lipids. The protective action is somewhat less at higher reaction times. This slight difference in the protective action of these two fractions of flour lipids against the improver effect of oxygen may be due to a difference in the degree of unsaturation of the two fractions; the iodine values of petroleum ether-extractable and "bound" lipid fraction were 111.4 and 103.0 respectively.

Addition of flour fatty acid fraction to defatted flour at 1% petroleum ether extract level gives an interesting effect (Fig. 2). The free fatty acid fraction produces a strong improver effect in the dough initially. Beyond this initial effect the graph for the doughs that contain fatty acids runs parallel to that for doughs that contain the lipid extract, indicating a subsequent protective effect of the free fatty acids similar to that of the lipid fraction.

In flour lipids another group of substances of interest to examine are the unsaponifiables, which include the natural antioxidants like tocopherols. They were mixed into defatted flour on a 1% petroleum ether extract basis and the results are shown in Fig. 2. The unsaponifiables show no protective action against the improver effect of oxygen; on the contrary, a slight improver effect in dough is indicated.

Effect of Fatty Acids. The effect of fatty acids in relation to their structure was investigated further. For this purpose palmitic representing a saturated acid, oleic with one double bond, which is normally not considered to be a substrate for lipoxidase, and linoleic acid which has a polyene structure required for lipoxidase (2) were selected. In addition, a mineral oil was included to represent a saturated compound having no carboxyl group. These substances were mixed with defatted flour on 1% flour-weight basis in air and in nitrogen. The results are shown in Fig. 3.

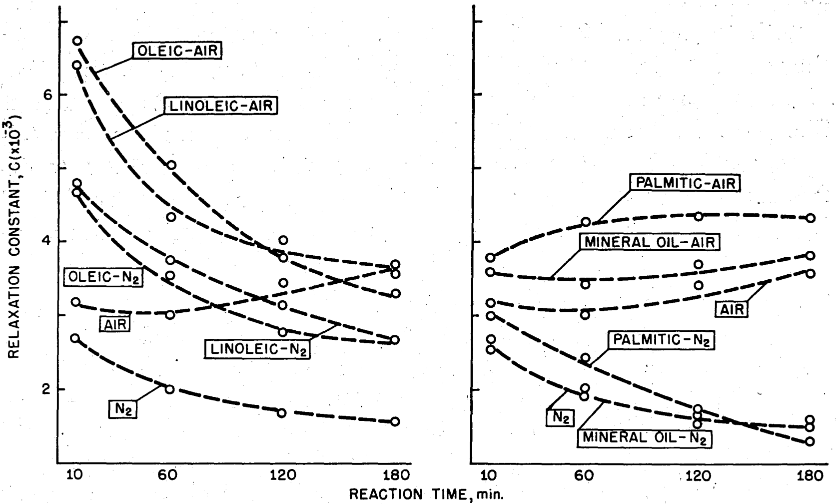

Fig. 3. Change in structural relaxation constant C with reaction time for doughs mixed from defatted flour in air and in nitrogen after addition of different fatty acids.

The upward displacement of the curves in Fig. 3, left, indicates that oleic and linoleic acids have a strong initial improving effect in doughs mixed in air, but with continued reaction time show a protective action similar to that already seen in Figs. 1 and 2. Because these unsaturated fatty acids were tested for peroxide content by the customary iodometric procedure and were found to be free of peroxides, the initial improving effect in doughs must be attributed to some other mechanism such as the effect of the acid group (16), interfacial effect, etc.

The time-dependent effect of these acids on structural relaxation constants of dough is likely associated with the peroxidation of these fatty acids through their double bonds. It is of interest that oleic and linoleic acids show a similar effect. But because only linoleic can serve as a substrate for enzymatic peroxidation through the lipoxidase system, another system, enzymatic or chemical, must be involved for the oleic acid.

Figure 3, right, shows that incorporation of palmitic acid with the flour and mixing the dough in nitrogen has little effect; the results are much like those for doughs from defatted flour, mixed in nitrogen as a control. When doughs from these flours were mixed in air, the effect of palmitic acid appeared to be to enhance somewhat the improving effect of atmospheric oxygen. The effect of mineral oil was intermediate between that of palmitic acid and the results of defatted flour control.

Effect of Antioxidants. The interesting action of unsaponifiables in the reconstitution experiments prompted us to study further the effect of adding pure tocopherol and also some commonly used antioxidants like propyl gallate, butylated hydroxyanisole, butylated hydroxytoluene, and nordihydroguaiaretic acid to flour. They were added to defatted and normal flours on 0.005% level and the doughs were mixed in nitrogen and in air. Figure 4 shows that tocopherol had a similar effect in dough to that obtained with unsaponifiables, i.e., a slight improving effect, rather than a protective action against the improver effect of oxygen during mixing in air. Incorporation of tocopherols

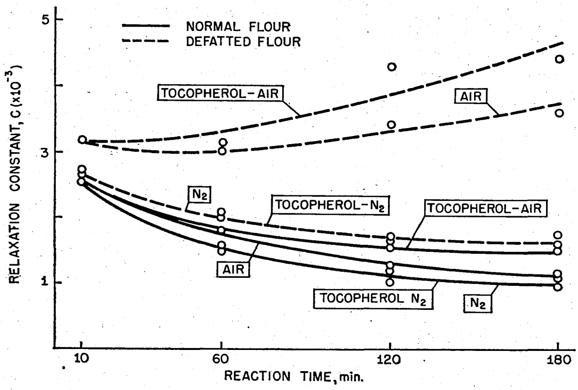

Fig. 4. Change in structural relaxation constant C with reaction time for doughs mixed from normal and defatted flours in air and in nitrogen, and containing 0.005% tocopherol.

into normal and defatted flours and mixing the doughs in nitrogen show little effect; the results are much like those for the corresponding doughs mixed in nitrogen as a control. Addition of other antioxidants to flour had the same effect, i.e., they behaved as improvers in dough during mixing in air. The relative effectiveness of these antioxidants as improvers in dough are BHA>NDGA>PG>BHT and tocopherols. They have little or no effect on doughs mixed in nitrogen.

The mechanism by which these antioxidants act as flour improvers is not well understood, but it is thought that these phenolic substances in the role of antioxidants might rearrange to give a semiquinone structure as described for BHA (17) or as in the case of the oxidation of BHT (5) which undergoes some degree of dimerization. These oxidized products may act as improvers in the dough, since quinones are known to react with thiol RSH (19). Moreover, compounds like methoxy and 2:6 dimethoxy-*p*-benzoquinones isolated from fermented wheat germ have been demonstrated to act as improvers in increasing loaf volume (12). To support this hypothesis, BHT was oxidized by alkaline ferricyanide (5) and the oxidized products were added to defatted flour. These doughs were mixed in nitrogen and in air to study the rheological effect of the oxidized products on dough. In order to compare the increasing effect of oxidation prod-

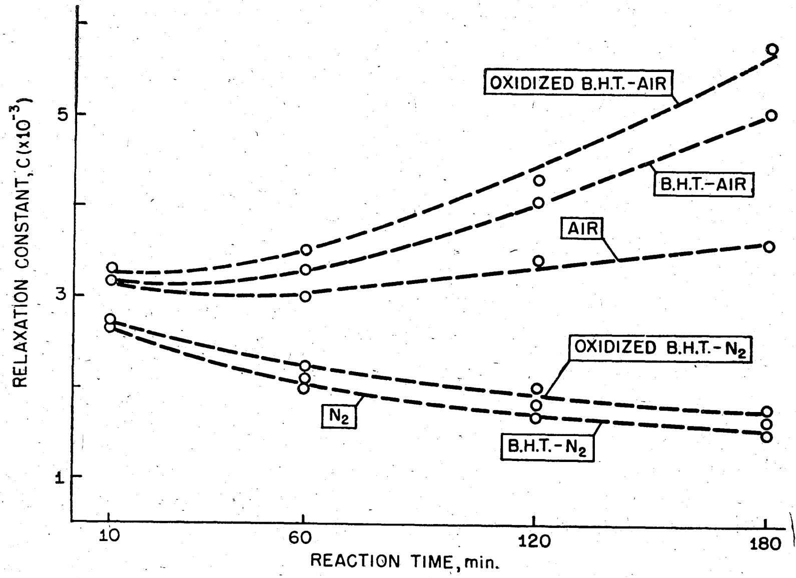

Fig. 5. Change in structural relaxation constant C with reaction time for doughs mixed from defatted flour in air and in nitrogen, and containing 0.005% of BHT and oxidized BHT.

ucts of BHT, the results for BHT before oxidation are also given in the same figure (Fig. 5). The graphs for changes in structural relaxation constants, C, with reaction time show that both BHT and oxidized BHT give improver effect in dough during mixing in air. Incorporation of BHT in defatted flour followed by mixing the dough in nitrogen shows hardly any effect, whereas for oxidized BHT there is a positive improver effect. From this it may be inferred that antioxidants in flour act as flour improvers through their oxidation products during mixing of the dough in air.

Effect of Improvers. The next phase of the investigation deals with the influence of lipids in relation to the response of flour to the improvers, bromate and iodate. For this purpose doughs from normal and defatted flours were mixed in air and in nitrogen with 5 and 15 p.p.m. of iodate and bromate respectively. The results for doughs treated with bromate are shown in the upper half and for doughs treated with iodate in the lower half of Fig. 6.

The effects of bromate and iodate on doughs prepared from normal flour are similar to results previously published (8,9). Of most interest is the curve for changes in structural relaxation constants for doughs from defatted flour with bromate mixed in air and in nitrogen, along with various controls (Fig. 6, top). For defatted flour the effect of mixing in air is superposed on the effect of bromate. This is in contrast to the previous results (4,7), that oxygen has an inhibitory effect on the reaction rate with bromate. One must conclude that the effect of oxygen in doughs from defatted flour is much greater than was expected.

Iodate (Fig. 3, bottom half) showed, as expected, a significant initial effect both for doughs mixed in nitrogen and in oxygen. Doughs from defatted flour mixed in nitrogen then showed no additional increase in the relaxation constant but rather a decrease. In contrast to doughs from normal flour, doughs from defatted flour showed a time-dependent increase in the relaxation constant. It is inferred that it was mainly oxygen that was effective, since iodate reacts completely in a short time. Again, the combined effect of iodate and oxygen in dough appears to be analogous to the effect of bromate and oxygen.

Baking Data. If flour lipids have a protective action against the improver effect of oxygen during mixing of dough, then defatted flour should achieve optimum dough properties at a shorter mixing time than normal flour. Preliminary data to support this hypothesis were obtained by baking tests. Duplicate loaves were baked from doughs mixed for 1.5, 3, 4.5, and 6 minutes. In general, as expected, samples

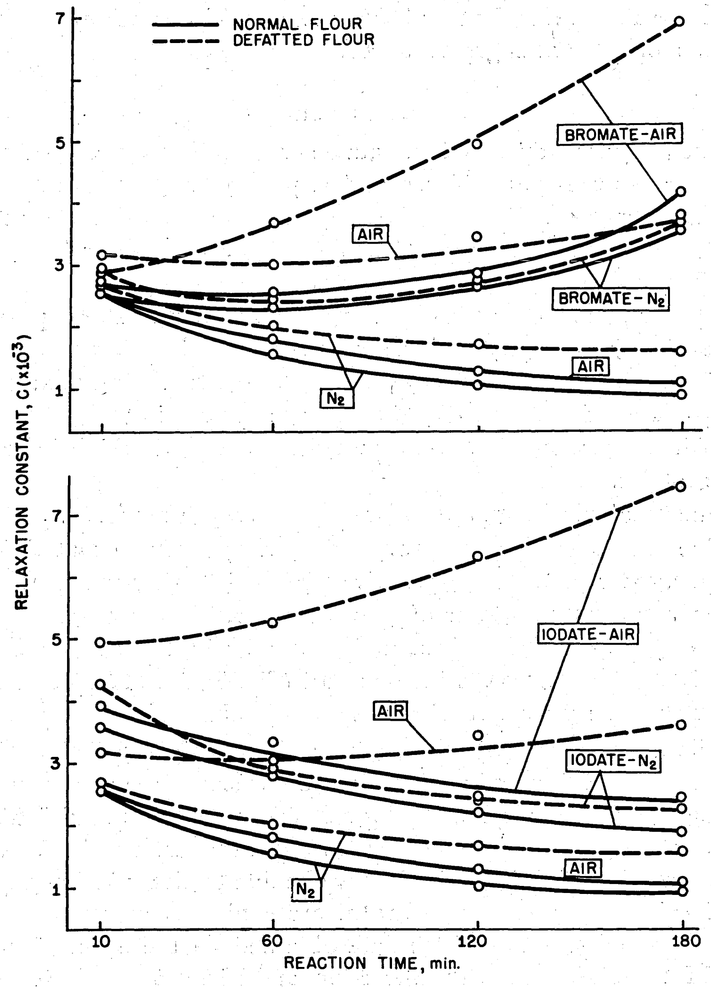

Fig. 6. Change in structural relaxation constant C with reaction time for doughs mixed from normal and defatted flours in air and in nitrogen, and containing 15 p.p.m. bromate and 5 p.p.m. iodate.

from defatted flour gave an optimum loaf volume at a shorter mixing time. The subsequent decrease in loaf volume for longer mixing times was more rapid for the bread from defatted flour as compared with normal flour. In other words, the doughs from normal flour had greater tolerance to mixing. With optimum mixing time, normal and defatted flours containing 50 p.p.m. propyl gallate or nordihydroguaiaretic acid gave greater loaf volume than the respective control flours. This suggests that antioxidants show an improving effect in the baking test. However, further baking tests will be necessary to explore this area of research in greater detail.

Discussion

The present study, based on data derived by rheological techniques, complements the work of Tsen and Hlynka (21) which was based on analytical chemical techniques. They suggested the following recognizable phases of the interrelations among oxygen, flour lipids, and sulfhydryl groups of the wheat proteins:

I. The reaction of molecular oxygen with unsaturated lipids and antioxidants to yield oxidized forms;
II. The reaction of lipid peroxides and oxidized antioxidants with protein –SH to form disulfide linkage in proteins;
III. Direct reaction of molecular oxygen to form disulfide linkage in proteins.

The rheological data are also consistent with reaction II.

The rheological results presented (Fig. 1) indicate that lipids in dough appear to have a protective action, i.e., they reduce the improver action of molecular oxygen in dough. This is reaction I, and the likely mechanism is through formation of fatty peroxides. Since oleic is also effective, as is linoleic acid (Fig. 3), we must assume that both enzymatic and chemical routes are possible.

The second phase (II) represents the effect of lipid peroxides as well as the oxidized form of antioxidants on wheat proteins, presumably through their sulfhydryl or thiol groups. Apparently the oxidized antioxidants seem to be more effective as improvers than lipid peroxides. This low effect of lipid peroxides may be due to either its low reaction itself or its other side reactions with carotenoids through lipoxidase in the process of bleaching. The data obtained on the effect of unsaturated fatty acids, neutral lipid fraction, and of antioxidants fit well into reactions I and II. The effect of palmitic acid, which is fully saturated, indicates that the rheological properties of dough may be strongly affected by lipid constituents apart from their participation in "dough oxidation."

The effect of well-known improving agents such as bromate and iodate is also associated with the lipids. Apparently these improvers give a greater response to doughs from defatted flour on mixing in air, and this is due to the marked effect of oxygen on defatted flour.

The third phase (III) in this work must be considered by inference. Earlier work by Tsen and Hlynka (21) used sulfhydryl-blocking agents, but this was not feasible in the rheological work because of the definite improver effect of sulfhydryl-blocking agents themselves (3) and the consequent difficulty of interpreting the rheological results.

As for the practical implications, obviously the work of this type suggests some of the background for an understanding of the addition of commercial shortening and fats in the baking process, as well as the function of lipids normally present in flour. Finally, it is appropriate to reiterate that rheological methods are useful in basic research and are likely to remain so in the field of dough chemistry.

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