A NOTE ON EFFECTS OF pH ON SULFHYDRYL GROUPS AND RHEOLOGICAL PROPERTIES OF DOUGH AND ITS IMPLICATION WITH THE SULFHYDRYL-DISULFIDE INTERCHANGE[1]

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The rheological properties of doughs are affected by changes in pH. A decrease in pH reduces the extensibility (1,2) and increases the relaxation constant of bromated and unbromated dough (3). This pH effect could be attributed to a number of factors. For example, changes in pH could modify the structure of dough proteins through electrostatic attraction or repulsion resulting from changes in the degree of ionization of ionizable groups on flour proteins, as suggested by Bennett and Ewart (2). Also, the changes could alter the rate of the sulfhydryl-disulfide interchange. It has been postulated by a number of investigators (4,5) that sulfhydryl-disulfide interchange reactions play a major role in determining the rheological properties of dough. A study was therefore undertaken to investigate in parallel experiments the effect of changes in pH on –SH groups and on extensigrams of dough, with an attempt to explain the pH effect in terms of sulfhydryl-disulfide interchange.

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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 13.7 and 0.50% (on 14% moisture basis). The –SH content was 1.07 μeq. per g. of flour. Doughs were prepared from 100 g. of flour (14% moisture) and sufficient salt solution (containing the other reagents used) to give an absorption of 55% and a salt content of 1% (flour basis). They were mixed in the GRL mixer (6). Precautions were taken to minimize the oxidation of dough by air. Flours were purged with nitrogen under alternate vacuum and pressure in an air-tight bowl before mixing. The solutions used for mixing were saturated with nitrogen. All the mixings were done under nitrogen for 2.5 min.

For extensigraph tests, doughs were given a reaction time of 10 min., rounded and shaped, and then stretched after a rest period of 20 or 135 min. During the reaction time and rest period the doughs were kept in a cabinet maintained at 30°C. and 95% r.h.

For the determination of –SH, 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 analysis of –SH. The –SH content of flour or dough was determined according to the modified method of Sokol *et al.* (7) as described previously (8). The pH of the dough was measured by a Beckman expanded-scale pH meter, with a piece of dough directly wrapping both electrodes.

Figure 1 shows the extensigrams for doughs of different pH. The extensigrams in the top half of the figure show that the reduction of pH from 5.8 to 4.8, resulting from additions of 15 and 30 μeq. hydrochloric acid per g. of flour, causes a progressive decrease in extensibility and increase in extensigram height for doughs rested for 20 or 135 min. This observation agrees with that of Bailey and Le Vesconte (1), and of Bennett and Ewart (2). The results are also in accord with the structural relaxation data of Hlynka and Chanin (3).

On the other hand, when the pH of dough is raised from 5.8 to 7.3 with the addition of 30 μeq. of sodium hydroxide per g. of flour, the dough becomes more extensible, as shown in the bottom half of the figure.

In all, these extensigrams show that the extensibility of dough increases with increasing pH from 4.8 to 7.3. Several experiments were also carried out with doughs of pH lower than 4.8 or higher than 7.3. The doughs of pH 4.0 and 1.9 showed a sharp decrease in extensibility; the doughs of pH 9.2 and 10.2 appeared short in extensibility as well as low in resistance to extension. Their extensigrams appear different from

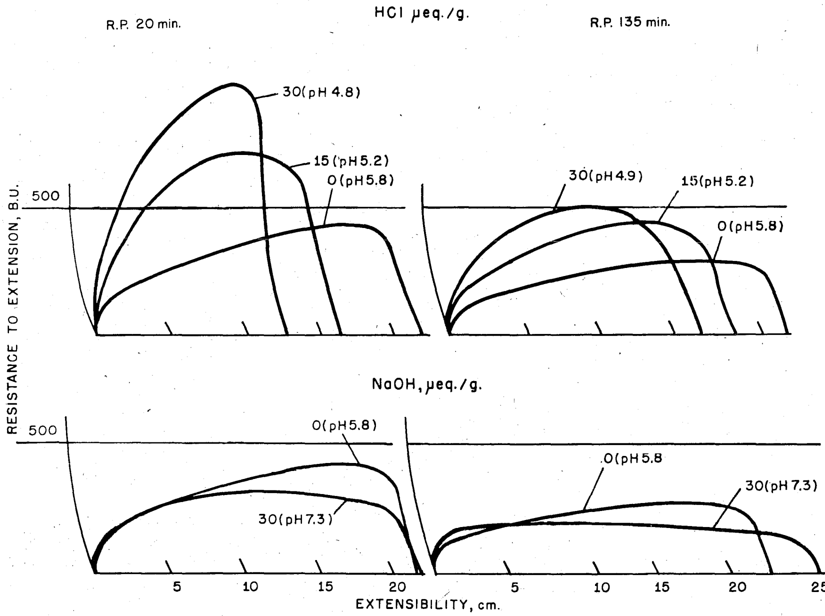

Fig. 1. Extensigrams of doughs at different pH values.

those shown in Fig. 1. The difference indicates that the large addition of hydrochloric acid or sodium hydroxide to adjust the pH of dough could induce some complicated changes in dough properties. Therefore, for this study the pH of dough is confined to a narrow practical range of 4.8 to 7.3.

The values of –SH content of doughs at different pH levels are given in Table I. There are no considerable differences in –SH content among these doughs. These results are not unexpected, since precautions were taken to minimize the oxidation of –SH by atmospheric oxygen. Without these precautions, of course, it could be expected that

TABLE I

EFFECT OF pH ON THE –SH CONTENTS OF DOUGHS (DRY BASIS) RESTED FOR 20 OR 135 MINUTES

Additive	Amount Added	Doughs Rested for 20 Min.		Doughs Rested for 135 Min.	
		pH	–SH	pH	–SH
	μeq./g.		μeq./g.		μeq./g.
HCl	30	4.8	0.89	4.9	0.84
HCl	15	5.2	0.89	5.2	0.83
O	0	5.8	0.93	5.8	0.86
NaOH	15	6.3	0.87	6.3	0.81
NaOH	30	7.3	0.81	7.3	0.76

the oxidation of –SH groups would increase with increasing pH, as demonstrated by Tsen and Tappel with various proteins and –SH compounds (9–11).

The main result obtained from this study of the changes in extensigrams (Fig. 1) and in –SH contents (Table I) of these doughs is that increase in pH lengthens the extensibility of dough without a significant change in –SH content. If, as postulated by Frater *et al.* (5), the rheological properties of dough depend largely on the rate of sulfhydryl-disulfide interchange, then the observed effect of pH might be incorporated into the postulated mechanism by invoking its effect on the ionization of –SH groups.

It is suggested that the disulfide interchange is catalyzed not by neutral thiols (RSH), but by mercaptide ions (RS^-)(12). Mercaptide ions are formed through the ionization of –SH groups, as shown in the following equation:

$$RSH \rightleftharpoons RS^- + H^+$$

This equation suggests that the concentration of the mercaptide ion may be controlled in several ways. A flour improver such as iodate, or an –SH-blocking agent such as N-ethylmaleimide, would decrease the concentration of RSH, and in turn of RS^- ion. Addition of acid would decrease the extent of ionization of RSH, and thus directly, the concentration of RS^- ion. Consequently, as far as extensigraph properties are concerned, the effect of lower pH is similar to that of improvers, in spite of their different primary chemical actions: inhibition of mercaptide formation by acid, and oxidation of –SH groups by flour improvers.

On the other hand, a base would promote the formation of mercaptide ions for catalyzing the interchange, accelerating the relaxation process of dough. In fact, the base-treated dough becomes more extensible than the untreated or acid-treated doughs, as reflected by the extensigrams in the bottom half of Fig. 1.

Studies of the various aspects of the "improver effect" have shown that a small number of –SH groups play a very important role in determining the rheological properties of dough (13,14). Here a slight change of pH alters dough properties as effectively as does a small addition of flour improver or –SH compound to reduce or increase –SH groups in dough. Accordingly, it seems that the observed pH effect on dough properties may be due to its effect on the ionization of –SH groups in dough, as explained above. The results reported in this communication also suggest that, in the evaluation of the sulfhydryl-disulfide interchange system and its effect on the rheological properties of dough, the role of the mercaptide ion should be studied in greater detail.

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