EFFECTS OF SOME ORGANIC SOLVENTS ON EXTENSIGRAPH CHARACTERISTICS OF DOUGH[1]

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ABSTRACT

The addition of alcohols from methanol to nonanol modify both the extensigraph extensibility and resistance, with the greatest effect at C_4 and C_5 irrespective of mixing in air or nitrogen.

When flour was extracted with water-saturated n-butanol it was irrevocably damaged, and reconstitution did not restore its original properties. Either gluten damage during extraction or an effect of residual butanol appears likely. Relaxation curves of dough indicate that SS-SH mechanism is not affected, but butanol and octanol retard normal dough autolysis during 3 hours' resting. At higher concentrations the effectiveness of benzene and octanol but not of butanol is increased by extended mixing. Synthetic doughs mixed from water, dried gluten, and polyvinylpolypyrrolidone appear to indicate that the effect of alcohols is due to a protein denaturation.

It will be shown in this paper that the addition of certain organic solvents to dough, or previous flour extraction by these solvents, affects its extensigraph characteristics. This is not surprising, since previous workers have observed phenomena from which some extensigraph response could be predicted.

Thus several workers found that flour extraction with certain organic solvents altered the baking characteristics of that flour. When diethyl ether was the solvent used, complete recovery was achieved after reconstitution of the flour (1,2). With butanol, the original properties of the flour could not be restored (3,4,5). Following extraction with water-saturated n-butanol, the gluten subsequently isolated from the flour was short (5,6,7), but it was not established whether this effect was due to the removal of the lipid or the change in the protein caused by the solvent. Grosskreutz (7) favored the first view; Rohrlich and Schoenmann (8) gave evidence for the second. After extraction, butanol is exceedingly difficult to remove from the flour and only Mecham and Mohammad (5) claim to have succeeded.

The addition of solvents to dough also affects its properties and those of the baked product. Ponte, Titcomb, and Cotton (9,10) found altered baking characteristics after the addition of several solvents; Kosutany (11) described the effect of ethanol on the stress-strain characteristics of dough; while Hess (12) observed a change in extensi-

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graph characteristics after the flour had been subjected to solvent vapor.

Theoretically, there are several possible assumptions of the mechanism of the solvent effect on dough. Dealing specifically with the lower aliphatic alcohols containing from 1 to 10 carbon atoms, an effect on the protein might be expected. Springall (13), discussing globular proteins, states that their precipitation is favored by a reduction in the dielectric constant of the medium (e.g., the addition of alcohols), and their dispersion, by an increase. The solvents might be expected to change the salt link-hydrophobic bond balance of the protein (14) and might involve the hydrogen bonding, although it is debatable whether the latter is of significance since a large amount of water is present in the dough.

On a macro scale it is known that when gluten is boiled in water it becomes short and inelastic, and its water-holding capacity is decreased. A similar change induced by solvents might by analogy be regarded as protein denaturation.

Peters and Woods (15) dealt with the effect of alcohols on the stress-strain curves of wool keratin and assumed that the effectiveness is dependent upon the molecular size of the alcohols. Methanol acts similarly to water, while the higher alcohols show an effect similar to that obtained by drying the fiber.

Dealing specifically with the disulfide-sulfhydryl interchange reaction of proteins, Halwer (16) and Straessle (17) showed that alcohol precipitation of certain proteins increased the interchange reaction. Thus in the present investigation relaxation experiments were designed to test this possibility.

Removal of flour fat and of bound lipid might give rise to a change in rheological properties of dough. The former would affect the hydrophilic-hydrophobic balance in which the protein expresses itself and would reduce the viscous component in the same way as the addition of any nonreactive liquid would decrease viscosity. The latter, the removal of bound lipid, would necessitate a rebonding of the protein links left vacant. Apart from this structural effect it is known that lipids act as oxygen carriers in the oxidation of sulfhydryl, competing with the direct oxidation of sulfhydryl by molecular oxygen (18). This mechanism was largely excluded in our experiments by mixing the dough under nitrogen.

Certain organic solvents have also a considerable influence upon the carbohydrate fraction. Therefore an attempt has been made to eliminate this aspect by studying the effect of alcohols on artificial "doughs" made from a mixture of gluten and polyvinylpolypyrrolidone. Denaturation of enzymes by organic solvents may play some role, and consequently time-effects have been studied by allowing the dough longer reaction times (0–3 hr.).

The present work examines various aspects of the effect of the homologous aliphatic alcohols (and other organic fat solvents) on dough properties.

Materials and Methods

The flour used in this study was unbleached, improver-free, straight grade, and commercially milled from a blend of Canadian hard red spring wheat. The protein content was 14.0% and ash 0.48% on a 14% moisture basis. At a consistency of 500 farinograph units the absorption was 64.1%; however, an absorption of 60.1% (less 4 percentage units) was used for the extensigraph work.

A portion of the flour was extracted with petroleum ether (Skellysolve F95, essentially pentane). The extraction was carried out in a glass column, 120 cm. in length and 8.5 cm. in diameter and constricted to 2.5 cm. at the bottom. A piece of stainless-steel screen supporting a layer of glass wool was placed inside the column to support the flour. Three-kilogram lots of flour were extracted with 6 liters of petroleum ether at both 25° and 2°C. The extensigraph characteristics showed no apparent difference between the flours extracted at these two temperatures. The extracted flours were air-dried until all odor of the solvent disappeared. The dried flours were passed through an 8xx sieve to reduce the particle size to that of the original flour. The moisture of the flours was 10.1% and the farinograph absorption (at 500 F.U.) was 66.5% at 14% moisture basis. As with the original flour, the absorption for the extracted flour used for the extensigraph work was decreased by 4 percentage units.

Doughs were prepared by mixing 200 g. flour (14% m.b.), salt solution (1% flour basis), and water equivalent to the calculated absorption. The alcohols of reagent grade were added separately, on the basis of 0.05 g. mole per 100 g. flour. The small volume of the alcohols added was not counted as "water" in the doughing liquid. The reason for this is that the higher-insoluble alcohols, for example, can more logically be included as part of the fat phase in dough. The consistency of the doughs was in general only slightly lowered by the addition of the reagents. Nevertheless, the compromise made in adopting this procedure was necessary. Doughs were mixed at 30° in a GRL mixer (19) and stored in a cabinet maintained at 30°C. and 95% r.h. In all tests the doughs were allowed a 5-min. reaction time and a 45-min. rest period or relaxation time; for tests summarized in Fig. 3 a

180-min. reaction time was also included. For the structural relaxation curves the values for the slope, L_A, and intercept, C, were calculated from the linear transformations of the relaxation data (20, Method 54-11) by the method of least squares.

Some extensigraph experiments were conducted with artificial doughs prepared by mixing 20 g. of commercial dry vital wheat gluten (protein 80.4% d.b.), 20 g. polyvinylpolypyrrolidone (PVP)[4] (washed with 2% NaOH, water, and ethanol), 0.006 g. sodium metabisulfite, and 87 ml. water in a Hobart-Swanson mixer for 20 min.

Results and Discussion

Effect of Aliphatic Alcohols on Extensigrams from Doughs Mixed in Air and in Nitrogen. The extensigraph resistance at 5 cm. extension (R_5) and the length of the base line of the extensigram, the extensibility (E) are plotted against the number of carbon atoms of the homologous aliphatic alcohol series (methanol to nonanol) in Figs. 1 and 2. The alcohols were used at a level of 0.05 g. mole per 100 g. of flour. It is apparent from both bar graphs that butanol (C_4) and pentanol (C_5) show the greatest effect.

Since it is known that lipids act as oxygen carriers competing in the direct oxidation of sulfhydryl and since the alcohols might interfere with this mechanism, the experiments were repeated with exclusion of air during the mixing process. The results obtained for doughs mixed in nitrogen are also shown in Figs. 1 and 2. It is apparent that there is no significant difference between mixing in air or nitrogen under the conditions of the test.

Effect of Alcohols on Extensigrams from Doughs Made from Petroleum Ether- and Butanol-Extracted Flours. Next, experiments were designed to determine whether the petroleum ether-soluble fraction was involved. Hence flour was extracted with petroleum ether by means of the column procedure described above, air-dried, and used for the test after due allowance had been made for the small change in water content of the flour. The results show (Figs. 1 and 2) that the resistance (R_5) is significantly decreased with butanol and petanol. The extensibility (E) is slightly decreased with butanol to nonanol. (Compare the histograms for the petroleum ether-extracted with the nonextracted flour mixed in nitrogen in each case.)

Next, an attempt was made to extract the flour with water-saturated n-butanol, since this solvent has been used extensively in connection with flour and since it removes rather more lipid than does petroleum

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[4] Polyvinylpolypyrrolidone, sold commercially under the trade name of Polyclar AT, was obtained from Chemical Developments of Canada, Montreal. In the form of a spray-dried powder it is widely used in the clarification of beer and wine.

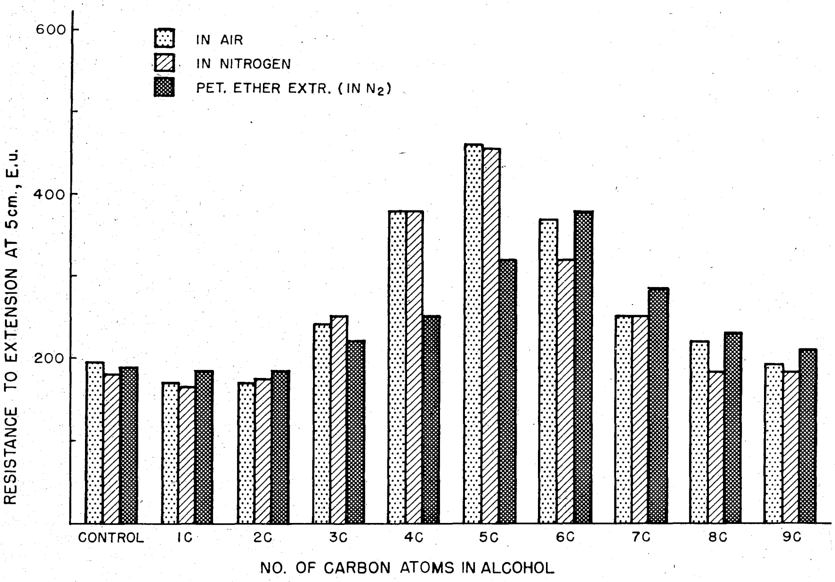

Fig. 1. Effect of addition of alcohols from methanol C_1 to nonanol C_9 on the extensigraph extensibility. Doughs were mixed from normal flour in air and nitrogen, and from petroleum ether-extracted flour mixed in nitrogen.

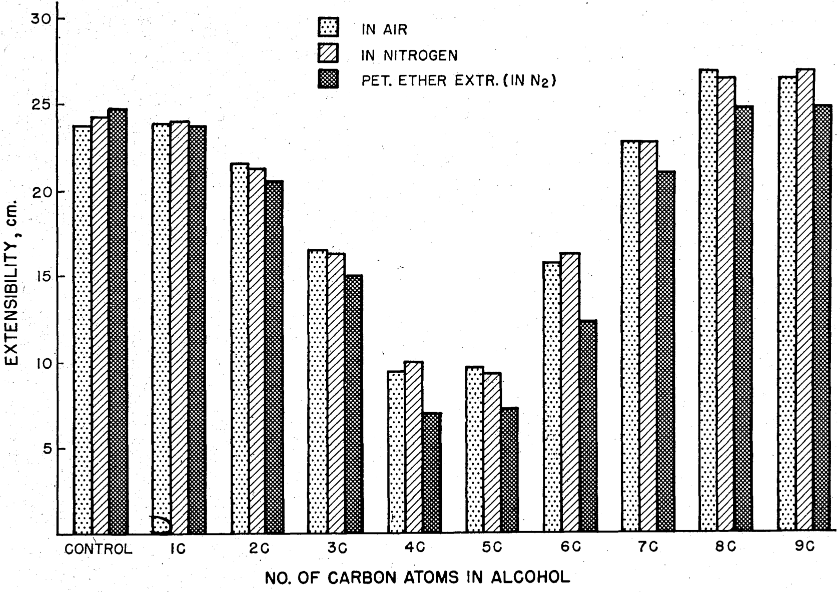

Fig. 2. Effect of addition of alcohols plotted against extensigraph resistance. Doughs were mixed from normal flour in air and nitrogen, and from petroleum ether-extracted flour mixed in nitrogen.

ether. The column extraction procedure proved unsuccessful because of an apparent separation of water and butanol phases on the flour column, with the formation of a doughlike mass. Thus the flour was extracted at room temperature by slurrying with wet butanol, shaking under nitrogen, and later separating by centrifugation. Several attempts were then made to remove all traces of butanol from the flour by various means. It was air-dried, humidified, vacuum-dried, and washed with methanol, ethanol, dry butanol, wet or dry ether, acetone, or a combination of these methods. Some odor of butanol, however, could be detected in the flour and more so when the flour was mixed into a dough. Subsequent extensigrams revealed in all instances that the butanol treatment had greatly decreased the extensibility and increased the resistance. Reconstitution, i.e. adding the correct quantity of extracted lipid material to the extracted flour, showed that the original properties of the flour could not be restored. Thus it would appear that after butanol extraction a mere reconstitution of the flour will not restore the original properties, an observation in agreement with that of Bloksma (3).

Effect of Butanol and Octanol on the Structural Relaxation of Doughs. Since it was apparent that butanol and octanol had rather different effects on dough (Figs. 1 and 2), butanol decreasing the extensibility and increasing the resistance and octanol affecting neither of these characteristics to any marked extent, both alcohols were studied further as representatives of their type of behavior.

Figure 3 shows a plot of resistance (R_5) against relaxation time (t) for the control dough and doughs containing 0.05 g. mole of butanol and octanol per 100 g. of flour. The upper limit of each curve represents the data for 5-min. reaction time, the lower limit for 180 min. Table I shows the structural relaxation constant, C, and the asymptotic load, L_A, determined in the usual way (20).

Considering jointly the results shown in Fig. 3 and Table I, three facts are apparent. First, the asymptotic load, L_A, at 5-min. reaction

TABLE I

Structural Relaxation Data for Doughs Containing Butanol and Octanol for 5- and 180-Min. Reaction Times

	Reaction Time	Control	Butanol	Octanol
	min.		*0.05 g. mole/100 g. flour*	
C	5	1,930	2,980	3,150
	180	1,000	2,420	2,380
L_A	5	152	252	184
	180	131	227	177

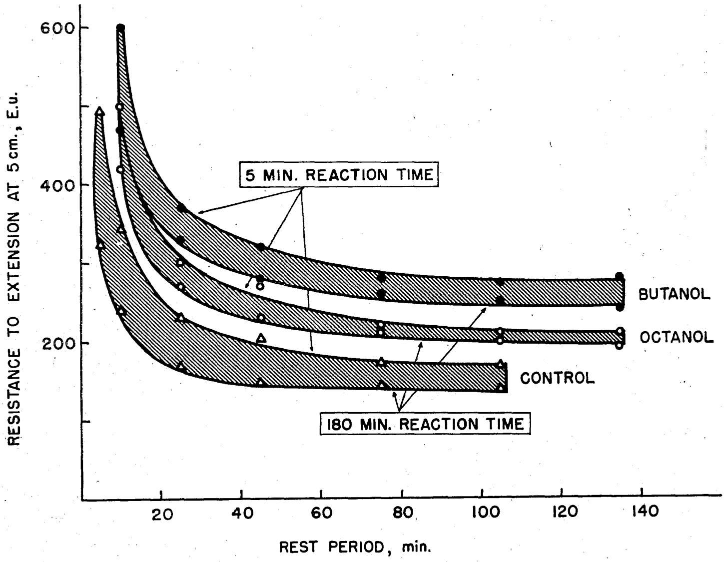

Fig. 3. Plot of resistance (R_5) against relaxation time (t) for the control dough and doughs containing 0.05 g. mole of butanol and octanol per 100 g. of flour. The upper limit of each shaded band represents the data obtained for 5-min. reaction time, the lower limit for 180 min.

time increases with the addition of both butanol and octanol. Hence the ultimate level at which the dough stabilizes is raised. Thus the alcohols appear to strengthen the dough, a physical effect somewhat analogous to the physical effect obtained with flour improvers. Second, the relaxation constant, C, is increased by the addition of the alcohols. This implies that in the presence of these alcohols the relaxation rate of the dough is decreased, the dough stabilizes less rapidly. If dough relaxation is due to an SS-SH interchange, then it would appear that this interchange is decelerated by the alcohols. Third, comparing the curves for 5- and 180-min. reaction time (the band width), it appears that the difference is greatest for the control dough. This band width represents the normal softening of the dough on standing. Butanol, and octanol even more so, decreases this band width (the softening effect), perhaps by inhibition of the enzymatic autolytic processes in the dough.

Effect of Concentrations of Butanol, Octanol, and Benzene on Extensigrams of Doughs Mixed for 2.5 and 20 Minutes. Figure 4 shows a plot of extensigraph resistance (R_5) *vs.* concentration of butanol and octanol, and of benzene for 2.5 and 20 min. of mixing in air and nitrogen. With butanol the increased mixing simply raises the resistance

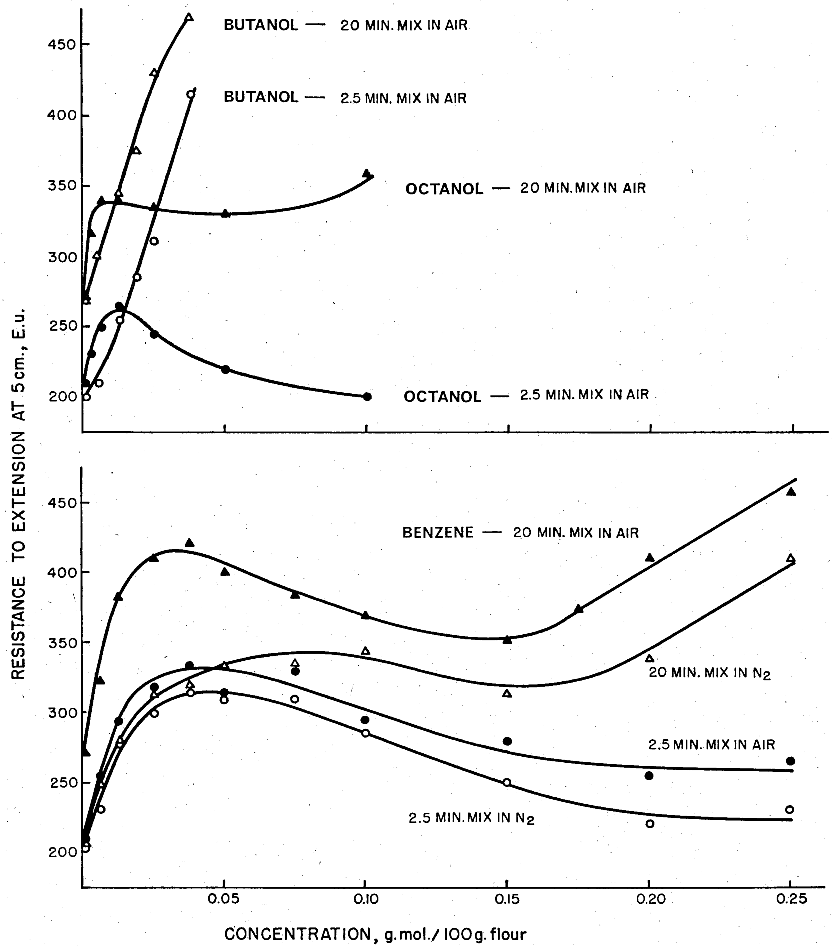

Fig. 4. Upper graph shows the effect of concentrations of butanol and octanol on extensigraph resistance for doughs mixed for 2.5 and 20 min. in air. Lower graph shows the effect of concentrations of benzene on the extensigraph resistance for doughs mixed for 2.5 and 20 min. in air and in nitrogen.

consistent with the known improver effect of air, which becomes increasingly apparent at longer mixing times. With octanol the curves showed a rapid initial rise followed by a leveling off, or even a decrease for the shorter mixing time. The curves are not parallel but diverge at higher concentration. The addition of higher concentrations than shown by the curves for butanol and octanol made the doughs very sticky and impractical to handle.

The curves for benzene, which represent another fat solvent of a different type, show a similar effect more fully over a wider range of concentrations. Here, both in air as well as in nitrogen, the effectiveness of benzene increases at higher concentrations with increased mixing time. One wonders whether this is due to the molecular size of the three chemicals used and is thus associated with the accessibility of the solvents to the protein macromolecules in dough, or to their solubility in water and consequently a different mode of action, either as part of the aqueous phase or part of the fat phase in dough.

Effect of Alcohols on Synthetic "Doughs" Made from Gluten and PVP Powder. Since it is known that certain alcohols react with starch to form inclusion complexes, an attempt was made to exclude starch from the experiment. Thus PVP powder was mixed with dry powdered gluten and water to obtain a "dough" which could be tested on the extensigraph. Considerable experimental difficulties were encountered. At first the farinograph proved ineffective for mixing a "dough" of the desired extensibility. Eventually the "doughs" were mixed in a Hobart-Swanson mixer for 20 min. with incorporation of some sodium metabisulfite to make the "doughs" more extensible. But another difficulty was encountered; it was impossible to control the temperature in this mixer during prolonged mixing. Moreover, a shortage of material did not allow sufficient replication. For these reasons the results are not reported here except for one qualitative observation which appeared significant and clear.

The control "dough" made from gluten, water, and PVP only was tough and elastic. It floated on water and bounced when dropped. (It appears that normal doughs do not rebound when dropped because the elasticity is insufficient to lift the dough's weight.) When dough was washed under running water the PVP was washed away in a milky suspension in a manner similar to the washing-out of starch from normal dough, leaving the gluten in both instances. When the "dough" was squeezed tightly by hand, free water could be obtained with neither the control "dough" nor the ones containing methanol, ethanol, propanol, or butanol. The dough with added pentanol appeared wet, and when it was squeezed a small amount of liquid appeared. With hexanol and heptanol a fair amount of liquid was freed; with octanol the amount was considerable.

This observation, then, appears to explain the original question contained in Figs. 1 and 2 as to why butanol and pentanol showed the greatest effect. It would appear that the alcohols denature the wheat protein progressively. At first the dough becomes tougher and less extensible. At the same time its water-binding capacity is progressively reduced. At pentanol (C_5) this water-binding capacity has been reduced to such an extent that free liquid appears. The rheological effect of free liquid would tend to reduce the extensigraph resistance and increase the extensibility, thus counterbalancing the progressive strengthening of the dough. In the PVP-gluten "doughs" this free liquid may be manually expressed; in the normal dough it is retained, presumably by flour components other than gluten. In this way normal dough shows increased extensibility and decreased resistance.

General Discussion

The data obtained in this investigation show that when the alcohol series — methanol to nonanol — are added to dough, the extensigraph resistance and extensibility are affected. Butanol and pentanol show the greatest effect. The bar graph of extensibility or resistance *vs.* alcohol is somewhat similar to that of Ponte *et al.* (10), who plotted farinograph peak time against alkane carbon chain length. What is the underlying mechanism?

The effect of the alcohols is independent of air-oxidation during mixing. Hence oxidation of the disulfide-sulfhydryl system by air, either directly or via the lipid, is not involved to any major extent.

Considering the effect of butanol and octanol on the structural relaxation of dough, it appears that both alcohols inhibit the autolytic processes of dough to some extent, but the effect is too small to explain the pronounced effect of some of the alcohols shown in the graphs in Figs. 1 and 2. There is also no evidence to suggest that the rate of the disulfide-sulfhydryl interchange reaction is affected by the alcohols.

Previous extraction with petroleum ether influences the subsequent effect of certain of the alcohols, but again not pronouncedly so. Extraction with butanol appears to produce an irreversible effect independent of the extracted material, since reconstitution will not restore the original properties. Whether this effect of butanol is permanent or would be alleviated by complete removal of the last traces of butanol after extraction is still an open question.

There is thus strong evidence that the alcohols act on that flour component of the dough to which the extensigraph characteristics are predominantly due — namely, the protein.

Grosskreutz (7), when postulating his lipoprotein model of wheat gluten structure, stated: "A simple manual inspection of the gluten extracted from doughs made from flour defatted with n-butanol reveals that the gluten is extremely fragile . . . it ruptures easily." Later he remarked: "It may be concluded that the removal of phospholipids does not destroy the basic protein platelet, but that it does seriously affect the ability of these platelets to bond into coherent sheets capable of large plastic deformation."

Rohrlich and Schoenmann (8) succeeded in removing from flour as much lipid with petroleum ether under dry conditions, as with wet butanol, and did not observe any gluten damage by this treatment. They concluded that the properties of the dough were based primarily on the gluten protein and were largely independent of the lipoprotein.

In the work reported here we have found no evidence to suggest that the extractable lipid plays any major role in the rheology of dough. The rheological observations of Grosskreutz (7) quoted above may be explained by a protein denaturation due to butanol or to butanol left in the gluten.

The observation that increased mixing influences, at higher concentrations, the effectiveness of octanol and benzene but not that of butanol poses a very fundamental question. Are the phenomena explicable on a molecular basis, i.e., does the small butanol molecule penetrate the protein freely, whereas the larger benzene or octanol molecules do not, or are the phenomena described based on changes of rheological units much larger than molecules? The concept of rheological unit (21,22) may be a useful one since there is no need to state the size of the unit involved. It may be a protein molecule, a piece of endosperm, or a large agglomerate formed during mixing. If the last two are involved, the effect of mixing may be explained by the different rates of penetration of the alcohols which in turn might depend on their solubility in water.

The fact that increasing amounts of liquid can be expressed manually from the dough containing the higher alcohols (C_5 and above) and that up to butanol the extensigraph resistance increases steadily appears to suggest a simple protein denaturation mechanism which may thus explain the typical shape of the bar graphs, Figs. 1 and 2. The experiments involving the synthetic gluten-PVP "doughs" thus suggest that any involvement of the starch in the mechanism is not of major importance.

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