STUDY OF GLUTEN PROPERTIES AS INFLUENCED BY CERTAIN ORGANIC SOLVENTS[1]

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ABSTRACT

Flour-water doughs made with methanol, chloroform, benzene, 1-hexanol, or different members of the n-alkane family (0.0074 g. moles/100 g. flour solids) yielded glutens exhibiting decreased extensibility, to various degrees, compared with control. Within the alkane family, the greatest decrease in extensibility occurred with C_6 or C_8 alkane; shorter or longer carbon chains had less effect on extensibility. Gluten expansion properties, determined by baking rehydrated commercial gluten containing the various solvents (0.037 g. moles/100 g. gluten solids) were also related to alkane chain length: hexane-containing glutens expanded more than any of the others tested. The baked hexane-gluten had a well-developed, uniform grain structure compared to the control gluten and to the glutens made with other solvents. Hexane- and benzene-containing gluten proteins were less soluble in various aqueous media than the control gluten protein; the solvents evidently generated the formation of relatively higher-molecular-weight aggregates in gluten. When defatted flour was employed as the source of gluten, however, hexane promoted greater protein solubility than control in contrast to the effects of benzene, implying different interactions among solvent, protein, and lipid, depending on whether hexane or benzene was employed.

Research done in this laboratory led to the observation that small quantities of certain aliphatic hydrocarbons, when added to dough, produced notable bread-improving effects (1). These improvements were related to grain structure and texture and did not involve increases in loaf volume, as is obtained by the usual oxidative-type improvers. Subsequent investigations revealed that bread-improvement capabilities were unique to members of the aliphatic hydrocarbons; certain other organic liquids caused "strenthening" effects measured by the farinograph (as did the aliphatic hydrocarbons), but these liquids failed to promote bread improvements (2). Work done on the lipid system of dough showed that the addition of hexane or heptane resulted in enhanced binding of lipids as well as protein (3), in the sense that these dough components were less extractable under given conditions.

The above studies suggested in a general way that the effects of solvents were related to fundamental changes in gluten properties; to obtain more specific information on this point, the work described herein was undertaken.

Materials and Methods

The flour used in these studies to prepare glutens and to perform baking tests was a commercially milled bakers' patent flour; this flour had protein and ash content of 12.0 and 0.44%, respectively, based on a moisture content of 14%. A sample of commercial vital gluten having protein, ash, and lipid (by acid hydrolysis) contents of, respectively, 74.1, 0.99, and 9.1%, all on dry-solids basis, was also utilized in certain studies.

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Viscoelastic Studies. Glutens were prepared as follows: 50 g. flour (dry-solids basis) and 40 ml. water were mixed for 2 min. in a beaker with a glass stirring rod. The resulting dough was allowed to rest in cold tap water for 30 min. The doughs were then hand-kneaded for 3 min. in each of three successive 500-ml. volumes of tap water. The resulting glutens were washed in a slow stream of running water until no milkiness was evident in the washings; then the glutens were gently rubbed between the hands to eliminate excess water. Portions of wet gluten weighing 2.85 g. were shaped into balls and were put in distilled water at 30°C. to rest for 15 min. before they were subjected to stress.

Gluten deformation was measured essentially as described by Kaminski and Halton (4). Briefly, the gluten pieces were impaled on a hook suspended from a large rubber stopper. Another hook, weighted to 5.70 g., was threaded through the same opening made for the first hook, then this whole assembly was fitted onto a 1-liter graduated cylinder filled with distilled water tempered to 30°C. The cylinder and contents were placed in a water bath maintained at 30°C. The deformation of the samples as a function of time was then measured. Results reported for these studies are the average of three independent preparations.

Solvents, where employed, were added immediately prior to mixing in sufficient volume to yield 0.0074 g. moles of solvent per 100 g. flour solids. The solvents were reagent-grade materials where available, and were all redistilled prior to use.

Gluten Expansion Studies. Twenty grams of commercial vital gluten (dry-solids basis) and 35 ml. distilled water were mixed for 1 min. in a National mixer. Solvents, where employed, were added just prior to mixing in sufficient volume to provide 0.037 g. moles solvent per 100 g. of gluten solids. After mixing, the gluten pieces were placed in a beaker of distilled water to rest for 15 min. The glutens were then shaped into balls, placed on watch glasses, and baked by one of the three following procedures: 100°C. overnight (about 16 hr.); 130°C. for 3 hr.; 213°C. for 1 hr. The volume of each baked gluten ball, when cool, was measured by rapeseed displacement. Values shown for glutens baked at 100° and 130°C. represent averages of three independent preparations; the values of glutens baked at 213° are the averages of five preparations.

Defatted gluten used in part of this work was prepared by extracting 100 parts of the commercial gluten solids with about 140 ml. of an ethanol-benzene (1:1, v./v.) mixture for about 5 min. at room temperature, followed by centrifugation to remove the extracted lipid. This was done 10 times, resulting in a total extraction of 6.9% lipid (petroleum ether-soluble material).

Baking Studies. A conventional laboratory sponge dough procedure yielding two 1-lb. loaves was employed. Details of this procedure have been described previously (2). The solvents where employed were added immediately before the dough was mixed, at a level of 0.0074 g. moles per 100 g. flour solids.

Gluten Solubility Studies. Glutens were prepared from flour as described

above, and also from the same flour that was first defatted by extraction with, successively, ethanol and petroleum ether on a Büchner funnel at room temperature. Solvents, where employed, were used at the rate of 0.0074 g. moles per 100 g. flour solids. Five grams of the wet glutens was dispersed in 100 ml. of the aqueous systems to be noted, with a Waring Blendor at low speed. The dispersions were centrifuged in a Sorvall centrifuge at 2,590 $\times$ *g* for 15 min; the supernatants were next filtered through glass wool, then an aliquot of the filtrate was analyzed for Kjeldahl nitrogen.

Results

Effects of Solvents on Viscoelastic Properties of Gluten. Under the testing procedure adopted in this study, all the solvents examined decreased the extensibility of gluten compared to control, as is shown in Fig. 1.

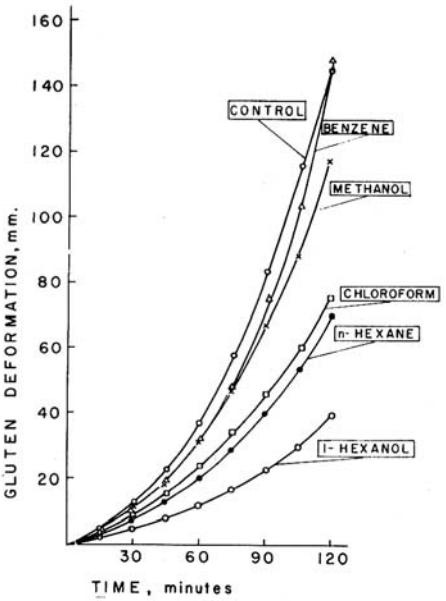

Fig. 1. Effect of various solvents on deformation of gluten. Solvents used at level of 0.0074 g. moles/100 g. flour solids from which glutens were prepared.

Hexanol had the greatest effect. Hexane and chloroform were intermediate in their action; methanol and benzene decreased extensibility only slightly more than control. A pronounced difference in the appearance of the glutens undergoing stress was observed: the control gluten was smooth and uniform, whereas the other glutens had a rough, somewhat lumpy character.

Within the alkane family, a relation between carbon chain length and extensibility was noted, as is indicated in Fig. 2.

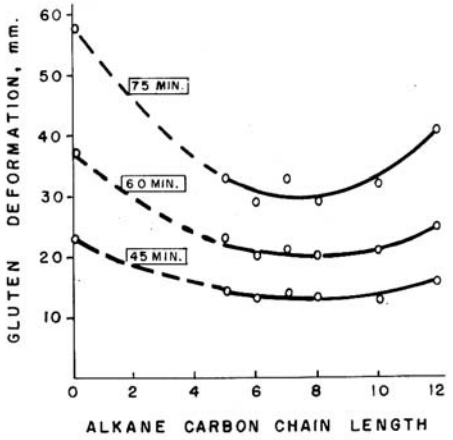

Fig. 2. Relation of n-alkane carbon chain length to gluten deformation.

This graph shows gluten deformation *vs.* the number of carbon atoms in the alkane studied, after various periods of stress. An inverse bell-shaped pattern was indicated between carbon chain length and effect on gluten extensibility. The greatest effect appeared between hexane and octane. This curve resembles one previously reported by this laboratory (2), where farinogram dimensions were plotted against alkane chain length, and also resembles a curve reported by Muller, Hlynka, and Kuzina (5) on the relation between alcohol chain length and extensigram dimensions.

Another manifestation of differences among these glutens was observed after small portions (about 9 g.) of the gluten were simply allowed to air-dry for several days. The control, methanol, benzene, chloroform, and 1-hexanol glutens were virtually identical in appearance; they were brown and rather glossy. The glutens made with the alkanes, on the other hand, were grayish and less intensely colored. Some of these effects are illustrated in Fig. 3.

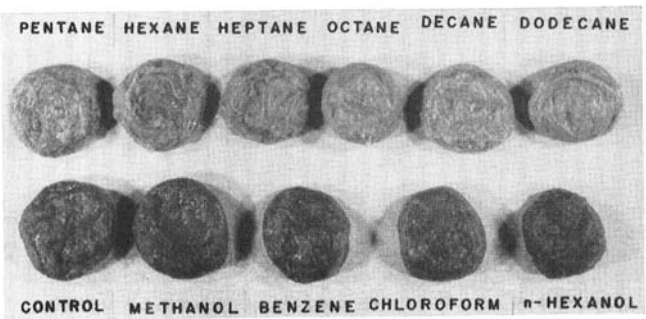

Fig. 3. Appearance of glutens after air-drying for several days (about 9 g. wet gluten employed).

Preliminary experiments to compare the extensibility of glutens prepared from defatted flour proved unsuccessful. Such glutens were evidently deprived of extensibility properties, since they exhibited virtually no deformation after prolonged periods of stress. Loss of gluten extensibility because of removal of lipids has been previously noted (6).

Effects of Solvents on Gluten Expansion. Incorporation of the various solvents had both quantitative and qualitative effects on the expansion of commercial gluten, when the gluten was subjected to several different heating conditions. Figure 4 considers the quantitative aspects.

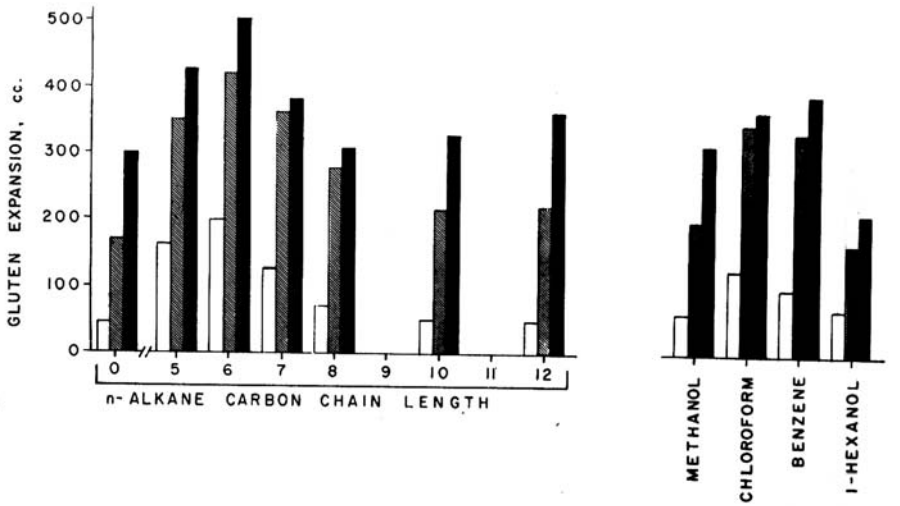

Fig. 4. Effect of various solvents on expansion of gluten when heated. Solvents used at level of 0.037 g. moles/100 g. gluten solids. White, hatched, and black areas indicate volumes after heating at, respectively, 100°, 130°, and 213°C.

The left side of the histogram presents the gluten-volume data for the alkane series. When glutens were baked at 100° and 130°C. a bell-shaped curve was again obtained, this time for the relation between alkane chain length and gluten expansion properties. A somewhat similar pattern existed for the glutens baked at 213°C., except that the curve appeared to rise again when decane and dodecane were employed. Peak volumes were obtained when hexane was the alkane added to the glutens.

Methanol, chloroform, and benzene induced greater volumes in the baked glutens than control, compared at the same baking temperature. 1-Hexanol produced slightly more volume at 100° and 130°C. and somewhat less at 213°C. than control at the respective temperatures.

Differences in color were also observed among the baked glutens. In the series baked at 130°C., for example, the glutens made with the alkanes, especially hexane, had a lighter, grayish color, compared to the brown color of the other glutens.

The unique qualities of the hexane gluten extended to the internal structure (Fig. 5) the hexane gluten obviously had a more highly developed, uniform grain structure compared to the others, which were quite coarse and had large holes to various degrees.

A more pronounced effect of the hexane was obtained when gluten, prepared from flour as described in the previous section, was dried overnight at about 75°C. under vacuum. Figure 6 shows that hexane-gluten under these

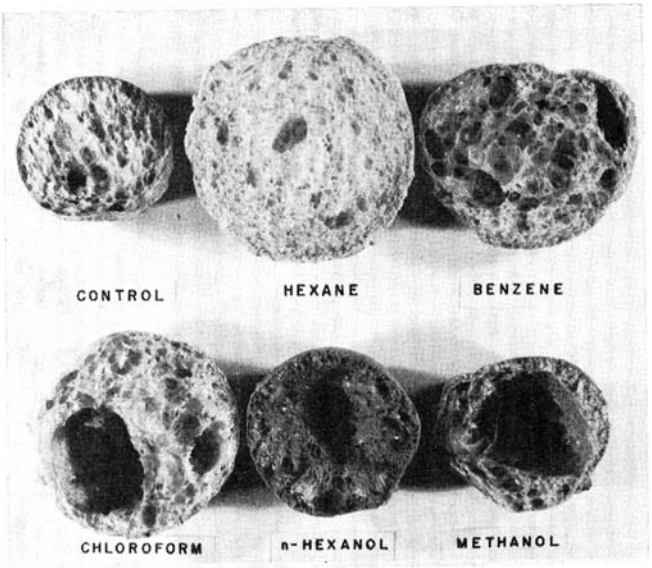

Fig. 5. Comparative effects of various solvents on internal structure of baked glutens.

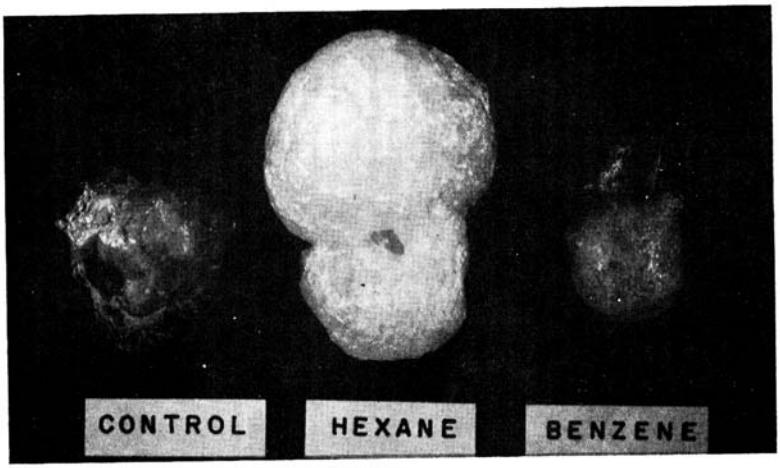

Fig. 6. Effects of hexane and benzene on expansion of glutens heated at about 75°C. overnight in a vacuum oven. Same level of solvents as indicated in Fig. 1.

conditions exhibited a remarkable ability to expand and produce a white, delicate film structure compared to the control and benzene glutens. The marked difference in color was probably attributable to density differences; portions of the three glutens pulverized in a mortar had about the same color.

Effects of the solvents on the expansion of defatted commercial gluten baked at 130°C. for 3 hr. are summarized in Table I. The volumes of these

TABLE I
EFFECT OF VARIOUS ORGANIC SOLVENTS ON EXPANSION OF DEFATTED COMMERCIAL GLUTEN

SOLVENT[a]	VOLUME OF GLUTEN[b]	SOLVENT[a]	VOLUME OF GLUTEN[b]
	cc.		*cc.*
Control	280	n-Decane	300
n-Pentane	248	n-Dodecane	280
n-Hexane	428	Methanol	262
n-Heptane	392	Benzene	390
n-Octane	380	Chloroform	330

[a] Organic solvents used at level of 0.037 g. moles/100 g. gluten solids.
[b] Glutens baked at 130°C. for 3 hr.

glutens were somewhat higher than that of the similarly treated, undefatted glutens (Fig. 4), except in the case of the chloroform gluten which was slightly lower. The over-all pattern of these data was roughly similar to the pattern shown in Fig. 4 with one exception: the pentane gluten had a lower volume than the control.

Effect of Solvents on Bread Quality. The effects of the solvents employed in the previous sections were studied in bake tests, with results as noted in Table II. Effects of some of these solvents were previously examined in bake tests (2); in the previous study, however, the solvents were employed on a constant-weight basis rather than on an equivalency basis as was done in the present experiment.

TABLE II
EFFECT OF VARIOUS ORGANIC SOLVENTS ON BREAD QUALITY

SOLVENT[a]	VOLUME	GRAIN SCORE	PROOF TIME
	cc.		*min.*
Control	2,668	8.2	62
n-Hexane	2,704	9.0	59
Methanol	2,704	7.8	64
Chloroform	2,660	7.8	64
Benzene	2,647	8.0	61
1-Hexanol	small[b]	7.0	[c]

[a] Quantity used: 0.0074 g. moles/100 g. flour.
[b] Volume was below range of loaf-volume-measuring apparatus.
[c] Proof was terminated after 120 min.

These data show that hexane produced a substantially finer grain and slightly faster proof compared to control and to doughs containing methanol, chloroform, and benzene, which yielded loaves with about similar character-

istics. The hexanol loaves did not reach standard proofing height after 120 min., possibly because of injury to the yeast, and consequently the loaves were of poor quality.

A comparison of the improved grain structure of the hexane bread to that of the control is given in Fig. 7.

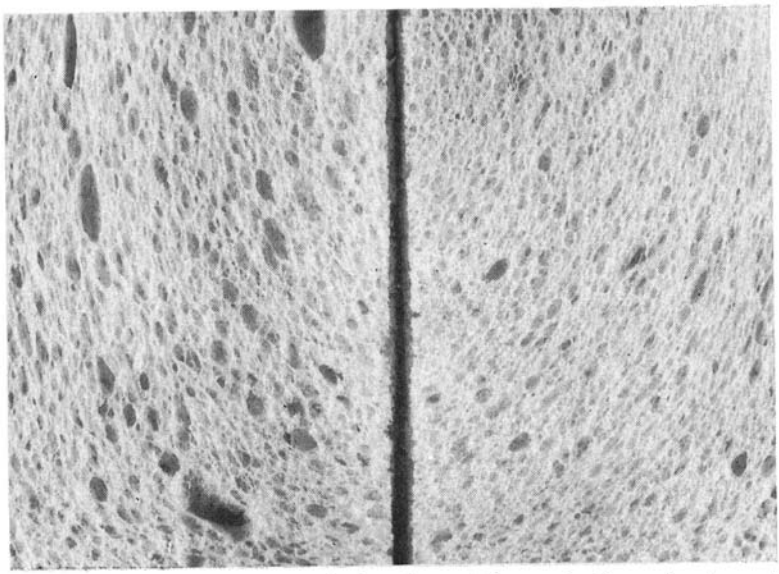

Fig. 7. Bread on left is control; bread on right made from dough containing 0.0074 g. moles n-hexane/100 g. flour solids.

Solubility of Glutens in Various Dispersing Media. Inasmuch as the preceding data indicated that certain physical properties of glutens were altered by the addition of solvents, the question arose as to what extent, if at all, the proteins of the gluten were affected. To obtain preliminary information, the solubility of the gluten proteins were compared, since any changes in solubility would probably reflect structural changes in the proteins. Solubilities of the gluten proteins in a number of dispersing media are summarized in Table III.

TABLE III

SOLUBILITIES OF CONTROL, HEXANE[a], AND BENZENE[a] GLUTENS IN VARIOUS DISPERSING MEDIA

	Protein Solubilized					
	Glutens Prepared from Control Flour			Glutens Prepared from Defatted Flour		
Dispersing Medium	Control	Hexane	Benzene	Control	Hexane	Benzene
	%	%	%	%	%	%
Water	7.8	4.8	5.8	5.7	7.2	5.4
0.1*N* Sodium chloride	5.6	4.4	4.1	4.0	4.4	4.3
0.05*N* Acetic acid	95.8	86.6	76.4	91.9	95.3	86.6
70% Ethanol	68.8	66.0	53.3	61.6	67.4	51.4
0.017*N* Aluminum lactate (pH 3.1)	91.2	87.5	84.1	88.1	88.6	83.1

[a] Organic solvents used at level of 0.0074 g. moles/100 g. flour from which glutens were prepared.

In these studies, the solvents were restricted to two: hexane and benzene. These are both nonpolar hydrocarbons; the first was shown to be a bread improver in the preceding section, the latter was not.

The data for the glutens prepared from intact flour showed similar trends. That is, more proteins were solubilized in the control gluten compared to the glutens made with hexane or benzene for the five different dispersing media. The benzene gluten was generally less soluble than the hexane gluten. It appears, therefore, that the presence of the solvents in gluten favored molecular aggregation leading to increased formation of gluteninlike proteins.

A somewhat different pattern emerged for the glutens obtained from defatted flour. Here, the benzene gluten was again less soluble than control, but the hexane gluten became more soluble than control. These results imply differing interactions among protein, lipids, and solvent, depending on the solvent employed in gluten-making.

Discussion

The data presented in this paper show that small quantities of certain organic solvents affected gluten extensibility and gluten expansion properties. The alkanes, especially hexane, seemed to exert unique effects as manifested by the physical appearance of glutens exposed to various drying conditions. Hexane and benzene both increased the level of acetic acid-insoluble proteins of gluten (i.e., protein "aggregates"). In this context, a recent paper by Mecham, Cole, and Pence (7) seems highly pertinent. These workers studied, in part, dough-mixing effects of crude gluten in comparison to glutens from which acetic acid-insoluble materials (i.e., protein aggregates) had been removed by centrifugation. The presence of the aggregates was found to be associated with increased dough elasticity and increased stability. The data shown in the present paper are parallel: glutens made with solvent contained higher levels of protein aggregates; they were less extensible (i.e., more elastic); and they produced films that were relatively more stable to expansion. These relationships suggest that the hexane-improving mechanism may involve the generation of protein aggregates, with the end result that more optimum film-forming properties are obtained. Thus, when the hexane glutens were developed, a superior film was formed around air cells trapped within the gluten, creating bubbles that were exceptionally stable when subjected to expansion in the oven; these bubbles did not coalesce or rupture as much as the bubbles in the other glutens. Greater rigidity and resistance to extension were found when the hexane glutens were subjected to stretching forces.

Obviously, many questions on these points need to be answered. For example, the fact that benzene, a nonimproving solvent, exerts at least some of the same effects on gluten as hexane, a marked bread improver, needs to be reconciled.

The above observation on the formation of protein aggregates complements the previously reported finding that solvents increase the binding of lipid in dough (3). Apparently, the solvents alter the associative forces of gluten components; the nature of the bonding forces involved remains an intriguing question to be answered. If a specific relation between the effect

of hexane and a particular gluten property could be found, knowledge of what constitutes gluten quality could be enhanced.

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