Some Properties of Dough and Gluten in D_2O[1]

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ABSTRACT

Extensigraph, farinograph, mixing, and baking properties of dough in D_2O are described and compared with properties of dough in H_2O. Farinograph mixing behavior of D_2O gluten was also examined. The results showed that a stronger dough-and-gluten is formed in D_2O than in H_2O. This behavior indicates that numerous weak hydrogen bonds play a very significant role in determining the physical structure and behavior of dough and gluten.

There are a large number of unsubstantiated reports in the literature that physical properties of dough and gluten are largely dependent on hydrogen-bonding of the gluten proteins. These reports are based on data such as the observation that urea is a strong gluten-dispersing reagent and that urea has a significant effect on dough properties (1). However, urea is an excellent dispersing reagent for all proteins, whether the proteins are hydrophobic or hydrophilic in character; and in addition, it has been shown recently by Tanford and his students that the dominant forces between amino acids and proteins, and urea, are probably hydrophobic in character (2). Support for two types of forces in gluten was given by studies of synthetic polypeptides containing side-chain amide groups which indicated that some of the physical properties of gluten are dependent on both hydrogen bonding and hydrophobic bonding (3,4). Additional evidence for nonpolar forces in gluten is provided by a report that some properties of gliadin and glutenin are better explained on the assumption that their large glutamine and asparagine side-group content should be considered to have nonpolar characteristics (5). From the work cited above, it is clear that a reasonable amount of experimental evidence is available to indicate that nonpolar forces are present in gluten and dough.

The present work is an attempt to present some direct evidence for the presence of hydrogen-bonding in dough by using D_2O as a dough constituent. The studies were prompted by observations reported by Kretovich and Vakar (6) and Sorena and Vakar (7), who studied the extension of gluten in H_2O and in D_2O. These authors found that gluten was mechanically stronger in D_2O than in H_2O, and they proposed that these differences were due to hydrogen bonds whose strength was increased by substituting deuterium for hydrogen. The present studies extend the studies of Vakar and co-workers by examining some extensigraph, farinograph, and baking properties of dough and gluten in D_2O.

MATERIALS AND METHODS

The hard red spring wheat flour used in this study was a straight-grade, untreated sample commercially milled from a blend of sound Canadian wheat. The white winter flour was a laboratory-milled sample from a composite of sound wheat. Durum flour was obtained by laboratory milling of a sound sample of durum wheat. Glutens were isolated from the flours as described

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previously (8). A description of the flours and glutens is given in Table I.

TABLE I
DESCRIPTION OF FLOURS AND GLUTENS STUDIED

MATERIAL	PROTEIN[a]	MOISTURE	ASH[b]	FARINOGRAPH ABSORPTION[c]	WATER CONTENT FOR MIXING CURVE[d]
	%	%	%	%	%
Flour					
Hard red spring	13.6	13.6	0.45	62.7	....
Amber durum	13.3	14.7	0.68	68.6	....
White winter	7.1	14.4	0.33	50.3	....
Gluten					
Hard red spring	73.4	11.1	0.25	....	100
White winter	69.2	10.5	0.44	....	100

[a] N × 5.7, 14% moisture basis.
[b] 14% moisture basis.
[c] 14% moisture basis, 500 B.U. consistency on a 1:1 sensitivity linkage ratio.
[d] Dry basis, farinograph consistency, using a 1:5 sensitivity linkage ratio.

D_2O, 99.75% by weight, was purchased from Atomic Energy of Canada Ltd.

To observe the behavior of dough in D_2O, freed from H_2O as much as possible, initial studies were carried out with freeze-dried samples of flour containing 1.1% H_2O. Work with flour of such a low moisture content had to be abandoned, owing to the large temperature increase (approximately 7°–8°C.) of the dough on addition of D_2O or H_2O. The temperature increase must be due to sorption and hydration phenomena. Accordingly, in all D_2O studies carried out in the present work, 99.7% D_2O was added to materials containing normal amounts of water (as indicated in Table I). In absolute terms, this means that D_2O doughs, or glutens, contained a solvent whose composition was approximately 81% D_2O and 19% H_2O.

D_2O was added to doughs or glutens in amounts equimolar to the quantity of H_2O used in the control experiments. From data on density and molecular weight (9), it can be calculated that at 25°C., 1 ml. of 99.75% (by weight) D_2O contains 55.17 and 0.15 mmoles of D_2O and H_2O, and 1 ml. of ordinary H_2O contains 55.34 mmoles of H_2O. Accordingly, to obtain equimolar amounts of D_2O and H_2O, equivalent volumes of the two solvents were used; the error of 0.04% introduced by this procedure was considered to be negligible.

Extensigraph Methods

Doughs were mixed in a GRL Mixer (10) at 68 r.p.m. for 2.5 min. at 30°C. An absorption of 58.7% (14% m.b.) was used for all samples. The reaction time was 5 min.; rest periods were 10 and 75 min. (Doughs were kept in a conditioning cabinet at 30°C. and 95% r.h.). Extensigrams were obtained with a Brabender Extensigraph.

Farinograph Methods

In all studies a Brabender Farinograph with a 50-g. stainless steel mixing bowl was used. The mixing speed was 63 r.p.m.; a 1:1 lever sensitivity linkage setting was used. With H_2O, an absorption of 62.7% was necessary to obtain a consistency of 500 B.U. at 30°C. for dough made from HRS flour.

For gluten studies, the procedure was as described previously (8,11) with a lever sensitivity linkage setting of 1:5. It should be recalled that the 1:5 farinograph sensitivity setting decreases the sensitivity in consistency by a factor of 5.

Baking Procedure

The doughs were mixed in a laboratory mixer attached to a power-input metering device (12). A rich baking formula was used (12). In the D_2O experiment all of the solutions were made up in D_2O except for the 0.3-ml. malt syrup which was made with H_2O.

Gassing Power

The AACC pressuremeter method was used to determine the gassing power; a modified pressuremeter was used (13).

RESULTS

Extensigraph Studies

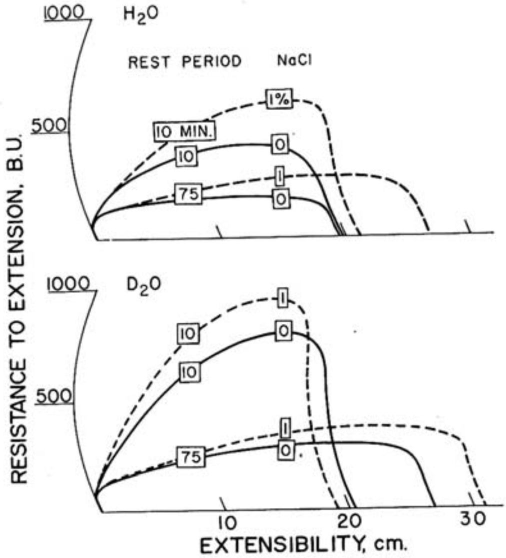

Fig. 1. Extensigrams of hard red spring flour mixed with H_2O and D_2O. Extensigram experiments with doughs containing 1% NaCl are also illustrated.

Some typical extensigraph curves of dough made from H_2O or D_2O are illustrated in Fig. 1. All experiments with D_2O doughs showed a very significant increase in resistance to extension, compared to doughs containing equimolar amounts of H_2O, since the extensigraph curves are much higher for the D_2O doughs. For example, with a 10-min. rest period, the maximum resistance to extension was 795, and 420 B.U. for the D_2O and H_2O doughs. Experiments with D_2O doughs containing NaCl, or bromate or iodate, showed analogous results. A summary of the extensigraph results is given in Table II.

TABLE II

EXTENSIGRAM DATA FOR HRS FLOUR DOUGHS MADE WITH H_2O OR D_2O

INGREDIENTS ADDED TO FLOUR	REST PERIOD (MINUTES)					
	10	75	10	75	10	75
	Resistance to Extension at 7 cm. (R7)[a]		Resistance to Extension at Max. (R max.)		Extensibility	
	B.U.	*B.U.*	*B.U.*	*B.U.*	*cm.*	*cm.*
H_2O	350	175	420	190	19.7	19.5
D_2O	550	215	795	305	20.8	27.2
H_2O + NaCl	430	200	615	285	21.3	27.1
D_2O + NaCl	700	225	940	375	19.0	31.2
H_2O + NaCl + KIO_3[b]	700	260	910	370	18.0	25.0
D_2O + NaCl + KIO_3[b]	885	260	995	435	15.2	34.2
H_2O + NaCl + $KBrO_3$[c]	590	200	745	310	20.2	29.0
D_2O + NaCl + $KBrO_3$[c]	800	245	905	400	16.5	31.5

[a] R7—values corrected with template.

[b] The amount of KIO_3 used was 8 p.p.m. (flour basis).

[c] $KBrO_3$—doughs given 3-hr. reaction times before rounding and shaping; all other doughs allowed 5-min. reaction times. The amount of $KBrO_3$ used was 15 p.p.m. (flour basis).

Baking Studies

Baking D_2O doughs produced a smaller loaf of 875 cc., since a comparable H_2O dough resulted in a 990-cc. loaf. During mixing of the doughs used for the baking studies, the work input was recorded. The D_2O dough required a higher work input of 6.5 w.-hr. for optimum development as compared with 5.5 w.-hr. for the H_2O dough. The decrease in loaf volume of the D_2O bread cannot be interpreted to result only from a stronger dough structure caused by substituting D_2O for H_2O, since the gassing power of doughs containing D_2O was significantly lower, as is illustrated in Fig. 2. Accordingly, the smaller loaf volume of D_2O bread may be due to both insufficient CO_2 production and a stronger dough structure.

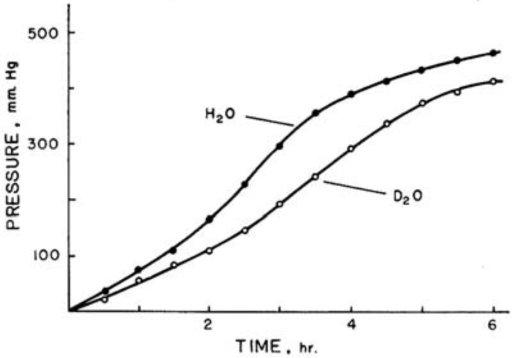

Fig. 2. Gassing-power curves of dough in H_2O and D_2O.

Farinograph Studies

Dough. Some typical examples of farinograms of D_2O doughs are shown in Fig. 3. The farinograph studies indicated that the D_2O doughs possessed higher values for development time, band width, and stability for all of the doughs examined, compared to doughs containing equimolar amounts of

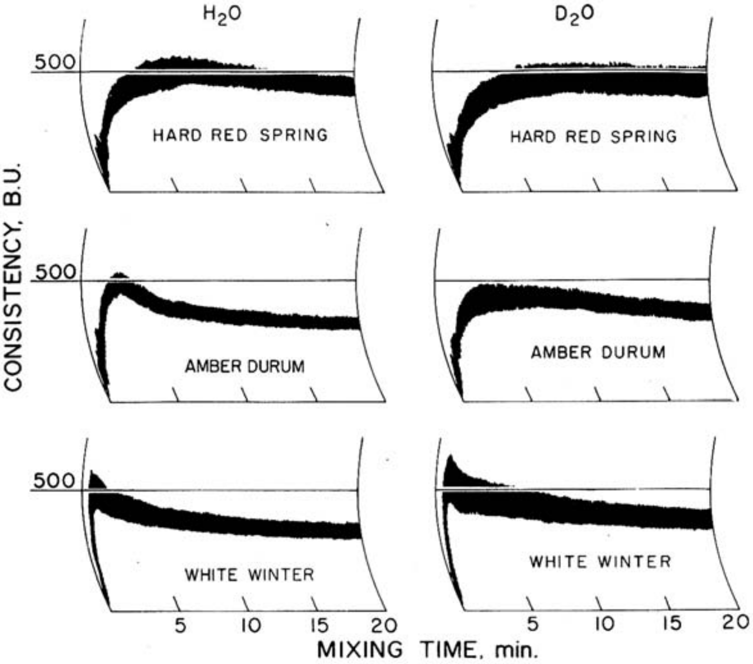

Fig. 3. Farinograms of three flours mixed with H_2O and D_2O.

H_2O. The changes in consistency, however, varied according to the sample being mixed, as there was a very slight decrease in consistency for HRS and durum doughs, and a slight increase in consistency for doughs from soft white winter wheat. A summary of the farinograph results is given in Table III.

TABLE III
Farinogram Data for Three Types of Flour Made with H_2O and D_2O

Flour Source	Experimental Conditions	Maximum Consistency	Development Time	Band Width (at Peak)	Tolerance Index (20 Min. Past Peak)
		B.U.	*min.*	*B.U.*	*B.U.*
Hard red spring wheat	H_2O	500	8.0	90	105
	D_2O	475	10.0	105	45
	H_2O + NaCl	440	10.0	100	45
	D_2O + NaCl	445	13.5	110	20
Amber durum wheat	H_2O	495	3.0	70	190
	D_2O	435	4.5	100	105
	H_2O + NaCl	445	4.0	70	145
	D_2O + NaCl	420	5.0	105	75
White winter wheat	H_2O	500	1.0	120	200
	D_2O	550	1.0	165	225
	H_2O + NaCl	450	1.0	120	135
	D_2O + NaCl	495	1.0	150	125

Some of the initial farinograms suggested that the behavior of D_2O doughs was similar to that of H_2O doughs containing NaCl. Accordingly, experiments with doughs containing NaCl were carried out. As the results in Table III indicate, the behavior of the HRS and durum wheat doughs in D_2O is similar to their behavior in H_2O containing 1% NaCl. The behavior of the soft white winter wheat dough in D_2O was, however, not similar, since the consistency of it in D_2O was higher than in H_2O alone, or in D_2O or H_2O containing NaCl.

Gluten. The behavior of gluten containing D_2O and H_2O is illustrated in Fig. 4. It is seen that there are two major differences in the behavior of gluten in D_2O as compared to H_2O. In D_2O there is a considerable increase in stability, and one notes that excessive oscillations are present in the farinograph mixing curves. The consistency of the glutens in D_2O was similar to that in H_2O.

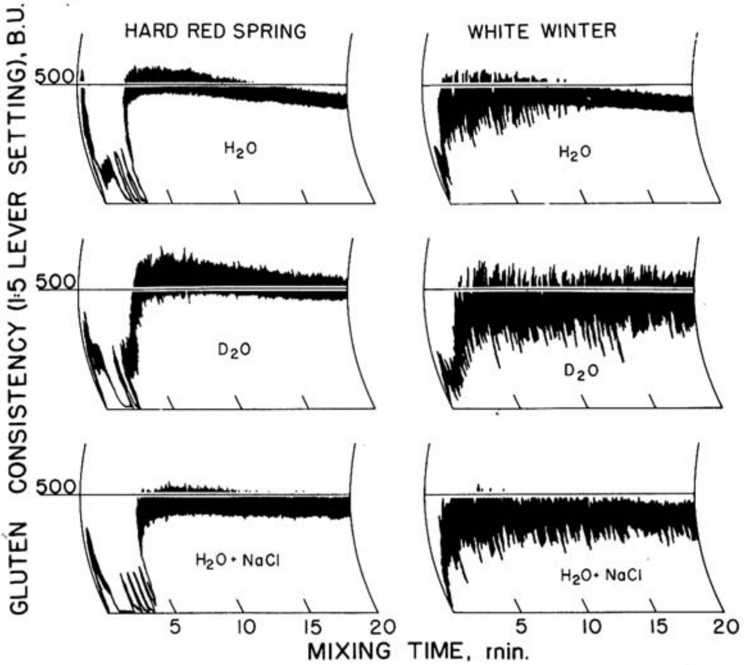

Fig. 4. Farinograms of two glutens mixed with H_2O and D_2O.

DISCUSSION

Our experiments have indicated that substituting D_2O for H_2O in dough results in stronger and tougher dough. These effects are probably due to a stronger gluten network, as indicated by our mixing studies with gluten and by the gluten extensibility studies of Kretovich and Vakar (6). The strengthening effect of D_2O on proteinaceous materials is further illustrated by the observations that wool exhibits stronger mechanical properties in D_2O than in H_2O (14).

Substituting D_2O for H_2O in mixtures containing proteins (or carbohydrates) results in the exchange for deuterium of hydrogen attached to oxygen, nitrogen and sulfur, and nonexchange of hydrogen bound directly to carbon (15). Accordingly, when a change in properties of a material is observed in D_2O, the phenomena must involve the strength of hydrogen bonds, and not hydrophobic bonds which generally involve the relatively nonionic side-chain, nonlabile hydrogen (15). Thus, it may be concluded that hydrogen bonds play an important role in dough structure.

While it has been justified theoretically that the O-D . . O bond is stronger than the analogous O-H . . O bond (16), one cannot assume that the N-H and N-D amide bonds of proteins form hydrogen bonds having different strengths, since the presence of the solvent water complicates the situation. Thus, the deuterium isotope effect in protein is linked with both solvent-solvent and solvent-protein interactions (15,17). Accordingly, upon deuteration in D_2O, a change in the strength of the peptide hydrogen bonds could be canceled by changes in the strengths of the hydrogen bonds between water molecules, and between water molecules and protein chains.

Although the identity of the hydrogen bonds involved cannot be determined, it is possible to predict something about strengths of the hydrogen bonds in dough, as is summarized in an excellent review article by Hvidt and Nielsen (15). The latter authors concluded that the effect of substituting deuterium for hydrogen on the strength of hydrogen bonds indicates that deuterium bonds are stronger than protium bonds when the original hydrogen bond is a weak one, and weaker than protium bonds when the original hydrogen bond considered is a strong one (15). Accordingly, since the deuterated dough-and-gluten is much stronger than normal protinated dough, one can conclude that the dough-and-gluten contains weak hydrogen bonds. However, even if these hydrogen bonds are weak, they play a very significant role in determining the physical properties of dough and gluten, as is directly demonstrated by the properties of dough and gluten in D_2O. Certainly, differences in hydrogen-bonding properties are probably present in different wheat species, as is demonstrated by the differences in behavior of the different doughs in the present work.

The present work is a presentation of only some of the phenomenological effects of dough made with D_2O. It should be possible to exploit further the remarkable effect caused by D_2O on dough properties. Thus, the rates of deuterium exchange and identity of the exchangeable protons could well provide significant evidence for the structure and structural differences of different doughs and glutens.

Acknowledgments

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