STUDIES OF WATER BINDING BY DIFFERENTIAL THERMAL ANALYSIS. I. DOUGH STUDIES USING THE BOILING MODE[1]

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ABSTRACT

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Water binding in dough was studied using the boiling mode of differential thermal analysis (DTA). The results showed that water binding in dough depends on the mixing strength of the flour (*i.e.*, wheat cultivar); stronger mixing flours bind water more strongly than weaker mixing flours. An increase in the water absorption of dough produced a curvilinear increase in water binding as measured by DTA. The binding energy decreased with increase in protein content of flour and increased linearly with increasing starch damage and water-soluble pentosan content. During dough-mixing water binding increased to a maximum and then decreased at about the same rate as farinograph consistency for the two stronger cultivars. For the weak cultivar investigated, the binding energy-mixing time curve was anomalous. Addition of sodium chloride, N-ethylmaleimide, cysteine, and potassium iodate decreased the energy of water binding, but ascorbic acid increased water binding.

Water is a normal constituent of wheat flour and is essential for the transformation of the flour to dough (1,2). Water is unique among known liquids in that it forms a viscoelastic dough and may be directly involved in the structure of dough by forming cross-links among the flour constituents (3). Webb *et al.* (4) suggested that, since water constitutes about 42% of the total weight of bread dough, it is unlikely that it is present simply as a diluent. They proposed that water in dough is attracted to polar groups with varying degrees of strength and that the distribution of water depends on the mechanical work input (during dough-mixing) used to develop the dough. The concept of free and bound water has also been introduced in considering the relation between water and the individual components of flour; bound water is an integral part of the structure of dough and free water is responsible for the fluidity or mobility of the dough (5,6).

This article reports some new information on the binding of water in dough obtained with the boiling mode of differential thermal analysis (DTA).

MATERIALS AND METHODS

Flours

The flours used in this study were milled on the Buhler experimental mill from grain of three wheat cultivars known to differ widely in mixing characteristics. The semidwarf hard red spring wheat, Red River 68, was selected as the overly strong cultivar; the Canadian hard red spring wheat, Manitou, was the medium strength cultivar; and the Ontario soft white winter wheat, Talbot, was used as the representative of weak-mixing cultivars. Grain of about the same protein content of the three cultivars was used for all experiments except in the

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experiment on the effect of protein content. For this experiment, grain of the cultivar Manitou of different protein was used.

Production of Damaged Starch

To obtain flours with varying levels of damaged starch, flour samples were ball milled and pin milled for varying periods. Prior to ball milling, the flour was dried for about 4 hr at 50° C (final moisture about 4%). Pin milling was achieved by passing the flour once and three times through the Alpine S.L. 160Z pin mill, running at 16,000 rpm. The level of starch damage was determined by the method of Farrand (7).

Preparation of Water-Soluble Pentosans

Water-soluble (WS) pentosan was isolated using the procedure of Jelaca and Hlynka (8). WS Pentosan content of the three flours was determined by the method of Baker *et al.* (9).

Preparation of Dough

Doughs were mixed on a constant-weight basis (80 g) at 30° C in a stainless-steel Brabender Farinograph® mixer. Mixing was done at the normal mixing speed of 63 rpm. Except in the case of the doughs with added chemicals or doughs used to study the effect of variable farinograph mixing time, the mixing time was 8 min. Doughs with added chemicals were mixed for 20 min to allow sufficient time for the chemicals to react with the flour constituents. The moisture content in all doughs (except in those used to study effect of variable moisture) was 46.2% (60% absorption).

N-Ethylmaleimide (NEMI), L-cysteine, L-ascorbic acid, and potassium iodate were added to doughs at the level of 2 μeq/g flour. This concentration was selected to represent approximately three times the molar equivalent of accessible -SH content of the flours used. Sodium chloride was added at the 2% level (flour basis). The total -SH contents (by amperometric titration) of the three flours used were 1.41, 1.30, and 0.98 μeq/g flour for Manitou, Red River 68, and Talbot, respectively.

Immediately after mixing, the dough samples were sealed in aluminum foil and kept in a closed container to prevent loss of moisture until subsamples were taken for analysis. Dough moisture content was determined from the weight loss after overnight drying at 105°.

For thermal analysis, dough subsamples of approximately 25 mg were cut from the center of each dough piece using a specially designed glass tube and plunger assembly (Fig. 1). The subsample was placed at the bottom of a DTA sample tube by means of the plunger assembly and was immediately weighed before analysis.

Differential Thermal Analysis

The DTA instrument used was the Dupont Model 990 equipped with the standard (500° C) DTA cell. Glass beads were used as the reference since glass has no thermal transitions in the temperature range used.

The sample tube containing the subsample of dough was placed in the DTA cell and heated from 30° to 160° C at a programmed rate of 5° C/min. The x- and y-axis sensitivities on the analyzer recorder were set at 50° and 5° C/in., respectively. x-Axis and y-axis represent the temperature of heating block and the difference in temperature of the sample and reference, respectively. At about 100° C, an endothermic peak appears (Fig. 1) due to the boiling-off of the dough moisture. In practice, each dough sample was heated only once; three to six replicate analyses with fresh subsamples were made for each dough. The area under the peak was measured with a planimeter. The results (average of all analyses) were expressed as area/mg dry flour.

RESULTS AND DISCUSSION

The analysis of water binding from the DTA boiling thermogram is based on the fact that, owing to the boiling-off of the dough moisture, the area of the endotherm peak at about 100° C is directly proportional to the amount of heat energy absorbed in the vaporization of the water, an endothermic process. Accordingly, at constant moisture, dough samples that give a larger peak area require more heat energy to boil-off the same quantity of water. It is assumed that the endothermic process at 100° C removes both bound and free water, since there were no other endothermic transitions up to 160° C. The boiling curve method gives only comparative results in terms of the energy of water binding. Accordingly, it cannot be used to determine the absolute amount of free or bound water for a particular biological material.

Effect of Mixing Strength of Flour

The areas of the endotherm peaks for doughs of the three wheat cultivars of

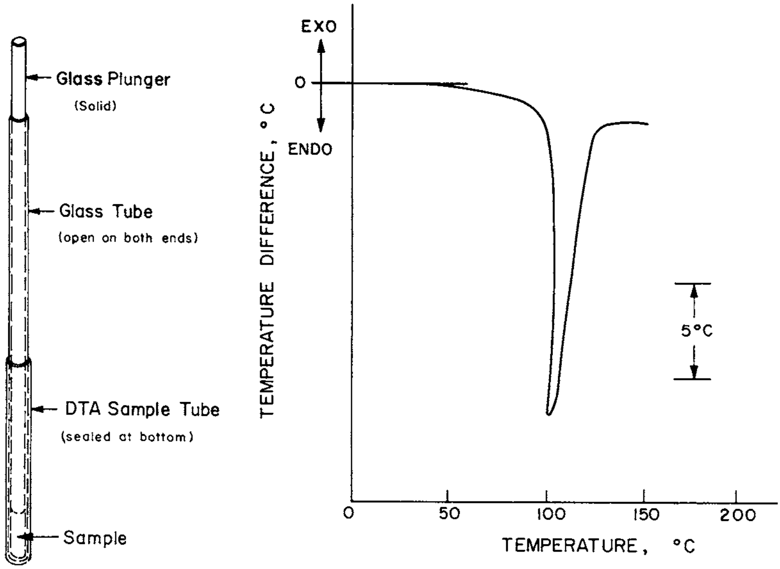

Fig. 1. Glass tube and plunger assembly for handling dough samples, and a typical boiling thermogram of a dough containing 46.2% moisture (exo indicates a release of heat and endo indicates an absorption of heat).

different mixing strength differed considerably, although the samples were given identical mixing treatment and contained the same amount of water. Red River 68, the strongest-mixing cultivar, had the largest peak area (0.44 cm^2/mg); Manitou, the medium strength cultivar, had an intermediate peak area (0.40 cm^2/mg); and Talbot, the weakest cultivar, had the smallest peak area (0.33 cm^2/mg). These results suggest that strong-mixing flours bind water more strongly than weaker-mixing flours. The actual properties of the flour and its constituents that determine the energy of water binding in dough remain to be established.

Effect of Water Content

Doughs of all three cultivars showed an increase in endotherm peak area with moisture content (Fig. 2) over the range investigated (35–52%). This can be explained by the assumption that water in dough uncovers binding sites that might not be accessible at a lower moisture content.

Over the entire range of moisture content investigated, Red River 68 (the strongest cultivar) showed a larger endotherm area compared with the areas for the two weaker cultivars. These results agree with the findings of the constant water content experiment (above) that showed water is bound more strongly in doughs of stronger-mixing cultivars than in those of weaker cultivars.

Effect of Flour Protein Content

The effect of protein content was examined for one cultivar, Manitou, for which grain samples of different protein content were available, and for all three cultivars by adding commercial vital wheat gluten (73% protein) at different levels to increase protein content.

For both the natural and gluten-supplemented flours, the peak area was inversely related to protein content (Fig. 3). These results suggest that flour proteins bind water with less energy than starch and, therefore, the total binding

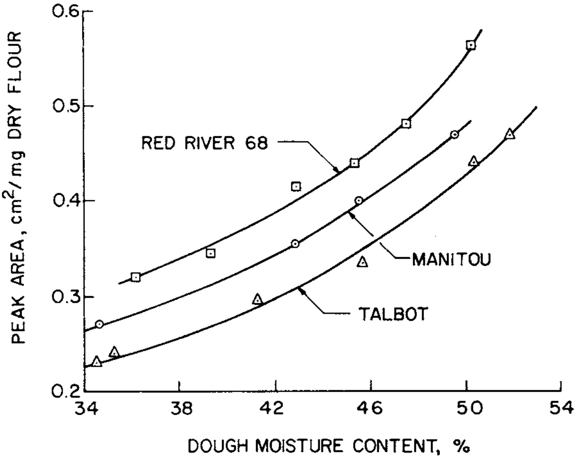

Fig. 2. Effect of dough moisture content (water absorption) on boiling endotherm peak area for flours of three wheat cultivars of different mixing strength.

energy in dough decreases with increasing protein content. This appears to be contrary to technological experience. When the flours used in this experiment were analyzed for damaged starch, it was found that the level of damaged starch (flour basis) decreased with increasing protein content. Accordingly, it would appear that the indirect relationship shown in Fig. 3 results from the decrease in the percentage of damaged starch which occurs with increasing protein content of the flour. These results imply that flour proteins bind water less strongly than damaged starch.

Effect of Starch Damage in Flour

The binding energy increased essentially linearly with increasing level of damaged starch, irrespective of the mixing strength of flour (Fig. 4). Damaged starch appears to be extremely important in the binding of water in dough as measured by DTA; this is in general agreement with technological experience.

Effect of WS Pentosans

The flours of the three cultivars used in the DTA study were analyzed for WS pentosans. The purpose of these analyses was to find out if differences in water-binding energy of the three flours could be related to their WS pentosans content, since the pentosan component of flour is considered to play an important role in the absorption of water by flour (8). The analyses showed that Red River 68 flour had 0.67%, Manitou 0.60%, and Talbot 0.51% WS pentosans. These results are, in general, consistent with those of Neukom *et al.* (10), who reported that the WS

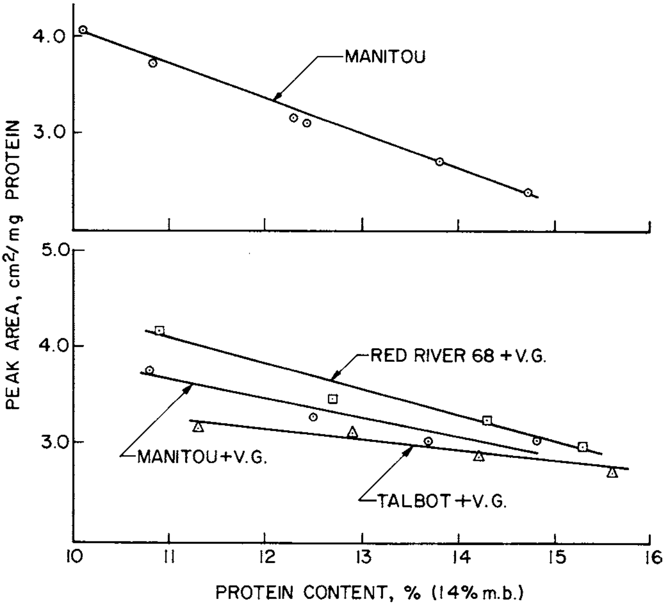

Fig. 3. Effect of protein content on boiling thermogram peak area: flours milled from Manitou wheat samples of different protein content (top), and flours of three wheat cultivars supplemented with vital gluten (V.G., bottom).

pentosans of wheat flours range between 0.5 and 0.8%.

Although the three cultivars did not differ markedly in pentosans content, the trend parallels the trend of the boiling endotherm peak areas. The cultivar that had the highest peak area also had highest WS pentosans content.

The effect of WS pentosans was examined further by incorporating varying amounts of WS pentosans into doughs from flours of the strongest and weakest cultivars (Red River 68 and Talbot). The amount of pentosans available was sufficient only for experiments with two of the three flours used in this study. Farinograph mixing results for both varieties showed that dough consistency (at

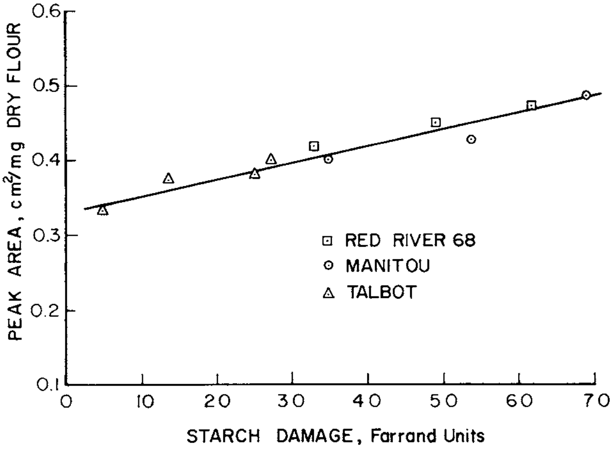

Fig. 4. Effect of damaged starch content on boiling thermogram peak area for flours of three wheat cultivars.

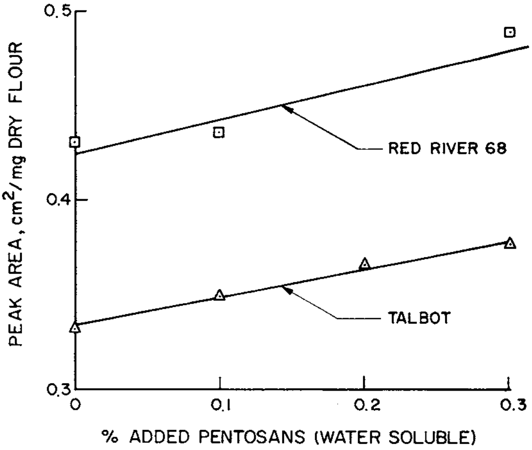

Fig. 5. Effect of added water-soluble pentosans on boiling thermogram peak area for flours of two wheat cultivars.

constant absorption) increased markedly with increasing amounts of pentosans added. At the higher consistencies, longer development times and greater stabilities (compared with the control doughs) were obtained. This effect of WS pentosans on farinograph properties has been reported by Jelaca and Hlynka (11).

The DTA boiling curve peak area increased essentially linearly with the amount of pentosan added (Fig. 5). The rate of increase for the two cultivars investigated was about the same. The stronger cultivar retained the initial advantage over the weaker cultivar at all levels of added pentosan. These results show that pentosans not only increase the water-binding capacity of flour in dough (as is known from studies by other techniques) but they also increase the binding energy.

Effect of Mixing Time

This experiment was included to investigate the hypothesis that mixing breakdown (overmixing) of dough produces an increase in the amount of free water (water of mobility) in dough (5).

The effect of mixing in the farinograph on the endotherm peak area is shown in Fig. 6. The results for the two stronger cultivars were as anticipated; the peak area curve followed the mixing curve, indicating that the energy of water binding at first increases with mixing time but later decreases with further mixing. The peak area-mixing time curve for these cultivars is analogous to the farinograph consistency-mixing time curve. In the case of Talbot, the weakest cultivar, the peak area decreased sharply from the beginning of mixing, later leveled off, and then showed a tendency to increase. There is no obvious explanation for this

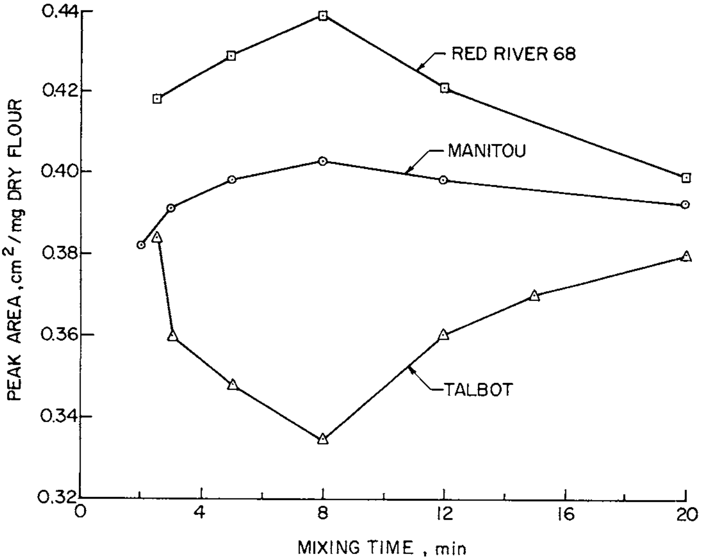

Fig. 6. Effect of dough-mixing time on boiling thermogram peak area for flours of three wheat cultivars.

behavior except that it may be related to the extremely low level of damaged starch in this flour. This point requires further investigation.

The results for the two stronger cultivars are generally consistent with the published work of Webb *et al.* (4), who showed (with the extensigraph) that the input of mechanical energy into dough by mixing decreased the resistance to extension and presumably decreased the binding of water, and thereby increased dough mobility. The actual relation between free and bound water in dough and its extensigraph properties has not been investigated.

Effect of Added Chemicals

Certain chemicals such as NEMI (a sulfhydryl (SH) blocking agent), L-cysteine (S-S reducing agent), potassium iodate (-SH oxidizing agent), ascorbic acid (shows both oxidizing and reducing actions in dough), and sodium chloride have pronounced effects on dough consistency during mixing. Accordingly, it was of interest to find out if these effects involve the binding of water in a way that could be detected by DTA.

Results for the three flours (Table I) showed a considerable drop (relative to the salt-free dough) in boiling curve peak area on the addition of 2% salt, indicating that salt decreases either the energy of water binding or the amount of bound water in dough. The effect was more pronounced in the dough of the stronger flours than in that of the weaker flour. The decreases in peak area for Talbot, Manitou, and Red River 68 were 8.5, 10.1, and 12.7%, respectively. It appears that the magnitude of the effect of added salt in doughs is strongly dependent on cultivar (*i.e.*, mixing characteristics). These results agree with the general knowledge that salt weakens the binding of water by polyelectrolytes (*e.g.*, proteins). This would lead to an increase in the proportion of free water in dough.

The addition of NEMI produced a small decrease in the endotherm peak area: 0.9, 3.2, and 3.2% for Talbot, Manitou, and Red River 68 doughs, respectively (Table I). This decrease in water binding in dough during mixing in the presence of NEMI can be attributed to the depolymerization of glutenin (12), with a concomitant decrease in water-binding capacity of this dough component.

Addition of cysteine produced the most extensive dough breakdown in the farinograph (results not shown) of the -SH and S-S related chemicals used in the present study. The decreases in the endotherm peak areas relative to untreated control were 3.1, 3.6, and 6.9% for Talbot, Manitou, and Red River 68, respectively.

TABLE I
Effect of Added Chemicals on Boiling Thermogram Peak Area of Doughs of Flours of Three Wheat Varieties

	% Decrease (−) or Increase (+)		
	Talbot	**Manitou**	**Red River 68**
NaCl	−8.5	−10.1	−12.7
NEMI	−0.9	− 3.2	− 3.2
Cysteine	−3.1	− 3.6	− 6.9
Iodate	−4.0	− 5.1	− 5.5
Ascorbic acid	+9.7	+ 7.2	+ 7.4

The observed decreases in peak area were greater with cysteine than with NEMI, on an equivalent concentration basis. However, this is not surprising since cysteine acts directly on S-S bonds, whereas NEMI reacts first with -SH and the excess NEMI is involved indirectly in the disruption of S-S bonds (12).

Doughs from the two stronger cultivars showed a higher decrease in peak area (greater decrease in energy of water binding) on addition of cysteine, suggesting that the proteins in stronger flours are more highly cross-linked (by disulfide bonds). Confirmation of this suggestion must await further results on the secondary and tertiary structures of gluten proteins and cultivar differences in these structures.

Addition of potassium iodate produced a 4–5% decrease in peak area in doughs of the three varieties (Table I). The effect of iodate (in the presence of atmospheric oxygen) on mixing and water-binding properties of dough is similar to that of NEMI and can be explained on the basis of oxidative cleavage of S-S bonds of glutenin (12).

Ascorbic acid is extensively used as a bread improver, especially in "no time" dough methods. It has certain advantages over other bread improvers such as potassium bromate and potassium iodate. Zentner (13) observed that doughs containing ascorbic acid are generally softer but feel drier to the touch than doughs treated with other dough improvers. Because of these differences between the effects of ascorbic acid and oxidizing improvers (*e.g.*, potassium iodate), it was of interest to compare the effects of these chemicals on water binding in dough as measured by DTA.

In contrast to the effects of other chemicals studied, the addition of ascorbic acid increased the DTA peak area for all three cultivars (Table I). The increase was slightly greater for the weakest flour than for the two stronger flours.

Relative to the difference in the effects of ascorbic acid and iodate (or NEMI) in dough, Zentner (13) showed that ascorbic acid does not reduce S-S bonds in flour proteins, nor does it block -SH groups. The present study suggests that it opens up some additional sites for binding of water in dough. The nature of these sites remains to be investigated.

The foregoing results on added chemicals (except ascorbic acid) on DTA water binding show that the magnitude of the effect of these chemicals depends on the mixing strength of the flour (wheat cultivar). In doughs of the weakest cultivar, the effects of the additives were generally slightly less than in the doughs of the two stronger cultivars. These findings are, in general, consistent with the hypothesis that there are qualitative differences in the proteins of different wheat cultivars that are partially responsible for differences in breadmaking quality.

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