

[Received June 2, 1988. Revision received November 29, 1988. Accepted December 1, 1988.]

## Effects of Neutral Salts of the Lyotropic Series on the Physical Dough Properties of a Canadian Red Spring Wheat Flour

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### ABSTRACT

Cereal Chem. 66(3):144-148

The effects of increasing concentrations of simple sodium salts of the lyotropic series ( $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{ClO}_4^-$ ,  $\text{I}^-$ ,  $\text{SCN}^-$ ) on the farinograph, extensigraph, and alveograph properties of a Canadian red spring wheat flour were investigated. Additions of low levels of salt (0.05–0.10M) increased dough strength properties compared to results obtained with pure water. These effects were more pronounced in the presence of the more chaotropic anions,  $\text{SCN}^-$  and  $\text{I}^-$ . At higher concentrations (0.5–1.0M), the presence of chaotropic anions reduced dough strength properties and increased farinograph water absorption, whereas nonchaotropic anions ( $\text{Cl}^-$ ,  $\text{Br}^-$ ) increased dough strength properties and had little effect upon absorption. Increasing the concentration of chaotropic anions decreased dough

extensibility whereas nonchaotropic anions increased dough extensibility. The general dough-strengthening effect at low salt concentrations compared to water can be attributed mainly to stronger interprotein interactions that result from electrostatic shielding of charged amino acids on the surface of the gluten proteins. The much larger effect of the chaotropic anions may be related to their ability to induce conformational changes in the gluten proteins that could further enhance interprotein interactions. At higher salt concentrations, the reduction in dough strength properties by chaotropic anions and the increase in dough strength properties by nonchaotropic anions can be attributed to their effects upon water structure that alter interprotein hydrophobic interactions.

The unique viscoelastic properties of wheat flour doughs that allow the production of leavened breads are closely associated with the properties of their gluten proteins. During mixing these proteins are hydrated and developed into a continuous matrix that entraps other flour components and imparts the required viscoelastic and related properties to dough that allow the retention of gas.

Several forces are believed to play important roles in the formation of the gluten protein matrix. The role of disulfide bonds is well known (Ewart 1985). In addition, ionic bonding, hydrogen bonding, hydrophobic interactions, and Van der Waals interactions are believed to make important contributions (Pomeranz 1971). However, one of the major problems facing cereal chemists in understanding the relative importance of these forces has been the lack of techniques of adequate specificity.

Studies indicate that hydrophobic interactions play an important role in determining the overall structure of gluten proteins and, consequently, varietal quality (Greene and Kasarda 1971, Caldwell 1979, Chung and Pomeranz 1979, Popineau and Pineau 1987, Kobrehel 1980, Huebner and Wall 1980). In our laboratory, we have been using simple neutral salts of the lyotropic series to specifically study hydrophobic interactions in wheat proteins. This presumption of specificity is based upon theoretical studies indicating that, at ionic strength levels sufficient to minimize electrostatic interactions, changes in protein properties due to variations in concentration and anion type can be attributed to hydrophobic interactions (Von Hippel and Schleich 1969, Melander and Horvath 1977). In previous studies (Preston 1981, 1984b) the extractability, turbidity, and gel filtration profiles of gluten proteins in simple neutral salts of the lyotropic series were found to be very sensitive to anion type and concentration. These results indicate that gluten proteins vary widely in their ability to undergo hydrophobic interactions. Preliminary studies presented by the author at the Second International Workshop on Gluten Proteins

(Preston 1984a) suggested that hydrophobic interactions were important in determining the physical properties of wheat flour doughs. Recent studies by Kinsella and Hale (1984), involving the effects of 1.0M neutral salts on farinograph properties have also shown the potential contribution of hydrophobic interactions to wheat flour dough properties. In the present paper, the effects of increasing concentrations of neutral salts of the lyotropic series on the farinograph, extensigraph, and alveograph properties of a hard red spring wheat flour were studied in relation to their effects upon electrostatic and hydrophobic interactions.

### MATERIALS AND METHODS

#### Materials

Sodium salts of the various anions (certified grade) were purchased from Fisher Scientific Co. (Fairlawn, NJ) and dissolved in distilled water at the desired concentrations. Straight-grade flours were prepared by milling a sample of no. 1 Canada western red spring wheat on the GRL pilot mill as previously described (Black 1980). The flour had a protein content of 12.7% (Kjeldahl N  $\times$  5.7), ash content of 0.39%, and a starch damage value of 31 Farrand units.

#### Physical Dough Properties

Farinograph and extensigraph properties were obtained using methods similar to AACC standard methods (1983) as outlined by Preston et al (1982). For each test, sufficient salt solution was added to obtain a peak centered on the 500 Brabender unit line. Alveograph properties were determined using the standard ICC method (1980). For all alveograph tests, salt solution was added to give an absorption of 50% (15% flour moisture basis). All tests were run in duplicate on different days and results are reported as averages of the duplicates.

### RESULTS

The effects of increasing concentrations of  $\text{Na}^+$  salts of the lyotropic series ( $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{ClO}_4^-$ ,  $\text{I}^-$ , and  $\text{SCN}^-$ ) upon the farinograph properties of the Canada western red spring (CWRS) wheat flour are shown in Figure 1. At low salt concentrations (0.05 and

<sup>1</sup>Paper no. 628 of the Grain Research Laboratory, Canadian Grain Commission, 1404-303 Main St., Winnipeg, MB R3C 3G8.

0.10M), absorption values at the 500 Brabender unit line were lower than those obtained with pure water. Further increases in sodium chloride concentration resulted in further decreases in water absorption. This effect has been shown previously by Hlynka (1962). However, increases in absorption were obtained with the other salts. The extent of these increases followed the lyotropic series with the chaotropic (structure-disrupting) thiocyanate and iodide ions showing the largest effects. At 1.0M concentrations these salts resulted in flour absorption values 9.4% higher than that obtained with water and 12.7% higher than that obtained with 1.0M sodium chloride.

As expected from previous studies (Hlynka 1962, Moore and Herman 1942), increasing concentrations of sodium chloride increased farinograph dough development time (Fig. 1). Addition of low levels (0.05M) of sodium bromide and sodium chlorate also increased dough development time compared to pure water. Higher levels had little further effect. In contrast, increasing levels of sodium thiocyanate and sodium iodide resulted in very large increases in dough development time at low concentrations (up to 0.10M) followed by very large decreases in dough development time at higher concentrations. At 1.0M concentration, dough development times with these chaotropic salts were much lower (by 6 min) than obtained with sodium chloride and considerably lower (by 2 min) than obtained with sodium bromide and sodium chlorate.

Increasing concentrations of sodium bromide and sodium chlorate had little effect on farinograph mixing tolerance index (MTI) values (Fig. 1). The effect of sodium chloride was similar to bromide and chlorate except at higher concentrations where a reduction in MTI was apparent. Increasing concentrations of the chaotropic salts (sodium thiocyanate and sodium iodide) resulted in increasing values of MTI indicating a reduction in the tolerance of the dough to overmixing.

The effects of salt type and concentration on the extensigraph properties of the red spring wheat flour are shown in Figure 2. At low concentrations all salts increased extensigraph maximum height compared with pure water. The effects of sodium thiocyanate and sodium iodide were much more pronounced than the effects of the other salts. At higher salt concentrations, sodium chloride caused large increases in maximum height as has been shown in previous studies (Fisher et al 1949). Minimal change occurred with sodium chlorate and sodium bromide. In contrast, large reductions in maximum height occurred with sodium thiocyanate and sodium iodide although values at 1.0M salt concentrations were still higher than obtained with pure water.

Increasing the concentration of the chaotropic salts resulted in decreasing extensigraph length values (Fig. 2). At low concentrations the nonchaotropic salts (sodium chloride and sodium bromide) increased extensigraph length, with small reductions occurring at higher concentrations. With sodium chlorate, low concentrations caused increases in curve length and at higher concentrations large decreases occurred.

At low concentrations all the salts tested caused increases in extensigraph area compared to the value obtained with pure water. The more chaotropic salts had the most pronounced effects (Fig. 2). In agreement with previous studies (Fisher et al 1949), higher concentrations of sodium chloride resulted in large increases in area. Little change in area occurred with higher concentrations of sodium bromide whereas reductions in area were evident with sodium chlorate, sodium iodide, and sodium thiocyanate in order of their chaotropic properties ( $SCN^- > I^- > ClO_4^-$ ).

Alveograph results, obtained at constant absorption, are shown in Figure 3. Alveograph maximum pressure increased with increasing concentrations of the more chaotropic salts ( $SCN^-$ ,  $I^-$ ,  $ClO_4^-$ ). The effects were most pronounced at higher concentrations. At concentrations above 0.50M sodium thiocyanate, the nature of the dough was such that it could not form the required bubble. The presence of nonchaotropic salts caused an initial decrease in pressure followed by small increases with increasing concentration. The effect of sodium bromide was greater than that of sodium chloride.

At low salt concentrations, alveograph curve length (Fig. 3)

generally showed increased values compared with pure water. At higher salt concentrations the effect on length followed the lyotropic series with the chaotropic salts reducing length and the nonchaotropic salts increasing length. The effects of salt type and concentration on alveograph *G* values were similar to those obtained with length (data not shown).

Low concentrations of chaotropic salts caused large increases in alveograph work (*W*) values compared with results obtained using pure water (Fig. 3). Changes at higher salt concentrations were less evident. Low concentrations of nonchaotropic salts had little effect on alveograph *W* values but high concentrations resulted in large increases.

## DISCUSSION

Gluten protein are primarily responsible for determining the physical properties of wheat flour doughs (Bloksma 1978). For most protein systems, changes occurring in their structure and

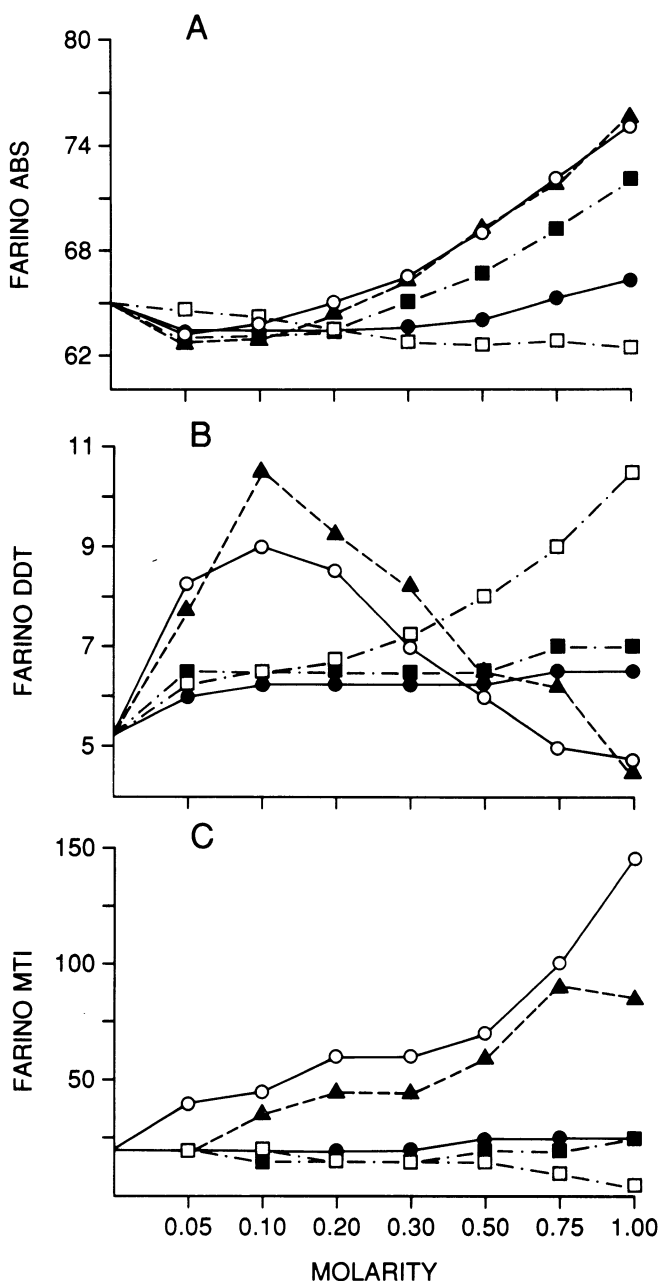


Fig. 1. Effects of increasing concentrations of neutral salts of the lyotropic series on farinograph (A) absorption, (B) dough development time, and (C) mixing tolerance index. NaCl (□—□), NaBr (●—●), NaClO<sub>4</sub> (■—■), NaI (○—○), NaSCN (▲—▲).

properties due to increasing concentrations of neutral salts can be attributed to effects upon electrostatic and hydrophobic interactions (Von Hippel and Schleich 1969, Dandliker and de Saussure 1971, Melander and Horvath 1977, Eagland 1978). At low salt concentrations ( $<0.15M$ ) these effects are primarily due to the large changes in electrostatic free energy associated with the ionic shielding of charged amino acids that reside on the protein surface. These changes in electrostatic free energy can strongly affect interprotein interactions resulting in "salting in" or "salting out" of hydrated proteins. The magnitude of this effect is dependent upon the ionic strength and the charge density on the surface of the protein and is normally independent of ion type.

At higher salt levels, where interactions between charged amino acids are minimized due to ionic shielding, the effects upon protein structure and properties are normally dependent upon salt type and concentration. Increasing concentrations of neutral salts result in an electrostatic "salting-in" effect that is dependent upon the salt concentration and the dipole moment of the protein (the protein acts as a neutral dipole at high salt concentration). A "salting-out"

effect also occurs that is associated with the effects of specific ions on water structure (i.e., the free energy of entropy is altered when apolar amino acids are exposed to an aqueous environment.) The latter factor is believed to be the basis for the effects of the lyotropic (Hofmeister) series of neutral salts. Thus, increasing concentrations of chaotropic anions such as  $SCN^-$  and  $I^-$ , which tend to reduce the free energy of entropy associated with the exposure of apolar residues, can reduce interprotein hydrophobic interactions and, in addition, disrupt the compact native structure of most proteins, which favors unfolding. In contrast, increasing concentrations of nonchaotropic anions such as  $Cl^-$  increase the free energy of entropy associated with the exposure of apolar residues and thus increase inter- and intraprotein hydrophobic interactions and generally tend to stabilize native protein structure.

The results from the present study showed that the effects of neutral monovalent salts of the lyotropic series on the physical dough properties of the hard red spring wheat flour were quite different at low and high salt concentrations. The addition of low concentrations (0.05 and 0.10M) of the various salts generally

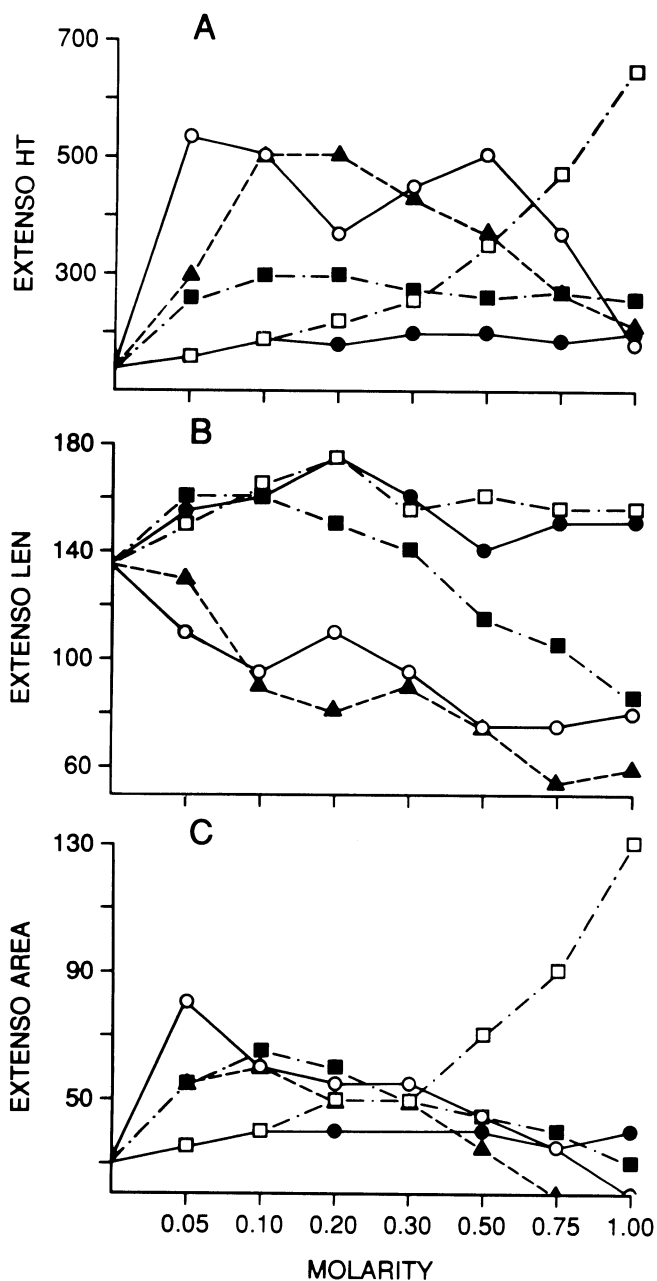


Fig. 2. Effects of increasing concentrations of neutral salts of the lyotropic series on extensigraph (A) maximum height, (B) length, and (C) area. NaCl (□—□), NaBr (●—●), NaClO<sub>4</sub> (■—■), NaI (○—○), NaSCN (▲—▲).

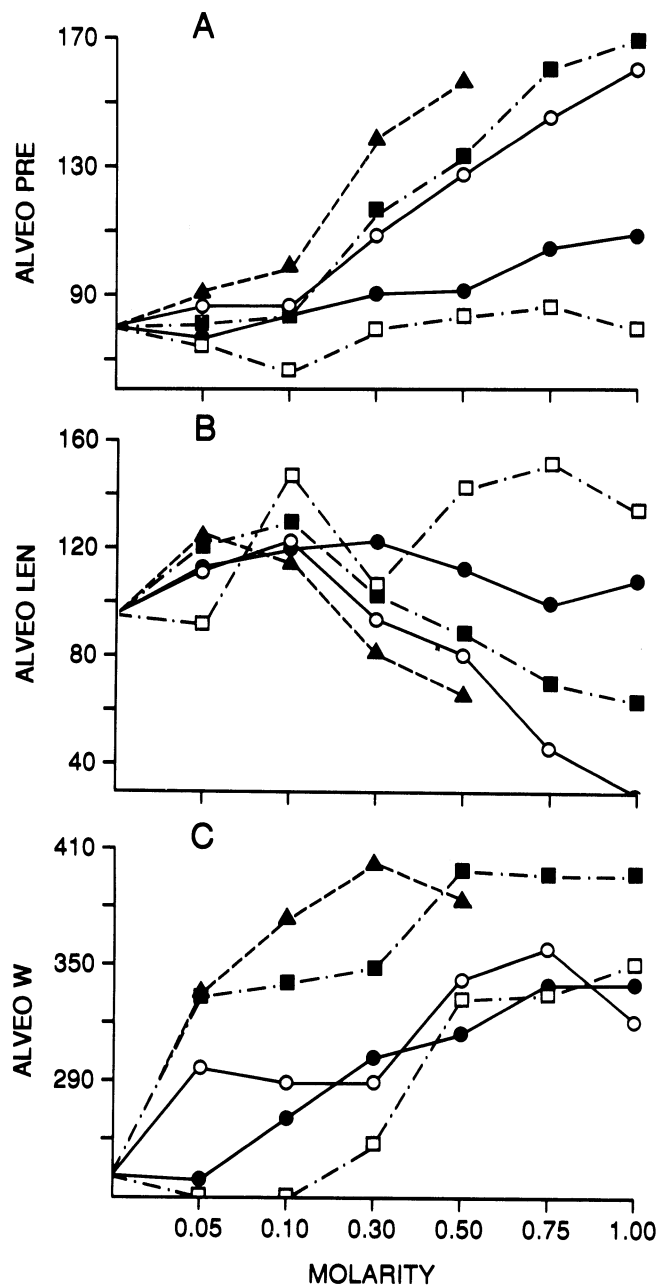


Fig. 3. Effect of increasing concentrations of neutral salts of the lyotropic series on alveograph (A) pressure, (B) length, and (C) work. NaCl (□—□), NaBr (●—●), NaClO<sub>4</sub> (■—■), NaI (○—○), NaSCN (▲—▲).

resulted in a decrease in farinograph absorption and an increase in dough strength properties compared to that obtained in pure water. In addition, specific anion effects were evident. Chaotropic (structure disrupting) anions such as  $\text{SCN}^-$  and  $\Gamma^-$  had larger positive effects upon dough strength properties than nonchaotropic anions such as  $\text{Cl}^-$ . At higher salt concentrations (0.5–1.0*M*), the effects upon absorption were strongly dependent upon anion type and generally followed their lyotropic (Hoffmeister) order. Higher concentrations of chaotropic anions increased absorption and decreased dough strength properties while higher concentrations of nonchaotropic anions had the opposite effect.

#### Effects at Low Salt Concentrations

The general tendency of neutral salts to strengthen dough properties and reduce absorption at low concentrations can be attributed to electrostatic shielding of ionic amino acids on the surface of the gluten proteins. This shielding effect would reduce electrostatic repulsions between individual gluten proteins, which normally have an excess of positively charged basic amino acids (Yoshino and Matsumoto 1966), and thus induce stronger interprotein hydrophobic and hydrophilic interactions resulting in increased aggregation (Bernardin 1978; Preston 1981, 1984b). Increased aggregation would increase the mixing time required to reduce these aggregates to obtain optimum (fully developed) dough properties. The increased interprotein interactions in the developed doughs would also result in higher extensigraph resistance, larger extensigraph area, and increased alveograph *W* values. Water absorption would be reduced due to increased competition for binding sites. These interpretations are consistent with previous studies that have shown that gluten proteins, and in particular glutenin, from strong flours interact more strongly through hydrophobic interaction (Caldwell 1979, Chung and Pomeranz 1979, Huebner and Wall 1980, Kobrehel 1980, Preston 1981, 1984b) and possess more strongly aggregated glutenin than weaker flours (Mecham et al 1965, Tsen 1969, Orth et al 1976).

In the present study the extent of dough strengthening due to low concentrations (0.05, 0.10*M*) of neutral salts was also dependent upon anion type. Therefore, the observed effects can't be attributed solely to ionic shielding of charged amino acids. This is not surprising in view of the fact that gluten proteins have low percentages of charged amino acids that can be neutralized with very low concentrations of salt (Yoshino and Matsumoto 1966, Bernardin 1978). However, the dough-strengthening effects of the anions were opposite to what was expected on theoretical grounds. Chaotropic or structure-disrupting anions ( $\text{SCN}^-$ ,  $\Gamma^-$ ) strengthened dough properties to a much greater extent than did the nonchaotropic anion,  $\text{Cl}^-$ , resulting in longer farinograph dough development times, higher extensigraph resistance and areas, and larger alveograph *P* and *W* values.

It may be possible to explain this effect on the basis of the relative effects of the anions on inter- and intraprotein interactions. At low salt concentrations, the relative effects of the anions of the lyotropic series on interprotein hydrophobic interactions would be much less evident than at higher salt concentrations. This has been demonstrated in studies of the properties of gluten proteins in solutions of neutral salt of the lyotropic series where it was shown that the extractability of these proteins at low salt concentrations was much less dependent on anion type than at higher concentrations (Preston 1981, 1984b). However, low concentrations of the chaotropic anions may be sufficient to cause conformational changes in these proteins. Normally these changes would involve the transfer of apolar residues from the interior of the protein to the surface (Von Hippel and Schleich 1969; Melander and Horvath 1977). The resulting decrease in the compactness of the gluten proteins would increase interprotein interactions and aggregation (i.e., increase the number and size of sites for hydrophobic, hydrogen, etc. interactions) and thus increase dough strength properties.

This hypothesis requires confirmation by techniques such as optical rotary dispersion that can measure conformation changes. Even so, it is interesting to note that previous studies involving the

effects of salt and pH on physical dough properties (Tanaka et al 1967, Galal et al 1978) and the effects of pH on the binding of 2-*p*-toluidinylnaphthalene-6-sulfate (TNS) to  $\alpha$ -gliadin (Greene and Kasarda 1971) were explained on the basis of conformation changes. In addition, results from our laboratory (Preston 1981), based upon turbidity measurements of gluten proteins extracted into 0.05*M* acetic acid, indicated that addition of low concentrations (0.05–0.2*M*) of chaotropic anions increased aggregation compared to nonchaotropic anions. These results are consistent with the effect of the anions on dough strength properties and with the hypothesis.

#### Effects at Higher Salt Concentrations

At higher salt levels (>0.2*M*), increasing concentrations of anions had effects upon dough strength properties and farinograph absorption values that closely followed their lyotropic properties. Increasing concentrations of chaotropic anions resulted in decreases in farinograph dough development time, extensigraph area, and extensigraph height and increases in farinograph absorption. Increasing concentrations of nonchaotropic salts had opposite effects. The largest difference in dough strength properties and farinograph absorption were evident at the highest anion concentration (1.0*M*). It should be noted that the effects of the anions on alveograph properties cannot be easily interpreted due to the sensitivity of this technique to changes in the water absorption potential of the doughs (Farrand 1964, Chen and D'Appollonia 1985, Preston et al 1987).

The lyotropic anion effects showed a similar order for farinograph mixing tolerance index and extensigraph length compared with dough strength properties. In the former case, the presence of chaotropic anions increased dough breakdown after peak development compared with nonchaotropic anions. In the latter case the chaotropic anions appeared to affect the cohesiveness of the dough producing a claylike texture that reduced extensibility. Previous studies with neutral salts of the lyotropic series showed similar effects on farinograph properties. Kinsella and Hale (1984) found that 1.0*M* chaotropic anions ( $\text{SCN}^-$ ) increased peak consistency at constant water absorption (increased water absorption at constant peak consistency), decreased dough development time, and increased the rate of dough breakdown after peak development, whereas nonchaotropic anions ( $\text{Cl}^-$ ,  $\text{Br}^-$ ) had opposite effects. Other salt concentrations were not tested.

The decrease in dough strength properties and dough extensibility with increasing concentrations of chaotropic anions above 0.2*M* can probably be attributed to decreases in interprotein hydrophobic interactions through their effects upon water structure. These decreases would reduce the tendency of the proteins to aggregate as shown previously (Preston 1981) and reduce elasticity and cohesiveness. Water absorption would also be increased due to the increased number of binding sites available. In contrast, increasing concentrations of nonchaotropic anions would increase interprotein hydrophobic interactions and lead to the opposite effects. However at salt concentrations up to 0.5*M* the dough strength properties in the presence of the chaotropic anions were still greater than the dough strength properties in the presence of nonchaotropic anions. As noted earlier, this may be attributable to intraprotein conformational changes induced by low concentrations of chaotropic anions. Above 0.5*M*, the weakening effect due to the reduction of interprotein interactions associated with changes in water structure appears to be sufficient to overcome the increased interprotein interactions resulting from conformational changes.

Overall, the large differences in the effects of the anions upon the various parameters studied suggest that hydrophobic and electrostatic interactions play a very important role in determining the physical dough properties of wheat flours. However, it should be noted that explanations involving only the gluten proteins put forward in this and previous studies (Kinsella and Hale 1984; Preston 1981, 1984b) are probably overly simplistic because interactions of the proteins with lipids and other flour components have not been considered.

## ACKNOWLEDGMENTS

The author would like to thank P. Lee and L. Minty for their technical assistance.

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[Received June 23, 1988. Revision received October 1, 1988. Accepted December 6, 1988.]