EFFECT OF NEUTRAL SALTS ON DOUGH AND BREAD

by

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INTRODUCTION

Work by Holmes and Hoseney (1987a) showed that chemical leaveners in frozen dough bread did not provide any positive benefits despite the additional source of carbon dioxide. The reasons for the lack of benefit were thought to be the result of the effect of specific ions and high total salt concentration on gas retention and production. The purpose of this study was to examine and better understand the effect of salts on dough and the resultant bread.

REVIEW OF LITERATURE

General effects on proteins

Hofmeister (1888) was reported to have been the first to quantitatively study the effects of neutral salts on proteins (Guy et al 1967 and Eagland 1975). He found, as reported by Guy et al (1967), that their effects were more than simply related to valency and charge density of the ions and that the specific ions present play a large role regarding the influence on a proteins solubility. The work of Hofmeister and others, as reported by von Hippel and Schleich (1969), have shown that ions can be generally related according to their effect on protein stability (Figure 1). As the stability of a molecule increases, its solubility decreases.

Electrostatic and hydrophobic interactions may be two of the most important interactions which affect the solubility of proteins. Theories suggest that low salt concentrations should increase proteins solubility largely because of electrostatic effects which can be accounted for by the Debye-Hückel theory (Arakawa and Timasheff, 1985). Kirkwood (1943) proposed that proteins may have a dipolar nature because of the presence of positively and negatively charged amino acid side chains. The effect of ions would then be related to the dipolar properties of the protein. Solubility of a protein were related to an activity coefficients which is derived from such properties as ionic strength, dielectric

Figure 1. Typical Hofmeister (lyotropic) series for anions and cations as determined for collagen-gelatin (von Hippel and Schleich 1969). Ions listed to the left promote protein stability or salting-out. Ions listed to the right promote protein instability or salting-in.

Anionic series

$SO_4^{2-} < CH_3COO^- < Cl^- < Br^- < NO_3^- < ClO_4^- < I^- < CNS^-$

Precipitating Solubilizing

Cationic series

$NH_4^+ < K^+ < Na^+ < Li^+ < Mg^{2+} < Ca^{2+}$

Precipitating Solubilizing

constant of the medium, and dipole moment of the dipolar ion. This is the only electrostatic theory which can account for the wide variation of the ability of different salts to salt-out proteins at high salt concentrations (Arakawa and Timasheff 1985).

As salt concentration increases, ions have an increasingly significant effect on hydrophobic interactions which play an important role in protein-protein interactions (Kauzmann 1959). At high salt concentrations the effect of the specific ion becomes predominant regarding the extent of the effect on the protein structure and solubility. Various ions either enhance or diminish hydrophobic interactions, thus causing salting-out or salting-in of the protein. The effect of the salt on the hydrophobic interaction is believed to be related to its effect on the structure of the water (von Hippel and Schleich 1969). Those salts that enhance the structure of water are regarded as nonchaotropic and promote hydrophobicity. Salts that diminish the structure of water are considered chaotropic and do not encourage protein association, and therefore promote solubility (Kinsella and Hale 1984).

Sínanoglu and Abdulnur (1964, 1965) proposed the cavity theory that relates hydrophobic interactions to the formation of cavities in the solvent by the solute. These interactions are affected by the surface tension of the solvent. Melander and Horváth (1977) utilized this theory together with electrostatic effects to interpret the effect of the salts on the precipitation of proteins. They concluded that molal surface tension increments quantify the effect of salts on hydrophobic interactions. A problem exists with the cavity theory and other theories based on it. Salts such as calcium chloride, magnesium chloride, and barium chloride cause a large increase in the surface tension of water and therefore should encourage insolubility of proteins, but these salts are known not to decrease protein solubility (Arakawa and Timasheff 1985). Also, this theory fails to explain the action of polyethylene glycol which is one of the strongest protein precipitating agents, but decreases water surface tension (Arakawa and Timasheff 1985).

The preferential interactions of salts with proteins has also been proposed to explain their effects on protein stability and solubility (Arakawa and Timasheff, 1982). These preferential interactions of the salt with the protein include the binding of the salt with the protein or as "the perturbation of the chemical potential of the protein by the co-solvent, salt" (Arakawa and Timasheff 1985). The preferential interactions of proteins and salts were proposed to come about from two factors: 1) The effects of the salt on the surface tension of water which contributes to an unfavorable change in free energy or salting-out. As the surface tension of water is increased, salt interactions with proteins decrease. 2) The salt binding to peptide bonds or certain side chains which contributes to a favorable change in free energy or salting-in (Arakawa and Timasheff 1984). Increases in free energy changes increase protein solubility and vice versa.

Based on this theory, as proteins are preferentially hydrated, they salt-out. Salts such a sodium chloride and sodium sulfate have a negative preferential interaction parameter, thus the protein is preferentially hydrated and salts-out (Arakawa and Timasheff 1982).

Salts having a preferential interaction parameter that is very small (becomes closer to zero) tend to bind to proteins. As salts bind to proteins, the net charge of the protein is increased to produce a repulsive force. The stability of the protein is then reduced. The electrostatic repulsive force prevents protein association and thus increases solubility (Arakawa and Timasheff 1982).

Recently a theory of thermodynamic linkages (Kumosinski 1988) has been used to explain the effect of salts on protein solubility that could not be explained by such theories as those proposed by Melander and Horváth (1977). In this theory, the solubility of proteins is linked to the free energy of salt binding. Protein solubility profiles are described using binding constants and solubilities. This theory was used to explain the decrease in protein solubility found for soy isolates in solutions of low salt concentrations (Shen 1981). Other theories, as discussed previously, indicate that protein solubility should decrease at low salt concentration based on the electrostatic principles employed.

Effect on solubility of wheat proteins

The effects of salts on the solubility of wheat proteins was first investigated by Gortner et al (1929). They found that one salt, when compared to another, may solubilize different amounts of protein from the same flour, and that the concentration of the salt was an important determinant of the amount of protein solubilized. Also, different flours varied in the amount of protein solubilized by the same salt solution. This raised the question of exactly what salt and what concentration should be used to determine the salt soluble proteins in flour. Answering this question was important in order to compare results of research related to the solubility of wheat protein.

More recently Preston (1981) used neutral salts to study the hydrophobic interactions of wheat proteins, and thus, their solubility. His findings showed that proteins decreased in solubility for all salts at low concentrations. The explanation given was that at the low salt concentration, the ions shielded the charged groups on the protein. The shielding allowed the proteins to associate, and thus salt-out. As salt concentrations increased, the solubility was either further decreased or increased depending on the salt used. The effect on wheat protein solubility of the salts at higher concentrations followed the lyotropic series.

Based on the results of the study, Preston (1981) described two characteristics related to the solubility of the proteins that make up wheat proteins: First, the acid-soluble proteins from wheat "have a strong inherent tendency to undergo interprotein hydrophobic bonding". This may be largely accounted for by the presence of high levels of apolar amino acids (Wu and Dimler 1963a & b) and the low proportion of ionic amino acids. The large number of uncharged residues creates large portions of the protein that are available for hydrophobic interactions. As ions shield the relatively few charged residues, there would be a reduction in repulsion by charged groups. The hydrophobic surfaces might then have a greater tendency to associate. Other attractive forces such as hydrogen bonding may also cause aggregation of the protein and, thus, decrease solubility. The increase in solubility that is found generally for other proteins, because of an electrostatic effect, may be largely overcome because of the relatively small number of charged groups in gluten protein, and those mostly having a positive charge. Second, gluten proteins appear to have a great variation in their surface hydrophobicities, and thus, the strength of interprotein interactions have the following order from strong to weak: glutenins, gliadins, albumins. The differences may be related to such factors as proportion of surface apolar groups, size and shape of proteins, and location and extent of disulfide bonding.

The character of the wheat proteins evaluated by Preston (1981) is similar to the soy isolate as examined by Shen (1981) in terms of the decrease in solubility at low salt concentrations. Therefore, it may be possible to use the theory proposed by Kumosinski (1988), which accounted for this decrease in solubility in terms of thermodynamic linkage.

Work has shown that the solubility of wheat protein is also affected by the presence of additives such as nonfat dried milk (NFDM). Lai et al (unpublished) found that the amount of soluble nitrogen in a dough of flour and NFDM was less than that in a flour dough, even though there was more total nitrogen present from both the flour and NFDM. A component of NFDM, such as the protein, may be interacting with the flour protein thus causing the proteins to be less soluble.

Effect on doughs

The effect of sodium chloride on dough rheology has been studied by many researchers (Skovholt and Bailey 1932, Moore and Herman 1942, Hlynka 1962, Fortmann et al. 1969, Danno and Hoseney 1982, and Guy 1985). In general, sodium chloride has been found to reduce optimum absorption, increase the optimum mixing time, and improve the mixing tolerance of the dough.

Skovholt and Bailey (1932) examined the effects of NFDM on breadmaking. The optimum absorption was found to increase with the addition of NFDM. This increase almost doubled when sodium chloride was also included in the formula. This suggests an interaction between the salt and NFDM.

Jankiewicz and Pomeranz (1965) reported that 0.01 M sodium pyrophosphate slightly widened the farinograph curve and significantly reduced the resistance to extension and total area under the curve in the extensigraph. Addition of 0.63 M magnesium sulfate to a fully developed dough containing the pyrophosphate buffer caused little observed change to the farinograph curve characteristics, other than its widening. Possibly, if the dough had been mixed for a longer time after the addition of the magnesium sulfate, some effects may have been observed, such as increased stability. Incorporation of magnesium sulfate at the beginning of the mixing process caused a great extension or delay in mix time. This seems to indicate that the salt does, indeed, have a large effect on the dough. This salt was used in conjunction with urea to determine if the action of urea could be reversed. Adding urea to a dough caused the dough to develop very rapidly compared to a control dough containing only the pyrophosphate buffer. The curve also showed great weakness (thinness and low stability) as mixing was continued through the development time of the control. Addition of 0.63 M magnesium sulfate after the dough was mixed to the peak resulted in the dough with the urea regaining almost normal farinograph characteristics.

If the presence of magnesium sulfate "reversed" the effect of urea, this raises questions regarding the mechanism of the effects of these substances on flour protein. Does urea affect the hydrogen bonding in flour proteins, as is often thought (Whitney and Tanford 1962)? If so, would the salt reverse the effect of urea if the salt is truly affecting hydrophobic interactions. Or, does urea actually affect hydrophobic interactions and not hydrogen bonding as suggested by Whitney and Tanford (1962)? Possibly, the salts affect hydrogen bonding rather than hydrophobic interaction. Urea and salts may be involved in different interactions which affect the mixing characteristics of the dough in opposite directions, and thus, negate each others effect. Finally, urea and/or salts may affect both hydrogen bonding and hydrophobic interactions.

Bennett and Ewart (1965) examined the effect of sodium chloride, sodium lactate, and other salts on the extensigraph characteristics of wheat flour doughs. One of their findings was that anions were a more important factor than cations with regard to the attributes examined. They suggested this may be due to the anions being more preferentially absorbed. The more significant effect by anions compared to cations was also found to be true by Guy et al (1967) in their farinograph study of the effect of neutral salts on flour and NFDM doughs. Bennett and Ewart (1965) proposed that the effect of ions was largely due to their direct interaction with the protein rather than the influence of the ions on the water structure, particularly at high salt concentrations, as has been proposed by later researchers (von Hippel and Schleich 1969, Melander and Horváth 1977, Preston 1981, Kinsella and Hale 1984,). The theory of preferential interactions by Arakawa and Timasheff (1982) does, in part, relate the effect of salts on protein solubility directly to interaction of the salt with the protein, but this is thought to affect the hydrophobic associations of the protein. The effect of the salt on water structure is also an integral part of the theory.

Bennett and Ewart (1965) found that as the proportion of salt soluble proteins increased, as in low protein flours, the breaking stress increased. They explained the increase in breaking stress of dough in the presence of ions as a result of increases in intermolecular forces such as salt links, ion-dipole bonds, polar interactions, hydrogen bonds, and Van der Waals forces. The presence of salts increased inter-chain bonding, and thus increased breaking stress. The reasoning of the effect of organic salts on dough by Bennett and Ewart (1965) does not propose hydrophobic interactions to be a major part of protein-protein interaction. The only suggestion of hydrophobic interactions was that between mandelate ions and the protein. They concluded that this type of hydrophobic effect would be small due to "interference with the close alignment of peptide chains as they extend under stress". The finding that salts had a greater effect on low protein flours may relate, at least in part, to the findings of Gortner et al. (1929). Gortner (1929) showed that a specific salt will affect differently the amount of soluble protein found in flours of various protein quality and quantity.

Guy et al (1967) examined the effect of salts of the lyotropic series on the rheology of flour and NFDM doughs using the farinograph. They noted that cations as compared to anions had a less marked effect on extensigraph characteristics, but were still significant, notably in terms of the effect on peak times and stability. Guy et al (1967) surmised that sites having a positive charge were primarily responsible for maintaining the structure favorable to water-binding because of the anions more obvious effect. An explanation of the effect of solubilizing neutral salts on the flour proteins was proposed to be related to increased binding of least-hydrated (solubilizing) ions which then promote introduction of water into the protein system because of osmotic forces. Cations were also found to influence farinograph properties. These workers suggested that the effect of the cations may be influenced more by valency than position in the lyotropic series.

Guy et al (1967) submitted that the changes in the rheological properties of a dough, as it relates to a colloid system, may be because of three factors singly or in combination, "an increase in hydration of suspended particles, an increase in asymmetry of the particles present, or particle size growth by interaction". As they suggested, the study of salts from the lyotropic series may provide a better understanding of the general effect of salts on these properties than is currently held. It may also provide some answers or insight with regard to how proteins affect rheological properties of a dough by better understanding of matters such as hydrophobic interactions.

Salovaara (1982a) found that partial replacement of sodium chloride by other salts affected the rheological properties of doughs as measured by the farinograph, extensigraph, and rotational viscometer. The discussion of the effects of the salts as a replacement was usually with reference to systems containing sodium chloride. This could be somewhat misleading. A salt, such as calcium chloride, was said to "reduce" the arrival time, peak time, or stability of a farinograph curve. These reductions were relative to doughs containing sodium chloride. In fact, the salt caused a longer arrival time, longer peak time, or increased stability relative to a dough with no salts. This shows that the salts had an effect on the dough, but possibly not to the same extent or in a different manner than the sodium chloride. The extensigraph and bake tests did not even include a control without a salt for comparison. In general, the tests showed that the salt effect on the rheological properties of dough paralleled the lyotropic series, i.e., as the salt caused greater protein solubility, the mix time or resistance to extension were reduced. Salovaara (1982a) did find that the effects of these salts on baking performance was not as clear as their effect on the rheological properties of the doughs.

Kinsella and Hale (1984) performed a study on the effects of neutral salt solutions on dough rheology as measured by the farinograph. Overall, their study reported that as the salt became more chaotropic, the mix time and stability were decreased while the consistency at peak development was increased. It was pointed out that the increase in dough consistency coincided with the increase in glutenin protein solubility (Preston 1981, 1984), as well as the decrease in water structure by the same ions (Hatefi and Hanstein, 1964).

Holmes and Hoseney (1987b) showed that various sodium salts affected the mixing properties (mix time and curve width) of dough as measured by the mixograph. It was reported that, on an equivalent mole basis, the amount of each sodium salt required to produce a given curve was as follows:

Chloride > Sulfate > Phosphate > Citrate

These results follow the lyotropic series, which relates salts according to their effect on the solubility of proteins. Of the salts tested, chloride is a more solubilizing salt and citrate a more precipitating one.

The most recent work concerning the effect of salts on dough was reported by Preston (1989). The effect of salts was evaluated with the farinograph, extensigraph, and alveograph. The comparisons were made with the sodium salts of anions from the lyotropic series. The findings showed that at low levels, all anions caused increases in both dough development time and absorption. These effects were found to be more pronounced for the chaotropic ions and were thought to be because of intra-protein conformational changes. Similar toughening effects were observed on the extensigraph as shown by a higher curve and a larger area. Doughs containing chaotropic ions gave shorter curve lengths than a dough without salt. In other words, the low levels of chaotropic salts seem to increases resistance to extension and total area under the curve (more strength value), but decreased total extension to a greater extent than nonchaotropic salts.

As the salt levels were increased, the effect of specific ion became more predominant. The results were supportive of those from previously mentioned workers. The relative effect of the ions followed the lyotropic series. As the ion was more nonchaotropic, the farinograph curve showed an increase in dough development and mixing tolerance, but a decrease in absorption. As the ion became more chaotropic, there was a decrease in mix time and mixing tolerance and an increase in optimum absorption.

The effect of salts at higher levels on the extensigraph showed the chaotropic ions caused a lower maximum height, area, and length values. Nonchaotropic ions generally caused an increase in maximum height and area, but small decreases in length.

Effect on resultant bread

Work with neutral salts on bread has been limited except for sodium chloride (Finney 1984 and Guy 1985) which is a basic bread ingredient. The work that has been conducted with other salts was often done with an interest in partial replacement of sodium chloride (Salovaara 1982, Stroh et al 1985, and Guy 1986). Studies have been reported on total replacement of sodium chloride by potassium chloride (Anon. 1984). The impetus for these studies has been to reduce sodium intake.

Holmes and Hoseney (1987b) removed the NFDM from the frozen dough bread formula to reduce the amount of oxidant required. It was found in bake tests, without NFDM in the bread formulation, that the optimum level of sodium chloride decreased from 1.5% to 0.5%. The work showed that the presence of NFDM had a negative effect which was, at least to certain extent, reversed by the addition of sodium chloride. This may support the findings of other workers that showed an apparent interaction between flour, NFDM, and sodium chloride (Skovholt and Bailey 1932, Lai et al unpublished).

Holmes and Hoseney (1987a) studied the addition of salts with regard to the use of chemical leaveners in bread. During the course of their work, salts of the lyotropic series were baked into bread to examine the effect of specific ions. The results showed that both chaotropic and nonchaotropic salts, as with sodium chloride, had optimum levels, at least in terms of their effect on loaf volume. These salts were both chaotropic and nonchaotropic. Overall, sodium chloride produced bread with the largest volume.

Holmes and Hoseney (1987a) also investigated the effects of the residual salts (RS) produced by the reaction between an equivalent amount of leavening acid (sodium aluminum sulfate (SAS), sodium aluminum phosphate (SALP), or potassium aluminum phosphate) and sodium bicarbonate. All of the leavening acids tested had a neutralizing value of 100, which means equal amounts of soda and acid were required. Holmes and Hoseney (1987a) determined that SAS showed the most potential for providing a benefit in bread. They reported that an optimum existed for the RS at 1.0 g SAS with 1.0 g soda per 100 g of flour. The RS from the other leavening acids and soda did not have an optimum level, but instead showed a continual decline in loaf volume as the level of leavening acid was increased from 0.25 to 2.0%.

Guy (1985) found that some sodium chloride was necessary to produce an acceptable loaf of bread in terms of both physical and sensory characteristics. Finney (1984) reported that there is an optimum level of sodium chloride relative to the physical characteristics of bread. Holmes and Hoseney (1987b) showed that the optimum level for sodium chloride decreased to between 0 and 0.5% when nonfat dried milk was removed from the formulation.

As stated earlier, Salovaara (1982a) did not find the effect of neutral salts on baked bread to be as predictable as their effects on solubility or rheological properties. It was suggested that this may be because, at least in part, the use of optimum mix time for each dough with a different salt, thus somewhat compensating for the action of the specific salt.

De Stefanis et al (1988) reported that there was an apparent interaction between certain metallic cations and bromate. Those found to have the most pertinent effects were the vanadium ion, most notably, but also, iron (Fe^{2+}), silver, and mercury. Vanadium and iron acted as a catalyst for bromate. Copper (copper sulfate) was shown to have an oxidizing effect when used alone which is in agreement with previous reported by Jørgensen (1945).

MATERIALS AND METHODS

Materials

The flour used in this study was provided by Ross Mills, Wichita, KS. Four different shipments (A,B,C, and D) were used. The protein, ash, and moisture content are given in Table I.

The shortening used was a partially hydrogenated and contained mono- and diglycerides (Crisco, Proctor and Gamble). The yeast used was Fermipan Instant Dry Yeast (Gist-Brocades, Charlotte, NC).

The sodium and potassium bicarbonates were provided by the Church and Dwight Co., Inc., Princeton, NJ. Sodium aluminum sulfate was obtained from Allied Chemical, Morristown, NJ. Sodium iodide, sodium chloride, sodium phosphate monobasic, sodium phosphate dibasic, sodium sulfate, sodium citrate, and monocalcium phosphate were all reagent grade. Sodium aluminum phosphate and sodium acid pyrophosphate were supplied by Stauffer Chemical Co. Westport, CT. The heat treated soy flour and the commercial samples of whey and sodium caseinate were supplied by ADM Arkady, Olathe, Kansas.

Table I. Analysis of flours[1]

Flour	Protein (%)	Ash (%)	Moisture (%)
A-Oct '87	11.9	0.48	12.7
B-Sept '88	12.0	0.48	11.4
C-Feb '89	12.3	0.49	11.8
D-June '89	11.5	0.47	12.8

[1] All percentages given on an "as is" moisture basis.

Methods

Mixograph

AACC method 54-40 (AACC 1983) was used. The sodium salts of citrate, phosphate, sulfate, and chloride were added to the flour in the dry form. Sodium iodide was added both in the dry form and in solution for comparison. Baking powder residual salts were added as a dispersion. Baking powders and other salts were added in the dry form.

Determination of pH

A 10 g sample of dough was placed into a blender jar containing 100 ml of distilled water. Two drops of octanol was added to prevent foaming. The mixture was blended for one minute on high speed. The dispersion was continually stirred during the pH measurement. The pH was determined with a calibrated Corning Model 125 pH meter. The reading was recorded when the value stabilized.

Preparation of chemical leavening residual salts

Neutralizing amounts of sodium bicarbonate and acid salts were combined in water. The amount of acid needed was found by the following equation:

$$\text{Acid (g)} = \frac{\text{Soda}}{\text{Neutralizing Value}} \times 100$$

The acid and soda were then boiled in excess water until all carbon dioxide was evolved, approximately 20 min. The residual salts were then pipetted into the doughs to deliver a given amount of residual salt based upon the concentration of the suspension. For a suspension containing residual salts at 0.05 g SAS per ml and 0.05 g soda per ml, 20 ml of the suspension would be added to 100 g flour to obtain 1% SAS and 1% soda (or 2% baking powder) based on flour weight.

Straight dough test bake procedure

Control formula- See Table II.

Test formula- As individual salts were evaluated, sodium chloride was removed from the formula unless otherwise stated. Removal of NFDM was also as indicated. Ingredient levels expressed as percentages throughout the remainder of this paper refers to percentages on a flour weight basis unless otherwise stated.

The straight dough bake test procedure followed was described by Finney (1984). The doughs were mixed to optimum (minimum mobility) in a National Special 100 Gram Pin Mixer (TMCO-National Mfg. Co., Lincoln, NE). The dough was fermented in a proof cabinet (TMCO-National Mfg. Co., Lincoln, NE) at 30°C and 90-95% RH for 180 min. During the fermentation the dough was sheeted at a gap of 3/16" after 105 and 155 min. At the end of the fermentation the dough was

Table II. Control bread formula

Ingredient	% (flour weight basis)
Flour (14% m.b.)	100.0
Nonfat dry milk (NFDM)	4.0
Shortening	3.0
Salt (NaCl)	1.5
Sucrose	6.0
Potassium bromate	optimum
Water	optimum
Yeast (Instant)	0.76

sheeted at a gap of 5/16" and then molded in a drum molder (Thomson Co., Beltsville, NJ). The dough piece was panned (dimensions = top-77 mm x 142 mm, bottom-62 mm x 126, depth-57 mm), then proofed at 30°C and 90-95% RH for 55 min. The proofed dough was baked at 218°C for 24 min. Immediately upon removal from the 12-1 pound electric reel oven (TMCO-National Mfg. Co., Lincoln, NE), the loaf was weighed and the volume was taken by rape seed displacement.

Short time test bake procedure

The same procedure was followed as in the straight dough except the fermentation time was reduced to 15 min. Punches were done at 3/16" after five and 10 min. The final punch (5/16") and panning were done after 15 min fermentation.

NFDM fractionation

NFDM was fractionated by two methods, isoelectric precipitation and dialysis. Isoelectric precipitation was done by reducing the pH of reconstituted NFDM with HCl to 4.6, precipitating the casein from the whey. After separation of the two fractions, the pH of each was returned to normal by the addition of NaOH. Dialysis was performed using a membrane. The fraction obtained were classified as the large dialyzed (LD) or retentate and small dialyzed (SD) fractions. The recovered fractions from each process were then lyophilized. Isoelectric precipitation produced 60% casein and 40% whey. The LD fraction was found to be 70% of the NFDM and the SD was 30%. These fractions were added separately to the bread formula based on the levels that they were found in the NFDM. For example, in the trial with casein, 2.4 g of the casein was used (60% of 4 grams of NFDM as used in the control bread formula).

Gas production

The Gasograph (D & S Instruments Ltd., Pullman, WA) was used to measure gas production. A 10 g (14% m.b.) flour sample was placed in the sample jars. Other ingredients (salt, sucrose, and NFDM) were added at the appropriate levels and dry blended with the flour. Just prior to connection to the Gasograph, instant dry yeast was added and mixed with the other dry ingredients. Fifteen ml of distilled water was added and the mixture was thoroughly blended with a glass stirring rod and then stoppered. The bottle was then placed into the water bath and the channel tube was attached. The gas production was continuously recorded on a strip chart during fermentation. The slurries were fermented for three or four hours.

Soluble nitrogen

Distilled water (100 ml) was added to a 20 g sample of flour (and appropriate salt level). The bottle was shaken by hand 50 times to blend the dry ingredients and water. An additional 100 ml of distilled water was added and the bottle was shaken 10 times. The capped bottle was placed on a wrist action shaker (Burrell Corp. Pittsburgh, PA) for one hour on high speed. The resultant mixture was centrifuged for 15 min at 2200 rpm. The supernatant was filtered through glass wool with suction. Fifty ml of the resultant filtrate was analyzed for nitrogen by the Kjeldahl method.

Statistical analysis

Analysis of variance with *t* tests (LSD) using the Statistical Analysis System (SAS Institute 1985) was used for evaluation of the data.

RESULTS AND DISCUSSION

Mixographs of sodium salts

Various sodium salts were evaluated in the current study at equal percentages based on flour weight. The results showed sulfate to produce a curve with the longest mix time, followed closely by citrate, and then by phosphate and chloride, which were similar. Sodium phosphate monobasic ($NaH_2PO_4 \cdot H_2O$) was used in this test. When converted to an equivalent mole basis the relation would be as follows:

Chloride > Phosphate > Sulfate > Citrate

It was not clear whether monobasic (F.W.=137.99) or dibasic (F.W.=141.96) sodium phosphate was used by Holmes and Hoseney (1987b). Therefore, a determination of the effect on pH was made with each of the phosphate salts. The pH of a dough containing 1.5% of the monobasic monohydrate salt immediately after mixing was 5.82. When the dibasic anhydrous salt was used at the same percent, the pH was 7.19. A control dough containing 1.5% sodium chloride had a pH of 6.21. The pH difference between the monobasic and dibasic salts could cause a change in the mixing properties of the dough. Hoseney and Brown (1983) showed that decreases in pH by lactic acid and increases in pH by sodium bicarbonate strongly affected the mixograph curve. In their study, they showed a change in pH from 5.58 to 7.58 caused a noticeable increase in mix time and width of curve.

Sodium iodide at low levels increased mix time and curve width (Table III). However, as concentration was increased those attributes were maximized and then began to decrease. This coincides with the salting-out effect of the protein at low chaotropic salt concentrations followed by salting-in at higher concentrations as found by Preston (1981). At 4 M, the absorption was increased to 140% The time to peak was reduced to 1 min and the peak height increased to 9.0 cm. The curve was narrow throughout. At high levels the curve produced depended upon whether the salt was added in solution or in dry form. When added as a solution, the increase in absorption to 140% was required and the narrow curve resulted. In dry form, at a salt level equal to the 4 M solution and with the absorption reduced to the level of the control, the resulting curve was very wide with a high peak as compared to the control. The salt may not be completely solubilized when added in this manner due to a lack of available water because of competition from other ingredients such as flour or NFDM. This would cause a different concentration of the salt, and thus, may affect the rheology of the dough. When the salt is added in solution, more protein may be solubilized which may cause the differences in the curves. Similar results were found by Sanchez et al (1973) with NaCl in that addition of

Table III. Effect of sodium iodide on mixograph time to peak and peak height[1]

Treatment[2]	Time to Peak (min)	Peak Height (cm)
Control-No Salt	3.48 B	6.6 C
0.05 M	4.23 A	7.0 BC
0.10 M	4.20 A	7.2 B
0.30 M	3.55 B	7.2 B
0.40 M	3.35 BC	7.2 B
0.50 M	2.93 CD	6.8 BC
1.00 M	2.50 D	5.9 D
4.00 M	1.23 E	9.0 A

[1] Values followed by different letters indicate statistically significant differences at the 5% level. Two replications for each treatments.

[2] 59% absorption for all variations except 4.00 M at 140%

the salt in a solution affected the mixing properties differently than adding the salt in the dry form.

Bake tests with sodium salts

Bake tests with various sodium salts showed that each salt gave an improving effect on loaf volume. The level producing the largest loaf volume for each salt was not the same. The optimum level for sodium chloride has been shown to be 1.5% (Finney 1984). In this study, the optimum level for sodium sulfate was found to be 0.75% (Table IV), and the optimum for sodium phosphate monobasic was 1.5% or less (Table V). Also, the maximum loaf volume obtained for each salt varied. At the optimum levels, sodium chloride provided the largest volume.

To determine the effect on loaf volume of a salt from the solubilizing end of the lyotropic series, sodium iodide was evaluated in a bread formulation. This salt was baked with two different flours, flour B and C. The results from the first test (Table VI) with flour B showed sodium iodide to produce bread with a slightly larger volume than sodium chloride, both salts at their optimum levels. The optimum for sodium iodide was also broader than that for sodium chloride; relatively constant from 0.5% to 1.5%. When baked without nonfat dried milk (NFDM), the optimum level of sodium iodide decreased to between 0.0 to 0.5% salt. This is similar to the optimum of sodium chloride with no NFDM. The test with

Table IV. Effect of sodium sulfate on loaf volume[1]

Treatment	Loaf Volume (cc)
Control-1.5% NaCl	895[2] A
No Salt	803[3] D
0.25% sodium sulfate	847[3] B
0.50% sodium sulfate	860[3] B
0.75% sodium sulfate	875[3] A
1.50% sodium sulfate	828[3] C

[1] Values followed by different letters indicate statistically significant differences at the 5% level.

[2] Average of 6 observations

[3] Average of 3 observations

Table V. Effect of sodium phosphate on loaf volume[1]

Treatment	Loaf Volume (cc)
Control-1.5% NaCl	896[2] A
No Salt	803[3] D
0.50% sodium phosphate	833[3] BC
0.75% sodium phosphate	850[3] B
1.00% sodium phosphate	850[3] B
1.50% sodium phosphate	842[4] BC
2.00% sodium phosphate	828[4] C

[1] Values followed by different letters indicate statistically significant differences at the 5% level.

[2] Average of 6 observations

[3] Average of 3 observations

[4] Average of 2 observations

Table VI. Effect of sodium iodide on loaf volume, flour B[1]

Treatment	Loaf Volume (cc)
Control-1.5% NaCl	884[2] A
No salt	848[3] B
0.5% sodium iodide	915[4] A
1.0% sodium iodide	902[4] A
1.5% sodium iodide	910[4] A

[1] Values followed by different letters indicate statistically significant differences at the 5% level.

[2] Average of 5 observations

[3] Average of 3 observations

[4] Average of 2 observations

flour C did not show sodium iodide to have as great an improving effect nor to have as broad of an optimum (Table VII) as previously found. A more definite optimum was found at 1.5% sodium iodide. At this optimum, the loaf volume was similar to that found with sodium chloride rather than larger as was found in the previous test. The fact that different flours were used in each test may account for some of the discrepancy between tests. There may have been variations in protein quality between the flours. Salts may cause different effects on flours of different qualities. This had been shown to be true in terms of peptization of proteins by Gortner et al (1929).

The internal characteristics of the loaves in the second bake test of sodium iodide appeared over oxidized at all levels. This was shown by thick cell walls and a slightly darker core in the center of the crumb. The appearance of over oxidation became more evident as the level of sodium iodide was increased. Externally, the appearance of over oxidation was indicated by less pan flow, i.e. rounded edges of the loaf and a more apparent seam. De Stefanis (1988) showed that certain metallic cations interacted with oxidants (potassium bromate) or caused oxidative effects on their own in bread. The sodium ion was not found to be one of these cations. Possibly, anions such as iodide may interact with the oxidant present in a manner similar to cations as shown by De Stefanis (1988).

Table VII. Effect of sodium iodide on loaf volume, flour C[1]

Treatment	Loaf Volume (cc)
Control	987[2] A
No salt	953[3] B
0.25% sodium iodide	955[3] B
1.00% sodium iodide	963[3] B
1.50% sodium iodide	980[4] A
2.00% sodium iodide	960[3] B

1 Values followed by different letters indicate statistically significant differences at the 5% level.

2 Average of 6 observations

3 Average of 3 observations

4 Average of 2 observations

Milk fractions

To better understand how the NFDM may be interacting with the salt as shown in bread by Holmes and Hoseney (1987b), the isoelectric precipitated and dialyzed NFDM fractions were evaluated in bread. The fractions were tested with sodium chloride levels at 0.75 and 1.5% based on flour. At 0.75% sodium chloride (Table VIII), the bread containing the LD fraction had a large decrease in loaf volume relative to the control loaf (810 vs. 972 cc) which contained 1.5% sodium chloride and no NFDM (Table VIII). The loaf with the SD fraction also had a decrease in loaf volume (907 vs. 972 cc). The casein fraction caused a decline in volume to 865 cc which was greater than the SD, but less than the LD. The whey caused a slight depression in volume to 940 cc.

The higher level of sodium chloride, 1.5% was examined with each fraction. With the LD fraction, a significant volume increase (810 to 880 cc) was obtained. There was no significant change in volume for the casein and SD fraction as the sodium chloride level was increased from 0.75 to 1.5%, nor for the bread with whey.

A more thorough examination of the LD fraction using several sodium chloride levels provided a better picture of the improving effect of this salt (Table IX). The bread without salt, NFDM, or the LD fraction had a lower volume than the control (873 vs. 927 cc). The lower volume may come about because of an absence of a nitrogen supplement for the yeast

Table VIII. Effect of NFDM fractions on loaf volume[1]

	Loaf volume (cc)	
Treatment	0.75 % NaCl	1.5 % NaCl
No NFDM	---	958[3] A
LD	810[2] E	865[2] D
SD	907[2] BCD	893[2] CD
Casein	865[2] D	873[2] CD
Whey	940[2] AB	920[2] ABC

1 Values followed by different letters indicate statistically significant differences at the 5% level.

2 Average of 2 observations

3 Average of 5 observations

Table IX. Effect of NaCl and LD fraction on loaf volume[1]

Treatment	Loaf Volume(cc)
Control	933[2] A
0.0% NFDM/0.0% NaCl	873[3] B
2.8% LD/0.00% NaCl	710[4] D
2.8% LD/0.75% NaCl	820[4] C
2.8% LD/1.50% NaCl	860[4] B
2.8% LD/2.25% NaCl	865[4] B

1 Values followed by different letters indicate statistically significant differences at the 5% level.

2 Average of 6 observations

3 Average of 3 observations

4 Average of 2 observations

in this treatment. NFDM provides a nitrogen source (Swortfiguer 1962) in the control formula and the LD fraction may also do the same when it was present. Relative to the control with NFDM, the LD fraction caused a large decline in volume (927 to 710 cc) when no salt was present. At 1.5 and 2.25% sodium chloride the volume recovered from 710 to 860 and 865 cc, respectively. The salt was shown to provide partial, but not complete, recovery of the deleterious effect of the LD milk fraction at the levels tested.

Commercially produced samples of sodium caseinate and whey were included in the baking trials (Table X) to compare their effects to those of the laboratory prepared fractions. When no salt was used in the bread formulation, the sodium caseinate produced a large decline in volume (645 cc) relative to the formula containing NFDM (815 cc) and no sodium chloride. The use of whey in a formula without salt resulted in a volume (980 cc) which was considerably greater than that of the control. Sodium chloride at the 1.5% level gave a volume increase for the bread with sodium caseinate (645 to 773 cc). The control with 4% NFDM also had a volume increase (815 to 868 cc). But, the additional salt resulted in a large decline in volume for the bread with whey (980 to 815 cc). These results indicate that when salt is not present, the negative affect of NFDM comes largely from the casein fraction and that the salts are providing a degree of protection against that fraction. The whey fraction did not provide any

Table X. Effect of commercial proteins on loaf volume[1]

Treatment	Loaf Volume (cc)	
	No NaCl	1.5% NaCl
Control-NFDM	815[2] CD	869[3] B
Soy flour	820[2] C	790[4] DE
Sodium caseinate	645[2] F	773[2] E
Whey	980[2] A	815[4] CD

[1] Values followed by different letters indicate statistically significant differences at the 5% level.

[2] Average of 3 observations

[3] Average of 5 observations

[4] Average of 2 observations

benefit when salt was present at the 1.5% level. This may indicate that when whey is used, little salt is required to reach the optimum, and as suggested previously, even 0.75% may be beyond that point.

Soy flour

A heat treated soy flour was studied to determine if it interacts with salt in a manner similar to NFDM in bread (Table X). Bread containing soy flour and no salt had a volume similar to the bread with NFDM and no salt, 820 and 815 cc, respectively. Upon addition of 1.5% sodium chloride, however, the volume of the bread containing soy flour showed a slight decrease in volume to 790 cc, while that with NFDM had an increase to 868 cc. In this test, the salt at 1.5% of the flour, did not decrease or prevent any deleterious effect of soy flour, but rather increased the deleterious effect. The soy flour may interact differently or at least not to the same extent as NFDM. Possibly, a lower level of salt would be optimum for use with soy flour as compared with NFDM.

Mixographs with baking powders and their residual salts

Work with salts was continued using baking powders and their residual salts (RS). This was done because of interest expressed by Church and Dwight (Princeton, NJ) in the use of chemical leavening in frozen doughs. The comparison between the two forms was done because it was not clear if the negative effects that had been found when using baking powder was the result of the action of the salts prior to or after reaction, and if in fact a difference existed. Mixographs of baking powder and its RS showed that their effects were similar, but not identical. The baking powder had a more pronounced affect on the rheology of the dough, at least in terms of the time to peak (Figure 2). This was the found to be true when NFDM was and was not present in the formula. This may be caused by an increase in pH when baking powder is incorporated in the unreacted form or possibly due to the difference in ions present or at least to their availability to interact.

Bake tests with residual salts of baking powders

Residual salts were further investigated in bake tests to determine possible interactions or a change in the optimum RS level at different NFDM levels and if this might improve loaf volume. There was an indication that as NFDM levels increased, the amount of RS that could be included also increased. Various levels of RS of SALP and soda were examined at different NFDM levels, 0, 4, and 8% (Table XI). Contrary to the findings of Holmes and Hoseney (1987a), the present study showed optimum SALP/soda RS levels at 0, 4 and 8% NFDM. The optimum level of salt increased as the level of NFDM increased. The largest loaf volume was obtained at 0% NFDM. Even so, the loaf volume (970 cc) at the optimum RS

Figure 2. Effect of sodium chloride, NFDM, SAS baking powder, and baking powder residual salts on mixograph time to peak. Error bars represent LSD value for statistically significant differences at the 5% level.

Control	- flour
1.5% NaCl	- flour and 1.5% NaCl
4% NFDM	- flour and 4% NFDM
1.5% NaCl/4% NFDM	- flour, 1.5% NaCl, and 4% NFDM
0.5/0.5% RS	- flour and residual salts of 0.5% SAS and 0.5% soda
1.0/1.0% RS	- flour and residual salts of 1.0% SAS and 1.0% soda
1.0/1.0% SAS BP	- flour and baking powder using 1.0% SAS and 1.0% soda
1.0/1.0% RS/4% NFDM	- flour, 4% NFDM, and residual salts of 0.5% SAS and 0.5% soda
1.0/1.0% SAS BP/4% NFDM	- flour, 4% NFDM, and baking powder using 1.0% SAS and 1.0% soda
1.5/1.5% RS	- flour and residual salts of 1.5% SAS and 1.5% soda

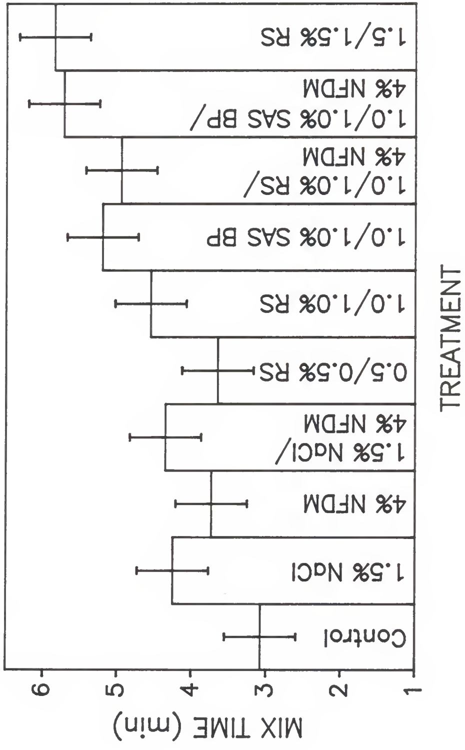

MIX TIME (min)
1
2
3
4
5
6
TREATMENT
Control
1.5% NaCl
4% NFDM
1.5% NaCl/
4% NFDM
0.5/0.5% RS
1.0/1.0% RS
1.0/1.0% SAS BP
1.0/1.0% RS/
4% NFDM
1.0/1.0% SAS BP/
4% NFDM
1.5/1.5% RS

Table XI. Effect of SALP/soda residual salts on loaf volume[1]

Treatment	Loaf Volume (cc) 0% NFDM	4% NFDM	8% NFDM
Control	---	956[2] CD	---
0.0% residual salts	958[3] CD	873[3] F	775[3] G
0.5% residual salts	1015[3] A	933[3] DE	915[3] E
1.0% residual salts	1013[3] A	973[3] BC	960[3] CD
2.0% residual salts	1000[3] AB	948[3] CD	970[4] C

[1] Values followed by different letters indicate statistically significant differences at the 5% level.

[2] Average of 6 observations

[3] Average of 2 observations

[4] 1 observation

level (2%) at 8% NFDM was similar to the loaf volume (973 cc) at the optimum RS level (1%) at 4% NFDM. This suggests the possibility that if NFDM content is increased, the addition of chemical leavener might be less deleterious to loaf volume.

There was also an indication that RS interact with or at least affect oxidation requirements. When NFDM was absent from the bread formula, a higher level of potassium bromate (20 ppm) was required for SALP RS than for SAS RS (5 ppm) (Table XII). In fact, at 5 ppm potassium bromate, the SALP RS caused a large decline (-200 cc) in volume relative to bread with no salt, but at 20 ppm the SALP caused a large increase (+130 cc) in loaf volume compared to the bread with no salt. Apparently the SALP/soda RS effects the potassium bromate requirement of dough. In these bake tests, which used a three hour fermentation, the SALP/soda RS along with the additional oxidation produced bread with as good a volume as the SAS/soda RS. This may simply be related to the higher level of potassium bromate used with the SALP/soda RS.

Bake tests with baking powders

Bake tests were also conducted with baking powders to compare their effects to those from residual salts and to determine if their actions the same as the residual salts. The unreacted sodium bicarbonate or leavening acids may affect the flour or NFDM proteins differently than did the RS. Sodium bicarbonate will cause an initial increase in pH, thus

Table XII. Effect of 5 ppm and 20 ppm potassium bromate with SALP/soda RS on loaf volume[1]

Treatment[2]	Loaf Volume (cc) 5 ppm	20 ppm
Control	---	958[3] D
No Salt	995[4] C	953[4] D
0.5% NaCl	1010[5] BC	---
1% SALP/soda RS	860[4] E	1010[4] BC
2% SALP/soda RS	853[4] E	1005[4] BC
3% SALP/soda RS	868[4] E	---
1% SAS/soda RS	1043[4] A	---
2% SAS/soda RS	1015[4] B	---

1 Control contained NFDM, other treatments did not

2 Values followed by different letters indicate statistically significant differences at the 5% level.

3 Average of 6 observations

4 Average of 2 observations

5 Average of 3 observations

affecting the rheology of the dough. Also, the carbon dioxide that is produced by the baking powders, but previously released in case of the residual salts, will contribute to the carbonic acid content of the dough and may slightly lower the pH which could affect the dough rheology or yeast activity. With interest focused on the use of chemical leaveners in frozen doughs, the tests were done at a short fermentation time (15 min) because frozen doughs generally has little or no fermentation prior to freezing. A comparison was made between SALP and glucono-delta-lactone (GDL) as leavening acids (Table XIII). GDL has a neutralizing value (NV) of 45, which required higher levels as compared to SALP, that has an NV of 100, to neutralize an equivalent amount of soda. When no NFDM was in the formulation, the GDL and soda produced bread with a larger volume than SALP and soda (980 vs. 885 cc). At 4% NFDM, the use of GDL and equivalent soda produced bread that was larger (928 cc) than the control (870 cc). Baking powder containing SALP showed no benefit, but it produced a smaller loaf volume at all NFDM levels tested, 0, 4, and 8%, and at both leavening acid levels, 0.5 and 1.0%, relative to GDL and control at the same NFDM levels.

Comparisons were made between the leavening acids SAS, GDL, and SAPP and equivalent amount of soda (Table XIV). The tests were performed with no NFDM in the formulation, except for the control. The best volume was obtained with bread containing no NFDM and no leaveners (973 cc). With leaveners

Table XIII. Effect of SALP and GDL baking powders at different NFDM levels on loaf volume using 15 minute fermentation[1]

Treatment	Loaf Volume (cc)		
	0% NFDM	4% NFDM	8% NFDM
Control	---	870[3] CD	---
0.5% SALP	885[2] BCD	758[3] E	---
1.0% SALP	853[3] D	763[3] E	763[3] E
1.1% GDL	980[3] A	895[3] BCD	---
2.2% GDL	970[3] A	928[3] ABC	940[3] AB

[1] Values followed by different letters indicate statistically significant differences at the 5% level.

[2] 1 observation

[3] Average of 2 observations

Table XIV. Effect of baking powder baking powders with GDL, SAS, and SALP on loaf volume using 15 min fermentation[1]

Treatment	Loaf Volume (cc) GDL	SAS	SAPP	Other
Control-4% NFDM and 1.5% NaCl	---	---	---	885[3] D
0.5% soda-no NFDM	940[2] ABC	958[2] AB	928[2] ABCD	---
1.0% soda- no NFDM	938[2] ABC	923[2] BCD	895[2] CD	---
0% acid salts-no NFDM	---	---	---	973[2] A

[1] Values followed by different letters indicate statistically significant differences at the 5% level.

[2] Average of 2 observations

[3] Average of 4 observations

in the formulation, the loaves containing the lower level (0.5%) of soda had better volumes than those with the higher (1.0%) level, possibly because of less negative effects from the salts. At the lower chemical leavening level, the SAS had the best volume (958 cc), with the GDL being slightly smaller (940 cc) and SAPP the smallest (928 cc). At the high level, GDL performed slightly better than the SAS as the leavening acid, 938 cc and 923 cc, respectively. Again, SAPP produced the smallest volume. The only benefit found with the baking powders was a slight, 5 to 10%, reduction in proof times.

Potassium bicarbonate was examined as a replacement for sodium bicarbonate (Table XV) at the short fermentation time. The leavening acid used was SAS. In general, there was no benefit of one salt over the other. In the presence of 4% NFDM, the potassium bicarbonate produced a slightly better volume than did the sodium bicarbonate. When NFDM was absent, however, the sodium bicarbonate produced a slightly larger volume than did the potassium bicarbonate. Bread volume was better, overall, without the chemical leaveners present. There may be a slight difference in how each salt interacts with the NFDM.

Effects of other salts

Salts containing cations other than sodium were evaluated with the mixograph, in bake tests, and by gas production (yeast). This was to determine if various cations affected

Table XV. Replacement of sodium bicarbonate with potassium bicarbonate at 0 and 4% NFDM using 15 minute fermentation[1]

Treatment	Loaf Volume[2] (cc)	
	0% NFDM	4% NFDM
No Salts	955 A	890 BC
No NaCl/0.5% $NaHCO_3$	873 BC	803 E
No NaCl/0.6% $KHCO_3$	858 CD	820 DE
1% NaCl	975 A	908 B
1% NaCl/0.5% $NaHCO_3$	888 BC	885 BC
1% NaCl/1.0% $NaHCO_3$	---	855 CD
1% NaCl/0.6% $KHCO_3$	868 BC	900 B
1% NaCl/1.2% $KHCO_3$	---	868 BC

[1] Values followed by different letters indicate statistically significant differences at the 5% level.

[2] Average of 2 observations

the bread differently. Perhaps positive divalent cations, such as calcium, form salt bridges with NFDM which has net negatively charged proteins at the pH characteristic of dough and bread. This type of interaction may affect the behavior of NFDM in a dough system, such as the NFDM proteins solubility or interaction with wheat proteins.

Mixographs

The mixograph results (Table XVI) showed that calcium chloride anhydrous did not significantly affect mix time at levels of 4% or lower. However, when this salt was present in the dough at 8%, there was a noticeable increase in mix time. Sodium chloride had little effect the low level (0.5%), but caused a significant increase in mix time at higher levels (1.5 and 4%). Lithium chloride (Table XVII) caused increased mix time, but not to the extent of sodium chloride at comparable levels. Potassium chloride caused a greater increase in mix time than did sodium chloride.

Bake Tests

Bake tests, using three hours fermentation, with calcium chloride (Table XVIII) did not show an optimum at any levels tested. Lower loaf volumes were produced at all salt levels compared to the control and the bread formulation without salt. Lithium chloride (Table XIX) resulted in even greater

Table XVI. Effect of calcium chloride and sodium chloride on mix time and peak height[1]

Salt	Mix Time (min)		Peak Height (cm)	
Control	3.50[2]	CDE	6.7[2]	D
0.5% sodium chloride	3.33[3]	C	5.8[3]	C
1.5% sodium chloride	4.25[3]	B	6.0[3]	B
4.0% sodium chloride	5.60[3]	A	6.1[3]	A
0.5% calcium chloride	3.28[4]	DE	7.0[4]	C
1.5% calcium chloride	3.28[4]	DE	7.0[4]	C
4.0% calcium chloride	3.15[4]	E	6.6[4]	D
8.0% calcium chloride	3.60[4]	CD	7.2[4]	BC

1 Values within a column followed by different letters indicate statistically significant differences at 5% level.

2 Average of 8 observations

3 Average of 4 observations

4 Average of 2 observations

Table XVII. Effect of LiCl, KCl, and NaCl at 0.005 moles on mixograph time to peak[1]

Salt	Mix Time (min)
Potassium chloride	5.35[2] A
Sodium chloride	4.88[2] A
Lithium chloride	4.20[2] B
No Salt	3.39[3] C

[1] Values followed by different letters indicate statistically significant differences at 5% level.

[2] Average of 2 observations

[3] Average of 5 observations

Table XVIII. Effect of calcium chloride on loaf volume[1]

Treatment	Loaf Volume (cc)
Control	890[2] A
No salt	835[3] B
0.5% calcium chloride	773[3] C
1.0% calcium chloride	705[3] D
1.5% calcium chloride	695[3] DE
2.0% calcium chloride	675[3] E
2.5% calcium chloride	622[3] F
3.0% calcium chloride	625[3] F
3.5% calcium chloride	610[3] FG
4.0% calcium chloride	593[3] G
5.0% calcium chloride	515[3] H

[1] Values followed by different letters indicate statistically significant differences at the 5% level.

[2] Average of 5 observations

[3] Average of 3 observations

Table XIX. Effect of lithium chloride on loaf volume[1]

Treatment	Loaf Volume (cc)
Control	904[2] A
No salt	825[3] B
0.5% lithium chloride	697[3] C
1.0% lithium chloride	622[3] D
1.5% lithium chloride	580[3] E
2.0% lithium chloride	530[3] F
2.5% lithium chloride	507[3] F
3.0% lithium chloride	463[3] G
3.5% lithium chloride	422[3] H
4.0% lithium chloride	342[3] I

[1] Values followed by different letters indicate statistically significant differences at the 5% level.

[2] Average of 5 observations

[3] Average of 3 observations

loaf volume depressions. Potassium chloride did show an optimum level with (Table XX) and without (Table XXI) NFDM. The optimum amount of potassium chloride with NFDM was slightly lower (1.0 to 1.5%) than that for sodium chloride (1.5%). Without NFDM, the optimum for potassium chloride was again at about 1.0 to 1.5%. This is in contrast to sodium chloride which shows an optimum without NFDM at 0.0 to 0.5%. The optimum level of potassium chloride gave a bread volume similar to that of the optimum level of sodium chloride when NFDM was present, and slightly larger when NFDM was not.

A bake test using calcium sulfate (0.0 to 4.0%) did not show any consistent positive or negative effects on loaf volume (Table XXII). This may be because of the low solubility of this salt.

Effect of various salts on gas production of yeast

Lithium chloride had a somewhat similar effect on the mixing characteristics of a dough as did sodium chloride. Therefore, it was thought that some other aspect which affects loaf volume might causing the deleterious effect on loaf volume that was found with sodium iodide, such as yeast gas production. Various salts were examined with regard to their effects on gas production of yeast. It was found that certain salts particularly lithium chloride, and calcium chloride, had an apparent toxic effect on yeast (Figure 3). This was supported by the negative effect that both lithium chloride

Table XX. Effect of potassium chloride with NFDM on loaf volume[1]

Treatment	Loaf Volume (cc)
Control	869[2] AB
No salt	827[3] CD
0.25% potassium chloride	842[3] BC
0.50% potassium chloride	858[3] ABC
1.00% potassium chloride	877[3] A
1.50% potassium chloride	875[4] A
2.00% potassium chloride	860[4] AB
2.50% potassium chloride	845[3] ABC
3.00% potassium chloride	838[4] BC
3.50% potassium chloride	798[3] D

[1] Values followed by different letters indicate statistically significant differences at the 5% level.

[2] Average of 5 observations

[3] Average of 3 observations

[4] Average of 2 observations

Table XXI. Effect of potassium chloride without NFDM on loaf volume[1]

Variation	Loaf Volume (cc)
Control	908[2] AB
No salt	907[3] AB
0.25% potassium chloride	882[3] B
0.50% potassium chloride	923[3] A
1.00% potassium chloride	928[3] A
1.50% potassium chloride	937[3] A
2.00% potassium chloride	915[3] AB
2.50% potassium chloride	845[3] C
3.00% potassium chloride	845[3] C
4.00% potassium chloride	820[3] C

[1] Values followed by different letters indicate statistically significant differences at the 5% level.

[2] Average of 5 observations

[3] Average of 3 observations

Table XXII. Effect of calcium sulfate on loaf volume[1]

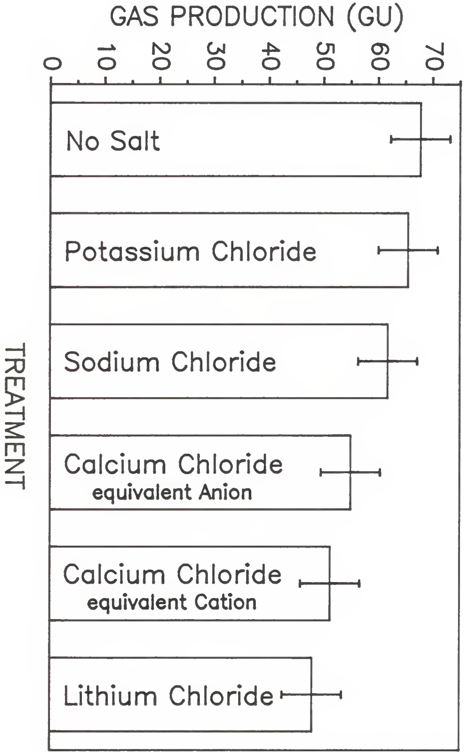
Treatment	Loaf Volume (cc)
Control	874[2] A
No salt	818[3] BCD
0.25% calcium sulfate	817[3] BCD
0.50% calcium sulfate	830[3] B
1.00% calcium sulfate	818[3] BCD
1.50% calcium sulfate	807[3] CD
2.00% calcium sulfate	823[3] BC
2.50% calcium sulfate	812[3] CD
3.00% calcium sulfate	802[3] D
4.00% calcium sulfate	805[3] D

1 Values followed by different letters indicate statistically significant differences at the 5% level.

2 Average of 6 observations

3 Average of 3 observations

Figure 3. Effect of potassium, sodium, calcium, and lithium chlorides on yeast gas production. Salts at same ionic level as 1.5% NaCl, except calcium chloride which was used at either the same anionic or cationic level as indicated. Error bars represent LSD value for statistically significant differences at the 5% level.


GAS PRODUCTION (GU)
0
10
20
30
40
50
60
70
No Salt
Potassium Chloride
Sodium Chloride
Calcium Chloride
equivalent Anion
Calcium Chloride
equivalent Cation
Lithium Chloride
TREATMENT

and calcium chloride had on loaf volume. The effect of the chloride salts on gas production was done at equal ion concentration except for calcium chloride which was done at either equal anion or cation as indicated on the figure.

Effects of salts on soluble nitrogen

Previous work (Lai and Hoseney, unpublished) showed that there was an apparent interaction between the NFDM protein and wheat proteins. This was again shown during the present work (Figure 4) by the fact that the sum of soluble nitrogen (SN) found in flour a flour slurry plus that found in a NFDM slurry was greater than the SN found when the two were combined into a single system. The effect of sodium chloride on this interaction was examined. The presence of sodium chloride did not increase the amount of SN in the individual protein sources. However, when sodium chloride was added to the combination of flour and NFDM, the SN was increased to near that of the sum of the SN of the components. The sodium chloride may shield the charges on both proteins and, thereby, stop the insolubilizing interaction between the flour and the NFDM proteins.

The effect of calcium chloride on soluble nitrogen was also studied (Figure 5). Calcium chloride did not affect the SN of NFDM. This salt was found to increase the amount of SN of the flour above that when no salt was present indicating a solubilizing effect on the flour proteins. However, the

Figure 4. Effect of sodium chloride on soluble nitrogen of NFDM, flour, and NFDM/flour slurries. NFDM was used at 4% and sodium chloride at 1.5% flour weight basis when either or both were included in a slurry. Error bars represent LSD value for statistically significant differences at the 5% level.

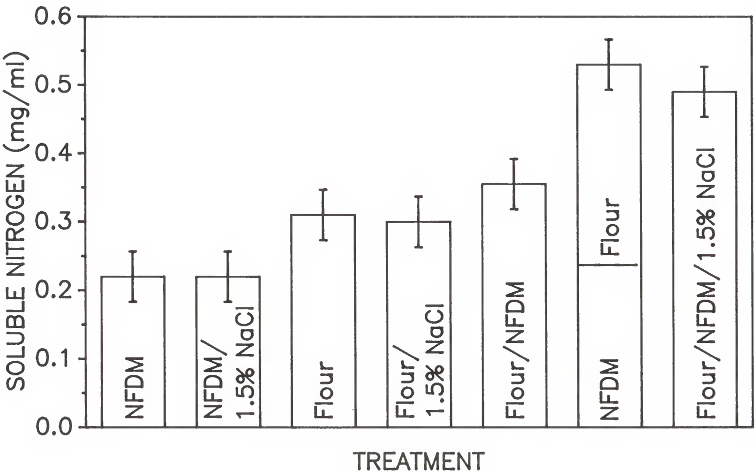
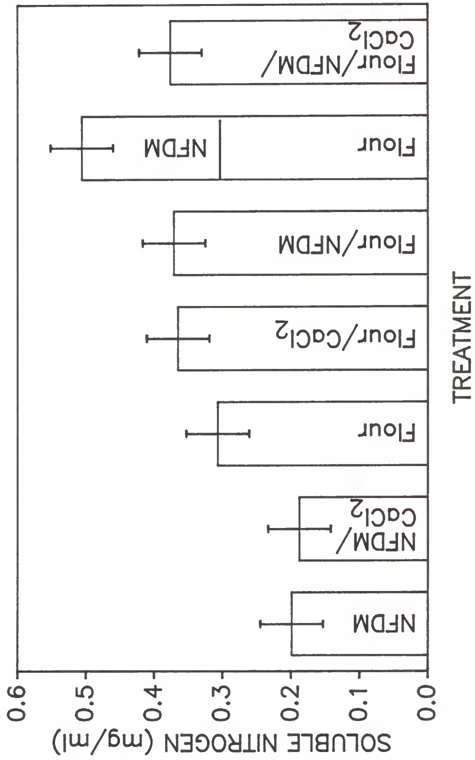

SOLUBLE NITROGEN (mg/ml)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
NFDM
NFDM/
1.5% NaCl
Flour
Flour/
1.5% NaCl
Flour/NFDM
NFDM
Flour
Flour/NFDM/1.5% NaCl
TREATMENT

Figure 5. Effect of calcium chloride on soluble of NFDM, flour, and NFDM/flour slurries. NFDM was used at 4% flour weight basis when included in the slurry. Calcium chloride was used at an equivalent anionic level of 1.5% sodium chloride when present in the slurry. Error bars represent LSD value for statistically significant differences at the 5% level.


SOLUBLE NITROGEN (mg/ml)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
TREATMENT
NFDM
NFDM/ CaCl2
Flour
Flour/CaCl2
Flour/NFDM
Flour
NFDM
Flour/NFDM/ CaCl2

use of this salt in the combination with flour and NFDM showed an increase over that without salt, but the increase found was only equal to the increase found with flour alone, and not equal to the sum of the SN of both components. Therefore, calcium chloride did not show the apparent protecting effect, at least to the same extent, as sodium chloride.

SUMMARY

This study has provided some interesting findings with regard to the effects that various salts have on dough properties and bread. The changes in mix times caused by salts appear to be related to their location in the lyotropic series. Salts from the precipitating end of the series generally produced an increase in mix time and curve width relative to dough without salts.

A solubilizing salt, such as sodium iodide, showed variable effects on the dough properties. At low salt concentrations (up to 0.1 M) mix times increased, but then declined as salt concentrations were further increased. The curve height did not follow the simple increase and decline pattern throughout the salt levels tested. Initially curve height increased at low salt concentration (up to 0.1 M) followed by a decline (up to 1 M). As the salt level was raised further (to 4 M), there was a dramatic increase in curve height. This may be related to the salt increasing the amount of soluble protein which may bind more of the available water causing a stiffer dough. Calcium chloride, a solubilizing salt, but less so than sodium iodide, did not greatly alter the mixograph characteristics at the concentration tested. The effect of salts on bread is not clear at this time, i.e., salts from both ends of the lyotropic series provided benefits in terms of loaf volume.

Sodium sulfate, sodium chloride and sodium iodide, all at various concentrations, produced better loaf volumes than when salts were not present.

The mechanism for the effect of salts on the dough and bread is not completely understood. Preston (1981) has suggested that at low concentrations, salts may be involved in electrostatic shielding of ionic amino acids on the protein surfaces. The effect of each salt would then be mainly dependent on ionic strength and charge density. He found all salts at low concentration decrease gluten protein solubility. This is contrary to the ideas presented in a review by Arakawa and Timasheff (1985) that suggest low concentrations of all salts should have a salting-in effect. Possibly the more recent theory by Kumosinski (1988) provides an alternate explanation by using thermodynamic linkage which accounts for the initial decrease in solubility.

The effects of the specific ion on protein solubility becomes increasingly predominant as the salt level is raised. Salts that decrease the solubility of proteins may do so by increasing the hydrogen bonding structure of water (von Hippel and Schleich 1969) thus increasing hydrophobic interaction between proteins which causes the greater insolubility. The salts that increase protein solubility disrupt the structure of water by interfering with hydrogen bonding causing reduced hydrophobic interactions (Kinsella and Hale 1984). These workers found that salts promoting hydrophobic bonding between protein required an increased mixing time which was supported by the results of this study.

Salts involved in chemical leavening, prior to and after reacting, may be more complex and present in a greater variety than the salts used in the previously mentioned studies. Generally, only one species of cation and anion were added to those systems. Effects of the chemical leaveners and their residual salts were, in many cases, similar to those of individual salts. For example, baking powders and their residual salts containing sodium aluminum sulfate caused an increase in mix time and loaf volume as was found with sodium sulfate. Determination of which ion or how a combination of ions affects dough and bread may be more difficult. However, this is something that should be pursued as long as there is an interest in the use of chemical leaveners in bread.

The beneficial effects of salts were demonstrated when NFDM was present in the formulation. NFDM, in the absence of salt, caused a decline in volume. Use of certain salts at the proper concentration resulted in recovery of some of this loss. Increasing the level of NFDM allowed the use of higher levels of salts in the bread making formula. This would be particularly valuable if chemical leaveners were used. At this point, however, the chemical leaveners examined were not found to provide a benefit in bread.

A striking difference in the interaction of salts with flour and NFDM proteins was shown by the effect of sodium chloride and calcium chloride on soluble nitrogen. The effect of salts on the interaction between flour and NFDM proteins may result from the same mechanisms which relate the solubility of an individual protein to the charge density and ionic strength of a salt and then to the specific ion present. The presence of sodium chloride in a flour/NFDM slurry caused an increase in soluble nitrogen, possibly because of a charge shielding effect. Further work should be done to determine the importance of the specific ion involved by testing other sodium salts.

The effect calcium chloride on soluble nitrogen was different than sodium chloride. The calcium ion is a divalent cation which may account for a large part of the differences found. Would other divalent cations react in the same manner? The positive divalent calcium ion may be forming salt-bridges between the NFDM proteins, which have a net negative charge at the pH of dough. The interactions may then render the NFDM proteins insoluble due to their increased association, and, therefore, less likely to react with flour proteins. This may explain the similarity in the soluble nitrogen content of a flour/NFDM slurry to that of flour alone, when both contained calcium chloride.

The findings of this study agreed with the conclusion of Holmes and Hoseney (1987b), that chemical leavening did not provide a benefit for frozen dough bread. Certain tests showed glucono-delta-lactone to have the greatest potential, or the least negative effect of the leavening acids examined. This may be because the salt effect of glucono-delta-lactone on the dough and bread is not as great as other acid salts. Use of coated sodium bicarbonate and/or acid salt might possibly prevent all or some of the effects reported in this study.

Additional research might lead to a better understanding of the effects of baking powder and/or its residual salts on bread.

This might include examination of the following:

1) The effect of residual salts on oxidation requirements for bread. An increase in the potassium bromate required when SALP rather than SAS was used as the leavening. The effect of salts and oxidants in bread making may not yet be optimized.

2) The effect of residual salts on the interaction between flour and NFDM proteins. Sodium and calcium chlorides were shown to have quite different effects on soluble nitrogen.

3) Effect of salts on dough and bread in conjunction with individual milk fractions and other proteins, such as soy flour.

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EFFECT OF NEUTRAL SALTS ON DOUGH AND BREAD

by

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ABSTRACT

Mixograph characteristics of wheat flour doughs were found to be affected by the addition of neutral salts. Changes in dough properties such as time to peak, peak height, or curve width was dependent upon the specific ions present and their concentration.

In bake tests, certain salts from both the precipitating and solubilizing portions of the lyotropic series increased loaf volume relative to bread without salt. This was not true for all salts, possibly because of a negative action on gas production of yeast. The extent of the improvement and optimum usage level varied for each salt.

An interaction among flour, nonfat dried milk (NFDM), and sodium chloride was suggested by a reduction in loaf volume when sodium chloride was omitted in the bread formula containing NFDM. Also, when NFDM was removed from the formula, the optimum level of salt was reduced to 0 or 0.5% from 1.5%.

The effect of salts on flour and NFDM protein interactions was further shown by their influence on soluble nitrogen (SN). The amount of SN found in a flour/NFDM slurry was less than the sum of that found in slurries of the individual components. Addition of sodium chloride to the flour/NFDM slurry caused the SN to be increased to approximately the sum of that found in the component slurries. Addition of sodium chloride to the individual components did

not increase their SN. Calcium chloride did not increase the SN as did sodium chloride.