Hydrophobic Associations and Gluten Consistency: Effects of Specific Anions

John E. Kinsella* and Mary Lee Hale

Anions of the Hofmeister series altered the classical farinograph patterns of bread flour dough. The consistency at peak development progressively increased, i.e., 450, 490, 540, 740, and 830 Brabender units (BU) in the presence of 1 molar concentrations of $F^- > Cl^- > Br^- > ClO_4^- > SCN^-$, respectively. This effect corresponded to the respective specific entropies and the glutenin solubilizing effects of these anions. However, whereas Cl^- and Br^- enhanced the stability of doughs, the chaotropic anions ClO_4^- and SCN^- caused rapid breakdown in a manner similar to that of urea. The effects of anions indicate the importance of hydration in the development of hydrophobic interactions and the associated cooperative hydrogen bonding in determining the viscoelastic properties of gluten.

The development of the requisite rheoelastic properties of gluten depends upon the hydration of the protein and concurrent mixing. Both, especially hydration, are needed to allow unraveling and unfolding of the tightly packed aggregated gluten (glutenin and gliadin) molecules from their anhydrous state and facilitate the rapid development of a viscoelastic film network of gluten throughout the dough mass. The rheomechanical properties (viscosity, plasticity, extensibility, elasticity, consistency, and strength) of this network are critical for the optimum leavening effect during baking (Bloksma, 1978; MacRitchie, 1980). The development and the properties of the gluten film depend upon a number of cooperative secondary interactions, i.e., electrostatic, van der Waals, hydrophobic, and dipole–dipole interactions and hydrogen bonding, in addition to covalent disulfide bond formation (Pomeranz, 1978; Kasarda et al., 1976; MacRitchie, 1980).

There is extensive evidence indicating the importance of disulfide bond formation in gluten development (Pomeranz, 1978). The role of hydrogen bonding has been indirectly indicated by the weakening effects of urea and the strengthening effects of deuterium (Krull et al., 1965; Pomeranz, 1978; Kasarda et al., 1976; Inda and Rha, 1981). Electrostatic interactions though weak may be involved as reflected by pH effects (Bloksma, 1978). There is limited information concerning the importance and magnitude of hydrophobic interactions in gluten development. It seems plausible, in view of the high contents of apolar amino acids, the low net charge (because most of the acidic amino acids are amidated), and the high proline content (favoring random coil conformation and maximum association), that hydrophobic interactions are critical to gluten development. Indirect evidence has been provided by hydrophobic chromatography (Caldwell, 1979; Chung and Pomeranz, 1979) and the effects of denaturants, fatty acid soaps, salts, urea, and guanidine hydrochloride on the solubility of gluten proteins (Kobrehel and Matignon, 1980; Huebner and Wall, 1980; Preston, 1981, 1984; Bernardin and Kasarda, 1973).

Water is essential for gluten development and also for hydrophobic effects, which can only be developed in the presence of free water (Franks, 1978). Approximately 50% of the water in dough exists as free water (Bloksma, 1978). Because hydrophobic interactions are enforced by the unique structure of water, agents that selectively perturb the structure of water may be useful in demonstrating that hydrophobic interactions affect the consistency and rheological properties of dough. Anions in the Hofmeister series alter water structure in a specific order (Franks, 1978; Hatefi and Hanstein, 1969; von Hippel and Schleich, 1969). Therefore, on the basis of their lyotropic and specific entropy properties, we studied the effects of selected anions on the consistency of doughs.

EXPERIMENTAL SECTION

Materials. The pure sodium salts of the various anions and urea (analytical grade) were purchased from Aldrich Chemical (Minneapolis, MN). Distilled water was used throughout. Commercial unbleached bread flour (11.5% protein) (Pillsbury Co., Minneapolis, MN) was used.

Methods. The effects of the various anions on the development and consistency and stability of dough gluten were evaluated by using a calibrated thermostated Brabender farinograph according to the method outlines by the American Association of Cereal Chemists (1962). Flour (300 g) was mixed with 200 mL of water (or salt solution) at 30 °C and the consistency monitored for at least 20 min.

RESULTS AND DISCUSSION

The different anions altered the typical Brabender consistency patterns normally obtained with flour (Figure 1). Whereas the normal flour when mixed with water gave farinograms that reached maximum consistencies somewhat above 500 BU (500–550) within 2.5 min and remained stable for 7–10 min, different anions decreased or increased dough consistency depending on their specific entropies (Table I).

The fluoride ion (F^-), which enhances the hydrogen-bonded structure of water (von Hippel and Schleich, 1969) and thus strengthens hydrophobic effects in proteins, reduced the maximum consistency; i.e., impaired normal gluten development though the stability of the dough was prolonged. In contrast, the chaotropic thiocyanate anion, (SCN^-), which greatly perturbs the normal structure of water (i.e., increases the specific entropy by disturbing hydrogen bonds and thereby reduces hydrophobic interactions), hastened the development of maximum consistency compared to the controls but was associated with accelerated breakdown of the gluten. The other anions had intermediate effects (Table I) according to their respective lyotropic properties (von Hippel and Schleich, 1969). Significantly, the increase in consistency at peak development paralleled a coincident increase in solubility of glutenin proteins in these salt solutions (Preston, 1981, 1984) and followed the increased water destructuring effects of these anions (Hatefi and Hanstein, 1964).

These ions may exert two effects; i.e., at relatively low concentrations, <0.15 M) they affect ionic interactions between charged groups and depending upon protein and pH cause salting in or salting out and protein aggregation (Damodaran and Kinsella, 1983). This has been observed with gliadin (Kasarda et al., 1967) and glutenin (Preston, 1981). At higher concentrations, >0.3 M, certain anions

Institute of Food Science, Cornell University, Ithaca, New York 14853.

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Table I. Relationships between the Properties of Anions and Their Effects on Gluten Solubility and Consistency of Flour Dough

ion (1 M)	specific[a] entropy	gluten[b] solubility, %	consistency[c] at max, BU	time at max, min	tolerance,[d] BU	softening[d]
water control		21	550	7	-20	-80
F^-	-2.3	trace	450	1	-40	-110
Cl^-	13.5	5	490	20+	0	20
Br^-	19.7	19	540	20	0	0
ClO_4^-	43.2	31	740	2	-100	-180
SCN^-	36.0	61	830	1	-230	-440
urea[a]			740	2	-230	-310

[a] Data of Hatefi and Hanstein (1969). [b] Solubility in 1 M concentration salt solution (Preston, 1981). Glutenin has limited solubility in 1 M urea. [c] Household flour (11.5% protein); mean value of three trials. [d] Change in consistency 5 and 12 min after attaining maximum consistency (BU).

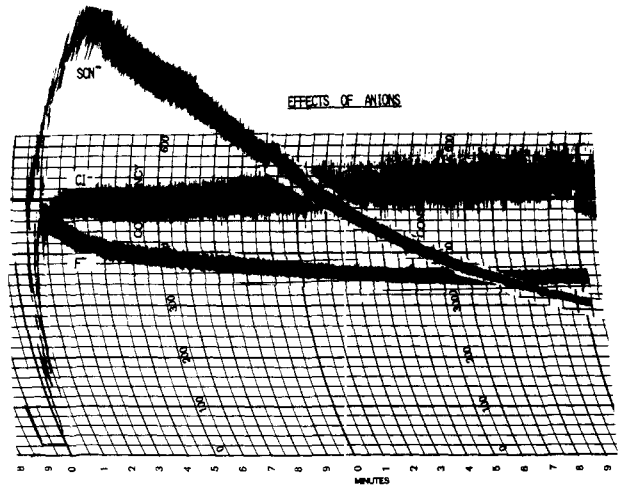

Figure 1. Brabender farinogram showing the effects of anions [fluoride (F^-), chloride (Cl^-), and thiocyanate (SCN^-)] at 1 M concentration on the consistency patterns of flour dough.

(i.e., chaotropic anions, like ClO_4^- and SCN^-) gradually enhance the solubility particularly of proteins with high contents of apolar amino acids (which enhance hydrophobic interactions), e.g., wheat glutenin (Preston, 1981). These anions weaken hydrophobic associations and facilitate unfolding, hydration, and solubilization of these polypeptides. Thus, while at low concentrations (0.1 M) all of these anions reduced the extensibility of the dough gluten in a comparable manner (Hale and Kinsella, 1984), at concentrations above 0.5 M the chaotropic effect of ClO_4^- and SCN^- became pronounced, whereas the other ions F^-, Cl^-, and Br^- exerted little additional effect. This indicates the improtance of hydrophobic interactions in gluten development.

During the formation of gluten the added water initially hydrates the compact tightly folded anhydrous gluten protein, which swells as hydrogen bonds and perhaps ionic interactions are progressively disrupted. With kneading, the polypeptides are unfolded and interpeptide interactions (via hydrophobic associations and consequent hydrogen bonds) are established. In addition, considerable physical entanglements and disulfide crosslinking occurs to give a continuous cohesive viscoelastic film (Pomeranz, 1978; MacRitchie, 1980; Kasarda et al., 1976). The addition of salts, depending upon their lyotropic properties apparently alters this sequence of events. In the presence of F^- and Cl^- hydrophobic interactions between gluten proteins are accentuated and the gluten proteins tend to remain more aggregated and be quite resistant to hydration and unraveling. Thus, the highly water structuring fluoride anion caused a reduction in dough consistency, presumably because compared to normal controls gluten unfolding was impaired and subsequent extensive protein–protein interactions and physical entanglements were reduced. This was less pronounced with Cl^- where development required extensive kneading (long development times) to induce unfolding and network formation. Similar effects of salts, i.e., increased mixing times, have been noted earlier (Galal et al., 1978; Danno and Hoseney, 1982).

The chaopropic anions, e.g., SCN^-, accelerated hydration and allowed more rapid unfolding of the apolar glutenin polypeptides as incdicated by the rapid increase in consistency. However, after a short peak the resistance of the dough decreased rapidly. This may reflect the fact that in the presence of SCN^- kneading resuls in more rapid disentanglement of the large glutenin polypeptides, which quickly become aligned in the direction of mixing with a concomitant decrease in consistency. Furthermore, in the presence of SCN^-, the normal hydrophobic–induced folding of apolar segments of polypeptides and associations between adjacent polypeptides are conceivably diminished. This, in turn, may decrease the number of hydrogen bonds (which need an anhydrous domain for maximum formation) and reduce entanglements. These result in a marked reduction in elasticity and decreased viscosity as evidenced by the farinograms. Of course, gradual solubilization of glutenin (Preston, 1981) would also result in decreased consistency. The perchlorate anion and urea, at 1 M concentrations, caused similar changes though not as extensive as with SCN (Hale and Kinsella, 1984). Jankeiwicz and Pomeranz (1965) obtained comparable consistency patterns with 3 M urea, which they ascribed to disruption of hydrogen bonds.

The specific action of chaotropic anions indicates the importance of hydrophobic interactions in contributing to the consistency of dough. Not only do these anions reduce hydrophobic interactions but also, when the latter are not established, the requisite hydrogen bonds cannot be formed (Ross and Subramanian, 1981), and thus the viscous flow properties of dough are altered. Furthermore, since the negative entropy effects of exposing apolar segments of glutenin polypeptides to the aqueous phase is decreased in the presence of chaotropic anions, dough elasticity may be weakened because of reduced protein–protein interactions.

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Registry No. F, 16984-48-8; Cl, 16887-00-6; Br, 24959-67-9; ClO_4, 14797-73-0; SCN, 302-04-5.

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Fast Atom Bombardment Mass Spectrometry of Macrocyclic Diester Pyrrolizidine Alkaloid *N*-Oxides

Joseph J. Karchesy, Max L. Deinzer,* and Donald A. Griffin

Fast atom bombardment mass spectra of macrocyclic diesters of retronecine *N*-oxide are characterized by an $(M + H)^+$ pseudomolecular ion base peak, an $(M + H - 16)^+$ ion, and a prominent ion series with m/z 136, 120, 118, 106, 94, and 80.

Pyrrolizidine alkaloid *N*-oxides represent one of the largest and better known groups of naturally occurring alkaloid *N*-oxides (Phillipson and Handa, 1978). Electron impact (EI) mass spectra of pyrrolizidine alkaloid *N*-oxides often do not exhibit a molecular ion (Culvenor et al., 1975). Abdullaev et al. (1974) have noted that even when the molecular ion is absent, a characteristic triplet of ion peaks at M – 16, M – 17, and M – 18 is usually encountered and may be used to identify the molecular weight of the compound. Methane chemical ionization (CI) mass spectra show a useful pseudomolecular ion $(M + H)^+$ with simple C-9 mono- and C-7, C-9 diesters of retronecine *N*-oxide; however, macrocyclic diesters such as monocrotaline *N*-oxide do not show a significant $(M + H)^+$ ion in their CI mass spectra. The macrocyclic diesters, which are commonly encountered, are representative of the most difficult pyrrolizidine alkaloid *N*-oxides to analyze by conventional mass spectrometry. This paper describes the preliminary results obtained from the fast atom bombardment (FAB) mass spectral analysis of five macrocyclic diesters of retronecine *N*-oxide.

EXPERIMENTAL SECTION

Parent pyrrolidizine alkaloids were obtained as previously described (Karchesy et al., 1984). Final purification was accomplished by preparative HPLC (Whatman C-8, methanol–0.1 M pH 6.0 phosphate buffer) and recrystallization from methanol. Identities were confirmed by melting points, GC–MS, and NMR. *N*-Oxides were prepared by oxidation of the parent pyrrolizidine alkaloid with hydrogen peroxide in methanol (Mattocks, 1969). The *N*-oxide products gave melting points in agreement with the literature (Bull et al., 1969; Culvenor et al., 1970). Samples for mass spectral analysis were prepared in glycerol and placed on a copper sample support. Spectra were obtained with a Varian CH-7 mass spectrometer modified to accept an Ion Tech, Ltd., saddle field ion source. Xe was used as the primary atom beam with the saddle field ion source operating at 7 keV. Spectra were calibrated with perfluorokerosene in the EI mode and recorded in the FAB mode with a System Industries 150 data system.

Department of Agricultural Chemistry and Environmental Health Sciences Center, Oregon State University, Corvallis, Oregon 97331.

Table I. Key Ions of Macrocyclic Diester Pyrrolizidine Alkaloid *N*-Oxides Investigated, m/z (Relative Intensity)

ion	*N*-oxide 1	2	3	4
$(M + H)^+$	342 (100)	368 (100)	352 (100)	350 (100)
$(M + H - 16)^+$	326 (5)	352 (15)	336 (14)	334 (18)
m/z 136	(24)	(27)	(22)	(33)
m/z 120	(30)	(56)	(28)	(55)
m/z 118	(30)	(50)	(33)	(55)
m/z 106	(14)	(24)	(13)	(26)
m/z 94	(17)	(28)	(16)	(29)
m/z 80	(12)	(24)	(14)	(26)

RESULTS AND DISCUSSION

FAB mass spectra of the *N*-oxides of the pyrrolizidine alkaloids monocrotaline (1), jacobine (2), senecionine (3), seneciphylline (4), and retrorsine (5) were readily obtained without special preparation or reagents to promote ionization. As shown in Table I and Figure 1, the spectra are characterized by an $(M + H)^+$ pseudomolecular ion as the base peak, an $(M + H - 16)^+$ ion, and a prominent ion series with m/z 136, 120, 118, 106, 94, and 80. The prominent ion series is similar to, but not identical with

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