Interfacial Properties of Gluten Monolayers Spread on Various Chloride Salt Solutions. Effects of Electrolytes, Salt Concentrations, and Temperature

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The interfacial behavior of gluten powder spread as a monolayer on aqueous phases containing various chloride salts was studied. The presence of electrolytes at low concentrations reduced the expansion and stability of the gluten monolayers compared to the results obtained with pure water. At low salt concentrations, no effect of the electrolyte nature was detectable (compression curves were superimposed for Na^+, K^+, and Ca^{2+}). However, when salt concentrations increased from 0.05 to 0.5 M, the influence of the electrolyte nature on gluten film expansion appeared clearly. Divalent cations (Ca^{2+}) gave films with greater expansion than monovalent cations (K^+, Na^+). Between the monovalent cations, Na^+ had a greater effect on gluten film expansion than did K^+. Gluten monolayer expansion evaluated by limiting the area (A_0) passed through a minimum when the salt concentrations increased from 0 to 0.5 M. The temperature also influenced the behavior of gluten monolayers as attested by A_0 and film elasticity values which decreased with temperature. The energy of compression (ΔG_c) that measures the intermolecular forces between film-forming molecules was generally higher on Ca^{2+} than on K^+ and Na^+, showing that Ca^{2+} induced stronger interactions than K^+ or Na^+. The $\Delta G_c - T$ plots showed that the compression of gluten films on various electrolytes led to ordered structures.

Keywords: *Gluten; monolayer; salts; interface; protein interactions*

INTRODUCTION

Wheat gluten is an elastic, coherent protein complex obtained by washing out starch and water-soluble proteins from a dough of wheat flour.

Several forces are involved in the formation of the gluten matrix. Among them, disulfide bonds play an important role (Shewry and Tatham, 1997). Ionic bonding, hydrogen bonding, hydrophobic interactions, and van der Waals interactions also have their contributions (Belitz *et al.,* 1986).

Several studies correlated the physicochemical properties of gluten with selected bread-making properties. In fact, use of gluten in any food product involves at least one heating step, during which gluten proteins are set. During baking, heating causes volume expansion, which influences the bread volume quality (Schofield *et al.*, 1983; Kokelaar *et al.*, 1995). Furthermore, it is well-known that the functional properties of gluten are changed by heat treatment. This treatment of gluten influences its interfacial behavior (Eliasson and Lundh, 1989). In addition, the gliadin−glutenin ratio influences the interfacial properties of gluten monolayers spread at the air−water interface (Balla *et al.*, 1997). In the bread-making process, chloride salts improve the flavor and dough-handling properties and moderate yeast activity during fermentation. The effects on the dough-handling properties are generally attributed to the interaction of salt ions with gluten proteins. Salt shields the charges on the gluten protein, allowing them to associate and, thus, produce a stronger dough (Hoseney, 1994).

However, the molecular basis of the salt effect on dough has not been elucidated in detail. Thus, the impact of salts on the heat-induced protein network formation is of interest for both practical application (inclusion of salts in food products) and fundamental investigations into molecular interactions within gluten proteins. As gas retention in dough is primarily related to the gluten proteins (MacRitchie, 1990), it is interesting to study the interfacial properties of gluten at the air−water interface under different physicochemical conditions. In this paper, we reported the effects of electrolytes, salt concentrations, and temperature on the behavior of gluten powder spread as monolayers at the air−water interface.

EXPERIMENTAL PROCEDURES

Gluten was purchased from Sigma Chemicals (Bornem, Belgium) (isolated from whole wheat). All saline solutions were made from reagents of analytical grade (Sigma). Ultrapure water was obtained using a Milli-Q system (Millipore Corp., Milford, MA).

Monolayer experiments were carried out using a Langmuir film balance system (Lauda, FW 2, Königshofen, Germany). In each experiment, an amount of 0.2 mg of gluten powder was spread dry at an area of 927 cm^2. The exact amount of spread material was calculated by weighing the weighing scoop before and after spreading of the sample. After spreading, the system was allowed to equilibrate for 30 min before compression started. The rate of compression was 0.515 cm^2/s. The

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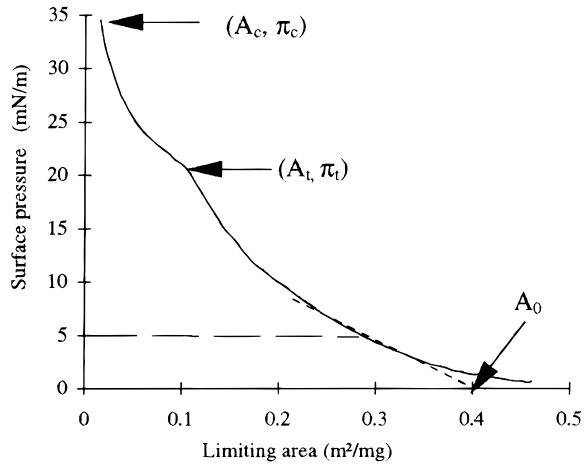

Figure 1. Schematic representation of an isotherm curve with indication of the parameters used in this study. A_c, π_c, end of compression point; A_t, π_t, transition point; A_0, limiting area.

trough was thermostated and the temperature of the subphase was maintained within ±0.5 °C.

The limiting area (A_0) was determined by extrapolation at the intersection of the abscise axis with the tangent of the $\pi-A$ isotherm at $\pi = 5$ mN/m. The transition surface pressure (π_t) was evaluated at the inflection of the $\pi-A$ isotherm. The film elasticity, $E = -A(d\pi/dA)$, was determined from the $\pi-A$ isotherm curve at high pressures (from 15 to 20 mN/m), and the mean value was considered. Compression isotherm reproducibility was carefully checked by making at least three measurement sets for each measure. The variation coefficients for each parameter (A_0, π_t, π_c) was less than 5%. The pH was measured using a Hanna pH meter (HANNA Instruments, 8418, Vila Doconde, Portugal). Figure 1 shows a schematic representation of an isotherm with indication of the parameters used in this study.

RESULTS AND DISCUSSION

Effects of Electrolytes and Salt Concentrations. The $\pi-A$ isotherms of the gluten powder were sigmoid-shaped curves. These curves presented a transition zone marked by an inflection rather than a plateau determined as the transition surface pressure (π_t). This transition zone was attributed to a change in the molecular arrangement at the air−water interface similar to other studies on various proteins (Graham and Phillips, 1979). Compression isotherms of gluten obtained at 23 °C on various saline subphases are compared in Figure 2. All isotherms recorded on the saline subphase are shifted to lower values of the film area in comparison with that established on pure water. These isotherms are slightly superimposed, no differences being observed in the film expansion among chloride salts at a low concentration (0.05 M) (Figure 2a). At high salt concentrations, differences in the monolayer curves were noted (Figure 2b). In this case, the gluten monolayer expansion was higher on $CaCl_2$ than on KCl or NaCl solution. These results show that at low salt concentrations, the nature of the electrolyte has no effect on monolayer overrun, but when the salt concentrations increase from 0.05 to 0.5 M, the monolayer properties are influenced by the nature of the cations.

The effects of chloride salts at low concentrations would result in a general increase in the intramolecular interactions within and among the polypeptide chains of the gluten proteins. This may induce gluten protein aggregation by suppression of the intramolecular repulsions of the gluten positive charges (Galal *et al.*, 1978). Indeed, water molecules are drawn away from the polypeptide chain structure to interact with the chloride salts. This could induce more compact gluten protein molecules compared to that spread on pure water. This may explain the shift of the limiting area of the film to lower values by the presence of chloride salts at low concentrations in the subphase, indicating that gluten film interacts less with water molecules. 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 salts (Preston, 1989).

The effects of additional salt in expanding the gluten protein film could result in intramolecular electrostatic repulsion within the protein molecules that induced unfolded conformation (Clements, 1973; Fu *et al.*, 1996). In addition to this electrostatic effect, high concentrations of salts also exert a nonspecific influence on the hydrophobic interactions, which induces a somewhat open structure. The importance of this effect depends on the salt properties (Arntfield *et al.*, 1990). The degree to which the water structure is affected depends on the nature of the cations or anions. For cations, the extent of the water structure breaking effect follows the order $Ca^{2+} > Mg^{2+} > NH_4^+ > Na^+ > K^+$, which is known as the lyotropic series (Cheftel *et al.*, 1985).

When the limiting area (A_0) which represents monolayer expansion was plotted against the subphase salt concentration, the curve representation in Figure 3 was obtained. A_0 decreases with increasing the concentration up to 0.05 M, and then it increases for NaCl and $CaCl_2$. On the other hand, the value of A_0 for KCl continues to decrease up to 0.3 M before increasing slightly. We propose the existence of a range of concentrations near the minimum point where masking of the net charges is dominant and further salt addition simply promotes intramolecular and intermolecular repulsions.

Elasticity relates to the change in surface pressure per unit change in interfacial area of film and is expressed as $E = -A(d\pi/dA)$, where A is the film area and π the surface pressure (Graham and Phillips, 1980). Therefore, the elasticity of a film measures its resistance to change in film area. Since gluten monolayers show no clear collapse behavior, the surface pressure at the end of compression, π_c, can be used as a stability indicator similarly to the collapse pressure. Table 1 shows the effects of the electrolytes on stability and elasticity of gluten films spread on various chloride salts at 23 °C. At low salt concentrations, the film elasticity and stability did not change notably in the presence of different electrolytes, but at high salt concentration (0.5 M), film properties differed on various cations. In general, gluten film stability increased when salt concentration increased while film elasticity decreased, except for gluten film compressed on $CaCl_2$. It is also noted that the elasticity and stability of gluten film are higher with subphase containing Ca^{2+} than Na^+, which in turn confers higher elasticity and stability than K^+.

Addition of low molecular weight divalent ions changes the rheological properties of the gluten film by strengthening the disulfide bonds which seem to have a great importance on gluten protein elasticity (Belitz *et al.*, 1986). Under the conditions of our experiments, which involved only compressional forces, it may be assumed that the presence of salt, especially $CaCl_2$, forms ad-

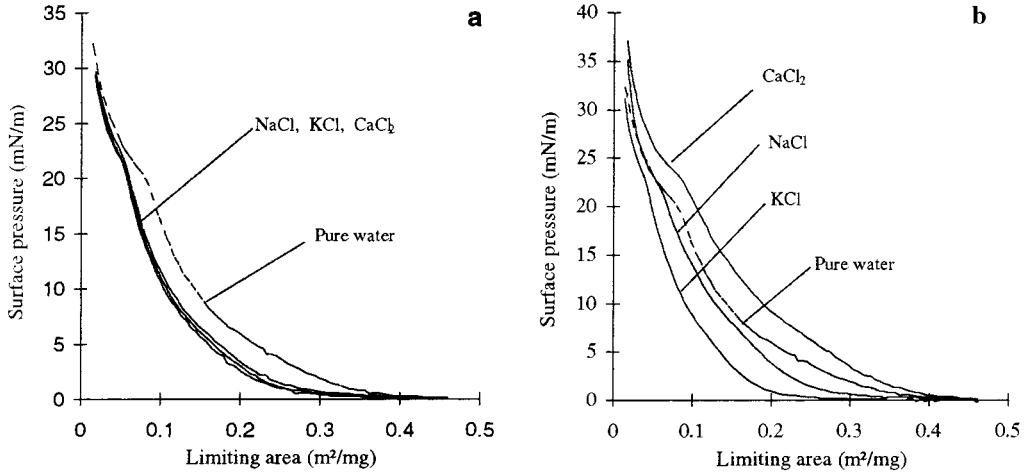

Figure 2. Isotherm curves of gluten powder spread on various saline subphases at 23 °C: (a) at salt concentration of 0.05 M; (b) at salt concentration of 0.5 M; (- - -) represents isotherm curve for gluten spread on pure water.

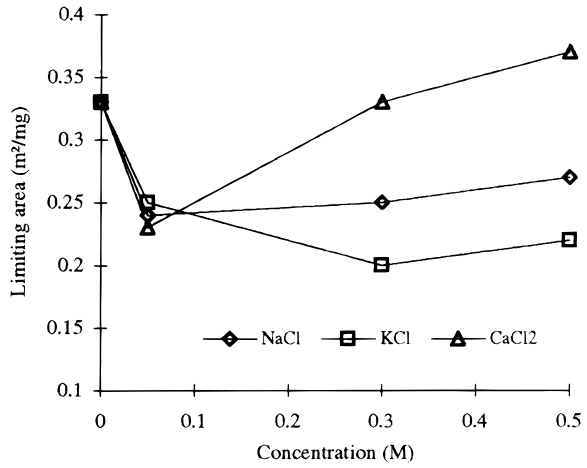

Figure 3. Effect of salt concentration at 23 °C on limiting area of gluten monolayer spread on various salt subphases.

Table 1. Effects of Electrolytes on Gluten Film Stability (π_c) and Elasticity (E) Spread on Various Chloride Salts at 0.05 and 0.5 M (±Standard Deviation)

subphase characteristics		E, mN/m	π_c, mN/m
0 M	pure water	20.55 ± 0.05	32.3 ± 0.5
0.05 M	NaCl	21.55 ± 0.04	28.4 ± 0.4
	KCl	21.55 ± 0.05	29.7 ± 0.5
	$CaCl_2$	21.65 ± 0.04	29.1 ± 0.4
0.5 M	NaCl	18.39 ± 0.04	35.2 ± 0.5
	KCl	17.46 ± 0.03	31.1 ± 0.4
	$CaCl_2$	21.32 ± 0.04	37.1 ± 0.3

ditional bonds that strengthen the gluten film, because they probably form additional cross-links.

In fact, gluten film elasticity is determined by the high molecular weight (HMW) subunits of glutenin. The structure of these subunits was controlled by the number and distribution of cysteine residues available to form intermolecular cross-links (Bloksma, 1990; Shewry and Tatham, 1997). The HMW subunits have cysteine residues predominantly at either end of the molecules, allowing deformation/reformation to occur in the central domain. Changes in the number of cross-links would be expected to have major effects on the physical properties of the glutenin polymers (Shewry *et al.*, 1992) and, thus, on the gluten film properties.

High film elasticity reflects a higher degree of inter- and intramolecular interactions which result in the formation of stronger, more cohesive films that are more resistant to compress as envisaged by Kim and Kinsella (1985) and by Damodaran (1994).

Finally, the pHs of the chloride salt solutions did not vary significantly from 0.05 to 0.5 M (from pH 5.3 to pH 5.8), which indicates that the change in the gluten film expansion observed in this study was not due to the pH effect.

The differences in monolayer expansion observed at 0.5 M but not at 0.05 M will provide us with a better evaluation of the importance of gluten film expansion, as lyotropic influence will not be a significant factor at the lower salt concentrations.

Nevertheless, the relationships between the gluten film properties and the position of a salt in the lyotropic series at concentrations of 0.5 and 0.05 M showed that hydrophobic interactions were not the only interactions which contributed in the stability of the gluten monolayer under compression. Salt addition to the gluten monolayer subphase would also cause structural changes in the gluten proteins.

Effects of Temperature. Compression isotherms of the gluten monolayers spread on chloride salts at 0.05 M and at different temperatures are shown in Figure 4a. When the temperature is increased from 10 to 35 °C, we observed greater expansion of the gluten monolayer on all electrolytes. A_0 varied from 0.18 to 0.31 m^2/mg in all cases, producing an increase of about 72%. At 10 °C, isotherm curves of gluten monolayers showed no evidence of a sharp transition zone which is similar to that of glutenin film at the air−water interface described by Tao *et al.* (1989). No clear difference in π_t values was observable among isotherm curves at 23, 30, and 35 °C in the presence of 0.05 M NaCl. Similar observations were made in the presence of 0.05 M KCl and $CaCl_2$ (figures not shown). The greater expansion of gluten films toward larger limiting area (A_0) values as temperature increases may result from the increase of thermal agitation that produces repulsion forces within gluten protein molecules.

Figure 4b shows that the elasticity of gluten films spread on those various salts at 0.05 M decreases; in other words, their compressibility (inverse of elasticity) increases with increasing temperature. These results could be also explained by the fact that temperature may affect gluten protein solubility. Indeed, the increase of gluten solubility is consistent with the disaggregation hypothesis, if it is assumed that the disruption of the insoluble gluten complex increases the flexibility

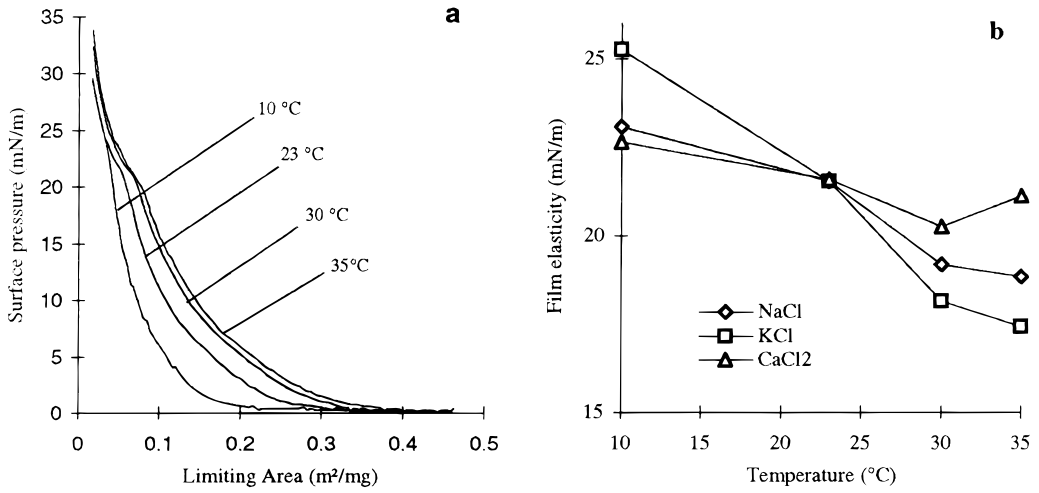

Figure 4. Effect of temperature at salt concentration of 0.05 M: (a) on isotherm curves of gluten powder spread on NaCl subphase; (b) on elasticity of gluten monolayer spread on various chloride salts.

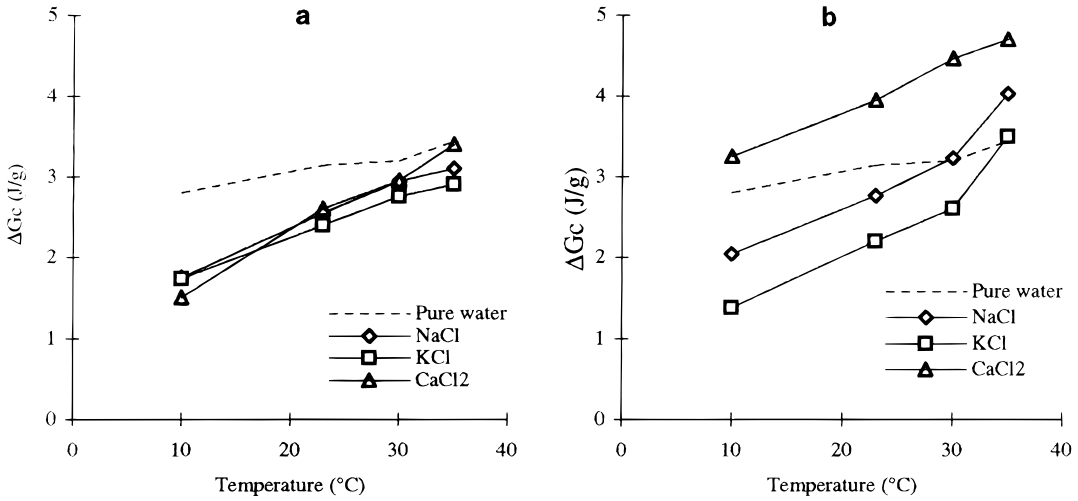

Figure 5. Temperature dependence of free energy of compression of gluten monolayers spread (a) on 0.05 M and (b) on 0.5 M chloride salts.

of the protein molecules in the film. This results from the reduction of intra- and intermolecular interactions within gluten protein molecules, which leads to more compresible films.

Thermodynamics of Gluten Film Compression. The free energy of compression, $\Delta G_c = -(\int_{A_1}^{A_2} \pi \, dA)$, represents the work required to compress a monolayer from a state where no molecular contacts occur (start of compression, A_1) to the end of compression (A_2). It measures the intermolecular forces in the film and depends on the interaction between the gluten protein molecules and the ions present in the subphase. Figure 5 shows the changes of the ΔG_c values as a function of temperature.

At 0.05 M, no difference in the ΔG_c values was observed at the same temperature for different cations (Figure 5a). At 0.5 M, ΔG_c values increase with temperature and change as a function of cation in the subphase (Figure 5b). Examination of these values indicates that it is generally easier to compress a gluten monolayer spread on monovalent cations than spread on divalent ones. Higher ΔG_c values observed on $CaCl_2$ reflect higher intermolecular cohesion in the film. ΔG_c values at 0.05 and 0.5 M of gluten films spread at 23 °C confirm the previous results on the effects of electrolytes which investigate possible protein structural changes.

From the slopes of the $\Delta G_c - \boldsymbol{T}$ plots, one can deduce the sign of the entropy of compression, $\Delta S_c = -\mathrm{d}(\Delta G_c)/\mathrm{d}T$, and then of the enthalpy, $\Delta H_c = \Delta G_c + T\Delta S_c$ (Maget-Dana *et al.*, 1992). The slopes given by the gluten monolayers spread on various electrolytes and at different concentrations of salt are positive. This leads to negative values of ΔS_c and ΔH_c, directly related to a more ordered arrangement of monolayers under compression. This suggests that molecules became more closely packed.

CONCLUSION

The main objective of our study was to investigate the importance of electrolytes, chloride salt concentrations, and subphase temperature on the surface properties of a gluten monolayer spread at the air−water interface.

Recent experiments indicated that minor changes in structure lead to notable differences in the conformation of the gluten proteins (MacRitchie, 1990; Damodaran, 1990). The results herein show that structural changes induced differences in the surface properties of gluten powder spread on various chloride salt subphases. In

fact, the presence of electrolytes in the monolayer subphase greatly modifies the interface parameters of a gluten film spread at the air−water interface. At a low concentration of chloride salts (0.05 M), gluten film overrun and stability decrease, independent of the cation type, while at a high salt concentration (0.5 M), film overrun and stability increase. They are highly dependent on the cation type and follow the lyotropic anion series (Hofmeister series).

Temperature also induced changes in the gluten film properties. In fact, gluten film expansion increases while film elasticity decreases with temperature. These changes are related to the increasing gluten protein solubility.

Thermodynamic measurements show that gluten monolayers are in ordered arrangement during compression and it is easier to compress the film spread on monovalent cations than on divalent cations.

Practically, in bread dough, salt is used at a concentration of 1.5−2% in flour weight basis, which represents roughly 0.5 M on a water basis assuming a dough water content of 33% (MacRitchie, 1976). The dough temperature is around 23 °C under normal conditions. Under these conditions, the results obtained with NaCl, which is the salt used in bread making, are intermediary between $CaCl_2$ and KCl.

ABBREVIATIONS USED

A_0, limiting area; π_t, transition surface pressure; π_c, surface pressure at the end of compression; E, film elasticity; ΔG_c, free energy of compression; ΔS_c, entropy of compression, ΔH_c, enthalpy of compression.

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