NOTE

PROTEINS

# Suitability of Lithium Chloride Solutions for Wheat Gliadin Extraction

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## ABSTRACT

Previous research suggested that solutions containing lithium chloride (LiCl) can extract gliadins more efficiently than can the traditional solvent, 70% ethanol. Those studies used sodium dodecyl sulfate gel electrophoresis to compare extracted fractions. In this study, reversed-phase high-performance liquid chromatography was used to further analyze and quantify such fractions. Results indicate that LiCl primarily extracts albumins, globulins, and $\omega$-gliadins, while most $\alpha$-, $\beta$-, and $\gamma$-gliadins, normally extracted with ethanol, are not solubilized.


Ethanol has long been used as a solvent for gliadin, usually at a concentration of 70%. It efficiently extracts most gliadins from flour, along with some albumins and globulins, plus a small amount of lower molecular weight glutenins (Bietz et al 1984, Burnouf and Bietz 1984, Huebner and Bietz 1993). However, a recent study by Kazemie and Bushuk (1992) suggested that 2*M* LiCl is a better gliadin extractant than is 70% ethanol.

This note reports a further investigation of the use of 2*M* LiCl as a wheat protein extractant. The results, however, indicate that LiCl solubilizes primarily $\omega$-gliadins and nongliadin proteins, while most $\alpha$-, $\beta$-, and $\gamma$-gliadins normally extracted with ethanol are not solubilized.

## MATERIALS AND METHODS

Flours (60 mg) from a Canadian hard red spring wheat cultivar (Neepawa) and a U.S. hard red winter wheat cultivar (Centurk) were extracted for 30 min with constant agitation with 0.8 ml of cold (2–5°C) water, and, after centrifugation (10 min at 12,000 $\times$ *g*) twice with 0.75 ml of 0.5*M* NaCl (extracts were subsequently combined) at 4–6°C to minimize possible proteolysis. The resulting centrifuged pellets were then sequentially extracted at room temperature for 30 min with constant agitation in 1.5-ml aliquots of 2*M* LiCl, 2*M* LiCl + 6*M* urea, 6*M* urea, and 70% ethanol in the order summarized in Table I. All extracts (typically 10-$\mu$l aliquots) were then analyzed by reversed-phase high-performance liquid chromatography (RP-HPLC) (Huebner and Bietz 1993) on a 4.6-mm (i.d.) $\times$ 15-cm Vydac $C_4$ column. Chromatographic solvent A was 10% acetonitrile (ACN) + 0.11% trifluoroacetic acid (TFA); solvent B was 90% ACN + 0.95% TFA. The gradient, applied at a flow rate of 0.9 ml/min, consisted of linear segments between: 0 min, 17% B; 3 min, 23% B; 50 min, 45% B; and 54 min, 46% B. Detection was at 210 nm (0.1 absorbance units full scale).

## RESULTS AND DISCUSSION

The extraction procedures summarized in Table I were designed to reveal types and amounts of proteins extracted with 0.5*M* NaCl, 2*M* LiCl, 6*M* urea, 2*M* LiCl + 6*M* urea, and 70% ethanol, in various sequences. Figure 1 shows representative RP-HPLC chromatograms of proteins extracted from Neepawa flour with NaCl, LiCl, urea, LiCl + urea, and 70% ethanol. Similar results (not shown) were obtained for the Centurk and Thatcher flours. Data in Figure 1 are normalized (i.e., the tallest peak in each chromatogram plotted to the same height) to more clearly reveal types of proteins present. Otherwise, many peaks in some chromatograms (e.g., of salt extracts) would be too small to be observed.

Examination and comparison of these chromatograms reveals the types of proteins extracted with each solvent. Previous studies have shown that, under these RP-HPLC conditions, most albumins and globulins, as well as $\omega$-gliadins, elute early, followed by $\alpha$- and $\beta$-gliadins, and finally by $\gamma$-gliadins (Bietz 1983, Bietz and Burnouf 1985)

NaCl solubilizes primarily globulins and albumins from wheat flour. However, it is well known that NaCl and other extraction solvents are not totally specific for individual protein classes. For example, Preston (1981) showed that different salts at different concentrations dissolve various percentages of gluten proteins. RP-HPLC (Fig. 1a) shows that most NaCl-soluble albumins and globulins elute relatively early (6–21 min). Some later-eluting NaCl-soluble proteins are also present, as observed by Bietz (1983). These differ from $\alpha$-, $\beta$-, and $\gamma$-gliadins extracted with 70% ethanol (Fig. 1e) (Huebner and Bietz 1993).

The predominant proteins extracted by 2*M* LiCl (Fig. 1b), after NaCl extraction, also elute relatively early (5–30 min) upon RP-HPLC. Proteins in this sample are poorly resolved as compared to those extracted with other solvents. It is apparent, however, that many 2*M* LiCl-soluble proteins have elution times corresponding to major peaks in the NaCl extract (Fig. 1a). Relatively few LiCl-soluble proteins coincide with those extracted with 70% ethanol (Fig. 1e). LiCl also appears to extract significant amounts

**TABLE I**
**Extraction Procedures and Yields**

| | Extraction Sequence | | | | | |
|---|---|---|---|---|---|---|
| Sample | $H_2O$ | 0.5*N* NaCl | 2*M* LiCl | 2*M* LiCl + 6*M* Urea | 6*M* Urea | 70% Ethanol |
| A | 1[a] | 2 | 4 | | | 3 |
| | (257) | (537) | (190) | | | (2,475) |
| B | 1 | 2 | 3 | | | 4 |
| | (257) | (537) | (297) | | | (2,565) |
| C | 1 | 2 | | 4 | 3 | |
| | (257) | (537) | | (482) | (3,037) | |
| D | 1 | 2 | | 3 | 4 | |
| | (257) | (537) | | (2,505) | (665) | |

[a] Upper numbers indicate order of extraction. Lower numbers (in parentheses) are reversed-phase high-performance liquid chromatography areas corresponding to 60 mg of flour, indicating amounts of protein extracted. All area data are averages of replicate extractions; the average relative standard deviation was 2.6%.

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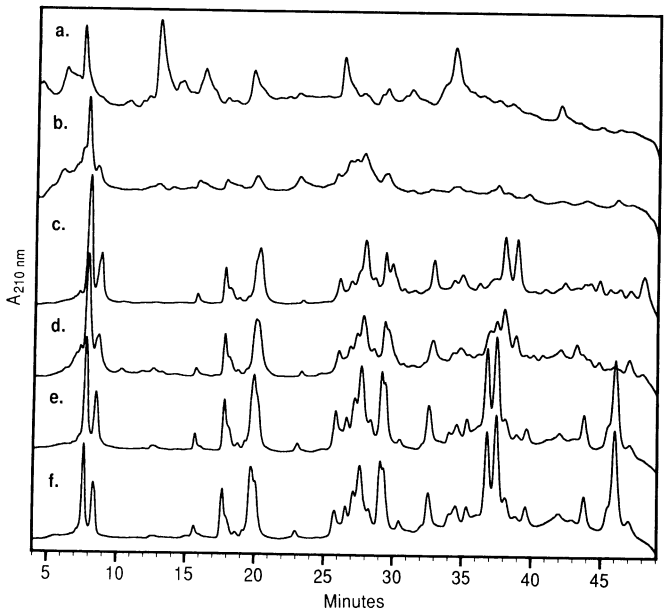


**Fig. 1.** Reversed-phase high-performance liquid chromatography of Neepawa flour proteins solubilized using the sequential extraction schemes of Table I. a, 0.5*M* NaCl (sample A1); b, 2*M* LiCl extract after NaCl extraction (sample B3); c, 6*M* urea extract after NaCl extraction (sample C3); d, 2*M* LiCl + 6*M* urea extract after NaCl extraction (sample D3); e, 70% ethanol extract after NaCl extraction (sample A3); and f, 70% ethanol extract after extraction with NaCl and 2*M* LiCl (sample B4).

of early-eluting (5–9 min) ω-gliadins (Fig. 1b); these are also the earliest eluting major peaks extracted by 70% ethanol or urea (Fig. 1e,c). Thus, LiCl extraction, after NaCl extraction, appears to extract primarily residual albumins and globulins, although some gliadins may be coextracted, as shown by SDS-PAGE (Kazemie and Bushuk 1992).

The poor extractability of most gliadins by 2*M* LiCl is apparent from the RP-HPLC pattern of flour proteins extracted by 70% ethanol after LiCl extraction (Fig. 1f). This sample is qualitatively identical to that of the 70% ethanol extract (Fig. 1e). However, when flour is extracted with 6*M* urea or 2*M* LiCl + 6*M* urea (Fig. 1c,d), RP-HPLC chromatograms of the extracted proteins appear qualitatively very similar to that of the 70% ethanol extract. Urea is, of course, an excellent solvent for gliadin.

Proteins extracted with 70% ethanol after 2*M* LiCl extraction (Fig. 1f) also appear identical to those extracted directly by ethanol (Fig. 1e). Thus, results of Figure 1 clearly show that much of the low molecular weight α-, β-, and γ-gliadins are not extractable by 2*M* LiCl alone.

Amounts of protein in each extract, estimated from total integrated RP-HPLC peak areas, are also shown in Figure 1. After initial water and NaCl extractions, 2*M* LiCl (sample B3) extracts only about 12% as much protein as does 70% ethanol (sample A3). The low extractability of proteins by LiCl is also apparent. Note that after LiCl extraction, 70% ethanol extracts as much protein (shown in many studies to be rich in α-, β-, and γ-gliadins) as it does before LiCl extraction (compare samples A3 and B4). Adding 6*M* urea to the LiCl solution (sample D3) results in slightly greater extraction of proteins than was achieved by ethanol alone (sample A3). Figure 1d suggests that these 6*M* urea + 2*M* LiCl soluble proteins are primarily gliadins. Urea-containing solvents are also known to extract some glutenin.

Thus, while these results confirm those of Kazemie and Bushuk (1992) that show 2*M* LiCl + 6*M* urea to be an efficient solvent for gliadins, they do not support the claims of those authors that 2*M* LiCl without urea extracts considerably (3×) more protein than does 70% ethanol. In fact, the present results show that LiCl alone solubilizes only a small amount of protein, shown by RP-HPLC to be primarily albumins, globulins, and ω-gliadins. Most α-, β-, and γ-gliadins are not solubilized by 2*M* LiCl but may subsequently be extracted by 70% ethanol. Nevertheless, the selectivity of LiCl may prove useful in some sequential extraction schemes, and LiCl + urea may be useful for extracting glutenin free of albumins, globulins, and gliadin.

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