NOTE

Use of Lithium Chloride for the Extraction of Flour Proteins[1]

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ABSTRACT

In this study, 2*M* lithium chloride (LiCl) appeared to be more effective than 70% ethanol solution for extracting gliadins from wheat flour. In addition, it was found that after extraction with solvents containing LiCl and urea or acetic acid and urea, a group of low molecular weight proteins (possibly reprecipitated gliadins) together with gelatinized starch could be removed from residue by gentle dispersion in double-distilled water. The protein in final residue appeared to be glutenin.

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Concentrated ethanol solutions have been widely used for the extraction of the gliadins from ground wheat or flour. However, review of the relevant literature revealed that this solvent is not very efficient in separating the gliadins from the glutenins that are not soluble in ethanol solution (for reviews, see Lasztity 1984 and Wrigley and Bietz 1988).

In this note we describe the use of 2*M* lithium chloride (LiCl) for the extraction of the storage proteins of wheat. Simple procedures for the preparation of glutenin from the LiCl-urea extract and the residue are also described.

MATERIALS AND METHODS

The flour was milled from a sample of grain of a pure variety (cv. Neepawa) of Canadian hard red spring wheat on a Buhler Laboratory Mill (MLU 202). Conditions for extraction of various protein fractions were described previously (Kazemie and Bushuk 1990); the procedures are summarized in Figure 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Ng and Bushuk (1987).

RESULTS AND DISCUSSION

Results of Figure 2A show that 2*M* LiCl (lane c) extracts additional gliadins from the residue of flour after conventional Osborne extraction with water, 0.5*M* NaCl, and 70% ethanol (lane b). Comparison of the patterns of the acetic acid-urea extracts after LiCl extraction (lane d) and after ethanol extraction (before extraction with LiCl) (lane e) confirmed the unique solubilizing ability of LiCl on flour proteins. For comparison, a direct SDS-mercaptoethanol extract of flour (lane a) and the residue (lane f) after extraction for lane d is shown.

In a separate experiment on gluten (results not shown), 2*M* LiCl extracted three times more protein than did 70% ethanol. Upon sequential extraction of gluten, 2*M* LiCl extracted twice as much protein from the residue remaining after the initial extraction with 70% ethanol.

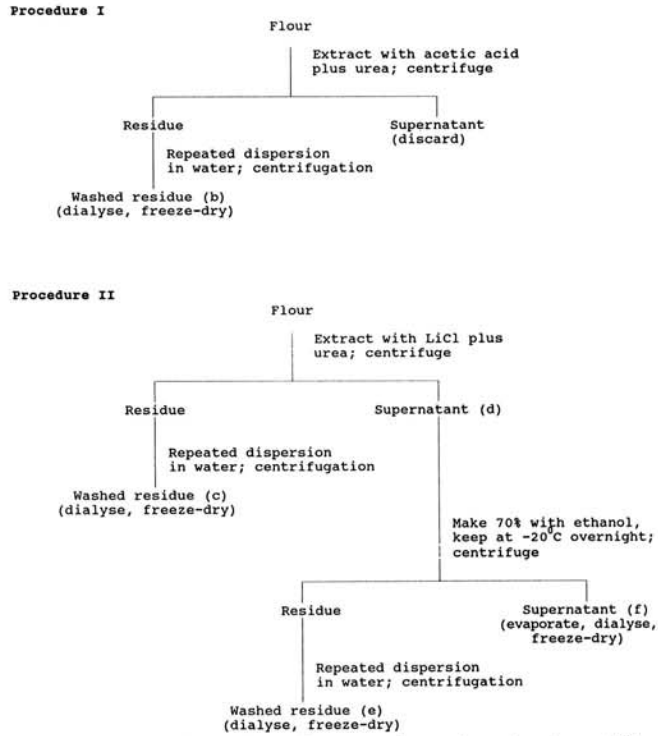

Fig. 1. Summary of procedures for preparing various fractions of flour proteins. Letters in parentheses represent the lanes in Figure 2B and C.

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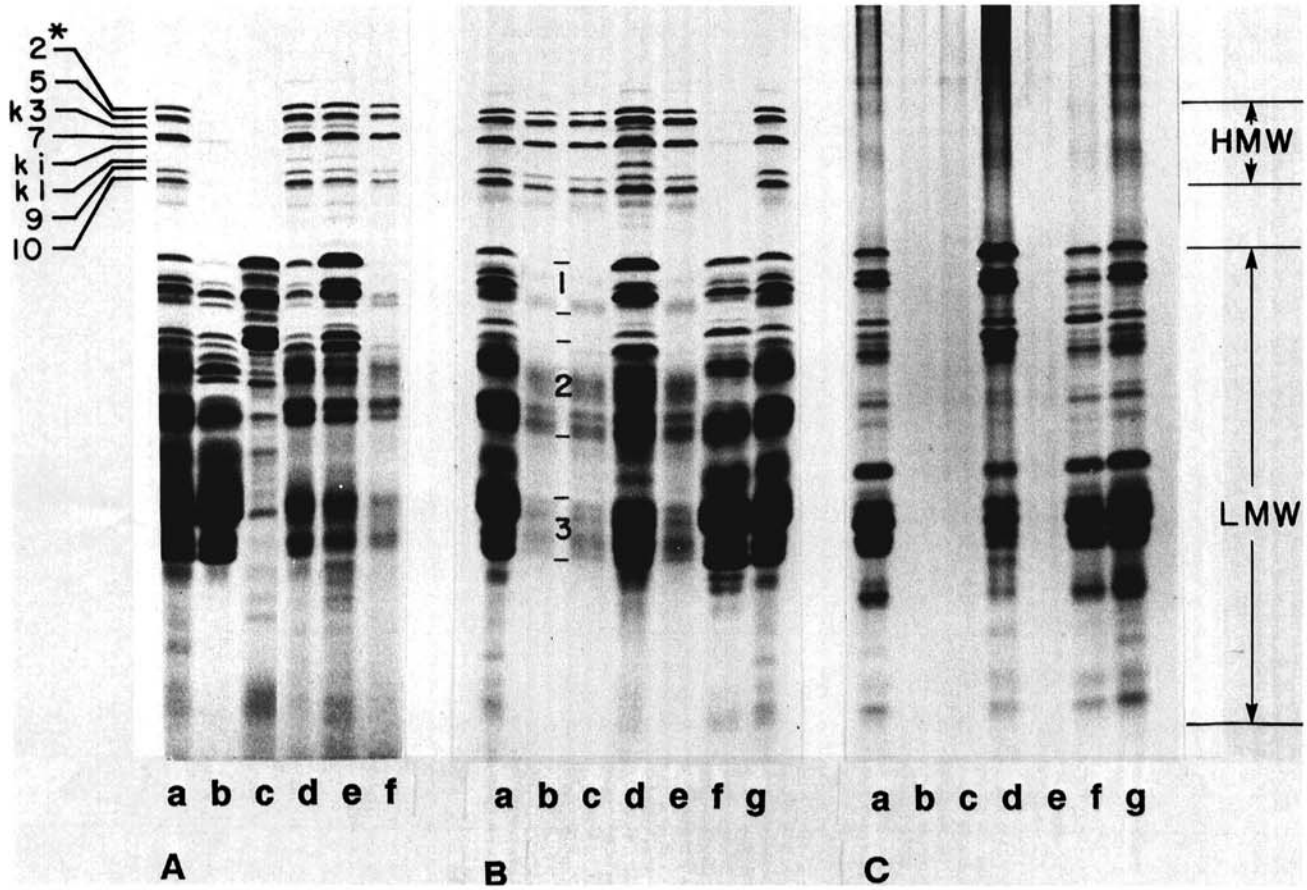

Fig. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) patterns of various protein fractions of Neepawa wheat flour. **A,** effect of lithium chloride (LiCl) vs. ethanol. a = direct SDS-mercaptoethanol extract of flour (control), b = 70% ethanol extract of flour after sequential extraction with water and 0.5*M* NaCl, c = 2*M* LiCl extract of residue from b, d = 0.1*M* acetic acid-6*M* urea extract of residue from c, e = 0.1*M* acetic acid-6*M* urea extract of residue from b, f = residue from d. **B** and **C,** analysis of fractions obtained by procedures shown in Figure 1 under reducing and nonreducing conditions, respectively. a and g = direct SDS-mercaptoethanol extract of flour (control), b = residue after extraction of flour with 0.1*M* acetic acid-6*M* urea (Fig. 1, procedure I). c = residue after extraction with 2*M* LiCl-6*M* urea, d = 2*M* LiCl-6*M* urea extract of flour (at twice the concentration of protein in the other lanes), e = precipitate from ethanol addition, f = proteins in ethanolic supernatant (Fig. 1, procedure II).

The utility of LiCl for solubilizing flour proteins is demonstrated further by the results (Fig. 2B and C) for fractions from the two procedures summarized in Figure 1.

The SDS-PAGE patterns for the three glutenin preparations (b, c, and e) contained all the five high molecular weight (HMW) glutenin subunits and bands that represent low molecular weight glutenin subunits. The sections in which the latter proteins are located are marked with 1, 2, and 3 in Fig. 2B (compare lanes b, c, and e). Under nonreducing conditions these proteins, together with the HMW glutenin subunits, remained on the top of the gels, which indicates that all three glutenin preparations were free of gliadins (compare lanes b, c, and e in Fig. 2B and C). It is also interesting to compare the pattern of the LiCl-urea extract with the pattern of the 70% ethanol extract (lanes d and f in Fig. 2B and C), which shows that the latter is free of HMW glutenin subunits. It is noteworthy that after ethanol precipitation, the K proteins, present in the LiCl-urea extract, are not seen in the patterns (compare lanes d and e in Fig. 2B). It is likely that ethanol, like some detergents (Kazemie and Bushuk 1990), might cause K proteins to aggregate and thereby prevent them from moving into the gel. These results suggest that for studies in which the structure of glutenin is the primary concern, the use of ethanol and detergents should be avoided. It would be misleading to attempt to interpret the results obtained after such treatments in terms of molecular structure.

Considering the solubility of glutenin, it is reasonable to assume that residues b and c in procedures I and II, respectively (Fig. 1), contain the largest molecules of glutenin. This characteristic would render the residue a more suitable candidate for structural studies.

The procedures described above provide a simple alternative to the extraction methods already in use for the preparation of soluble and insoluble (residual) glutenin. LiCl plus urea represents one of the good solvents for protein extraction, especially when it was necessary to obtain active structural proteins for reconstitutional and functional studies (Kazemie 1975, 1976).

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