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Agarose Gel Isoelectric Focusing of Gliadins in the Presence of Ethanol

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ABSTRACT

Conditions for the analysis of gliadins by isoelectric focusing in the presence of ethanol were worked out. Comparison of the electrophoretic band patterns obtained in aqueous and ethanolic media showed that the resolution was highly improved in the presence of ethanol. The gliadins were identified by immunological detection in nitrocellulose blots obtained from the agarose gels.

INTRODUCTION

Gliadins constitute the gluten protein fraction that is usually extracted from wheat flour with 70% ethanol (Osborne, 1907). Other alcohols have been used as well (Byers *et al.*, 1983). Currently, these proteins are analysed by polyacrylamide gel electrophoresis (PAGE) at pH 3·1 in the absence (Maier & Wagner, 1980) or in the presence (Byers *et al.*, 1983) of sodium-dodecylsulphate (SDS). Isoelectric focusing (IEF), possibly followed by PAGE at right angles to it, has also been used for the analysis of gliadins (Wrigley, 1970). These analyses are performed in aqueous media where

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gliadins are poorly soluble. Therefore, it would be of advantage to perform the electrophoretic analysis in less polar solvents. The present paper describes a method for the analysis of gliadins by IEF in agarose gels in the presence of ethanol.

MATERIALS AND METHODS

The wheat used, of the Drabant variety, was kindly supplied by Weibulls (Landskrona, Sweden). Agarose IEF, Pharmalytes 3-10, gelbond film, paper applicators and pI calibration kit 3-10 were from Pharmacia AB (Sweden); nitrocellulose filter, 0·22 μm, was from Biorad (Richmond, CA, USA), blotting paper from Stora (Sweden); and the non-fat milk powder from Semper (Sweden). Rabbit antibodies (IgG) against gliadins (product No. 53940) were from Calbiochem (San Diego, CA, USA) and porcine peroxidase conjugated antirabbit antibodies were from Dakopatts (Denmark).

Extractions

The extraction procedure of Osborne (1907), modified by Chen and Bushuk (1970), was used to remove the albumins and globulins and to isolate the gliadins. The extraction of 3·5 g of wheat flour with 35 ml of liquid was performed by magnetic stirring in a centrifuge bottle: twice with 0·5M NaCl and twice with distilled water, in each case for 1 h at 4°C; and, finally, twice with 70% (v/v) ethanol for 30 min at room temperature (about 20°C). The centrifugations were performed at 5000 × *g* for 30 min. The alcoholic gliadin fractions were pooled and stored as a frozen solution at −20°C or in a freeze-dried state.

Isoelectric focusing

IEF was run in 1% agarose with 6·3% Pharmalyte 3-10 in 45% (v/v) ethanol, which was found to be the highest possible ethanol concentration not causing precipitation of agarose or Pharmalyte. The gel was prepared by dispersing 0·3 g of agarose in 15 ml of boiling water. The mixture was cooled to 75°C, after which 15 ml of preheated 99·5% ethanol and 1·9 ml of Pharmalyte 3-10 were added. The suspension was poured on to prewarmed gelbond film (24 cm × 12 cm), cooled to 4°C and left for at least 1 h to complete the gelation. The gel was stored in a humid chamber, saturated with 45% ethanol, overnight at 4°C. It could also be used directly. All

samples were applied on paper applicators at the cathodic side of the gel. The freeze-dried samples were dissolved in 45% (v/v) ethanol, and the frozen extracts were applied directly after thawing. The electrode solutions were 1 M NaOH and 50 mM H_2SO_4 in 45% (v/v) ethanol for the cathode and anode, respectively. The IEF was carried out at 5 W for 3000 Vh on an LKB Multiphore electrophoresis apparatus at 10°C. The pH gradient was then measured with a surface pH electrode (Multiphor Electrode, LKB, Bromma, Sweden). As a control, the experiment was also performed in agarose gels prepared without ethanol.

Immunological detection

The proteins of the IEF gel were transferred to a nitrocellulose (NC) filter by overlaying the gel with a stack consisting of a wet NC filter, a wet blotting paper, eight layers of dry blotting paper, a glass plate and, on top, a 3 kg weight applied for 20 min at room temperature. The immunological detection of gliadins on the NC filters was carried out according to Towbin *et al.*, as described by Andrews (1986). Instead of bovine serum albumin, 10% non-fat milk in Tris buffer saline (TBS), 10 mM Tris-HCl, pH 7·4, containing 0·9% NaCl, was used. The immunoglobulins were diluted 1:500 with 10% non-fat milk in TBS supplemented with 10% (v/v) cow sera. The peroxidase staining was performed according to Hawkes *et al.*, as described by Andrews (1986). The immunoblots were scanned with an LKB 222 laser gel scanner. Reference proteins of the calibration kit were stained with 0·2% Coomassie Brilliant Blue R 250 in 30% ethanol and 10% acetic acid.

RESULTS

Ethanol concentrations exceeding 45% (v/v) caused precipitation of the agarose during the preparation of the gel. Therefore, 45% (v/v) ethanol was chosen for the analysis of gliadins. The freeze-dried gliadins, dissolved in ethanol, and gliadins kept as frozen extracts produced similar IEF band patterns when immunoblotted, showing more than 20 discrete bands (Fig. 1 (a) and (b)). A blurred and poorly resolved band pattern was obtained when IEF was performed in the absence of ethanol (Fig. 1(c)). The scanning of the immunoblots (Fig. 2) showed that, upon IEF in the presence of ethanol, reproducible and well-resolved peaks of the gliadins were obtained. In 45% ethanol Pharmalyte 3–10 produced a pH gradient in the range 1–9 from the anodic to the cathodic side, as measured with the surface electrode. The pI values in 45% ethanol for the IEF protein markers of the calibration kit did not coincide with their expected values in water.

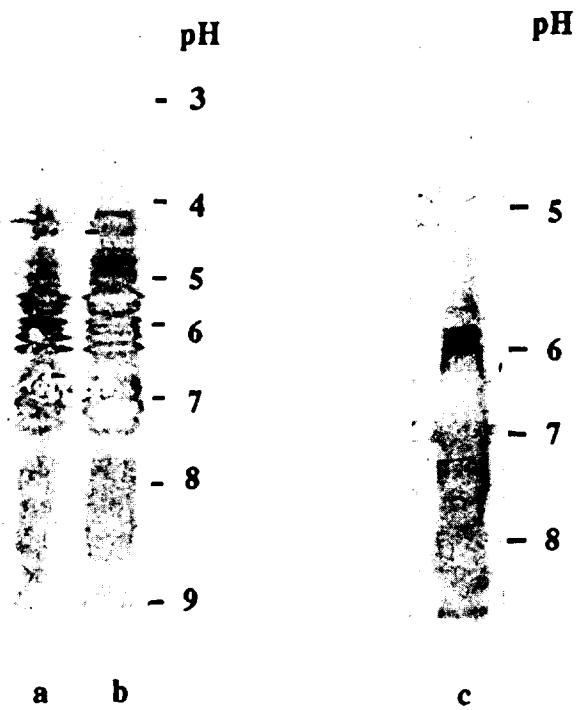

Fig. 1. Immunoblots of the gliadins after IEF in agarose gels in the presence of 45% ethanol (a and b) and in the absence of ethanol (c). The gliadins were extracted with 70% ethanol from albumin and globulin depleted wheat flour. (a) frozen extract, (b) freeze-dried extract, (c) freeze-dried extract.

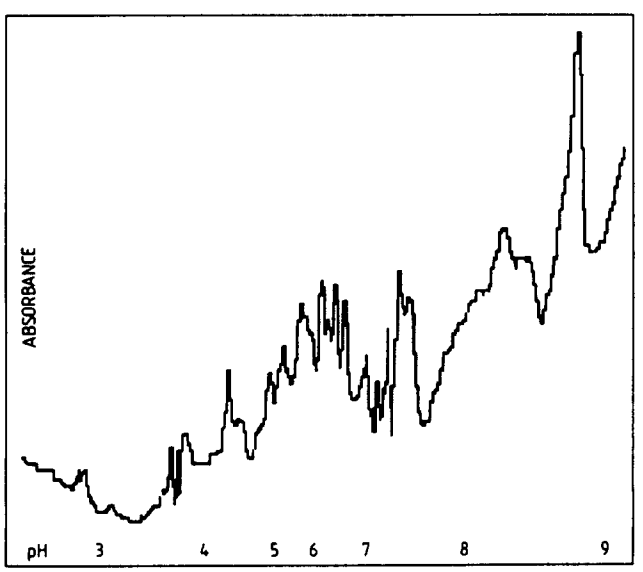

Fig. 2. Gel scan of the immunoblot illustrated in Fig. 1(b).

DISCUSSION

From the results it is evident that, in the analyses of gliadins by IEF, a better resolution could be achieved if 45% ethanol was included in the separation medium.

The pK values of ionizable groups depend upon the polarity of the solvent; therefore, the pI values of the various gliadins in 45% ethanol are expected to differ from their values in water. It is reasonable to suggest that these pK shifts, together with the weakened hydrophobic interactions between the proteins, are decisive factors in achieving a better separation of the gliadins by IEF if ethanol is included in the separation medium.

Vecchio *et al.* (1984) and Artoni *et al.* (1984) previously worked out conditions for electrophoresis of hydrophobic proteins using acryl-morpholine polymers as the matrix and sulfolane as the solvent. In our work, agarose gels combined with ethanol as solvent offer a mild environment for the separation of gliadins by IEF. Moreover, agarose gels are easy to handle; they are suitable for immunological and preparative purposes and they are non-toxic. IEF in the presence of ethanol allows high resolution of the various gliadins in one single step. In combination with immunoblotting and gel scanning, improved possibilities are obtained for identification of wheat varieties and for quantitative and qualitative analyses of the gliadin components. These parameters are important for the industrial use of wheat flour.

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REFERENCES

Andrews, A. T. (1986). *Electrophoresis, Theory, Techniques, and Biochemical and Clinical Applications* (2nd edn). Oxford University Press, New York.

Artoni, G., Gianazza, E., Zanoni, M., Gelfi, C., Tanzi, M. C., Barozzi, C., Ferruti, P. & Righetti, P. G. (1984). Fractionation techniques in a hydro-organic environment II. Acryloyl-morpholine polymers as a matrix for electrophoresis in hydro-organic solvents. *Analy. Biochem.*, **137**, 420–8.

Byers, M., Miflin, B. J. & Smith, S. J. (1983). A quantitative comparison of the extraction of protein fractions from wheat grain by different solvents, and of the polypeptide and amino acid composition of the alcohol-soluble proteins. *J. Sci. Food Agric.*, **34**, 447–62.

Chen, C. H. & Bushuk, W. (1970). Nature of proteins in *Triticale* and its parental species. I: Solubility characteristics and amino acid composition of endosperm proteins. *Can. J. Plant Sci.*, **50**, 9–14.

Maier, G. & Wagner, K. (1980). Routinemethode zur Identifizierung von Weizensorten mit Hilfe der Polyacrylamidgelelectrophorese. *Z. Lebensm. Unters. Forsch.*, **170**, 343–5.

Osborne, T. B. (1907). *The Proteins of the Wheat Kernel.* Publication no. 84, Carnegie Inst., Washington, DC, pp. 1–119.

Vecchio, G., Righetti, P. G., Zanoni, M., Artoni, G. & Gianazza, E. (1984). Fractionation techniques in a hydro-organic environment I. Sulfolane as a solvent for hydrophobic proteins. *Analy. Biochem.*, **137**, 410–19.

Wrigley, C. W. (1970). Protein mapping by combined gel electrofocusing and electrophoresis: Application to the study of genotypic variations in wheat gliadins. *Biochemical Genetics*, **4**, 509–16.