Short Communications

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Zone electrophoresis of wheat gluten on polyacrylamide gels

The electrophoresis of wheat gluten on starch gel has been reported by a number of workers[1–3] and up to 20 bands have been reported using aluminium lactate–lactic acid buffers at pH 3–3.3. PENCE AND MECHAM[4] believe that not all components reported arise from gluten but that some are water-soluble proteins resulting from incomplete extraction with sodium pyrophosphate. One of the more serious drawbacks of the starch-gel technique is the difficulty encountered in attempting to quantitate results. This difficulty is largely overcome by the use of acrylamide gels which are transparent and can readily be scanned in a densitometer after staining. Starch gel can also be rendered transparent for scanning[5,6] but the technique requires thin sections and is very time consuming. The system described here differs from those used previously with gluten not only in the nature of the gel but also in that the pH is on the alkaline side of the isoelectric point.

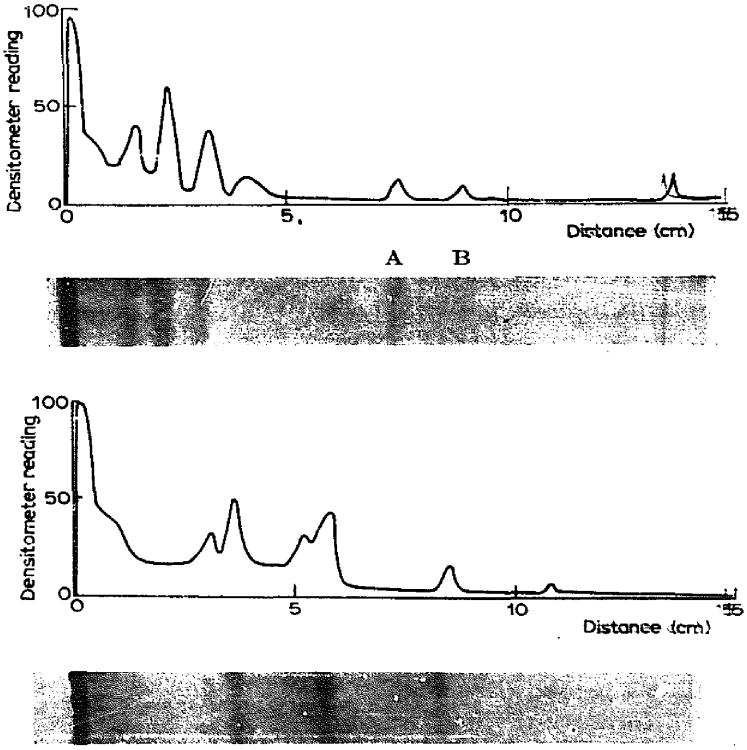

Fig. 1. Polyacrylamide gel electropherograms of wheat gluten. Gels run at pH 8.6 in 2 *M* urea, 50 V/cm, 10°. Upper run, 90 min; lower, 300 min. The corresponding densitometer curve is above each photograph.

Acetic acid-soluble protein (gluten) was extracted from flour or from wheat meal by the procedure of COATES AND SIMMONDS[7] omitting the preliminary *n*-butanol treatment. For electrophoresis freeze-dried samples (2.5 %, w/v) were dispersed in 2.5 *M* urea. The buffer used in both the gels and the electrode vessels was 0.015 *M* Tris (Sigma Chemical Company, St. Louis, Mo.), 3 m*M* citric acid, 8 m*M* boric acid with sufficient LiOH to give a final pH of 8.6 and made 2 *M* with respect to urea. Gels containing 5 % (w/v) Cyanogum 41 (American Cyanamid Company, New York, N.Y.) were prepared as described by RAYMOND AND WANG[8]. Electrophoresis was carried out in a vertical gel apparatus (E-C Apparatus Company, Swarthmore, Pa.). Runs were conducted for either 90 or 300 min at approx. 50 V/cm. With a voltage gradient of this order the current carried by the gel dropped from 200 mA at the beginning of the run to approx. 60 mA after 1 h and then remained relatively constant. After completion of the run the gels were removed from the apparatus and stained with water-soluble nigrosin (0.0125 %) in methanol–water–acetic acid (4:5:1) for 3 h with constant agitation. Nigrosin stains gluten proteins much more clearly and more permanently than the commonly used amido black. Destaining was carried out first with methanol, water, acetic acid then with 5 % aqueous acetic acid which restored the gel to its original size. Before scanning the gels were soaked in 20 % aq. glycerol to prevent the gel drying out. A Photovolt recording densitometer modified to handle gels was used for scanning. The recorder was set on logarithmic response to give readings essentially in terms of absorbance.

Fig. 1 shows 2 gels run for 90 and 300 min, respectively. Above each gel is the corresponding densitometer curve.

In the 90-min run, two relatively fast moving bands labelled A and B can be seen. These bands were absent when butanol was included in the protein extraction method. In the 300-min run these bands have passed right through the gel but better resolution is observable in the slower-moving components. When fractions of the gluten proteins obtained by chromatography on carboxymethyl-cellulose were run on acrylamide gel many bands were observed which could not be seen in the whole extract because of their relatively low concentration. The characterization of these chromatographic fractions is to be published shortly.

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[1] J. H. WOYCHIK, R. J. DIMLER AND F. R. SENTI, *Arch. Biochem. Biophys.*, 94 (1961) 477.
[2] G. A. H. ELTON AND J. A. D. EWART, *J. Sci. Food Agr.*, 13 (1962) 62.
[3] C. B. COULSON AND A. K. SIM, *Biochem. J.*, 80 (1961) 46P.
[4] J. W. PENCE AND D. K. MECHAM, *Wallerstein Lab. Commun.*, 25 (1962) 37.
[5] J. GROULADE, J. M. FINE AND C. OLLIVIER, *Nature*, 191 (1961) 72.
[6] E. VAHVASELKÄ, *Nature*, 193 (1962) 474.
[7] J. H. COATES AND D. H. SIMMONDS, *Cereal Chem.*, 38 (1961) 256.
[8] S. RAYMOND AND Y. WANG, *Anal. Biochem.*, 1 (1960) 391.

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