Visualising Wheat Gluten Proteins

Staining Profile Depends on the Method Used

Gluten proteins from wheat are important for the determination of dough quality concerning bread making. A negative aspect of these proteins is that they can contain peptide sequences that can cause coeliac disease. These gluten proteins are composed of high amounts of the amino acids proline and glutamine, which can influence the staining behaviour. A combination of staining methods is necessary to identify proteins involved in celiac disease.

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Bread Wheat

Wheat is a major food crop used for the preparation of bread and pasta. Wheat gluten extracts are increasingly used as an additive in many food products such as soups, sauces, candies, ice cream etc. Nowadays, wheat is bred for high quality, high yield, and disease resistance, which has led to thousands of different wheat varieties. High quality of bread wheat is determined by composition of gluten proteins. These proteins have the capability to form a matrix by inter- and intra-molecular disulfide linkages. During dough preparation, air is captured within this matrix, which gives bread its volume and structure [1].

Gluten Proteins

Gluten proteins from wheat are composed of monomeric gliadins and polymeric glutenins. Gluten proteins are composed of a large number of the amino acids proline and glutamine and only a small number of arginine, lysine, and histidine [2]. Gliadins have the characteristic to be soluble in alcohol solutions and glutenins are soluble in alcohol solutions containing a reducing agent and in diluted acid or alkali [3]. A negative aspect of gluten proteins from wheat, rye,

and barley is that they can contain immune responsive peptide sequences that can cause coeliac disease (CD). After consumption of gluten-containing foods, these peptides trigger an immune response that causes damage to the small intestine in CD-patients. This leads to a range of symptoms such as diarrhoea and malabsorption [4].

Protein Staining Methods

Protein staining methods differ in the interaction of the dye with the amino acids of the protein, the detection sensitivity, whether the method is end-staining, how the stain is detected and in the complexity of the method. Most methods are based on staining proteins after separation by gel electrophoresis, either by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) or by 2-dimensional gel electrophoresis (2-DE). Traditional Coomassie Brilliant Blue (CBB) R-250 staining is a simple method and interacts with lysine and arginine residues [5]. The procedure has a low detection sensitivity but is compatible with mass spectrometry. Colloidal staining with CBB G-250 (PageBlue) is much more sensitive than traditional CBB R-250 staining and does not require a destaining step. It is an end-point staining and interacts with lysine, arginine, histidine, and tyrosine residues [5]. The silver staining procedure is quite complex and over-development can occur. It interacts with lysine residues [5] and provides high sensitivity. Sypro Ruby is a fluorescence-based end-point stain with a high sensitivity and is compatible with mass spectrometry. The method is easy and interacts with lysine, arginine, and histidine [5]. An image is obtained by using a fluorescence scanner. CyDye DIGE fluor minimal dyes (Cy2, Cy3, and Cy5) are used in Difference in Gel Electrophoresis (DIGE) for labelling different protein samples prior to analysing them on the same gel [6]. This makes it possible to detect and quantify differences between experimental pairs of samples resolved on the same gel, and between gels. The method labels lysine residues, is very sensitive [7], and images are obtained by scanning the different fluorescence dyes. Immunoblotting is also a very sensitive detection method. Proteins are transferred to a membrane and specific proteins can be stained by using antibodies raised against these proteins.

Staining Efficiency of Wheat Gluten Proteins

Gluten proteins were extracted from different wheat varieties and separated by gel electrophoresis. For details of the used methods, see [8]. Because of similar molecular masses, many gliadins share a mobility region with low molecular weight glutenin subunits (LMW-GS) if analysed by SDS-PAGE (fig. 1). 2-DE results in increased resolution of these proteins (fig. 2). All CBB R-250 stained gluten proteins destained to some extent, as was already shown before [9]. The duration of destaining showed to be important for the resulting pattern (fig. 1A and 1B, boxed protein bands). When using CBB G-250, some proteins were poorly stained (fig. 1C, marked with arrows). Using silver nitrate staining, high molecular weight glutenin subunits (HMW-GS) were poorly stained compared to other gluten proteins (fig. 1D). The duration of development had to be increased, leading to over-development. Sypro Ruby resulted in better staining of HMW-GS (fig. 1E), however, with a speckled background staining.

As can be expected, immunoblotting using specific antibodies against CD immune responsive peptides showed that similarly abundant proteins of the same molecular weight can react different in immune staining, and that

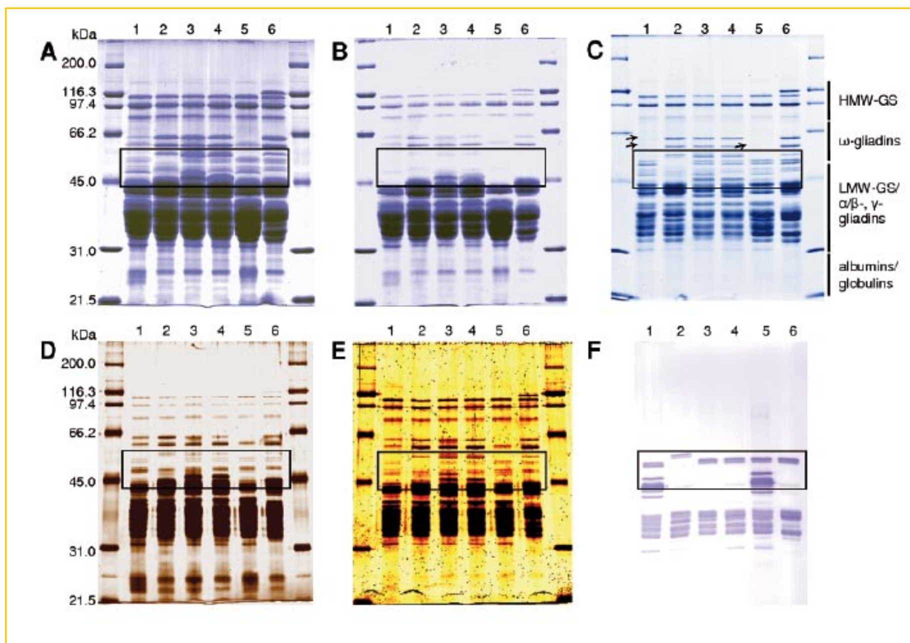

Fig. 1: SDS-PAGE (10 %) analyses of gluten protein extracts from hexaploid wheat varieties. 1. Bovictus; 2. Combi; 3. Sperber; 4. Rektor; 5. Toronto; 6. Ambras. Stained with: A) 15 µg protein, CBB R-250 and 2 hours destaining. B) 15 µg protein, CBB R-250 and 16 hours destaining. C) 2 µg protein, CBB G-250 (PageBlue). Arrows indicate poorly stained proteins. D) 1 µg protein, silver nitrate. E) 1 µg protein, Sypro Ruby. F) Immunoblot using monoclonal antibody against T-cell epitope Glia-α20.

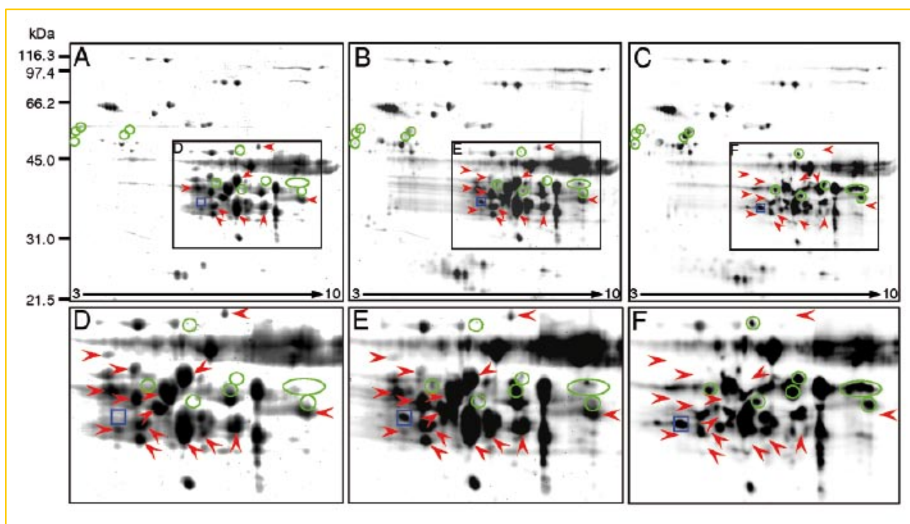

Fig. 2: 2-DE of gluten proteins from hexaploid wheat variety Rektor, 50 µg protein labelled with Cy3 combined with 100 µg unlabeled protein. IEF performed on 24 cm linear IPG strip pH 3-10, followed by SDS-PAGE (10 %). A) CBB R-250. B) Sypro Ruby. C) Cy3 signal. Red arrowheads represent spots selectively visualised with CBB R-250 and Sypro Ruby. Green circles represent spots detected with Cy3. The spot indicated with the blue square is detected with Sypro Ruby and Cy3, but not with CBB R-250. D, E, and F) Enlargements of boxes in A, B, and C, respectively.

some low abundant proteins have a strong immune response (fig. 1F).

Because gluten proteins contain only small amounts of lysine, arginine, and histidine, the efficiency of CyDye labelling of specific protein spots showed to be affected (fig. 2). Some gluten proteins were not labelled (fig. 2) and, on the other hand, some other proteins were labelled that were not visualised by Sypro Ruby or CBB R-250.

Conclusion

For visualising gluten proteins, all staining methods described here are applicable, though prominent selective staining is observed for each method. Because of the aberrant amino acid composition and solubility of wheat gluten proteins, the staining method used to visualise the proteins in 1D or 2D gels affects the resulting protein pattern. In CD research the most important criterion in the selection of the staining method is that as many gluten proteins as possible should be visualised, including proteins present in small amounts, as all of them may contain CD immune responsive epitopes.

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