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Rapid identification and quantitation of high-molecular-weight glutenin subunits

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Abstract

Knowledge of glutenin-subunit composition is important for the prediction of the genetic potential of breeding lines for dough quality. In screening for quality using the *Glu-1* scoring system, the high-molecular-weight glutenin subunits (HMW-GS) are especially valuable. This information is needed at the earliest stages of breeding to ensure that poor-quality lines are not propagated. Conventionally, glutenin polypeptides have been identified by SDS gel electrophoresis, but this method is slow, labour-intensive and only semi-quantitative. The recent Lab-on-a-Chip technology provides faster micro-fluidic analysis of these proteins at 1 min per analysis (3 min, given instrument conditioning time). To screen breeding lines for dough quality, the Lab-on-a-Chip approach offers quick quantification of specific glutenin subunits with computerized interpretation. To achieve these objectives, we have allocated subunit identities to the peaks in the Lab-on-a-Chip sample profiles, using multiple-deletion lines of wheat and varieties of known composition. The positions of HMW-GS can be used to identify the composition of unknown varieties and breeders' lines by computerized comparison against this established library of profiles from the Lab-on-a-Chip.

Keywords: Wheat-protein composition; Dough-quality prediction; Capillary electrophoresis; Glutenin subunits; Lab-on-a-Chip; High-molecular-weight glutenin subunits

1. Introduction

The glutenin fractions of the wheat storage proteins are known to be responsible for the main differences in bread-making quality. Native glutenin is a macropolymer built from subunits by the formation of intermolecular disulfide bonds. Reduction of the disulfide bonds facilitates the study of component proteins. The amounts, the size distribution of the glutenin subunits and the type of high-molecular-weight glutenin-subunit (HMW-GS) present influence end-product quality (Gupta et al., 1993; Huang and Khan, 1997; Payne et al., 1987). Both HMW-GS and low molecular weight glutenin-subunit (LMW-GS) composition are valuable for predicting the genetic potential of breeding lines for dough quality. For many years, HMW-GS have been especially important for quality screening, using the *Glu-1* scoring system (Payne et al., 1987). Breeders dealing with large numbers of lines particularly need this information to predict the dough-strength potential at the earliest stages of breeding, to ensure that poor-quality lines are not propagated unnecessarily.

For several decades, SDS gel electrophoresis has been used extensively for analysing glutenin-subunit composition, but the procedure is slow, labour-intensive, non-quantitative and difficult to interpret (especially for the LMW subunits). Reversed-phase high-performance liquid chromatography (RP-HPLC) has also been used (Marchylo et al., 1989). More recently, size-based capillary electrophoresis has been used to separate HMW-GS (Bean and Lookhart, 1998, 1999; Sutton and Bietz, 1997; Werner et al., 1994; Zhu and Khan, 2001). Because of their complexity, each of these analytical methods are suited only to well equipped laboratories, and in addition back-up resources and trained operators are needed. SDS-PAGE and RP-HPLC are slow. Automatic sampling improves the efficiency of HPLC and capillary electrophoresis by permitting 24-h operation. Those interested in subunit composition would be well served if more efficient methods of analysis could be devised, especially if the equipment could

Abbreviations: DMSO, dimethyl sulfoxide; DTT, dithiothreitol; HMW-GS, high-molecular-weight glutenin subunits; KDa, kilo Dalton; LMW-GS, low-molecular-weight glutenin subunits; RSD, relative standard deviation; RP-HPLC, reversed-phase high-performance liquid chromatography; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis.

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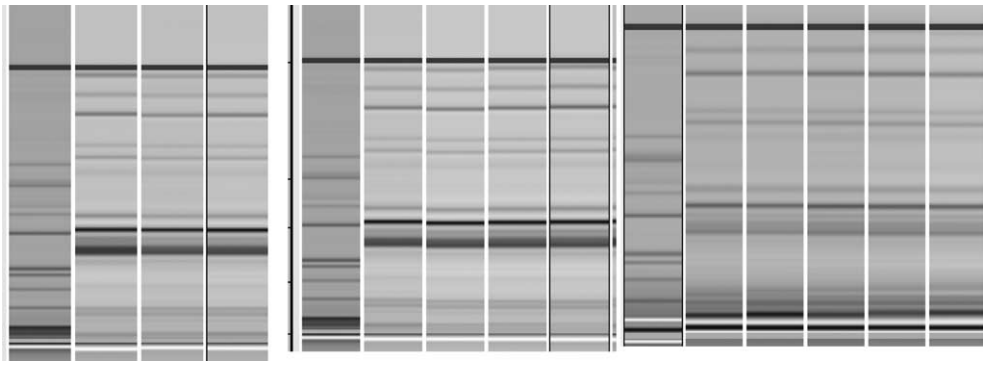

Fig. 1. Lab-on-a-Chip capillary electrophoresis of wheat-flour proteins (all Rosella variety) showing the reproducibility of the procedure. The group at the left shows patterns for Rosella extracts, grown at three different sites. The central set shows patterns for four different extracts of the same sample. The set at the right shows patterns for five applications of the same extract on five different chips.

be deployed beyond the conventional laboratory. Such innovation should deliver quantitative results quickly, with automatic and immediate interpretation, involving equipment that is simple to operate. The Lab-on-a-Chip procedure (Uthayakumaran et al., 2005), a micro-fluidic method based on the principles of size-based capillary electrophoresis, has been explored as a possible tool to fulfil these specifications.

2. Materials and methods

2.1. Wheat samples

Authentic samples of Australian varieties, kindly supplied by Australian Winter Cereals Collection, Tamworth, NSW, were used as a basis for identifying peaks in the glutenin-subunit profiles. Additional assistance in peak identification was provided by use of multi-null lines lacking specific glutenin subunits (Lawrence et al., 1987). Well characterised breeders' lines were also used to evaluate the system.

2.2. Extraction of HMW-GS

Wholemeal or flour samples (20 mg) were extracted once with 1 ml dimethyl sulfoxide (DMSO) and twice with 1 ml 50% propan-1-ol to remove gliadin, albumin and globulin proteins (mixing each time on a vortex mixer for 10 s and centrifuging 10 min at 16,000 *g*). The full range of glutenin subunits was then extracted at 65 °C for 30 min with 50, 100, 200, 300 and 400 µl of 1% SDS solution containing 1% dithiothreitol (DTT) followed by centrifugation for 10 min. A final extraction volume of 300 µl was selected as optimal, based on the resolution and intensities of peaks reported by the Agilent software, especially in the LMW region. Each clarified extract (4 µl) was mixed with 2 µl of Agilent sample buffer and 84 µl of deionised water. This mixture (6 µl) was applied to one of the 10 sample wells on the Agilent LabChip.

2.3. Analysis of samples

Proteins, extracted as described in Section 2.2, were analysed in an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA) with a Protein 200+ chip. Each sample contained an internal standard comprising an upper marker of 227 kDa and a lower marker of 6 kDa. Each chip included a ladder comprising reference proteins of 9, 14.5, 28, 46, 63.5, 95, 158 kDa, plus the upper and the lower markers (6 and 227 kDa), against which protein mobilities were compared for each analysis.

3. Results

3.1. Electrophoretic patterns and quantitative profiles of glutenin subunits

The Lab-on-a-Chip system provides results similar to size-based capillary electrophoresis, so that the HMW and LMW subunits appear as two groups of bands in the simulated gel patterns (Figs. 1 and 2) or as two distinct groups of peaks in the elution profiles (Fig. 3). Preliminary extraction with DMSO–propanol is required to remove non-glutenin proteins, which

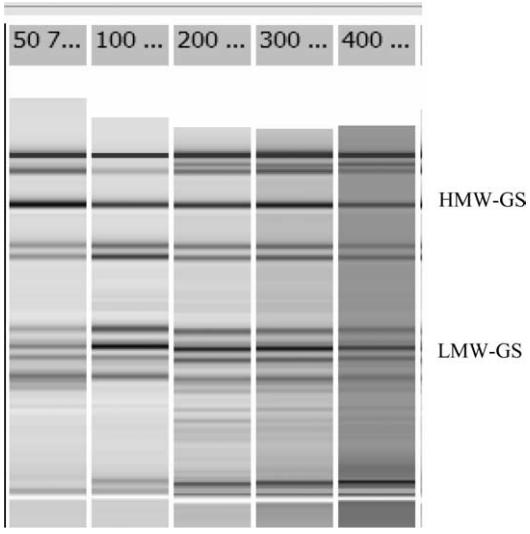

Fig. 2. Lab-on-a-Chip capillary electrophoresis of glutenin subunits extracted with different volumes (as shown) of 1% SDS + 1% DTT (after extracting non-glutenin proteins with DMSO). Elution profiles have been computer-manipulated to simulate gel-electrophoresis patterns.