Rapid wheat varietal identification using the Agilent 2100 bioanalyzer and automated pattern-matching

Application Note

Dr Dhan Bhandari

Abstract

Accurate identification of wheat varieties is of paramount importance to the milling industry in many countries. This Application Note describes how the Agilent 2100 bioanalyzer and the Protein 230 assay can be used in conjunction with a third-party software, to analyze wheat proteins for varietal identification.

Agilent Equipment:

• 2100 Bioanalyzer Protein 230 Kit

Application Area:

• Food analysis

Agilent Technologies

Introduction

Authentication of varieties is important for the cereal industry for maintenance and testing of grain quality to meet market requirements. The charge-based separation of proteins by the acid-PAGE (polyacrylamide gel electrophoresis) technique is widely used for wheat varietal identification. However, this requires highly skilled operators to prepare, run and scan the gels and interpret band patterns. Also, there are safety concerns regarding the toxicity of unpolymerised acrylamide. While acid-PAGE is effective in analytical laboratories, the routine method can take up to two days. This is too slow for use at mill intake, which requires assessment of the wheat shipment during the period of delivery – typically under one hour. In this Application Note we demonstrate the use of the Agilent 2100 assay, with bioanalyzer and Protein 230 the Nonlinear Dynamics' Phoretix 1D Advanced (TotalLab TL120 DM) computerized pattern-recognition software to provide a better alternative to acid-PAGE. The aim of this study was to develop a robust, automated method for rapid identification of wheat varieties.

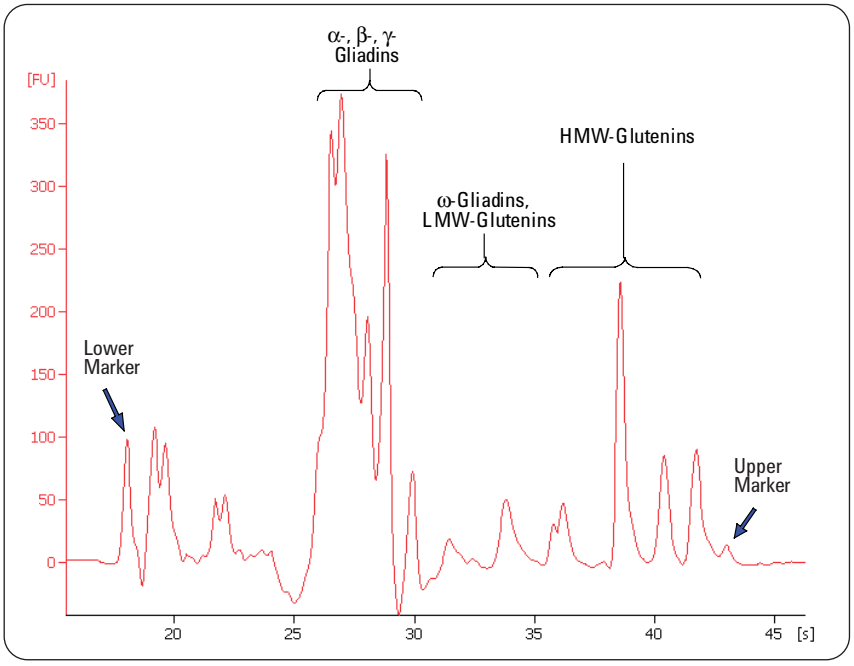

Figure 1
Typical wheat protein separation by the Protein 230 assay.

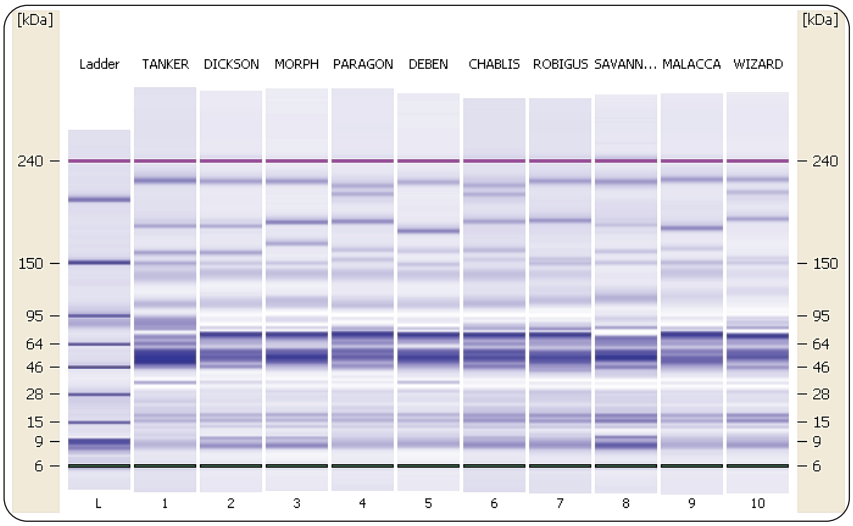

Figure 2
Bioanalyzer gel-like image of MW standards and 10 different wheat varieties.

Methods

Total wheat proteins (including glutenins) were extracted from individual grains in 0.4 mL of 2M urea, 15 % glycerol, 0.1 M DTT and 0.1 M Tris/HCl, pH 8.8, using an ultra-sonic water bath for 15 minutes. Extracts were centrifuged at 11,000 g for 5 minutes and treated with the Protein 230 assay reagents in according to the assay protocol. The samples were separated on the Agilent 2100 bioanalyzer, with each analysis of 10 samples plus ladder taking less than 25 minutes. Replicates of 34 wheat cultivars representing the 2004/5 UK Recommended List varieties were analyzed. The electropheromram profiles were processed using the Phoretix 1D Advanced and 1D Database (Nonlinear Dynamics) software for pattern-matching purposes.

Results

The Protein 230 assay produced well-resolved protein profiles, suitable for varietal discrimination (figures 1 & 2). The Phoretix software was able to compare the electropherogram profiles. Figure 3 shows an example of a dendrogram where all the replicates of three different varieties are correctly grouped. A prototype wheat library was developed by selecting the most representative varietal profile. Results showed that 90 % of test samples could be identified within the top three matches. Work is in progress to optimize the performance of this library. The practicality of the method and the robustness of the system is bourne out by the fact that the system is now in routine use in UK commercial mill intake laboratories.

Conclusions

Our study has demonstrated that using the Agilent 2100 bioanalyzer with the Phoretix system offers a standardized, objective method for rapid varietal discrimination. The ease of use and total analysis time of less than 50 minutes makes it most suitable for mill intake use. The optimized system will enable millers to make more confident decisions in accepting grain consignments, and could become widely adopted as an effective policing tool within the grain industry. A number of UK mills have purchased the combined systems for screening wheat deliveries at intake.

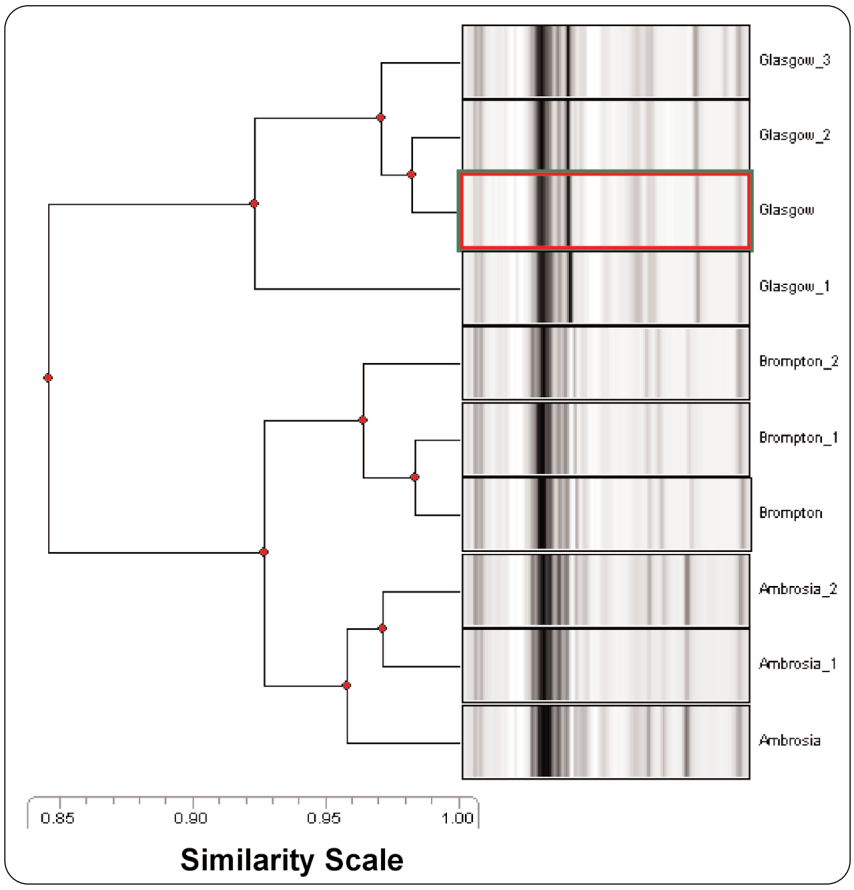

Figure 3
Dendrogram illustrating pattern-matching of replicate profiles of 3 wheat varieties.

Acknowledgements

CCFRA is grateful to the National Association of British and Irish Millers (nabim) for sponsoring this study.

Dr Dhan Bhandari is Senior Scientist at Campden & Chorleywood Food Research Association (CCFRA), Chipping Campden, Gloucestershire, GL55 6LD, UK.

---

www.agilent.com/chem/2100

Published March 1, 2016
Publication Number 5989-7735EN

Agilent Technologies