Multivariate RP-HPLC Analysis as a Tool in Quality Studies in Durum Wheat*

W. J. Rogers[1,4], J. L. Robutti[2], S. E. Lerner[1], A. M. Di Martino[2], N. R. Ponzio[1], F. S. Borrás[2], M. Cogliatti[1], M. L. Seghezzo[3] and E. R. Molfese[3]

[1]Facultad de Agronomía, Universidad Nacional del Centro de la Provincia de Buenos Aires, Azul, Provincia de Buenos Aires, República Argentina; [2]Estación Experimental Agropecuaria Pergamino – INTA, Pergamino, Provincia de Buenos Aires, República Argentina; [3]Laboratorio de Calidad Industrial de Granos, Chacra Experimental Integrada Barrow MAGyAI – INTA, Tres Arroyos, Provincia de Buenos Aires, República Argentina; [4]Researcher of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) of Argentina, e-mail: rogers@faa.unicen.edu.ar (WJR), slerner@faa.unicen.edu.ar (SEL)

Abstract: Multivariate analysis of RP-HPLC data has been used in this study as a means of relating information derived from gliadin chromatograms to quality characteristics in durum wheat. In a study involving seven cultivars grown under distinct nitrogen and sulphur regimes, it was shown that for (*i*) grain sulphur content, (*ii*) grain protein content and (*iii*) Farinograph tolerance, strong correlations were detected between the observed values and those predicted from the most influential principal components (PC), whereas for a range of other quality characters no such correlations were seen. For (*i*) grain sulphur content, r^2 for the regression between observed mean values and the predicted values was 0.739 before normalising the data for total gliadin content and 0.710 after normalisation, meaning that total gliadin content was not significantly involved in the observed relationship for this trait; the highest loadings for the first PC were located at 23.6, 32.5 and 39.5 min in the positive sense and at 36.1 min in the negative, indicating that these were the gliadins that most varied with the variation observed in sulphur content. For (*ii*) grain protein content, there was an expected effect of total gliadin content, reflected in the difference in r^2 values obtained before and after normalisation: 0.780 and 0.411, respectively. Nonetheless, a significant correlation, though of intermediate magnitude, remained for the normalised data, where the principal gliadins involved eluted at 23.6, 26.9 and 39.5 min. For (*iii*) tolerance, r^2 before and after normalisation was 0.595 and 0.576, respectively, indicating that, as for sulphur content, total gliadin content did not strongly influence the observed relationships. In this case, the gliadins eluting at 23.3, 36.1 min (positively) and 32.5 min (negatively) were those with the highest loadings for the first PC. The three characters appear to be related in that they showed some common gliadin peaks with major influences. In contrast, the gliadin differences observed in this study did not appear to affect characters involved in evaluating gluten strength (gluten index and Farinograph energy level), and neither appeared to affect gluten content. These latter observations are consistent with previous evidence that it is the glutenins that exert a greater influence upon this aspect in durum wheat than the gliadins. It is concluded that multivariate PC analysis of gliadin chromatograms provides the means to identify which gliadin components are important in the determination of particular quality characteristics in durum wheat.

Keywords: RP-HPLC; gliadin; principal components; industrial quality; grain sulphur content; grain protein content; Farinograph tolerance; *Triticum turgidum* var. *durum* L.

*Mention of trademarks has been necessary for the HPLC analyses in order to enable comparison of the findings reported here with other work, but this does not indicate their endorsement over other products by the participating institutions.

Protein chromatograms generated by RP-HPLC of cereal flour extracts provide a wealth of information, as demonstrated by the pioneering work of Bietz (1983) and many subsequent publications (Bietz1990, for review). The interpretation of this information is demanding due to the complexity of the protein profiles obtained, and considerable debate has taken place regarding the statistical methods that should be employed in their analysis (Bietz *et al.* 1990). As a result of this debate, the use of multivariate approaches involving principal component (PC) analysis has emerged as a powerful tool in data interpretation, as shown in various crops: for example, in bread wheat (Simpson *et al.* 1989; Bietz *et al.* 1990; Bietz & Simpson 1992), in durum wheat (Bietz *et al.* 1990) and in maize (Robutti *et al.* 2000). A further example of the utility of PC analysis in durum wheat (*Triticum turgidum* L. var. *durum*) has recently been given by Rogers *et al.* (manuscript submitted), who applied these methods to gliadin chromatograms and characterised not only qualitative differences between cultivars, but also quantitative differences between fertiliser regimes. Furthermore, the specific gliadin peaks responsible for the differences were identified in each case, from consideration of the magnitude of their factor loadings.

The gliadins are not only of interest in their capacity to distinguish between cultivars or in their differential response to distinct fertiliser treatments; they are also of interest for their direct or indirect affects on quality, as has been repeatedly shown in both bread wheat (Rogers *et al.* 1989) and durum wheat (Payne *et al.* 1984). Indeed, Burnouf and Bietz (1984) developed a method based upon RP-HPLC for distinguishing durum wheats carrying γ-gliadin 42 from those carrying γ-gliadin 45, motivated by the observation that these variants are, albeit indirectly, associated with poor quality and good quality, respectively (Payne *et al.* 1984; Pogna *et al.* 1988).

The current article seeks to explore further the relationships between gliadin composition and quality by extending the previous multivariate analyses of Rogers *et al.* (manuscript submitted) to include the elucidation of the relationships between the gliadin profiles obtained in the durum wheat samples analysed and industrial quality characteristics. For each quality character, the most influential PCs were used to obtain a set of predicted values for the samples under study, which were then related to the observed values through regression analysis. Factor loadings were again utilised, this time as the basis to determine the gliadin peaks of major influence on quality.

MATERIALS AND METHODS

Cultivars. The cultivars of durum wheat (*Triticum turgidum* var. *durum* L.) studied were as follows, where the full name that includes the breeder of origin is followed in parentheses by a shortened form subsequently used in the text: the Argentinean cultivars Bonaerense Quilacó (Quilacó), Bonaerense Valverde (Valverde), Bonaerense INTA Cumenay (Cumenay), Bonaerense INTA Facón (Facón), Buck Ambar (Ambar) and Buck Topacio (Topacio), and the Chilean cultivar Chagual INIA (Chagual). All analyses were carried out using original seed kindly provided by the relevant breeder.

Experimental field trial. A field trial sown to achieve a final plant density of approximately 350 plants/m^2 was grown in 1998 towards the end of July in a typical Argiudol soil type in three replicate blocks in a randomised complete block design on the Experimental Farm of the Faculty of Agronomy, Universidad Nacional del Centro de la Provincia de Buenos Aires, Azul, Province of Buenos Aires, Argentina, map coordinates 36°49′53″ south, 59º53′23″ west. The following fertiliser treatments were applied to each cultivar: N0S0 (no applied nitrogen or sulphur fertiliser), N1S0 (applied nitrogen fertiliser only), N1S1 (both applied nitrogen and sulphur fertiliser) and N0S1 (applied sulphur fertiliser only). The amount of nitrogen fertiliser applied for the treatments N1S0 and N1S1 was adjusted according to soil analyses and aimed at achieving a grain yield of 6000 kg/ha, according to the model (kg/ha): applied N-$N0_3$ = 150 kg/ha N-$N0_3$ – available soil N-$N0_3$ at sowing, in two applications as urea, 30% at sowing and 70% at the end of tillering (Zadoks 31) (Zadoks *et al.* 1979). Together with the second application of nitrogen, 40 kg/ha of K_2SO_4 were applied for the treatments N1S1 and N0S1. Disease was controlled by fungicide application, particularly for fusarium at the heading stage (Zadoks 59). Weeds were controlled by early application of herbicides at the four/five leaf stage (Zadoks 14/15). Soil analyses gave an organic material content of 4.8%.

RP-HPLC. Sample Preparation and Extraction Procedures – Harvested grain from each plot (cultivar × fertiliser × replicate block combination) was ground in a Udy cyclone sample mill equipped with

a 1 mm sieve. Nondefatted flour samples (200 mg) were extracted with 3 ml of 70% ethanol (v/v) with agitation using a vortex for 1h in 10 ml polypropylene centrifuge tubes, followed by centrifugation for 30 min at 3000 × g. Supernatants were filtered through 0.22 μ filters prior to RP-HPLC.

Chromatography – Gliadins were characterised by RP-HPLC as described by Huebner and Bietz (1993). A Hewlett-Packard 1050 HPLC system with a quaternary pump, autosampler and UV detector at 210 nm was used. The column was a Vydac C18 (5 μm particles, 300Å pores, 250 × 4.6 mm) at 60°C, preceded by a 22 × 3.5 mm precolumn. Quantitative analyses were performed using HP ChemStation 3.0 software. Single extracts were analysed, since earlier experiments indicated reproducible retention times with maximum differences of 0.24 min and coefficient of variation < 2% for absolute peak areas.

Chromatograms were analysed using the Grams 32 software (Galactic Industries, Salem, NH, USA). The blank chromatogram from each run was subtracted from the chromatogram of each sample. All chromatograms were aligned to peak 23.3 min and truncated to comprise retention times between 9 and 42 min. Chromatograms were normalised by total chromatogram area, so all peak areas are expressed as relative proportions. A multivariate PC analysis was run on all chromatograms and also on those corresponding to the mean values of the three plots using the above mentioned software programme.

Quality tests. For the analysis of industrial quality, the samples were conditioned to 15% humidity for 20 hours and a Brabender Quadrumat Junior mill with a 335 μm sieve used to obtain semolina. The analyses of industrial quality carried out were: grain protein content (%P, using NIR with a Infralizer 400 apparatus), grain sulphur content (%S, using inductively coupled plasma atomic emission spectroscopy), wet gluten content (%G, following standard method ICC No. 137), gluten index (GI, following standard method ICC No. 155), energy level (EL) from the Farinograph (carried out with constant water absorption and fixed mixing time), Farinograph tolerance (tolerance), pasta colour, pasta stickiness and pasta visco-elasticity.

Regression analysis was applied to relate the observed quality values to predicted values derived from the PCs obtained from the above analysis of chromatogram data. Plot values were used for %S whereas mean values of the three plots were used for the remaining characters.

RESULTS AND DISCUSSION

As implied in the Introduction to this article, detailed multivariate RP-HPLC analysis of differences between fertiliser treatments and cultivars in their qualitative and quantitative gliadin profiles have been described by Rogers *et al.* (submitted). The current paper relates the PC components derived in this previous study to the industrial quality characters described above (Materials and Methods).

For the characters %S, %P and tolerance, strong correlations were detected between the observed values and those predicted from the most influential PCs derived from the gliadin chromatogram profiles. The remaining characters did not show such correlations.

The number of PCs involved in deriving the predicted values varied over the three characters and for normalised against non-normalised data: %P – non-normalised 3 PCs, normalised 5 PCs; %S – non-normalised 8 PCs, normalised 10 PCs; tolerance – non-normalised 3 PCs, normalised 2 PCs.

For %S, r^2 for the regression between observed mean values and the predicted values was 0.739 before normalising the data for total gliadin content and 0.710 after normalisation, meaning that total gliadin content was not significantly involved in the observed relationship for this trait; the highest loadings for the first PC were located at 23.6, 32.5 and 39.5 min in the positive sense and at 36.1 min in the negative (Figure 1), indicating that these were the gliadins that most varied with the variation observed in sulphur content.

For %P, there was an expected effect of total gliadin content, reflected in the difference in r^2 values obtained before and after normalisation: 0.780 and 0.411, respectively. Nonetheless, a significant correlation, though of intermediate magnitude, remained for the normalised data, where the principal gliadins involved eluted at 23.6, 26.9 and 39.5 min.

For tolerance, r^2 before and after normalisation was 0.595 and 0.576, respectively, indicating that, as for %S, total gliadin content did not strongly influence the observed relationships. In this case, the gliadins eluting at 23.3, 36.1 min (positively) and 32.5 min (negatively) were those with the highest loadings for the first PC.

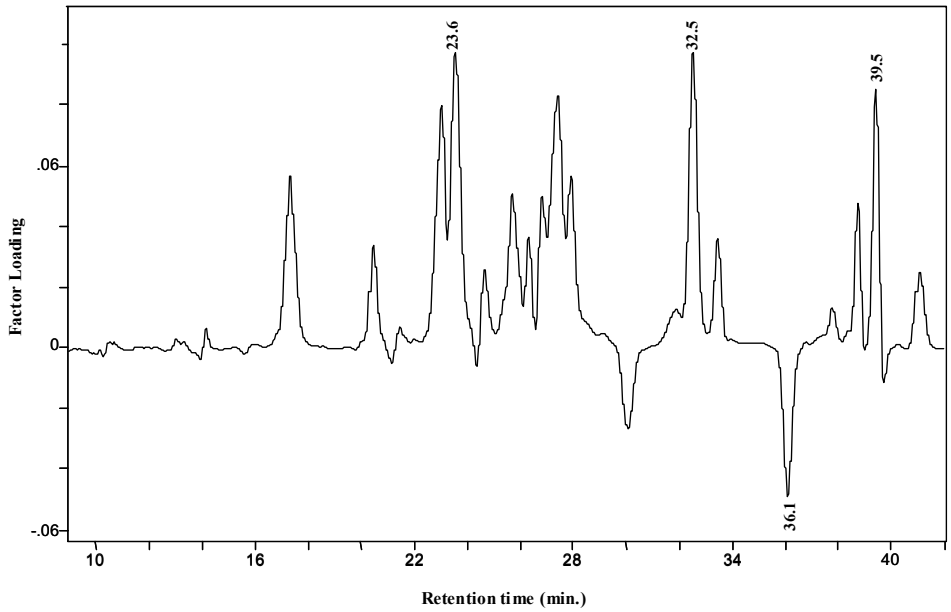

Figure 1. Factor Loadings of the first PC for the %S prediction equation. Marked retention times are those with the highest loadings

Figure 2 shows that the gliadin peaks at 32.5 and 36.1 min, important in influencing %S and tolerance as shown above, are those involved in distinguishing one of the cultivars, Chagual, from the remaining cultivars (cv. Cumenay given as an example, for %S).

The following conclusions can be derived from the results described here:

(1) The three characters appeared to be related in that they showed some common gliadin peaks with major influences;

(2) The effect of the gliadins on %S and tolerance was independent of total gliadin content;

(3) It is interesting that the strongest regressions were observed for %S, since, based upon other results previously reported, some of the differences in grain composition detected between samples may have been due to differences in the relative proportion of S-rich to S-poor gliadins provoked by the distinct fertiliser treatments applied (Rogers *et al.* submitted);

(4) The gliadin differences observed in this study did not appear to affect characters involved in evaluating gluten strength (GI and EL), and neither appeared to affect gluten content (%G). This is consistent with previous evidence that it is the glutenins that exert a greater influence upon this aspect in durum wheat than gliadins (Burnouf & Bietz 1984; Payne *et al.* 1984; Pogna

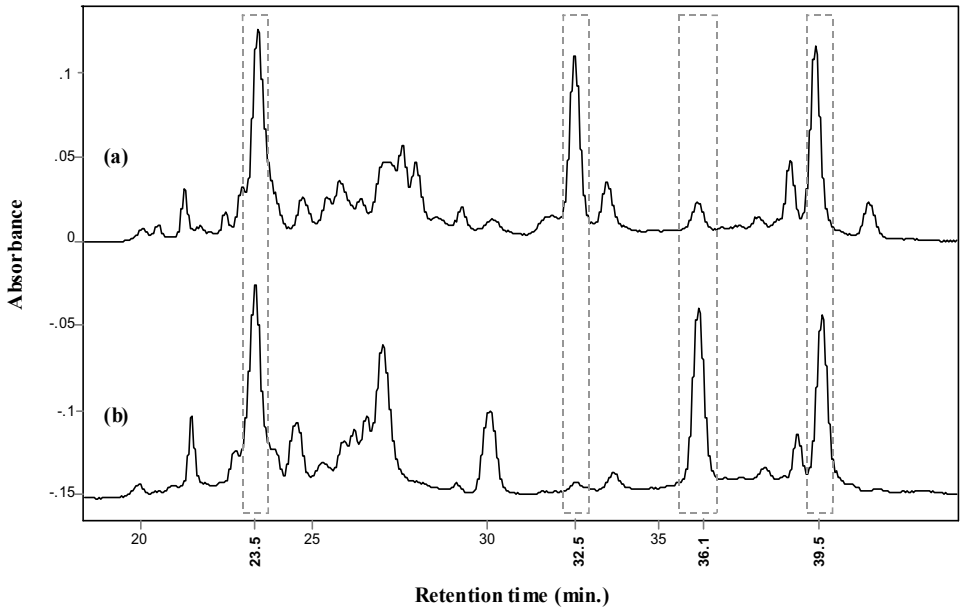

Figure 2. Gliadin chromatograms of cvs. Cumenay (a) and Chagual (b) showing the retention times of the largest loadings of PC1 for the prediction of %S. Note the difference between cvs. for retention times at 32.5 and 36.1 min

et al. 1988; Carrillo *et al.* 1990, 2000; Ruiz & Carrillo 1995);

(5) These observations indirectly imply that there is a relationship between %S and tolerance in the context of gliadin effects.

Regarding the methodology utilised in this article: it can be concluded that multivariate PC analysis of gliadin chromatograms provides the means, through regression analysis of predicted and observed values for specific quality characteristics, coupled with consideration of factor loadings, to identify which gliadin components are the most influential in determining particular quality characteristics in durum wheat.

Acknowledgements. This work was funded by the Agencia Nacional de Promoción Científica y Tecnológica (National Agency for Scientific and Technological Promotion) of the Secretaría de Ciencia, Técnología e Innovación Productiva, Ministerio de Educación, Ciencia y Tecnología (Secretariat of Science, Technology and Productive Innovation, Ministry of Education, Science and Technology) of Argentina, Project FONCyT-PICT No. 08-03196, and by the Programa Institucional de Investigación y Transferencia Tecnológica de la Facultad de Agronomía (Institutional Programme of Research and Technological Transfer of the Faculty of Agronomy), Azul, UNCPBA, Argentina, Project No. 03/A118.

References

Bietz J.A. (1983): Separation of cereal proteins by reversed-phase high-performance liquid chromatography. Journal of Chromatography, **255**: 219–238.

Bietz J.A. (1990): HPLC of cereal endosperm storage proteins. In: Gooding K., Regnier F. (eds): HPLC of Biological Macromolecules; Methods and Applications. Marcel Dekker, Inc., NY, U.S.A: 429–455.

Bietz J.A., Huebner F.R., Nelsen T.C., Simpson D.G., Guo S., Sacks J. (1990): Statistical interpretation of data from high-performance liquid chromatography of wheat proteins. In: Bushuk W., Tkachuk R. (eds): Gluten Proteins 1990. In: Proc. of the 4th Int. Workshop on Gluten Proteins, AACC, St. Paul, MN, June 27–29, 1990: 420–432.

Bietz J.A., Simpson D.G. (1992): Electrophoresis and chromatography of wheat proteins: Available methods and procedures for statistical evaluation of the data. Journal of Chromatography, **624**: 53–80.

Burnouf T., Bietz J.A. (1984): Reversed-phase high-performance liquid chromatography of durum wheat gliadins: relationships to durum wheat quality. Journal of Cereal Science, **2**: 3–14.

Carrillo J.M., Vazquez J.F., Orellana J. (1990): Relationship between gluten strength and glutenin proteins in durum wheat cultivars. Plant Breeding, **104**: 325–333.

Carrillo J.M., Martinez M.C., Moita C., Brites M.T., Nieto Taladriz M.T., Vazquez J.F. (2000): Relationship between endosperm proteins and quality in durum wheat (*Triticum turgidum* L. var. *durum*). Options Mediterranees, **40**: 463–467.

Dick J.W., Quick J.S. (1983): A modified screening test for rapid estimation of gluten strength in early-generation durum wheat breeding lines. Cereal Chemistry, **60**: 315–318.

Huebner F.R., Bietz J.A. (1993): Improved chromatographic separation and characterization of ethanol-soluble wheat proteins. Cereal Chemistry, **70**: 506–511.

Payne P.I., Jackson E.A., Holt L.M. (1984): The association between γ-gliadin 45 and gluten strength in durum varieties: a direct causal effect or the result of genetic linkage? Journal of Cereal Science, **2**: 73–81.

Pogna N., Lafiandra D., Feillets P., Autran C. (1988): Evidence for a direct causal effect of low molecular weight subunits of glutenins on gluten viscoelasticity in durum wheats. Journal of Cereal Science, **7**: 211–214.

Robutti J.L., Borrás F.S., Ferrer M.E., Bietz J.A. (2000): Grouping and identification of Argentine maize races by principal component analysis of zein reversed-phase HPLC data. Cereal Chemistry, **77**: 91–95.

Rogers W.J., Cogliatti M., Lerner S.E., Ponzio N.R., Robutti J.L., Di Martino A.M., Borrás F.S., Seghezzo M.L., Molfese E.R.: Densitometric SDS-PAGE and multivariate RP-HPLC analyses of N-S fertilisation and cultivar effects on grain protein composition in durum wheat. Submitted.

Rogers W.J., Payne P.I., Harinder K. (1989): The HMW glutenin subunit and gliadin compositions of German-grown wheat varieties and their relationship with bread-making quality. Plant Breeding, **103**: 89–100.

Ruiz M., Carrillo J.M. (1995): Relationships between different prolamin proteins and some quality properties in durum wheat. Plant Breeding, **114**: 40–44.

Simpson D.G., Guo S., Sacks J., Bietz J.A., Huebner F.R., Nelsen T. (1989): Relating chromatographic data to measurements of wheat quality: Case studies in dimension reduction. Chemometrics and Intelligent Laboratory Systems, **10**: 155–167.

Zadocks J.C., Chang T.T., Konza C.F. (1979): A decimal code for the growth stages of cereals, with illustrations. Annals of Applied Biology, **93**: 221–234.