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Spatial Patterns of Gluten Protein and Polymer Distribution in Wheat Grain

Jibin He,[†] Simon Penson,[‡] Stephen J. Powers,[†] Chris Hawes,[§] Peter R. Shewry,[†] and Paola Tosi*[,†]

[†]Department of Plant Biology and Crop Science, Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, United Kingdom

[‡]Campden BRI, Station Road, Chipping Campden, Gloucestershire GL55 6LD, United Kingdom

[§]Department of Biological and Medical Sciences, Oxford Brookes University, Oxford, Oxfordshire OX3 0BP, United Kingdom

Supporting Information

ABSTRACT: The starchy endosperm is the major storage tissue in the mature wheat grain and exhibits quantitative and qualitative gradients in composition, with the outermost cell layers being rich in protein, mainly gliadins, and the inner cells being low in protein but enriched in high-molecular-weight (HMW) subunits of glutenin. We have used sequential pearling to produce flour fractions enriched in particular cell layers to determine the protein gradients in four different cultivars grown at two nitrogen levels. The results show that the steepness of the protein gradient is determined by both genetic and nutritional factors, with three high-protein breadmaking cultivars being more responsive to the N treatment than a low-protein cultivar suitable for livestock feed. Nitrogen also affected the relative abundances of the three main classes of wheat prolamins: the sulfur-poor ω-gliadins showed the greatest response to nitrogen and increased evenly across the grain; the HMW subunits also increased in response to nitrogen but proportionally more in the outer layers of the starchy endosperm than near the core, while the sulfur-rich prolamins showed the opposite trend.

KEYWORDS: *wheat, gluten protein, bread wheat, wheat quality, pearling, compositional gradient*

INTRODUCTION

Cereals are a major source of calories and protein for human nutrition, contributing to over 45% of the global food supply (kcal capita^{-1} day^{-1}) and over 40% of the global protein supply (g capita^{-1} day^{-1}) over the past decade (http://faostat.fao.org). The production of wheat (*Triticum aestivum*) exceeded 650 million tonnes in 2010, ranking it as the third highest production among cereal crops after maize (*Zea mays*) and rice (*Oryza sativa*).

The most important wheat product in most areas of the world is bread, with about 60% of the wheat flour produced in the U.K. being used for bread production. Typically, a minimum grain protein content of 13% is required for breadmaking in the U.K. as well as in other countries that use the Chorleywood Breadmaking Process (CBP), and at least 12% protein is required for other breadmaking processes.[1] However, protein quality is also important for breadmaking, and in particular, the ability of the gluten proteins to form a dough with the appropriate viscoelastic properties. Strong dough is generally required for breadmaking, and consequently, a vast amount of research has been directed toward understanding the genetic and biochemical basis for this property. The gluten proteins are classically divided into two fractions, with the monomeric gliadins providing viscous flow and extensibility to the dough and the polymeric glutenins determining the elasticity (also called strength). Furthermore, one group of glutenin components, called the high-molecular-weight (HMW) subunits, are particularly important in conferring dough strength, by determining the proportion of large glutenin polymers.[2,3]

The starchy endosperm is the major storage tissue in wheat, comprising about 83% of the whole grain,[4] and gives rise to the white flour fraction upon milling. However, starchy endosperm cells are not homogeneous in composition, resulting in well-established compositional gradients. In particular, the two or three layers of cells immediately below the aleurone layer (the sub-aleurone cells) are richer in protein with fewer starch granules than those in the central starchy endosperm,[5,6] and similar gradients have been reported for other cereals.[7−11] Gradients also exist in the proportions of different types of gluten protein in the wheat endosperm[12−16] and in the concentration of amino acids.[17]

At present, nothing is known about whether these gradients differ between genotypes and, in particular, between high-protein breadmaking wheats and low-protein feed wheats and whether they are affected by the level of nitrogen fertilization (and, hence, the grain protein content). Similarly, although it would be predicted that flours obtained from different parts of the grain will differ in their functional properties, including their quality for breadmaking, this has not been determined. The present study was therefore designed to answer these questions, using pearling to prepare fractions from grain samples of four cultivars (three breadmaking and one feed cultivar) grown at two nitrogen levels, to determine their protein contents and compositions and their proportions of high- and low-molecular-

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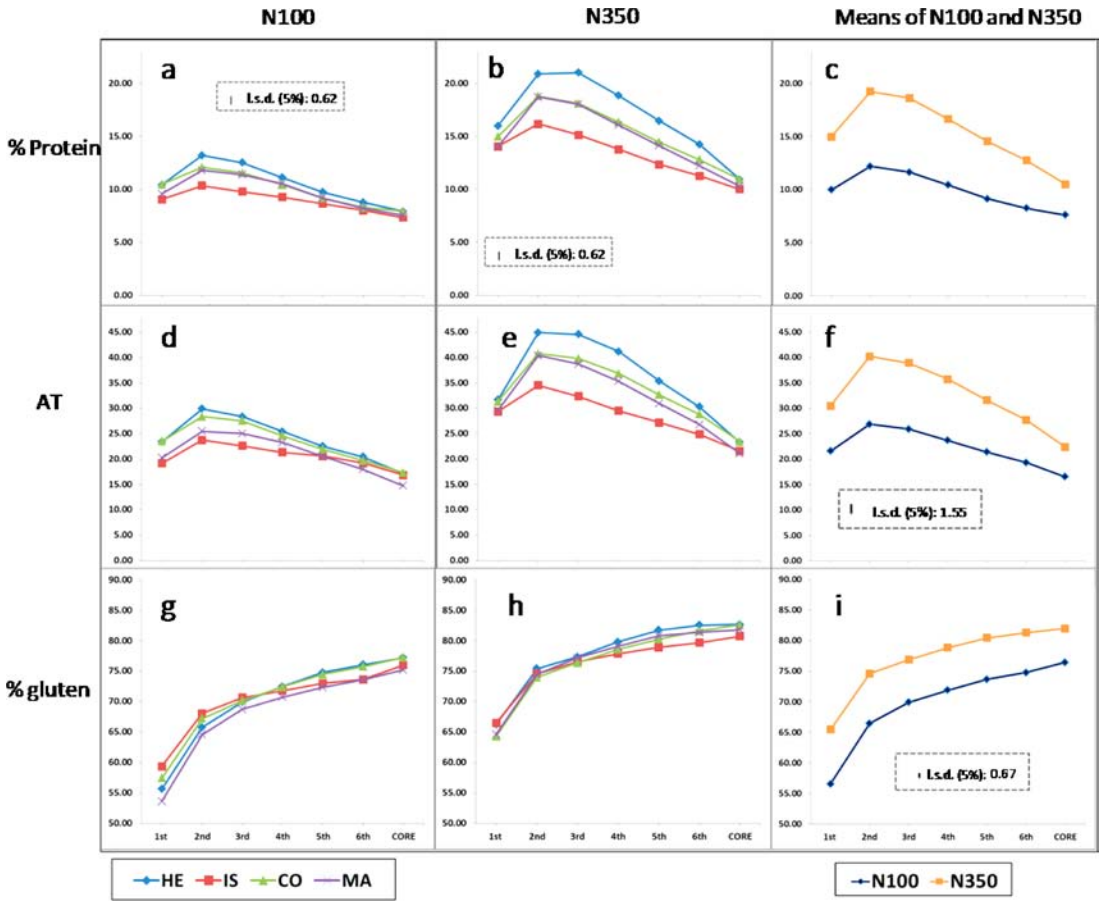

Figure 1. Contents of total protein determined as N × 5.7 (percent protein) and SE-HPLC (AT, total area) and total gluten proteins determined by SE-HPLC (percent gluten) in the pearling fraction of the four wheat cultivars. There was a significant ($p < 0.05$; F test) interaction between cultivar, N, and fraction for percent protein and a significant ($p < 0.05$; F test) interaction between N and fraction for AT and percent gluten: (a) percent protein of fresh grain weight (12% moisture) of N100 lines; LSD (5%), 0.62; n, 3; and df, 110; (b) percent protein of fresh grain weight (12% moisture) of N350 lines; LSD (5%), 0.62, n, 3; and df, 110; (c) means of N100 and N350 protein; n, 12; (d) total area (AT) of SE-HPLC chromatogram of N100 lines; (e) total area (AT) of SE-HPLC chromatogram of N350 lines; (f) means of N100 and N350 total area (AT); LSD (5%), 1.55; n, 4; and df, 18; (g) percent gluten of total protein of N100 lines; (h) percent gluten of total protein of N350 lines; and (i) means of N100 and N350 gluten (percentage of total protein); LSD (5%), 0.67; n, 4; and df, 18. Note that, for panels d, e, g, and h, there is no biological replication for the three-way factorial structure ($n = 1$) and, therefore, no LSD values; Panels f and i are used for comparison of means.

weight gluten polymers by size-exclusion high-performance liquid chromatography (SE-HPLC).

MATERIALS AND METHODS

Samples. Samples of four wheat cultivars (Hereward, Cordiale, Malacca, and Istabraq) were obtained from the Wheat Genetic Improvement Network (WGIN) nitrogen use field trial grown at Rothamsted Research in 2009. These were grown with 100 and 350 kg/ha of N fertilizer, to represent unusually high and low levels, with most U.K. farmers applying about 200 kg of N/ha. The nitrogen treatments were split in March, April, and May, corresponding approximately to growth stage (GS) 24, 31, and 32 (Zadoks scale); doses applied were of 50/50/0 kg/ha for N100 lines and 50/250/50 kg/ha for N350 lines. The plants were also treated with sulfate (111 kg/ha) in March. All plots were randomized with three replicates.

All samples comprised equal proportions of grain harvested from the central parts of three replicate plots (each 3 × 10 m) of each cultivar by N combination, making 24 samples in total from the randomized trial.

Hereward, Malacca, and Cordiale are breadmaking winter wheats, with Hereward being the most successful breadmaking wheat grown in the U.K. over the past 20 years in terms of consistency of quality, while Istabraq is a feed quality winter wheat.

Pearling. Grain samples were abraded using a previously published pearling method.[16] The remaining cores after pearling and whole grains were milled into fine flour using a laboratory ball mill (SPEX 8000M, SPEX Industries, Inc., Metuchen, NJ).

Nitrogen. Total nitrogen was determined for all samples using the American Society for Testing and Materials (ASTM) standard protocol E1019 using a Leco Combustion analysis system based on the Dumas method. No technical replicates were included because internal standards were used to ensure accuracy according to the protocol.

Determination of the Gluten Protein Composition. Gluten proteins were extracted by a method modified from van den Broeck et al.[18] Samples were extracted by stirring for 1 h with 50% (v/v) propan-1-ol without dithiothreitol (DTT) and then extracted by stirring twice with 50% (v/v) propan-1-ol plus 4.5% (w/v) dithiothreitol (1 mg of flour/10 μL of buffer). The supernatants were pooled and dried in a Speedy Vac to remove propan-1-ol and then diluted in loading buffer [0.0625 M Tris-HCl at pH 6.8, 10% (v/v) glycerol, 0.002% (w/v) Bromophenol Blue, and 2% (w/v) sodium dodecyl sulfate (SDS)] based on the same flour/buffer ratio. The residue was then extracted with loading buffer plus 1.5% (w/v) DTT using the same flour/buffer ratio. One replicate extraction was made for each sample.

Each set of samples, including one extract and one residue extract, were denatured at 80 °C for 3 min and then separated for 57 min at 200 V on a precast 4−12% Bis-Tris Nu-Page gel (Invitrogen, Paisley,

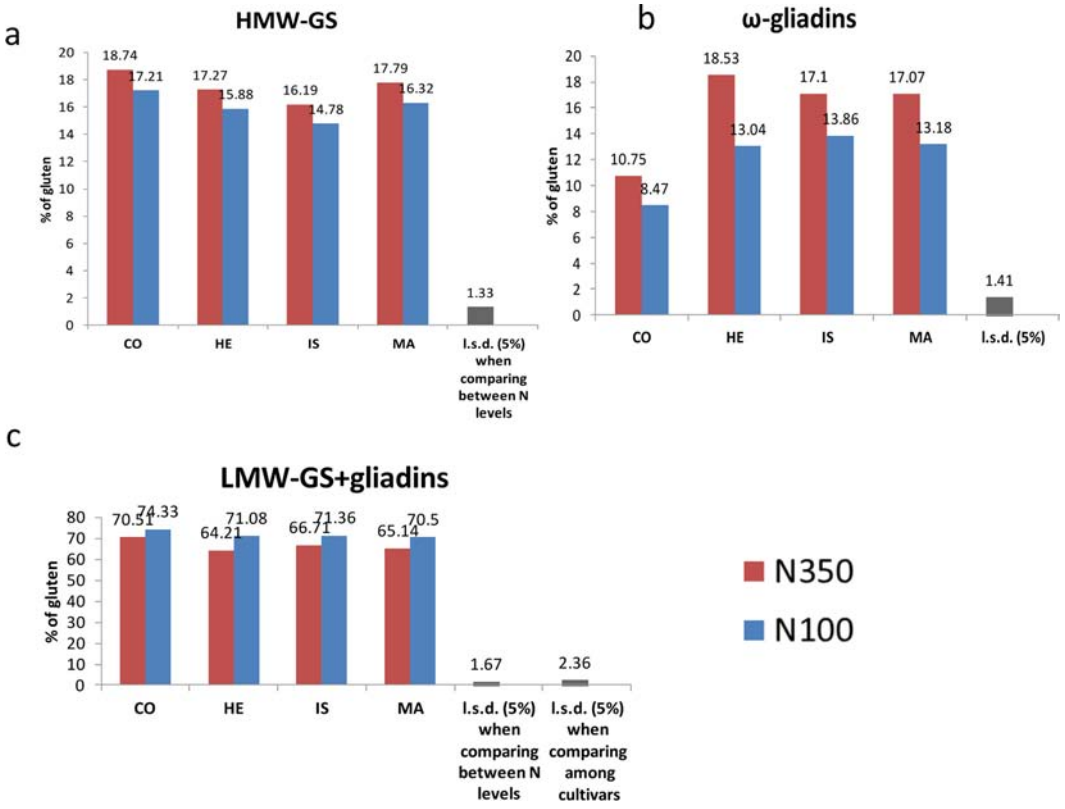

Figure 2. Gel scanning quantification of HMW-GS, ω-gliadins, and LMW-GS + gliadins (percent gluten) in wholemeal flour from the four wheat cultivars and at the two nitrogen levels: (a) percent HMW-GS (in gluten) of whole grains; LSD (5%), 1.33; *n*, 12; and df, 14; when comparing between N levels to means of 100 kg of N/ha at 16.05 and 350 kg of N/ha at 17.50, non-significant ($p > 0.05$; *F* test) differences among cultivars and for the N by cultivar interaction were found; (b) percent ω-gliadins (in gluten) of whole grains; LSD (5%), 1.41; *n*, 3; and df, 14; and (c) percent LMW-GS + gliadins (in gluten) of whole grains; LSD (5%), 1.67; *n*, 12; df, 14; when comparing between N levels with means of 100 kg of N/ha at 71.82 and 350 kg of N/ha at 66.64; LSD (5%), 2.36; *n*, 6; and df, 14; when comparing among cultivars with means of CO, 72.42; HE, 67.65; IS, 69.03; and MA, 67.82, no significant ($p < 0.05$; *F* test) interaction was found.

U.K.). Two technical replicates were included for each sample set. The staining, destaining, gel scanning, and gel quantification methods have been reported previously.[16] The gluten protein bands were divided into three subgroups corresponding to HMW-GS, ω-gliadins, and low-molecular-weight (LMW)-GS + gliadins (α-/γ-gliadins), according to their apparent molecular weight and on the basis of the results of western blot analysis carried out on replicates of these gels using antibodies specific for the three subgroups (data not shown).

SE-HPLC. Pearling fractions were analyzed using the Profilblé method developed jointly by ARVALIS and l'Institut National de la Recherche Agronomique (INRA). Details of the method can also be found in the literature.[19,20] Because of the quantity of material required within limited time constraints, only one biological replicate of each line by N combination was analyzed, with two technical replicates.[2]

Statistical Analysis. Biologically replicated data (for total nitrogen and gluten protein composition) from assays were analyzed using analysis of variance (ANOVA), taking into account the design structure (randomized plots from the field trial) and the treatment structure of main effects and interactions between cultivars, N levels, and grain fractions. Non-biologically replicated data (for SE-HPLC) derived from the cultivars by N levels by grain fraction factorial treatment structure were similarly analyzed but by taking the three-way interaction as the residual term, thus allowing for a test of the main effects and two-way interactions between the factors. Checks of residuals revealed that no transformation of data was required. Given *F* test results from ANOVA, relevant means were output for display and comparison using the appropriate least significant difference (LSD) values at the 5% level of significance. The GenStat (14th edition, VSN International, Ltd., Hemel Hempstead, U.K.) statistical package was used for this analysis.

RESULTS

Preparation of Pearling Fractions. All samples were pearled to remove six sequential fractions, which corresponded to 7, 6, 7, 10, 10 and 10% of the grain weight (measured at approximately 12% moisture) and were enriched in pericarp tissue, the aleurone layer, the sub-aleurone layer, and three progressively more central areas of the starchy endosperm, respectively.[4,21] These fractions, flours produced by ball milling the remaining cores (about 50% of the grain fresh weight), and whole grain were analyzed to determine their content and composition of gluten protein subunits and polymers.

Protein Distribution. The distribution of total protein (calculated as nitrogen × 5.7) in the fractions of the four cultivars at 100 and 350 kg of N/ha is shown in panels a and b of Figure 1. There was a significant ($p < 0.05$; *F* test) interaction between cultivar, N, and fraction, with a clear gradient from fraction 2 (which contains much of the protein-rich aleurone) to fraction 6. However, this gradient is much steeper in the grain grown at 350 kg of N/ha, as illustrated by the curves representing the mean protein content (mean of all four cultivars) in each fraction at the two nitrogen levels (Figure 1c)

Differences in the total protein contents of the fractions were also determined as the total area under the peaks (AT) separated by SE-HPLC of total protein extracts, expressed in arbitrary units (panels d–f of Figure 1). Although it was not possible to test the three-way interaction for these data, there

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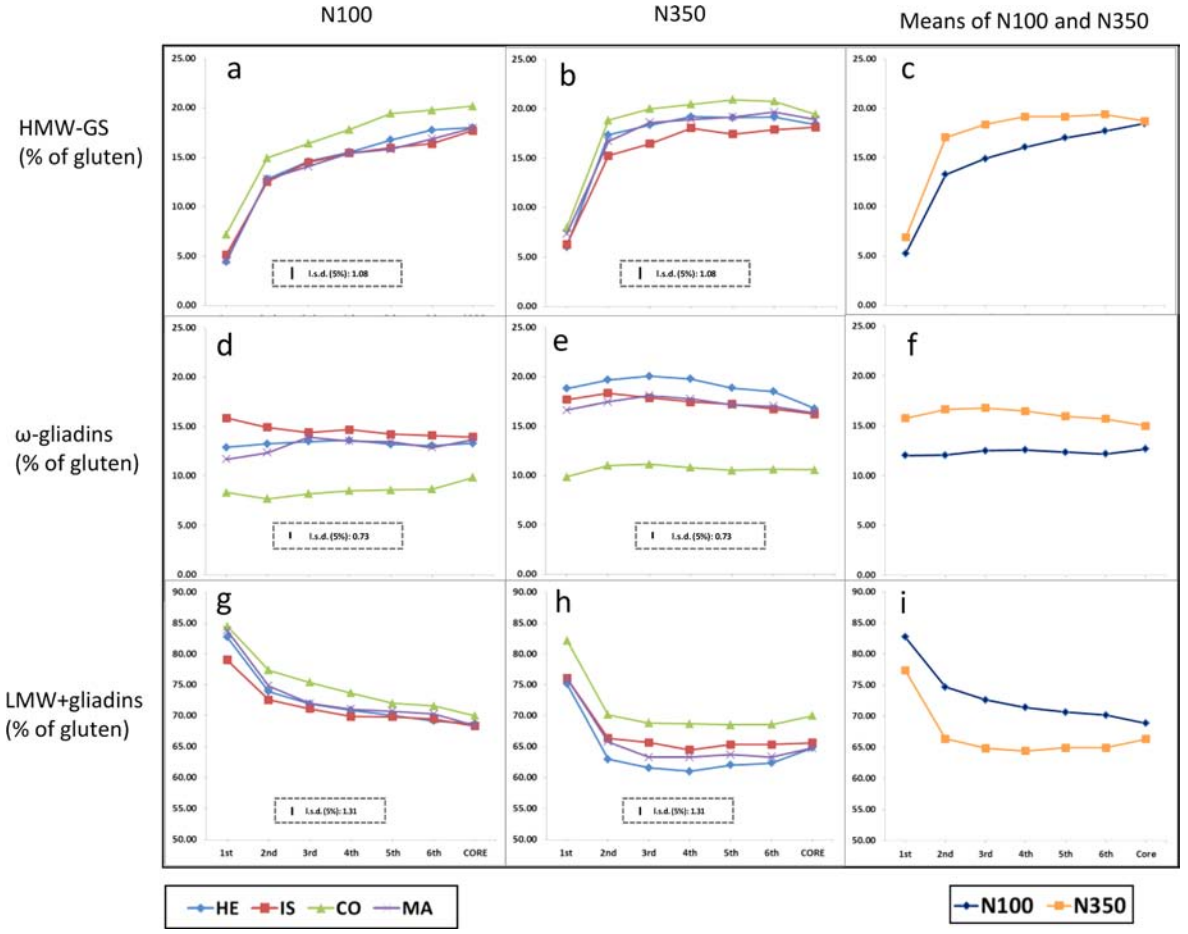

Figure 3. Proportions of HMW-GS, ω-gliadins, and LMW-GS + gliadins (expressed as a percentage of total gluten proteins) determined by scanning of SDS−PAGE separations of protein extracts from the pearling fractions. There was a significant ($p < 0.05$; F test) interaction between cultivar, N, and fraction: (a) percent HMW-GS of N100 samples; LSD (5%), 1.08; n, 3; and df, 110; (b) percent HMW-GS of N350 samples; LSD (5%), 1.08; n, 3; and df, 110; (c) means of percent HMW-GS in N100 and N350 samples; n, 12; (d) percent ω-gliadins of N100 samples; LSD (5%), 0.73; n, 3; and df, 110; (e) percent ω-gliadins of N350 samples; LSD (5%), 0.73; n, 3; and df, 110; (f) means of percent ω-gliadins in N100 and N350 samples; n, 12; (g) percent LMW-GS + gliadins of N100 samples; LSD (5%), 1.31; n, 3; and df, 110; (h) percent LMW-GS + gliadins of N350 samples; LSD (5%), 1.31; n, 3; df, 110; and (i) means of percent LMW-GS + gliadins in N100 and N350 samples; n, 12.

was a significant ($p < 0.05$; F test) interaction between N and fraction. Furthermore, the curves over the fractions for these two estimates of total protein are remarkably similar with Hereward having the highest protein content and steepest gradients and Istabraq having the lowest total protein and shallowest gradients for both measures, to and from a maximum at the second fraction. Hereward also shows the greatest response to applied N, and Istabraq shows the smallest. This is consistent with the known properties of these cultivars, with Istabraq being a high-yielding feed wheat (and consequently having a lower protein concentration in the grain) and Hereward having the highest quality of the three breadmaking cultivars. Hence, the slope of the gradient over the fractions is determined by both genetic and nutritional effects on grain protein content.

It is well-established that the gluten proteins of wheat are only present in the starchy endosperm cells of the grain, whereas the aleurone contains other proteins, including globulins related to the 7S vicilin-type storage globulins characteristic of seeds of legumes and other dicotyledonous plants (reviewed by ref 22). The proportion of gluten proteins in the total proteins was estimated as the combined proportions of the first four peaks separated by SE-HPLC of total protein extracts, which have been shown to correspond mainly to glutenin polymers (F1 and F2) and gliadins (F3 and F4) (panels g−i of Figure 1). There was a significant ($p < 0.05$; F test) interaction between N and fraction, with the four cultivars showing similar curves at both 100 and 350 kg of N/ha and with the proportion of gluten proteins increasing from the outside of the grain to the core (panels g and h of Figure 1).

Gluten Protein Composition. Two analytical systems were used to determine gluten protein composition. Sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) was initially used to determine the proportions of HMW subunits of glutenin, ω-gliadins, and a broad group of subunits comprising LMW subunits of glutenin, α-gliadins and γ-gliadins, using a three-stage extraction procedure. Aqueous propan-1-ol (50%) with and without the reducing agent DTT were initially used to extract reduced gliadins and glutenin subunits, and the two extracts combined. However, this procedure did not completely extract the HMW subunits. A third fraction was therefore extracted with SDS and DTT. The combined first and second extracts and the third extract were then quantified by SDS−PAGE and gel scanning, and the results combined to give an accurate determination of gluten protein subunit composition.

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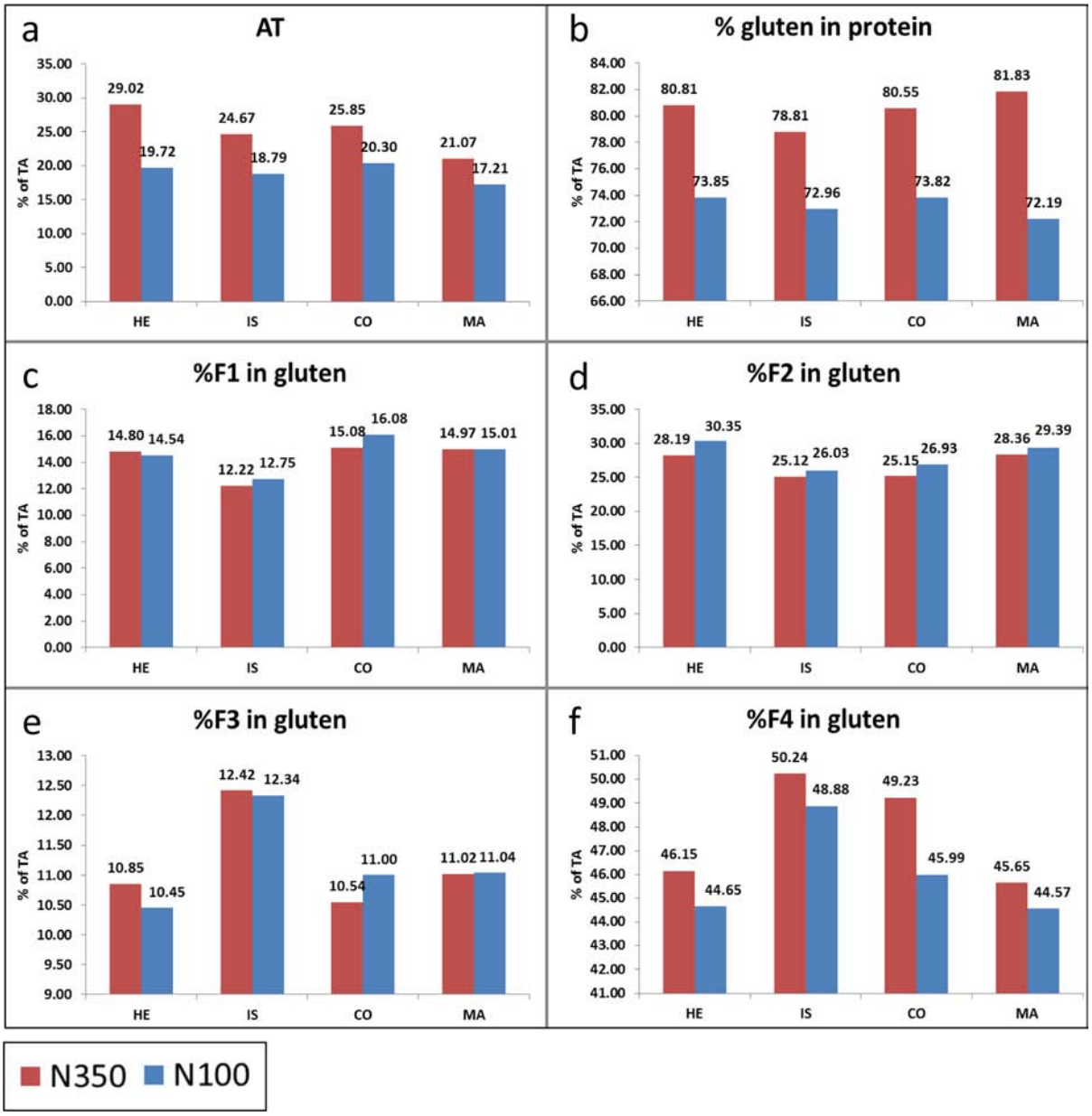

Figure 4. SE-HPLC analysis of flours from four cultivars at two nitrogen levels: (a) total area (AT) of SE-HPLC chromatogram of whole grains; (b) percent gluten of total protein of whole grains; (c) percent F1 in gluten of whole grains; (d) percent F2 in gluten of whole grains; (e) percent F3 in gluten of whole grains; and (f) percent F4 in gluten of whole grains. Note that the combinations of cultivar by N for these data were not biologically replicated.

SDS−PAGE analyses of the separated fractions are shown in Supplemental Figure 1 of the Supporting Information. The protein bands were quantified as three groups, two of which corresponded to the HMW subunits of glutenin and the ω-gliadins and the third corresponding to a mixture of LMW subunits of glutenin, α-gliadins and γ-gliadins (which are not clearly resolved by SDS−PAGE). These three groups correspond to the three types of wheat prolamin defined according to their molecular and genetic relationships: the HMW, sulfur-poor, and sulfur-rich prolamins.[23]

The cultivars contained either four or five HMW subunits of glutenin: 1Dx3 + 1Dy12 and 1Bx7 + 1By9 in Hereward, 1Dx2 + 1Dy12 and 1Bx7 + 1By9 in Istabraq, 1Ax1, 1Dx5 + 1Dy10, and 1Bx14 + 1By15 in Malacca, and 1Ax1, 1Dx5 + 1Dy10, and 1Bx17 + 1By18 in Cordiale. Similarly, they differed in their patterns of ω-gliadins. Whereas Hereward, Malacca, and Istabraq had similar patterns with four major ω-gliadin bands resolved by SDS−PAGE, only two bands were present in Cordiale.

Analyses of the wholemeal flours (Figure 2) revealed a significant ($p < 0.05$; F test) main effect of N for HMW-GS, an interaction between N and cultivar for ω-gliadins, and independent main effects of N and cultivar for LMW-GS + gliadins. Hereward and Istabraq had slightly lower proportions of HMW subunits than Cordiale and Malacca, 15.88 and 14.78% compared to 17.21 and 16.32% at 100 kg of N/ha, which is consistent with the presence of four and five HMW subunits in these two pairs of cultivars, respectively.[24] Similarly, Cordiale had a significantly ($p < 0.05$; LSD) lower proportion of ω-gliadins than the other cultivars, 8.47% at 100 kg of N/ha

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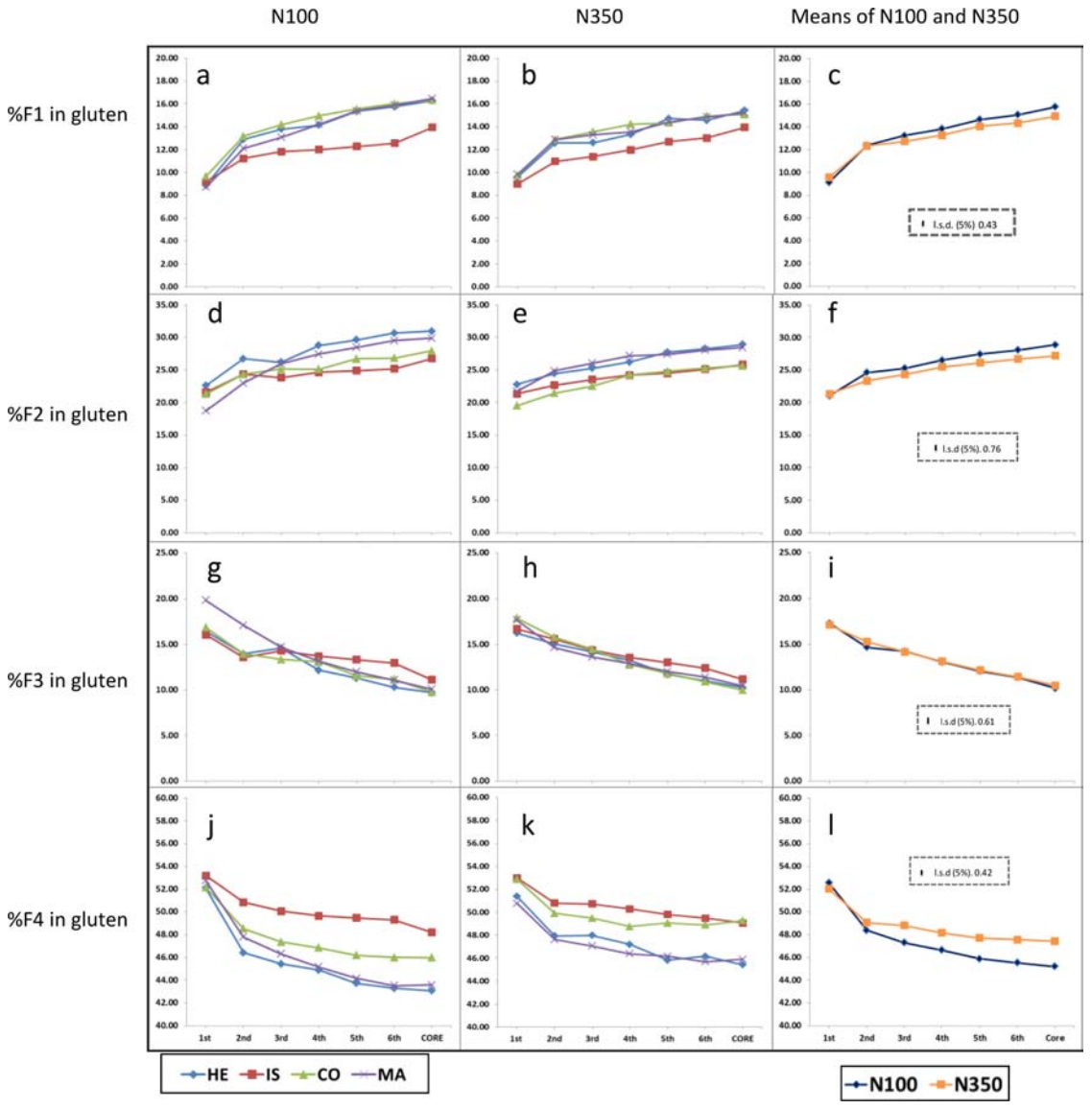

Figure 5. Proportions of polymeric (F1 and F2) and monomeric (F3 and F4) gluten proteins separated by SE-HPLC. There was a significant ($p < 0.05$; F test) interaction between N and fraction: (a) percent F1 in gluten of N100 samples; (b) percent F1 in gluten of N350 samples; (c) means of percent F1 in gluten of N100 and N350 samples; LSD, 0.43; n, 4; and df, 18; (d) percent F2 in gluten of N100 samples; (e) percent F2 in gluten of N350 samples; (f) means of percent F2 in gluten of N100 and N350 samples; LSD, 0.76; n, 4; and df, 18; (g) percent F3 in gluten of N100 samples; (h) percent F3 in gluten of N350 samples; (i) means of percent F3 in gluten of N100 and N350 samples; LSD, 0.61; n, 4; and df, 18; (j) percent F4 in gluten of N100 samples; (k) percent F4 in gluten of N350 samples; and (l) means of percent F4 in gluten of N100 and N350 samples; LSD, 0.42; n, 4; and df, 18. Note that for panels a, b, d, e, g, h, j, and k, there is no biological replication for the three-way factorial structure ($n = 1$) and, therefore, no LSD values; panels c, f, i, and l are used for comparison of means.

compared to 13.04% (Hereward), 13.86% (Istabraq), and 13.18% (Malacca), which reflects the presence of either two or four ω-gliadin bands. A comparison of the samples grown at 100 and 350 kg of N/ha showed that nitrogen application resulted in increases in the proportions of HMW subunits and ω-gliadins and a decreased proportion of LMW subunits + α-/γ-gliadins in the grain grown at 350 kg of N/ha compared to that grown at 100 kg of N/ha, by mean values (over cultivars) of +1.45, +3.72, and −5.17%, respectively. Similar effects have been reported in previous studies.[25−27]

The proportions of the three groups of gluten protein in the pearling fractions are shown in Figure 3. There was a significant ($p < 0.05$; F test) interaction between cultivar, N, and fraction for all three groups. Clear gradients are observed for the proportions of HMW subunits and LMW subunits + α-/γ-gliadins, which increase and decrease, respectively, from the outer to inner parts of the grain (panels a−c and g−i of Figure 3). In contrast, at 100 kg of N/ha, the percent ω-gliadins changed little from fraction 1 to the core and, at 350 kg of N/ha, decreased slightly from fractions 3 to 6 (panels d−f of Figure 3).

Gluten Protein Polymers. SE-HPLC was used to fractionate the gluten proteins into four groups corresponding to HMW glutenin polymers (which are enriched in HMW subunits) (F1), LMW glutenin polymers (which are enriched in LMW subunits) (F2), and two fractions that are enriched in ω-gliadins (F3) and α-/γ-gliadins (F4).[19,20] A further peak, F5, contains non-gluten proteins, and F1−F5 together therefore provide a measure of the total protein content (called AT),

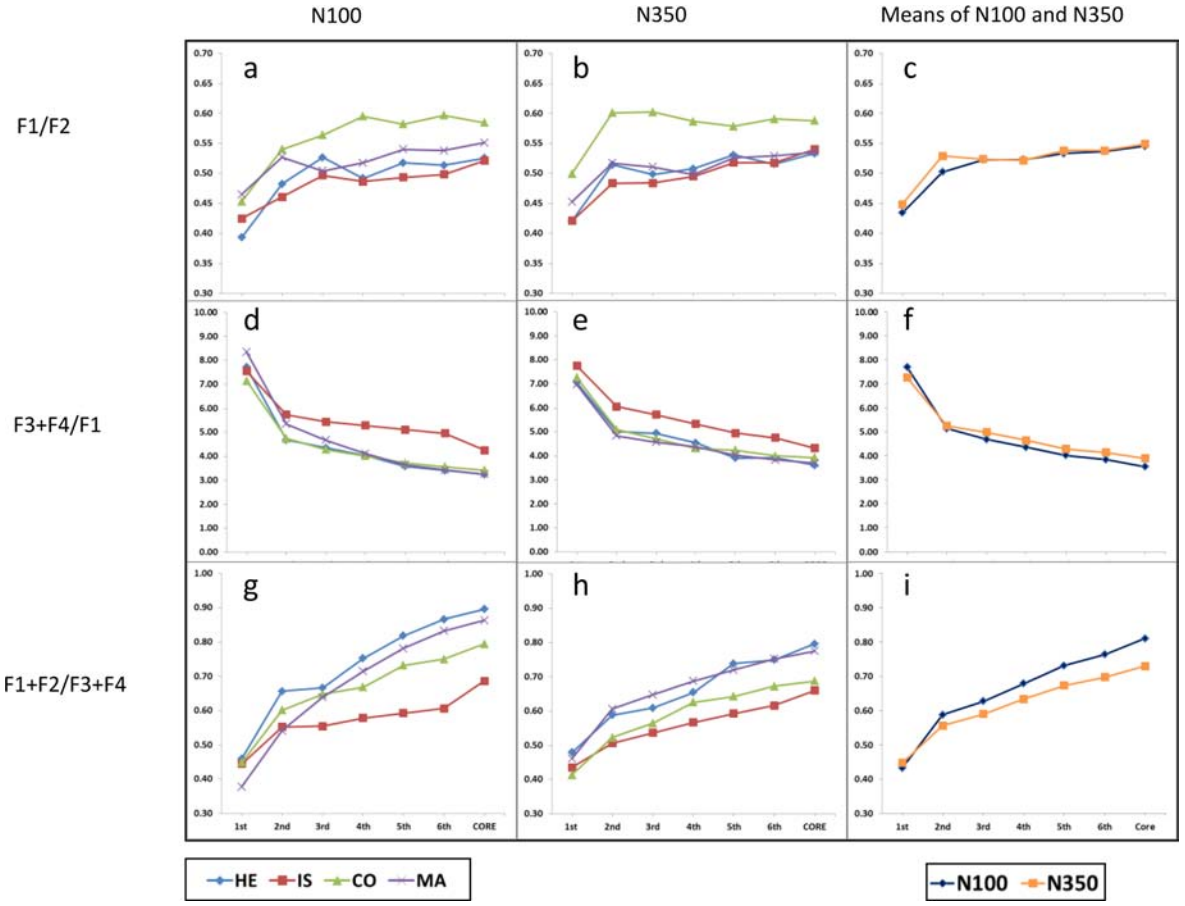

Figure 6. Ratios of fractions separated by SE-HPLC: (a) F1/F2 ratio of N100 samples; (b) F1/F2 ratio of N350 samples; (c) means of F1/F2 ratio of N100 and N350 samples; (d) F3 + F4/F1 ratio of N100 samples; (e) F3 + F4/F1 ratio of N350 samples; (f) means of F3 + F4/F1 ratio of N100 and N350 samples; (g) F1 + F2/F3 + F4 ratio of N100 samples; (h) F1 + F2/F3 + F4 ratio of N350 samples; and (i) means of F1 + F2/F3 + F4 of N100 and N350 samples. See Figure 5 for variation in the comparison of treatments for individual fractions, having applied ANOVA.

with F1−F4 providing a measure of the gluten protein content (as discussed above).

The quality of wheat for breadmaking is related to the proportion of HMW glutenin polymers and the ratio of glutenin/gliadin (F1 + F2/F3 + F4), with the ratios F1/F2 and F3 + F4/F1 showing good correlations with breadmaking quality.[2] These ratios are independent of the contribution of peak F5, and the proportions of peaks F1−F4 discussed below are calculated as percentages of the total gluten proteins (F1 + F2 + F3 + F4).

Wholemeal flours (Figure 4) showed small differences in the proportions of the four fractions between the 100 and 350 kg of N/ha samples, with small increases in the percent F4 (between 1.08 and 3.24% for the four cultivars, with a mean of 1.80%) accompanied by similarly small decreases in the percent F2 (between 0.91 and 2.16%, with a mean of 1.47%). The effects of N treatment on the F1 and F3 fractions were even smaller and differed between the cultivars. The F3 + F4/F1 ratio (see Supplemental Figure 2 of the Supporting Information) was greater and the F1 + F2/F3 + F4 ratio was smaller in the 350 kg of N/ha samples, while the F1/F2 ratio was either unchanged (Istabraq and Cordiale) or increased slightly (Hereward and Malacca) with high N. It was not possible to analyze data from wholemeal flours statistically, because there was only one replicate with two factors (N and cultivar). However, analysis of data from the pearling fractions allows for the three-way interaction of factors to be the residual in the ANOVA, and this revealed a significant ($p < 0.05$; F test) interaction of N and fraction for each percentage of F1−F4 (Figure 5). There were clear increases in the proportions of F1 and F2 from the outside to the inside of the grain, with the proportions being slightly higher in the grain grown with 100 kg of N/ha than with 350 kg of N/ha (panels a and b and d and e of Figure 5). These trends are most clearly demonstrated by the average curves for the two nitrogen levels shown in panels c and f of Figure 5. In contrast, the proportions of the F3 and F4 peaks decreased to similar extents from the outside to the inside of the grain (panels g−l of Figure 5). However, whereas the percent F3 was not affected by the nitrogen level, the percent F4 was slightly higher with 350 kg of N/ha than with 100 kg of N/ha after the first fraction. There were also some differences between the four cultivars, with Istabraq having lower values for percent F1 and higher values for percent F4 in all fractions and at both nitrogen levels.

The ratio of F1/F2 generally increased from fraction 1 to 6 and through to the core in all cultivars but was highest in Cordiale and lowest in Istabraq compared to the other two cultivars (panels a and b of Figure 6), while the ratio F3 + F4/F1 decreased and was highest in cultivar Istabraq (panels d and e of Figure 6). The ratio F1 + F2/F3 + F4 (glutenin/gliadin ratio) was also lowest in Istabraq (panels g and h of Figure 6) and lower with 350 kg of N/ha than with 100 kg of N/ha (Figure 6i).

When the analyses in Figures 3 and 5 are compared, it is notable that the percent ω-gliadins determined by gel scanning

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(panels d and e of Figure 3) did not show a similar decrease in proportion to the percent F3 determined by SE-HPLC, which may indicate the presence of other proteins (possibly glutenin oligomers) in the F3 peak. Similarly, the different effects of the nitrogen level on the percent LMW subunits and α-/γ-gliadins (a decrease) and the percent F4 (an increase) and percent F2 (a decrease) probably reflect the fact that the latter fractions comprised only gliadins and mainly LMW subunits, respectively.

DISCUSSION

Our studies, on the basis of the analysis of wheat pearling fractions, clearly demonstrate the occurrence of quantitative and qualitative protein gradient in the starchy endosperm of wheat, a phenomenon that has been suggested on the basis of the different compositions and, consequently, functional properties of millstreams.[28,29] Patent flours are the purest flours (with the lowest ash contents) that are obtained from the first streams and have lower protein contents than straight flours, which contain the whole product of milling, except for the bran and shorts. Patent flours, however, have the highest commercial value, because they give stronger dough than the corresponding straight flours.

HMW glutenin subunits are the major determinant of dough strength, being the main components of the largest glutenin polymers; our studies, on the basis of gel scanning of SDS−PAGE analyses of pearling fractions, show that this class of proteins represents a higher proportion of the total gluten protein of the central endosperm cells compared to cells from the outer layers of the endosperm. The proportion of the largest glutenin polymers (defined as percent F1 in our SE-HPLC analysis) varies accordingly, increasing progressively from the outer to the inner pearling fractions, while the total protein content and the total gluten protein content are higher in the pearling fractions corresponding to more peripheral areas of the endosperm. Because of the shape of the wheat grain, the pearling method does not allow for the production of "pure fractions" (fractions containing only specific cell layers). However, the good reproducibility of the milling procedure combined with extensive data analysis allows for the identification of statistically significant differences in composition between the pearling fractions.

The first two pearling fractions, although enriched in pericarp and aleurone tissues, also contain the starchy endosperm-specific gluten proteins, while the decreasing contents of "contaminating" aleurone-specific proteins (albumin and globulin types) are responsible for the inverse gradients observed for percent gluten proteins when compared to total protein (Figure 1) in fractions 3−6.

The combined data from gel scanning analysis and SE-HPLC also suggest that the progressive enrichment in the HMW subunit observed in the pearling fractions from outer to inner parts of the endosperm is accompanied by a decrease in the proportions of LMW glutenin components. ω-gliadins, on the other hand, appear to be fairly evenly distributed across the grain.

Nitrogen had a positive effect on the total amount of protein and on the percent gluten proteins in all four cultivars, both increasing at the higher nitrogen level, although the increase was greater in the three breadmaking cultivars than in the single feed wheat cultivar. Nitrogen also affected the relative abundances of the three main classes of wheat prolamins: the sulfur-poor ω-gliadins showed the highest response to nitrogen, increasing fairly evenly across the grain; the HMW subunits also increased but proportionally more in the outer layers of the starchy endosperm than near the core (compare the means in Figure 3c), while the sulfur-rich prolamins showed an almost exact opposite trend. A comparison of the SE-HPLC analyses for the two nitrogen levels suggests that the decrease in sulfur-rich prolamins observed at a higher nitrogen level is mainly due to a decrease in the LMW glutenin subunit components (decrease in peak 2), because the peak relative to monomeric gliadins (peak 4) is proportionally higher at 350 kg of N/ha than at 100 kg of N/ha and the F1 + F2/F3 + F4 ratio is lower.

Our data are also consistent with previous reports that the increased grain protein content is associated with a disproportionate proportion of gliadins, resulting in dilution of the glutenin polymers associated with dough strength and good breadmaking performance.[30−33] Our study therefore reiterates the need for a balanced nitrogen regime to achieve the best quality.

ASSOCIATED CONTENT

Supporting Information

SDS−PAGE of gluten protein (50% propan-1-al) and residual protein fractions from the four wheat cultivars, indicating the groups of HMW-GS, ω-gliadins, and LMW-GS + gliadins that were quantified by gel scanning (Supplemental Figure 1) and comparison of SE-HPLC fraction ratios for the different cultivars at the two nitrogen levels: (a) F1/F2 ratio of whole grains, (b) F3 + F4/F1 ratio of whole grains, and (c) F1 + F2/F3 + F4 ratio of whole grains (Supplemental Figure 2). This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail: paola.tosi@rothamsted.ac.uk.

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ABBREVIATIONS USED

ASTM, American Society for Testing and Materials; CBP, Chorleywood Breadmaking Process; DTT, dithiothreitol; HMW, high molecular weight; LMW, low molecular weight; SDS, sodium dodecyl sulfate; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; SE-HPLC, size-exclusion high-performance liquid chromatography; WGIN, Wheat Genetic Improvement Network; GS, growth stage

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