Relationships Between Polymeric Glutenin and the Quality Characteristics for Seven Common Wheats (*Triticum aestivum*) Grown in the Field and Greenhouse

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ABSTRACT

Seven common wheat cultivars were grown under greenhouse and field conditions. Sodium dodecyl sulfate (SDS) extraction and size exclusion high-performance liquid chromatography (SE-HPLC) were used to separate proteins according to size (pF1–pF5 and pFi). The technological parameters such as alveograph index (*W, P, G*) and Zeleny volume (*Z*) of each genotype for each agronomic condition were measured. A high genetic variability was observed for all parameters. Differences between the two agronomic conditions were significant for most parameters, however, the interactions between genotype and agronomic conditions were significant only for a few protein fractions. The amount of certain polymeric glutenin classifies the genotypes in the same way as quality criteria (Zeleny and alveograph) do. The ratio of the amount of soluble polymeric glutenin excluded by column of SE-HPLC (pF1) to the amount of non-excluded ones (pF2) divides genotypes into two groups, one with HMW-glutenin subunits 5+10 and the other with HMW-glutenin subunits 2+12 (*Glu-D1* allelic composition).

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Glutenins, which are one of the main constituents of the gluten, appear to have an important effect on the technological quality of common wheats (Shewry et al 1992, Melas et al 1993). The relationship between the composition of high-molecular-weight glutenin subunits (HMW-G) and certain technological parameters was first established by Payne et al (1987). Kolster (1992) reported on the variability in the quantity of glutenins in different wheat varieties grown under variable agronomic conditions. He showed the influence of the amount of some HMW-G subunits on the technological quality of various genotypes. It is difficult to quantify polymeric protein by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Kolster et al 1992). Reversed-phase high-performance liquid chromatography (RP-HPLC), developed by Bietz (1983) for wheat proteins, is far more precise for quantification (Bietz et al 1992, Lew et al 1992) and allows the study of this source of variability at the intervarietal or intravarietal level. The amount of some polymeric glutenin appears to be correlated with gluten baking strength (*W* and *P* alveograph indexes) and with loaf volume (Dachkevitch and Autran 1989). Size-exclusion high-performance liquid chromatography (SE-HPLC) (Bietz 1984) allows the quantification of the polymers solubilized by SDS. Huebner and Bietz (1985, 1986) showed that the ratio of the excluded polymers (>800 kDa) to the soluble protein fraction was directly related to the mixing time. Autran (1987) also reported that the ratio of calibrated polymers to the soluble protein is related to the baking score. The importance of the glutenin for quality has also been shown by Singh et al (1990, 1991) and by Gupta et al (1992). Both found correlations between the total quantity of polymers and the technological parameters linked with the mixing. Gupta et al (1993) reported that the characteristics of the dough were affected more by the SDS-insoluble proteins than by the total polymeric proteins. So, it is possible that the different ways polymeric proteins rely on culture conditions and induce technological quality variations.

Dachkevitch et al (1989) found a negative correlation between the studied polymers and the technological quality parameters using SDS extraction and ignored the insoluble polymers. Singh et al (1990) reported the opposite result using a complete ultrasonic extraction of the polymeric glutenin. They showed a positive correlation between glutenin and technological parameters. The difficulty in extracting and assessing polymers may explain a positive or a negative correlation between polymeric glutenin and quality.

To evaluate the genetic variability and the influence of environmental factors (temperature and water disponibility) on the polymerization phenomena, we studied the distribution of three different types of polymers (SDS-insoluble, SDS-soluble but non-calibrated by SE-HPLC, and SDS-soluble and calibrated by SE-HPLC) in seven varieties of common wheat with very different bread quality characteristics, grown under two conditions.

MATERIALS AND METHODS

Plant Material

Seven common wheat cultivars (all used for bread production in France) were grown in the field and greenhouse. Plants were not irrigated in the field but were irrigated once a week in the greenhouse. Greenhouse temperatures were higher than field ones, especially during the post-anthesis period. Plots consisted of four 1.5-m rows in the greenhouse and 10 1.5-m rows in the field. Plants were at 10-cm intervals with rows spaced 25 cm apart. The experimental design was a randomized complete block with four replications. The technological quality and HMW-G subunits compositions of cultivars is presented in Table I.

Sample Preparation, Bread Quality, and Nitrogen Determination

Plants were harvested at maturity. Grain samples (20 g/cultivar/replication) were milled in a Janke A1O grinder fitted with a 200-µm screen and the remainder sample was used for the alveograph and Zeleny tests (Afnor 1991). The total nitrogen content of the flour and of the insoluble nitrogen fraction (Fi) were determined by a Perkin-Elmer PE 2410 series II analyzer; 100-mg sample dried at 80°C for 12 hr was used for each test. The protein content (mp) was calculated using 5.7 as the factor to convert N to protein (Tkachuk 1966).

SE-HPLC Analysis

Flour samples (240 mg) were stirred for 2 hr at room temperature (25°C) with 30 ml of the 0.1*M* sodium-phosphate buffer (pH

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6.9) containing 2% SDS (w/w). Extraction was followed by the denaturation of enzymes (5 min at 90°C) (McDonald et al 1964) and by centrifugation at 10,000 × *g* for 30 min at 15°C. The supernatant (soluble fraction) was then filtered through a nylon filter (0.45 µm) and subjected to SE-HPLC analysis. The HPLC system consisted of a LDC/Milton Roy ConstatMetric 3000 pump, which delivered the eluant (0.1% SDS phosphate buffer, pH 6.9) with a 0.7-ml output and a 200-psi pressure, an injection loop of 20 µl, and a TSK G4000 SW (7.5 mm × 300 mm, 450 Å) column with a TSK 3000 SW (7.5 mm × 75 mm) guard column. The detection was made by an ABI 1000 Diode array detector (210 nm). The chromatograms were analyzed by the Lab-Calc II software (Galactic Industries Corporation). The chromatograms (Fig. 1) were divided into five peaks (the elution pattern SE-HPLC of all genotypes studied is presented in Fig. 2). The first fraction (F1) corresponded to large polymeric glutenin, which was eluted at the void volume. Fraction 2 (F2) consisted of smaller polymers of glutenin that were calibrated by the column. Fractions 3 and 4 (F3 and F4) corresponded essentially to monomeric proteins: gliadins and salt-soluble proteins (albumins and globulins), respectively. Fraction 5 (F5) was supposed to contain protein precursors. The apparent molecular weight of major peaks was estimated by calibrating the column with four unreduced protein standards, thyroglobulin (669,000), bovine serum albumin (66,000), chymotrypsin A (25,700), and cytochrome C (11,700).

The percentages of the five fractions (F1–F5) were obtained by dividing the specific area of the fraction by total area of SE-HPLC chromatogram. The quantity of protein in each fraction per 100 mg of kernels (pF1–pF5) was calculated after determining the quantity of protein in insoluble fractions (Fi) and in the grain (mp).

A highly significant correlation was found between the estimated soluble proteins quantity determined by SE-HPLC chromatogram and the total amount of protein obtained through the nitrogen analyzer ($r = 0.85^{***}$, $n = 56$). This correlation allows the calculation of many useful sums or ratios, for instance the total glutenin, pF1 + pF2 + pFi, or the ratio of soluble glutenin to insoluble ones, (pF1 + pF2)/pFi.

TABLE I
Technological Characteristic and the Allelic Types of High-Molecular-Weight Glutenin Subunits in Wheat Genotypes

Genotype	C.T.P.S. Classification[a]	French Miller Union Appreciation	Glutenin Band		
			glu A1	*glu B1*	*glu D1*
Qualital	A	Improver	2*	7–9	5–10
Recital	B1	Good	2*	6–8	5–10
Capet	B1	Good	---	7–9	5–10
Soissons	B2	Good	2*	7–8	5–10
Goelent	B2	Good	---	6–8	5–10
Thesee	B2	Poor	---	6–8	2–12
Apollo[b]	D2	Poor	---	6–8	2–12

[a] Classification by the French Permanent Technical Committee for Plant Selection. Group A contains the improver bread, group B (subgroup B1 and B2) contains the good breadmaking varieties, and group D (subgroup D1 and D2) contains the poor breadmaking varieties.
[b] Variety with wheat/rye (1BL/1RS) translocation.

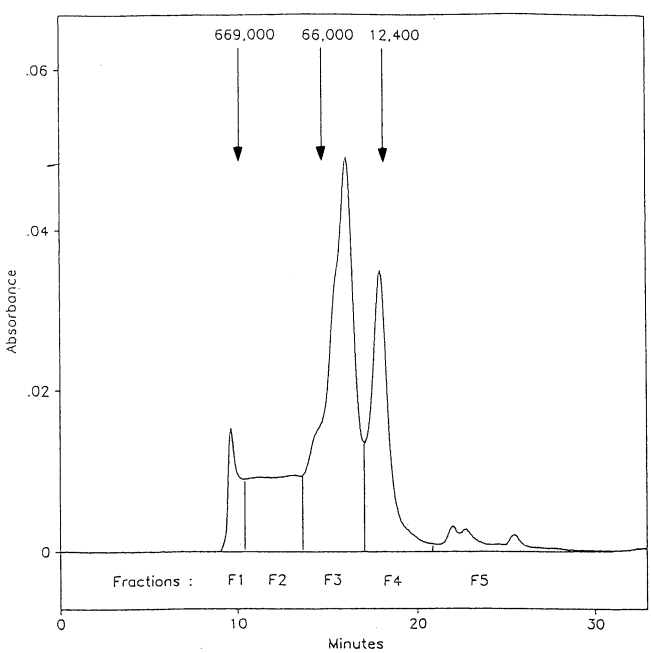

Fig. 1. Typical elution pattern of unreduced common wheat proteins extracted by phosphate-sodium dodecyl sulfate buffer. Arrows indicate the positions of three reference proteins. Five chromatographic fractions correspond to different sizes of proteins F1 to F5 (See materials and methods for description of fractions).

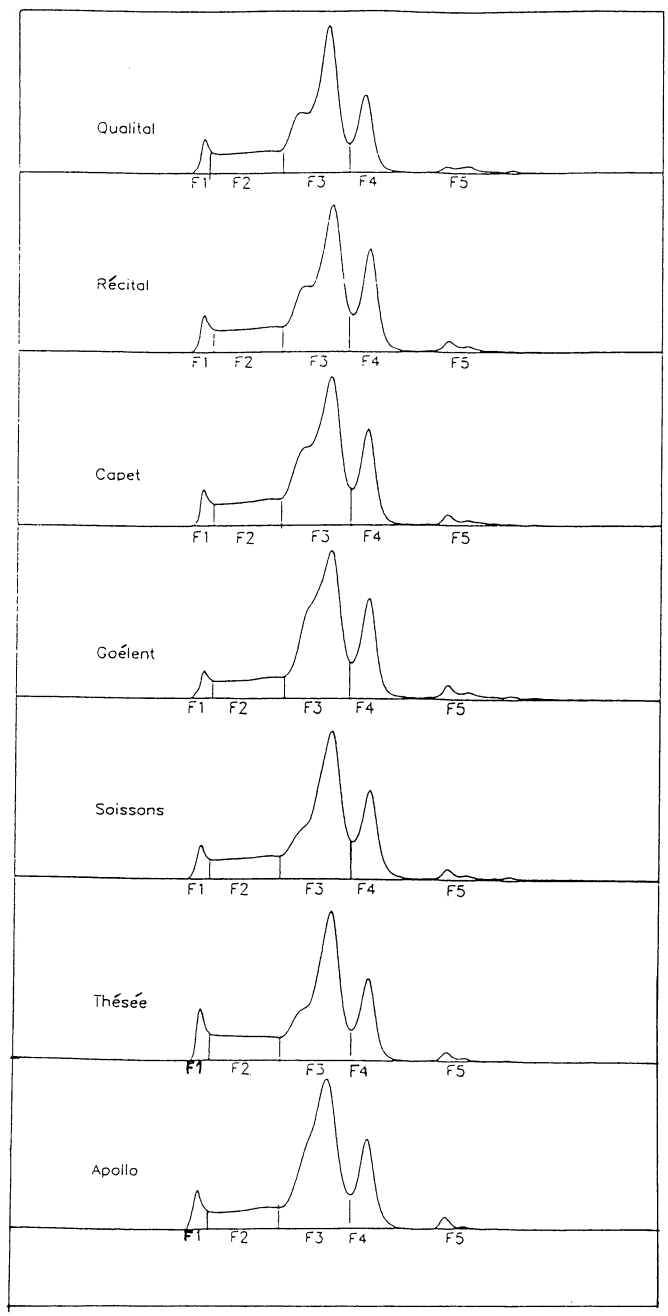

Fig. 2. Elution pattern size-exclusion high-performance liquid chromatography (SE-HPLC) of the seven genotypes studied (Apollo, Capet, Goélent, Qualital, Récital, Soissons, and Thésée).

Statistical Analysis

Analysis of variance for each trait was calculated to determine the main effects of genotype, agronomic conditions, and their interaction. To normalize the distributions, all data concerning SE-HPLC analysis were transformed by the arcsin $\sqrt{x}$ function before statistical analysis. A comparison between cultivars, culture conditions, and their interactions was done for diverse groups of protein analyzed. The Newman-Keuls test was used for comparing the means of the measured traits.

RESULTS AND DISCUSSION

Results of analysis of variance showed significant differences between genotypes in all measured traits. The two agronomic conditions (greenhouse and field) were significantly different at $P = 0.001$ level for total grain protein (mp), insoluble glutenin (pFi), excluded soluble glutenin (pF2), gliadin quantity (pF3), protein fraction F5 (pF5), total glutenin quantity (pF1 +pF2 + pFi), the ratio of soluble glutenin to insoluble ones ([pF1 + pF2]/pFi), the ratio of excluded soluble glutenin to non excluded soluble glutenin (pF1/pF2), and for P alveograph index ($P = 0.01$) and Z Zeleny volume ($P = 0.001$). The interactions between genotypes and agronomic conditions were not significant except for mp and pF3 ($P = 0.001$) and for (pF1 +pF2 + pFi) ($P = 0.01$).

The response of genotypes and the agronomic conditions and their effect on technological parameters are reported in Table II. There were significant increases in some technological parameters in the greenhouse when compared with the field (P = 37% and Z = 55%).

The protein fractions (except pF1 and pF4) and total protein (mp), were genotype dependent and were affected by agronomic conditions ($P = 0.001$). The independence of pF1 for agronomic conditions may not have any significance as this fraction is linked with the other glutenin fractions (Fi, F2). But the independence of pF4 shows that in the two agronomic conditions, all genotypes produce roughly the same amount of metabolic proteins for an equal mass of grain. The average protein content of the cultivars is significantly higher in the greenhouse (11.99) than in the field (10.09) and all cultivars have higher values for this trait in the greenhouse than in the field (Table III).

The total grain protein discriminated all genotypes ($\bar{X}G$) except Apollo, like the French C.T.P.S. classification does (Tables I and III). The amount of insoluble glutenin per 100 mg of dry kernels (pFi) also classified the genotypes according to their usual technological classification, (Table III). The effect of growing conditions was highly significant for pFi (1.44 and 2.78 in greenhouse

TABLE II
Mean Performance of Genotypes and Culture Conditions for Technological Tests

Genotype	Alveograph Index			Zeleny Volume (Z)
	W	*G*	*P*	
Qualital	475.25 a[a]	21.27 a	118.88 a	69.33 a
Recital	369.00 ab	22.20 a	102.00 ab	50.50 b
Capet	321.50 b	20.05 a	96.50 ab	54.00 b
Goelent	146.50 c	12.85 b	99.00 ab	47.50 b
Soissons	221.50 bc	18.40 a	73.00 b	47.00 b
Thesee	254.50 bc	19.15 a	100.50 ab	46.50 b
Apollo	130.50 c	19.15 a	48.75 c	30.50 c
Greenhouse	300.43	18.47	105.57 a	60.00 a
Field	247.79	19.55	76.89 b	38.67 b

[a] Means with the same letter do not differ significantly at the $P = 0.05$ level (Newman-Keuls test).

TABLE III
Mean Performance of Genotypes, Agronomic Conditions, and Their Interaction for Total Protein Content (mp), Protein Chromatographic Fractions (pF1–pF5), Insoluble Protein Residue (pFi), and Some Protein Fractions and Their Ratio

Genotype		mp	pFi	pF1	pF2	pF3	1[a]	2[b]	3[c]	4[d]
Qualital	Gh[e]	13.48 a[f]	3.00	0.52	1.68	5.95 a	5.20 a	0.74	0.31	0.88
	F[g]	12.75 ab	2.55	0.54	1.63	5.69 ab	4.72 b	0.88	0.33	0.83
	$\bar{X}$(G)[h]	13.12 a	2.78 a	0.53 b	1.65 a	5.82 a	4.96 a	0.81c	0.32 b	0.85 a
Recital	Gh	11.73 bcd	2.35	0.49	1.52	5.02 bc	4.36 bc	0.87	0.32	0.87
	F	10.75 de	2.01	0.52	1.44	4.46 cd	3.98 cd	1.00	0.36	0.90
	$\bar{X}$(G)	11.24 b	2.18 b	0.51 b	1.48 bc	4.74 bc	4.17 b	0.93 c	0.34 b	0.88 a
Capet	Gh	12.02 bc	2.50	0.50	1.48	5.24 b	4.48 bc	0.81	0.34	0.86
	F	10.32 ef	2.05	0.44	1.28	4.29 d	3.77 de	0.84	0.34	0.88
	$\bar{X}$(G)	11.17 b	2.27 b	0.47 b	1.38 cd	4.76 bc	4.12 b	0.83 c	0.34 b	0.87 a
Goelent	Gh	11.51 cd	2.69	0.35	1.14	5.16 b	4.19 cd	0.56	0.31	0.81
	F	9.52 fg	2.03	0.36	1.05	4.02 de	3.44 ef	0.69	0.34	0.86
	$\bar{X}$(G)	10.51 c	2.36 b	0.35 c	1.10 f	4.59 c	3.81 c	0.63 c	0.33 b	0.84 a
Soissons	Gh	11.58 cd	2.57	0.45	1.35	5.03 bc	4.38 bc	0.72	0.33	0.88
	F	8.75 g	1.55	0.45	1.15	3.62 e	3.15 f	1.04	0.39	0.87
	$\bar{X}$(G)	10.17 c	2.06 b	0.45 b	1.25 e	4.32 c	3.76 c	0.88 c	0.36 b	0.87 a
Thesee	Gh	11.40 cd	1.64	0.68	1.71	5.05 bc	4.03 cd	1.47	0.40	0.80
	F	9.45 fg	1.23	0.62	1.40	4.02 de	3.25 f	1.81	0.44	0.81
	$\bar{X}$(G)	10.42 c	1.44 c	0.65 a	1.55 ab	4.54 c	3.64 c	1.64 a	0.42 a	0.80 a
Apollo	Gh	12.23 bc	1.79	0.57	1.46	5.95 a	3.82 de	1.14	0.40	0.64
	F	9.09 g	1.09	0.48	1.14	4.14 de	2.71 g	1.48	0.42	0.66
	$\bar{X}$(G)	10.66 bc	1.44 c	0.53 b	1.30 de	5.04 b	3.27 d	1.31 b	0.41 a	0.65 b
Greenhouse	$\bar{X}$(Gh)[i]	11.99 a	2.36 a	0.51	1.48 a	5.34 a	4.35 a	0.90 b	0.34 b	0.82
Field	$\bar{X}$(F)[j]	10.09 b	1.79 b	0.49	1.30 b	4.32 b	3.57 b	1.11a	0.37 a	0.83

[a] Total glutenin (pF1 + pF2 + pFi).
[b] Ratio of soluble glutenin to insoluble glutenin ([pF1 + pF2]/pFi).
[c] Ratio of excluded soluble glutenin to non-excluded soluble glutenin by size-exclusion high-performance liquid chromatography (pF1/pF2).
[d] Ratio of total glutenin to gliadin ([pF1 + pF2 + pFi]/pF3).
[e] Greenhouse.
[f] Means with the same letter (comparing main effects or interactions separately) do not differ significantly at $P = 0.05$ (Newman-Keuls test).
[g] Field.
[h] Main effect of genotype.
[i] Main effect of greenhouse.
[j] Main effect of field.

and field, respectively) with no genotype and culture condition interactions.

The influence of culture conditions was important for insoluble glutenin (pFi). We noted an increase of 32% of the average value when comparing greenhouse (2.36) and field (1.79). Bekes et al (1994) demonstrated with recombined flours that many rheological parameters are linked with the insoluble glutenin (Fi) quantity.

To evaluate the influence of genotypes and of culture conditions on the polymers, analysis of variance was calculated from sums and ratios of protein fraction quantities (Table III).

The ratio of total glutenin to gliadins (pF1+pF2+pFi)/pF3 appears to be constant over genotype, except for Apollo. This stability supports the hypothesis of a pool synthesis of both gliadins and glutenins (Pernollet 1985, Johansson et al 1994). The smaller ratio for Apollo might be explained by the substitution of chromosome *1BL* coding for HMW-glutenin subunits by the chromosome *1RS,* which is known to code mainly for gliadins (Shepherd 1973, Lawrence et al 1981) and for few LMW-glutenin subunits (Gupta 1989).

The total glutenin quantity (pF1+pF2+pFi) also classified the studied genotypes like the French C.T.P.S. classification. This result is in agreement with that of Gupta et al (1992). They also found some technological parameters correlated with the amounts of glutenins.

The ratio of soluble glutenin to insoluble ones, (pF1 + pF2)/pFi classified the seven genotypes ($\overline{XG}$) into three groups: group a contains Thésée, group b contains Apollo, and group c contains Qualital, Récital, Capet, Goélent, and Soissons. The c group contains the breadmaking varieties. The inversion between Apollo (b) and Thésée (a) can be explained by Thésée synthesizing more soluble polymers than Apollo.

The pF1/pF2 ratio appeared to depend on the genotypes and the growing conditions. This result is different from those obtained by Dachkevitch (1989). This ratio (pF1/pF2) divided the seven genotypes into two groups: the high quality genotypes and the poor quality ones.

Our overall results showed that there are important variations for the glutenin quantities among genotypes and for both culture conditions. We found that the insoluble fraction protein, the ratio of excluded soluble glutenin to non-excluded soluble glutenin (pF1/pF2) and the ratio of soluble glutenin to insoluble glutenin [(pF1+pF2)/pFi] are linked with some technological parameters and result in the same classification as the quality scores.

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LITERATURE CITED

AFNOR 1991. (recueil de normes françaises) Contrôle de la qualité des produits alimentaires, céréales et produits céréaliers, alveograph test NF V O3-710, and Zeleny test NF ISO 5529.

AUTRAN, J. C. 1987. Biochemical tests for the evaluation of wheat technological quality: Their potential in breeding programs. Pages 19-36 in: Agriculture Hard Wheat: Agronomic, Technological, Biochemical, and Genetic Aspects. Rep. EUR 11172. B. Borghi, ed. Commission of the European Communities: Luxembourg.

BEKES, F., GRAS, P. W., GUPTA, R. B., HICKMAN, D. R., and TATHAM, A. S. 1994. Effects of a high M_r glutenin subunit (1Bx20) on the dough mixing properties of wheat flour. J. Cereal Sci. 19:3-7.

BIETZ, J. A. 1983. Separation of cereal proteins by reversed-phase high-performance liquid chromatography. J. Chromatogr. 255:219-238.

BIETZ, J. A. 1984. Analysis of wheat gluten proteins by high-performance liquid-chromatography. II. Baker's Dig. 58(2):22-24.

BIETZ, J. A., and SIMPSON, D. G. 1992. Electrophoresis and chromatography of wheat proteins, available methods and procedure of the statistical evaluation of the data. J. Chromatogr. 624:53-80.

DACHKEVITCH, T. 1989. Etude des complexes protéiques de blé tendre par chromatographie liquide à haute performance de tamisage moléculaire (SE-HPLC): Relation avec la qualité technologique. Thèse, Université des Sciences et Techniques du Languedoc: Montpellier, France.

DACHKEVITCH, T., and AUTRAN, J. C. 1989. Predicting of baking quality of bread wheats in breeding programs by size-exclusion high-performance liquid chromatography. Cereal Chem. 66:448-456.

GUPTA, R. B. 1989. Low-molecular-weight subunits of glutenin in wheat and related species: Their characterization, genetics, and relation to bread-making quality. PhD thesis. University of Adelaide: Australia.

GUPTA, R. B., BATEY, I. L., and MacRITCHIE, F. 1992. Relationships between protein composition and functional properties of wheat flour. Cereal Chem. 69:125-131.

GUPTA, R. B., KHAN, K., and MacRITCHIE, F. 1993. Biochemical basis of flour properties in bread wheats. I. Effects of variation in the quantity and size distribution of polymeric protein. J. Cereal Sci. 18:23-41.

HUEBNER, F. R., and, BIETZ, J. A. 1985. Detection of quality differences among wheats by high-performance liquid chromatography. J. Chromatogr. 327:333-342.

HUEBNER, F. R., and BIETZ, J. A. 1986. Assessment of the potential breadmaking quality of hard wheats by reversed-phase high-performance liquid chromatography of gliadins. J. Cereal Sci. 4:379-388.

JOHANSSON, E., OSCARSON, P., HENEEN, W. K., and LUNDBORG, T. 1994. Differences in accumulation of storage proteins between wheat cultivars during development. J. Sci. Food Agric. 64:305-313.

KOLSTER, P. 1992. High molecular weight glutenin subunits of wheat: Qualitative and quantitative variation in relation to breadmaking quality. PhD thesis. University of Wageningen: The Netherlands.

KOLSTER, P., KRECHTING, C. F., and VAN GELDER, M. J. 1992. Quantification of individual high molecular weight subunits of wheat glutenin using SDS-PAGE and scanning densitometry. J. Cereal Sci. 15:49-61.

LAWRANCE, G. J., and SHEPHERD, K. W. 1981. Chromosomal location of genes controlling seed proteins in species related to wheat. Theor. Appl. Genet. 59:25-31.

LEW, E. J. L., KUZMICKY, D. D., and KASARDA, D. D. 1992. Characterisation of low molecular weight subunits by reversed-phase high-performance liquid chromatography, sodium dodecyl sulfate polyacrylamide gel electrophoresis, and N-terminal amino acid sequencing. Cereal Chem. 69:508-515.

McDONALD, C. E., and CHEN, L. L. 1964. Properties of wheat flour proteinases. Cereal Chem. 41:443-455.

MELAS, V., MOREL, M. H., and FEILLET, P. 1993. Les sous-unités gluténines de faible poids moléculaire: des protéines d'avenir? Ind. Céréales 84:3-14.

PAYNE, P. I., NIGHTINGALE, M. A., KRATTIGER, A. F., and HOLT, L. M. 1987. The relationship between the composition and the breadmaking quality of British grown wheat varieties. J. Sci. Food Agric. 40:51-65.

PERNOLLET, J. C. 1985. Biosynthesis and accumulation of storage proteins in seeds. Physiol. Veg. 23:45-59.

SHEWRY, P. R., HALFORD N. G., and TATHAM, A. S. 1992. High molecular weight subunits of wheat glutenin. J. Cereal Sci. 15:105-120.

SHEPHERD, K. W. 1973. Homoeology of wheat and alien chromosomes controlling endosperm protein phenotypes. Pages 745-760 in: Proc. Int. Wheat Genet Symp., 4th. Columbia, MO.

SINGH, N. K., DONOVAN, R., and MacRITCHIE, F. 1990. Use of sonication and size-exclusion high-performance liquid chromatography in the study of wheat flour proteins. I. Relative quantity of glutenin as a measure of breadmaking quality. Cereal Chem. 67:161-170.

SINGH, N. K., SHEPHERD, K. W., GUPTA, R. B., MOSS, J. H., and MacRITCHIE, F. 1991. Proportion of glutenin in the flour protein as a measure of dough strength. Pages 129-144 in: Gluten Proteins 1990. W. Bushuk and R. Tkachuk, eds. Am. Assoc. Cereal Chem.: St. Paul, MN.

TKACHUK, R. 1966. Factor of conversion of nitrogen to protein. Cereal Chem. 43:207-203.

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