Protein Composition and Agglomeration Tendency of Gluten Isolated from European Wheats (*Triticum aestivum* L.) in a Batter System

S. P. Roels,*,† M. Sindic,‡ C. Deroanne,‡ and J. A. Delcour†

Laboratory of Food Chemistry, Katholieke Universiteit Leuven, Kardinaal Mercierlaan 92, B-3001 Heverlee, Belgium, and Department of Agro-Industries, Agricultural State University, Passage des Déportés, B-5030 Gembloux, Belgium

Pilot scale isolation of gluten (with recovery of gluten on 400, 250, and 125 μ sieves) from flour prepared from six European wheat varieties (Apollo, Slejpner, Sperber, Camp Remy, Minaret, and Soissons) resulted in, on average, gluten yields of 9.6% (4.7−13.2% range). Gluten protein recoveries averaged 63.0% (34.5−85.7% range). Gluten yields and gluten protein recoveries were linearly related and increased when mixing times and baking absorptions required for optimal dough development of the parent flours increased, indicating that there is a relationship between the agglomeration properties of gluten proteins in a batter system and the optimal technological conditions necessary for processing the flours in breadmaking. The Osborne protein fractions in the gluten fractions were determined. The 0.05 M acetic acid soluble (glutenin) fraction was quantitatively the most important fraction (40−46% of gluten Kjeldahl nitrogen) while comparable levels of 70% ethanol soluble (gliadins) and 0.05 M acetic acid insoluble (residue protein) were found (17.9−22.3% of gluten Kjeldahl nitrogen and 22.0−29.0% of gluten Kjeldahl nitrogen for gliadins and residue protein, respectively). With decreasing pore size of the sieves, the level of glutenin in the gluten decreased while the level of gliadins increased. This indicates that, in gluten with good agglomeration properties, the level of glutenins is high and that the agglomeration properties of such proteins (i.e. their tendency to aggregate) strongly determines the agglomeration behavior of the gluten as a whole.

Keywords: *Gluten agglomeration; gluten composition; Triticum aestivum*

INTRODUCTION

The European industrial capacity for starch and starch-derived hydrolyzates is on the rise. In the past decades there has been a shift from maize to wheat starch processing. Generally, wheat starch and gluten are isolated by a batter process, where wheat flour is blended with water to produce a smooth flowable batter and aged and gluten agglomerates are isolated on vibrating sieves. The crude gluten and starch are refined essentially as in the Martin process. The vital wheat gluten mostly finds its application as a flour fortification agent. Despite the fast growth of this industry, relatively few reports (Hamer et al., 1989; Kelfkens and Hamer, 1991a,b; Prieto et al., 1992; Sindic et al., 1993) deal with the factors governing gluten agglomeration in batter systems, a step essential for the efficient separation of wheat flour into starch and gluten.

To gain more insight into the factors determining gluten agglomeration, vital wheat gluten was isolated from wheat flour samples of different varieties (Apollo, Slejpner, Camp Remy, Sperber, Minaret, and Soissons) which were thoroughly characterized by Roels et al. (1993) in terms of their intrinsic breadmaking quality. The flours were processed into vital wheat gluten and starch using a batter system. The agglomerated gluten was fractionated by sieving and recovered by freeze-drying. The proteins in the resulting fractions were submitted to an Osborne type of fractionation, i.e., their composition of albumins and globulins, gliadins, glutenin, and glutenin residue were estimated. In the accompanying paper (Roels et al., 1998), the carbohydrate composition of the gluten fractions is examined. We hope these efforts would contribute to the understanding of wheat gluten agglomeration and increase our insight into the extent to which flour breadmaking variables such as optimum mixing time and baking absorption would be relevant for gluten agglomeration.

MATERIALS AND METHODS

Gluten Isolation. Six wheat flours (Roels et al., 1993) were used for the gluten isolation. The batter procedure isolation device used was comparable to that by Weegels et al. (1988). Briefly, 2.0 kg of flour was mixed with 70% (flour weight basis) water for 2 min (Weegels et al., 1989) in a Hobart A200 mixer equipped with a dough hook. The resulting dough was allowed to rest for 8 min. Water (2.0 L) was then added, and the suspension was stirred with a flat beater for 25 min. The mixture was transferred into a vessel with 8.0 L of water. The diluted suspension was then stirred continuously uniaxially for 35 min to agglomerate the gluten. The mixture and 10 L of water were then pumped over vibrating sieves with decreasing pore size (400, 250, 125, 90, and 50 μ, respectively). Gluten was recovered from the first three sieves, lyophilized ,and ground in a IKA M20 mill for 1 min (4 × 15 s with 30 s

* Author to whom correspondence should be addressed [telephone (+32) 16 32 15 82; fax (+32) 16 32 19 97; e-mail [Stefaan.Roels@agr.kuleuven.ac.be].

† Katholieke Universiteit Leuven.

‡ Agricultural State University.

S0021-8561(97)00566-9 CCC: $15.00
Published on Web 03/28/1998

intermediate waiting times). Isolations were performed at room temperature and in duplicate.

Gluten Composition. Moisture and protein contents were determined according to AACC Methods 44-19 and 46-11A (N × 5.7), respectively (AACC, 1995). The level of glucose (Glc) was determined following acid hydrolysis and gas chromatography of the alditol acetate by the modified procedure of Englyst and Cummings (1984) as described elsewhere (Veraverbeke, 1995).

Gluten Protein Composition. An Osborne type of fractionation was performed to determine the relative amounts of trapped albumins and globulins (considered as one fraction), gliadins, and glutenins present in the fractions recovered from different sieves. All extractions were performed at 4 °C with continuous shaking. After each extraction, suspensions were centrifuged at 12000*g* for 10 min. About 10 mg of gluten (weighed to the nearest 0.01 mg) was extracted successively with 1.0 mL portions of 0.5 M NaCl for 15 and 4 h, respectively, and then with 1.0 mL of water for 2 h to remove residual salt. The extracts were recovered with a pipet and combined. Residues were then extracted 3 times with 1.0 mL portions of 70% ethanol for 15, 4, and 2 h, respectively. The extracts were recovered and combined in a similar manner. Finally, the residues were extracted with 1.0 mL of 0.05 M acetic acid for 15 h at 6 °C. The mixtures were allowed to rest for 72 h at 6 °C and were then centrifuged (12000*g* for 10 min). Aliquots of the extracts prepared as described above containing an appropriate amount of nitrogen were used for protein determinations. Glutenin residue (unextracted nitrogen) was calculated as the difference between total Kjeldahl nitrogen and the amount of nitrogen extracted. Extractions were performed in triplicate.

Protein content in the supernatants was determined colorimetrically as described by Bradford (1976). Extinction at 595 nm was read exactly 20 min after the addition of the Coomassie Brilliant Blue (CBB) dye reagent, and results were expressed as Kjeldahl nitrogen by using appropriate protein standards which were prepared as described below. Commercial wheat gluten (N. V. Amylum, Aalst, Belgium) were extracted twice with 0.5 M NaCl (1/12 w/v) and once with water for 15, 4, and 2 h, respectively. After each extraction, suspensions were centrifuged at 10000*g* for 10 min at 6 °C. The supernatants were combined, filtered (Schleicher & Schuell $597^{1/2}$), dialyzed against deionized water for 72 h, and freeze-dried, yielding the albumin/globulin fraction. The gluten residue was handcut, frozen in liquid nitrogen, and freeze-dried. The dry material was ground with a mortar and pestle to pass a 250 μ sieve and extracted three times with 70% ethanol for 15, 4, and 2 h as described above. The extracts were combined and filtered (Schleicher & Schuell $597^{1/2}$). Ethanol was removed by rotary evaporation (40 °C). The resulting mixture was freeze-dried, yielding the gliadin fraction. Finally, the residue from the alcoholic extractions was extracted twice with 0.05 M acetic acid as described above. Supernatants were combined, filtered (Schleicher & Schuell $597^{1/2}$), and freeze-dried, yielding the glutenin fraction. Nitrogen contents of the protein standards were determined with a micro-Kjeldahl method (Jones, 1991).

Zeleny Sedimentation. Zeleny sedimentation values of the wheat flours were determined according to Roels et al. (1993).

Statistical Analysis. Hypothesis testing based on two-sided *t* tests with unequal standard deviations was used to analyze differences between means of observations (Wonnacott and Wonnacott, 1985).

Definitions. Prior to presenting the results and their discussion, it is useful to define the following terms: (1) gluten yield (GY), g of dry matter (dm) retained on three consecutive sieves from 100 g of flour (dm); (2) gluten protein recovery (GPR), g of protein (dm) agglomerated on three consecutive sieves from 100 g of flour protein (dm); (3) gluten protein agglomeration index (GPAI), the ratio (in percent) of protein agglomerated on the first sieve to the total amount of protein recovered from three consecutive sieves; and (4) reconstituted gluten samples (recon), blends of gluten from any given cultivar that contain the same ratio of dm as originally recovered from the sieves.

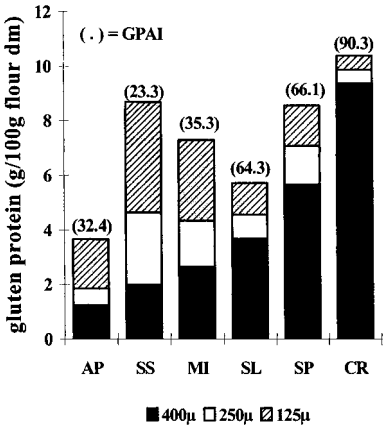

Figure 1. Gluten protein retained on individual sieves and gluten protein agglomeration indices (GPAI, between brackets) of six European wheat cultivars with varying breadmaking potential (AP, Apollo; SS, Soissons; MI, Minaret; SL, Slejpner; SP, Sperber; CR, Camp Remy).

RESULTS AND DISCUSSION

Gluten Recovery and Gluten Protein Agglomeration Index. For different varieties, large differences in GY can be observed as well as for the amounts of protein agglomerated on the individual sieves (Figure 1). GY's for Apollo, Slejpner, Sperber, Camp Remy, Minaret, and Soissons were 4.7, 7.3, 11.0, 13.2, 9.8, and 11.6%, respectively. GPR's for the six varieties were 34.5, 49.6, 70.0, 85.7, 65.4, and 72.7%, respectively. For the two series mentioned by Roels et al. (1993), i.e., Apollo, Slejpner, Sperber, Camp Remy on the one hand and Apollo, Slejpner, Minaret, Soissons on the other, GY's increased when mixing times and baking absorptions required for optimal dough development were increased, indicating that the gluten agglomerating properties are related to the mixing and absorption and, hence, baking characteristics of the flours. As shown in Figure 2a and eq 1, GY and GPR were strongly correlated. The large differences in GPR indicate that

$$\mathrm{GPR} = 5.84\mathrm{GY} + 6.91 \qquad (1)$$

$$(r^2 = 0.994,\ n = 6,\ P < 0.001)$$

the ratios of gluten/nongluten proteins in the flours are different, that the gluten proteins present exert a completely different agglomeration behavior, or that this agglomeration behavior is affected by other wheat flour components. The differences in agglomeration behavior are reflected in the GPAI. GPAI values for the varieties Apollo, Slejpner, Minaret, Soissons, Sperber, and Camp Remy used in this study were 32.4, 64.3, 35.3, 23.3, 66.1, and 90.3, respectively. From a technological point of view, the GPR as well as the GPAI appear to be important in terms of yield and processing ability. Gluten proteins from Camp Remy agglomerated readily, resulting in large aggregates mainly retained on the first sieve (GPAI 90.3) with a high recovery (85.7%). On the other hand, gluten proteins from the variety Soissons formed smaller aggregates that were mainly retained on the last sieve (GPAI 23.3) with a high recovery (72.7%). For Apollo, the low GPAI (32.4) and recovery (34.5%) reflect poor processing properties. GPAI's for Minaret and Soissons were very low, al-

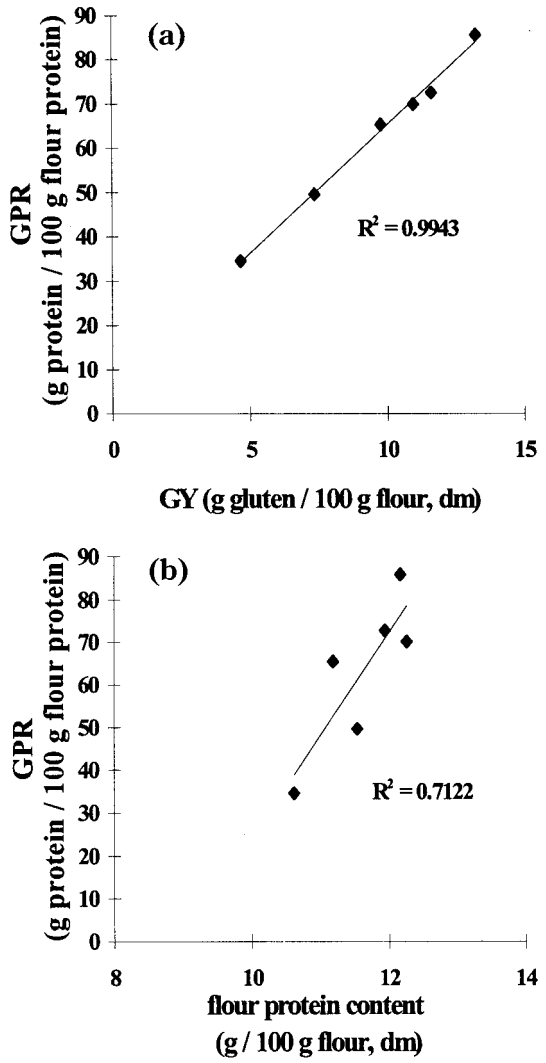

Figure 2. (a) Relation between gluten protein recovery (GPR) and gluten yield (GY). (b) Relation between GPR and flour protein content.

though the GY's were acceptable due to a substantial amount of slowly agglomerating gluten that was recovered from the >125 μ sieve.

The GPAI was not correlated with the flour protein content, as also observed by Hamer et al. (1989) but not by Sindic et al. (1993) who reported a good correlation between flour protein content and the GPAI ($r^2 = 0.757$) for six wheats grown in 1991. However, for five samples grown in 1990, the correlation between these two parameters was substantially lower ($r^2 = 0.475$). It therefore seems that this relationship, if any, varies from one year to another. Thus, the amount of flour protein cannot provide sufficient information on the agglomeration tendencies of gluten. Hamer et al. (1989) reported a fair ($r^2 = 0.624$, $n = 10$) correlation between the flour protein content (% of dm) and GY. For the set of our samples, the relationship can be described by eq 2.

$$\text{GY} = 4.25(\text{flour protein content}) - 39.76 \quad (2)$$

$$(r^2 = 0.753,\ n = 6,\ P < 0.05)$$

In view of the high correlation between GY and GPR, it is not surprising that GPR is also correlated with the flour protein content (eq 3).

$$\text{GPR} = 24.07(\text{flour protein content}) - 216.55 \quad (3)$$

$$(r^2 = 0.713,\ n = 6,\ P < 0.05)$$

From Figure 2b it is clear that flour protein content is important for the amount of gluten (GY and GPR) that can be recovered as also follows from the work by Hamer et al. (1989) and Sindic et al. (1993). This would also be expected in view of the work by Gupta et al. (1992) and the fact that, with increasing nitrogen content, the relative proportion of the storage proteins in the total protein increases (Hoseney, 1994).

The reported correlation (Sindic et al., 1993) between Zeleny sedimentation value of the flour and GPAI ($r^2 = 0.656$) was not observed in this study ($r^2 = 0.035$). However, for the present sample set, the Zeleny sedimentation value of the flour previously shown (Roels et al., 1993) to be inversely related to the water-extractable flour nonstarch polysaccharide content, is well correlated ($r^2 = 0.903$) to the GPR.

Breadmaking versus Gluten Agglomeration. The highest loaf volumes of the breads obtained for each variety in their respective areas of manageability with Response Surface Methodology (Roels et al., 1993) were correlated with the agglomerating properties (GPR and GPAI) of the gluten isolated from the parent flours. As indicated in Table 1, loaf volume was not significantly correlated with the GPR or the GPAI, indicating that neither the amount of protein that can be recovered from 100 g of flour protein (indicated by the GPR) nor the rate of gluten agglomeration (indicated by the GPAI) can explain the observed variations in breadmaking quality. However, when these parameters were taken together, a highly significant linear equation could be derived (Table 1). From this equation, it can be concluded that bread volume increases when the amount of gluten protein that agglomerates in a batter system increases, while a negative correlation between bread volume and GPAI is observed. Thus, with regard to bread volume (obtained after baking the flours under optimum conditions of water absorption and mixing time), the determining factor is the amount of gluten protein that can be recovered (in a batter system), while the rate of agglomeration seems of secondary importance. This can be explained by the fact that for dough preparation in breadmaking, optimum mixing times were applied (Roels et al., 1993), thus allowing the gluten proteins of every cultivar to develop ideally.

Gluten Protein Composition. The protein compositions of gluten fractions recovered from different sieves as well as the compositions of reconstituted samples are summarized in Table 2. Also given are the gliadin/glutenin (Gli/Glu) ratios. For each variety, a systematic statistical analysis was performed in order to determine the significance of differences in analytical results

Table 1. Equations Relating Loaf Volume (Roels et al., 1993) to Gluten Agglomerating Properties (GPR and GPAI) with Correlation Coefficients (r^2) and Probability Levels[a]

equation	r^2	probability (P)
BV = 0.2142GPR + 37.28	0.6059	0.0680
BV = −0.0413GPAI + 52.91	0.0449	0.6869
BV = 0.2999GPR − 0.136GPAI + 38.94	0.9960	0.0002

[a] BV, bread volume (in cc/10 g); GPR, gluten protein recovery (in %); GPAI, gluten protein agglomeration index.

Table 2. Protein Composition (in wt % of Gluten Kjeldahl Nitrogen) of Gluten Fractions Recovered from Different Sieves and Reconstituted Samples (Averages of Two Isolations) and Gli/Glu Ratios

cultivar	sieve	Alb + Glob[a]	gliadin	glutenin	residue	Gli/Glu
Apollo	>400 μ	12.0	19.8	46.7	21.5	0.42
	>250 μ	12.2	19.6	45.7	22.5	0.43
	>125 μ	12.2	24.7	39.0	24.1	0.63
	recon	**12.1**	**22.3**	**42.3**	**23.3**	**0.53**
Slejpner	>400 μ	15.3	16.7	46.2	21.8	0.36
	>250 μ	12.6	18.5	49.2	19.8	0.38
	>125 μ	15.1	28.3	32.3	24.4	0.88
	recon	**14.8**	**19.4**	**43.8**	**22.0**	**0.44**
Sperber	>400 μ	11.9	16.5	43.3	28.3	0.38
	>250 μ	10.9	20.5	42.1	26.5	0.49
	>125 μ	12.9	30.0	33.1	23.9	0.91
	recon	**11.9**	**19.5**	**41.4**	**27.2**	**0.47**
Camp Remy	>400 μ	12.9	17.5	47.1	22.5	0.37
	>250 μ	9.3	15.6	47.3	27.8	0.33
	>125 μ	12.0	28.5	24.8	34.8	1.15
	recon	**12.7**	**17.9**	**46.0**	**23.3**	**0.39**
Minaret	>400 μ	11.2	16.1	44.9	27.9	0.36
	>250 μ	10.8	19.9	42.5	26.9	0.47
	>125 μ	10.9	22.3	35.3	31.5	0.63
	recon	**11.1**	**20.0**	**40.0**	**29.0**	**0.50**
Soissons	>400 μ	9.5	18.2	49.0	23.3	0.37
	>250 μ	10.1	19.1	48.3	22.5	0.40
	>125 μ	11.0	25.7	40.0	23.3	0.64
	recon	**10.5**	**22.1**	**44.5**	**23.0**	**0.50**
6 cultivars	mean	12.2	20.2	43.0	24.6	0.47

[a] Alb + Glob, albumins and globulins; Gli/Glu, gliadin-to-glutenin ratio; recon, reconstituted samples.

obtained for the different protein classes and different sieves (Table 3).

Glutenins. For the different varieties, differences as well as similarities can be observed in the protein agglomeration behavior. For all varieties studied, the glutenin (in percentages of gluten Kjeldahl nitrogen) is quantitatively the most important component in the fractions recovered from different sieves (except for the Camp Remy >125 μ fraction). The glutenin in the fractions recovered from the first two sieves (>400 and >250 μ) did not vary significantly ($P < 0.05$) but was significantly lower (Table 3) in the fraction recovered from the third sieve (>125 μ). The difference between the amount of glutenin recovered from the first sieve and that from the third sieve is most pronounced for Camp Remy and less pronounced for Apollo. Thus, for Camp Remy, large gluten aggregates are easily formed that are mainly recovered from the first sieve (GPAI = 90.3) while a drastic decrease is observed in the level of glutenin recovered from the last sieve. For Apollo, the formation of such large aggregates is much slower (GPAI = 32.4) and glutenin levels decrease only gradually. The difference between the amounts of glutenin recovered from the first and the third sieves correlates well with the GPAI ($r^2 = 0.8083$; $n = 6$) of different varieties. These results indicate that gluten agglomeration largely depends on the glutenin fraction. It can therefore be concluded that, for varieties with high GPAI, glutenin molecules tend to readily aggregate and form the backbone for further agglomeration. Thus, these results show the importance of glutenins, not only with regard to their functionality in breadmaking (Orth and Bushuk, 1972) but also in gluten agglomeration.

Gliadins. In contrast to glutenin, gliadins behave in an opposite way during agglomeration. The level of gliadin increased in the fractions recovered from the sieves with decreasing pore size. For all varieties studied, the level found in fractions recovered from the third sieve was significantly higher ($P < 0.05$ or higher) than the level in fractions recovered from the first and second sieves (except the nonsignificant difference between the gliadin level on sieves two and three from Minaret). Differences in gliadin content between fractions collected from the first and second sieves are not significant ($P < 0.05$) for the varieties Apollo, Slejpner, and Soissons but are significant for Sperber, Camp Remy, and Minaret (Table 3). Gliadin and glutenin contents are inversely related ($r^2 = 0.742$; $n = 18$). These observations are also illustrated in Figure 3, where the gliadin and glutenin contents of the fractions recovered from the different sieves are plotted as a function of their protein content. A linear relationship exists between glutenin ($r^2 = 0.875$) or gliadin ($r^2 = 0.777$) content and protein content that is independent of wheat variety. Thus, fractions with a high protein content contain proportionally more glutenin, again illustrating that gluten agglomeration is mainly determined by the glutenin fraction. Since fractions with a high protein content contain proportionally more glutenin and less gliadin, the Gli/Glu ratio consequently decreases with increasing protein content (Table 2).

Albumins and Globulins. Differences in agglomeration behavior for different varieties are more pronounced for the albumin + globulin fraction. For Apollo and Minaret, the relative amount remains constant in the gluten recovered from the three sieves. For Camp

Table 3. Significance of Differences in Analytical Results Obtained for the Different Protein Classes and Different Sieves at the $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***) Levels

		Alb + Glob[a]		gliadin		glutenin		residue		Gli/Glu	
cultivar	sieve	400 μ	250 μ	400 μ	250 μ	400 μ	250 μ	400 μ	250 μ	400 μ	250 μ
Apollo	250 μ	ns	/	ns	/	ns	/	ns	/	ns	/
	125 μ	ns	ns	***	***	***	***	*	ns	***	***
Slejpner	250 μ	*	/	ns	/	ns	/	ns	/	ns	/
	125 μ	ns	*	***	***	***	***	ns	ns	***	***
Sperber	250 μ	***	/	**	/	ns	/	ns	/	*	/
	125 μ	***	***	***	***	***	***	ns	ns	***	***
Camp Remy	250 μ	***	/	**	/	ns	/	**	/	*	/
	125 μ	ns	**	***	***	***	***	***	*	***	***
Minaret	250 μ	ns	/	***	/	ns	/	ns	/	**	/
	125 μ	ns	ns	*	ns	***	***	ns	ns	**	*
Soissons	250 μ	*	/	ns	/	ns	/	ns	/	ns	/
	125 μ	***	**	***	***	***	***	ns	ns	***	***

[a] Alb + Glob, albumins and globulins; Gli/Glu, gliadin-to-glutenin ratio; ns, not significant at $P = 0.05$; /, sieves of equal dimensions.

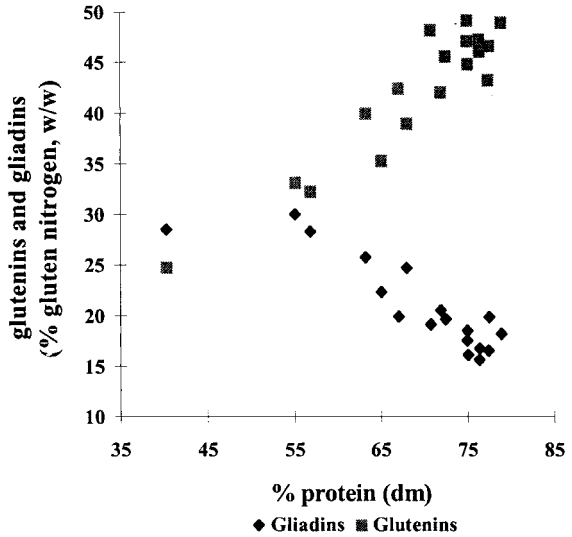

Figure 3. Variation in gliadin and glutenin levels with protein content in gluten samples isolated from six European wheat cultivars. Samples were recovered from 400, 250, and 125 μ sieves.

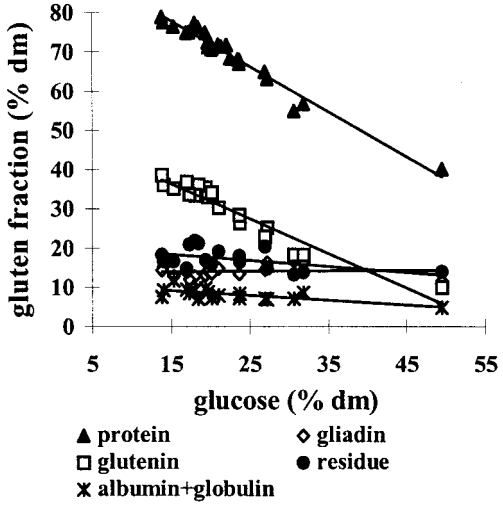

Figure 4. Relationships between protein levels and glucose levels and between levels of different protein classes and glucose levels for gluten fractions isolated from six European wheat cultivars.

Remy, Sperber, and Slejpner, the relative amount first decreases and then again increases. For Soissons an increase of the albumin + globulin content was observed in gluten fractions recovered from sieves with decreasing pore size. As the albumins and globulins are incorporated in the gluten matrix in different manners for different varieties, one can conclude that their role in the gluten agglomeration is rather aspecific.

The levels of albumins + globulins in the reconstituted gluten samples and their concentration ranges are slightly higher than those reported by Wadhawan and Bushuk (1989) for 27 commercial wheat gluten samples where the levels varied between ca. 6.9 and 8.9%. This is probably due to a greater washing efficiency in industrial practice than in the small scale procedure used in this work. The proportion of gliadin in their samples varied largely from 15.3 to 36.0% but no such differences were observed in the gluten from our six wheat varieties (17.9−22.3%). The largest differences observed in protein composition between the commercial wheat gluten and our samples are for the glutenin and residue fractions. Wadhawan and Bushuk (1989) reported values ranging from ca. 19.2 to 36.0% for the soluble (in 0.05 M acetic acid) glutenin fraction and from ca. 32.4 to 46.7% for the insoluble (residue) fraction, whereas in our samples, these levels varied from 40.0 to 46.0% and from 22.0 to 29.0%, respectively. The observed differences may well be due to the reduced solubility of the glutenins upon heating as shown by Wadhawan and Bushuk (1989), since the two laboratory-prepared hand-washed gluten samples included in their study also contained a higher glutenin content and a lower amount of insoluble glutenin than the commercial samples.

Osborne Protein Fractions versus Starch. Since gluten mainly consists of protein and starch, it is not surprising that a good correlation ($r^2 = 0.967$) exists between the protein content (% of dm) and the amount of Glc (% of dm) found in the fractions recovered from different sieves or in reconstituted samples (Figure 4). Since protein content and glutenin are closely related, a similar relation exists between the level of glutenin and the level of Glc ($r^2 = 0.917$). The level of gliadin is independent of the Glc level ($r^2 = 0.002$). The levels of albumins + globulins and the residue fractions decrease when Glc levels increase ($r^2 = 0.462$ and 0.237, respectively). The highly significant relationship between glutenin and Glc found in the different fractions again shows the importance of the former in gluten agglomeration. Indeed, it can be speculated that the more readily glutenin interacts with itself to form a "gluten backbone" with incorporation of gliadins (and albumins and globulins), the less starch will be included.

CONCLUSIONS

Gluten was isolated from wheat flours with different breadmaking performance in a batter system. The processing ability of the flours greatly differed as reflected by the GPR's and GPAI's. The differences in gluten agglomeration behavior of the varieties were ascribed to differences in glutenin aggregation. The aggregation tendencies of the acetic acid soluble glutenin fraction mainly determines the purity of the obtained gluten fraction. The gluten fractions with the best agglomerating properties contain the lowest levels of glucose and the highest levels of acetic acid soluble glutenin. Different aggregation tendencies of glutenins therefore are not only important with regard to their functionality in breadmaking but also in gluten agglomeration. Gliadins exert an opposite behavior in gluten agglomeration compared to the acetic acid soluble glutenin fraction and their proportions gradually increase as gluten fractions agglomerate more slowly. Albumins and globulins are incorporated nonspecifically in the gluten network.

ABBREVIATIONS USED

CBB, Coomassie Brilliant Blue; dm, dry matter; Glc, glucose; Gli/Glu, gliadin-to-glutenin ratio; GPAI, gluten protein agglomeration index; GPR, gluten protein recovery; GY, gluten yield; recon, reconstituted gluten samples.

ACKNOWLEDGMENT

The technical assistance of L. Van Den Ende and the help with the statistical analysis by Prof. P. Darius are greatly appreciated.

LITERATURE CITED

American Association of Cereal Chemists. *Approved Methods of the AACC*, 9th ed.; AACC: St. Paul, MN, 1995; Method 44-19, approved April 1961; Method 46-11A, approved October 1976.

Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein−dye binding. *Anal. Biochem*. **1976**, *72*, 248−254.

Englyst, H. N.; Cummings, J. H. Simplified method for the measurement of total nonstarch polysaccharides by gas−liquid chromatography of constituent sugars as alditol acetates. *Analyst* **1984**, *109*, 937−942.

Gupta, R. B.; Batey, I. L.; MacRitchie F. Relationships between protein composition and functional properties of wheat flours. *Cereal Chem*. **1992**, *69*, 125−131.

Hamer, R. J.; Weegels, P. L.; Marseille, J. P.; Kelfkens M. A study of the factors affecting the separation of wheat flour into starch and gluten. In *Wheat is Unique*; Pomeranz, Y., Ed.; American Association of Cereal Chemists: St. Paul, MN, 1989; pp 467−477.

Hoseney, R. C. Proteins of cereals. In *Principles of Cereal Science and Technology* (2nd ed.); American Association of Cereal Chemists: St. Paul, MN, 1994; pp 65−79.

Jones, J. B. Kjeldahl method for nitrogen (N) determination. Jones, J. B., Ed.; Micro-Macro Publishing: Athens, GA, 1991.

Kelfkens, M.; Hamer, R. J. Agronomic factors related to the quality of wheat for the starch industry. Part I: Sprout damage. *Starch* **1991a**, *43*, 340−343.

Kelfkens, M.; Hamer, R. J. Agronomic factors related to the quality of wheat for the starch industry. Part II: Nitrogen fertilisation and overall conclusions. *Starch* **1991b**, *43*, 344−347.

Orth, R. A.; Bushuk, W. A comparative study of the proteins of wheats of diverse baking qualities. *Cereal Chem*. **1972**, *49*, 268−275.

Prieto, J. A.; Kelfkens, M.; Weegels, P. L.; Hamer, R. J. Variations in the gliadin pattern of flour and isolated gluten on nitrogen application. Implications for baking potential and rheological properties. *Z. Lebensm. Unters. Forsch*. **1992**, *194*, 337−343.

Roels, S. P.; Cleemput, G.; Vandewalle, X.; Nys, M.; Delcour, J. A. The bread volume potential of variable quality flours of constant protein level as determined by factors governing the mixing time and baking absorption levels. *Cereal Chem*. **1993**, *70*, 318−323.

Roels, S. P.; Grobet, P. J.; Delcour, J. A. The distribution of carbohydrates in gluten fractions isolated from European wheats (*Triticum aestivum* L.) in a batter system. *J. Agric. Food Chem*. **1998**, *43*, 1334−1343.

Sindic, M.; Chevalier, O.; Duculot, J.; Foucart, M.; Deroanne C. Fractionnement du froment d'hiver: Influences variétale et phytotechnique. *Ind. Céréales* **1993**, *2*, 3−7.

Veraverbeke, W. Samenstelling en functionaliteit van vitale gluten. Thesis, Katholieke Universiteit Leuven, Belgium, 1995.

Wadhawan, C. K.; Bushuk, W. Studies on vitality of commercial gluten. II. Solubility, fractionation, electrophoresis, and fluorescence results. *Cereal Chem*. **1989**, *66*, 461−466.

Weegels, P. L.; Marseille, J. P.; Hamer, R. J. Small scale separation of wheat flour in starch and gluten. *Starch* **1988**, *40*, 342−346.

Weegels, P. L.; Marseille, J. P.; Hamer, R. J. De scheiding van tarwebloem in zetmeel en gluten. *Voedingsmiddelentechnologie* **1989**, *4*, 17−20.

Wonnacott, R. J.; Wonnacott, T. H. Hypothesis testing. In *Introductory Statistics*; John Wiley and Sons: New York, 1985; pp 257−289.

Received for review July 2, 1997. Revised manuscript received January 28, 1998. Accepted January 30, 1998.

JF970566F