

Contribution of Hydrophobic Soluble Gluten Proteins, Fractionated by Hydrophobic Interaction Chromatography in Highly Acetylated Agarose, to Dough Rheological Properties

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ABSTRACT

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Hydrophobic interaction chromatography with highly acetylated agarose in 1-mL columns was used to fractionate gliadins and acid-soluble glutenins. Proteins were eluted in two fractions, the first with acetate buffer (pH 3.6) containing 35% propanol, and the second with Tris buffer in 8M urea. The proportion of eluted protein in the second fraction was called the surface hydrophobicity index. The study included 20 wheat samples of different baking qualities. Multiple regression analysis using the general linear model combined with the stepwise technique was used to relate the surface hydrophobicity index of soluble gluten proteins to specific dough

rheological characteristics. Surface hydrophobicity index of gliadins and acetic acid soluble glutenins explained part of the variability of swelling index, extensibility, and work of deformation (dough strength) measured with the alveograph, and part of the farinograph water absorption variability, but showed no relationship to dough mixing characteristics. Hydrophobic soluble gluten proteins fractionated by hydrophobic interaction chromatography (HIC) explained a part of the variability of dough rheological properties.

Important characteristics of gluten proteins are aggregation properties and low solubility in water. The high proportion (30–40%) of amino acids residues with nonpolar and neutral chains and the low amount of ionizable amino acids are responsible for this low solubility (MacRitchie 1992). Differences in solubility of gliadins and soluble glutenins in alcohols of different alkyl chain length (propanol > ethanol > methanol) (Bean et al 1998) clearly show the importance of the hydrophobicity of these proteins. The hydrophobic interactions, along with hydrogen bonds and covalent disulfide bonds, define the aggregation properties of gluten proteins and make an important contribution to the rheological properties of gluten and dough (Popineau and Pineau 1987).

The surface hydrophobicity of gluten proteins has been studied using different methods. Greene and Kasarda (1971) and Popineau and Pineau (1987) used hydrophobic probes to study gliadins. Preston (1984) studied the aggregation of gluten proteins in the presence of neutral salts of the lyotropic series. In a recent study (Bean and Lookhart 1998), the selective extraction of HMW glutenin subunits in the presence of a nonchaotropic salt was explained by the aggregation of monomeric gliadins to a backbone of polymeric glutenin, possibly through hydrophobic interactions.

Reversed-phase (RP) HPLC has been used to study the hydrophobicity of gluten proteins (Burnouf and Bietz 1985). The most important contribution of this methodology has been the identification and quantitation of the protein species. It is useful in varietal identification (Huebner and Bietz 1994a) and in relating protein groups to quality (Huebner and Bietz 1994, Wieser et al 1994b). Van Lonkhuijsen (1992) used RP-HPLC to relate a highly hydrophobic γ -gliadin to loaf volume and Zeleny sedimentation value. Huebner et al (1997) also found a very high correlation (0.93) between γ -gliadin peaks and loaf volume. Reconstitution studies show the beneficial effects of hydrophobic gliadins on bread-making quality of flours (Weegels et al 1996).

Hydrophobic interaction chromatography (HIC) has also been used to study gluten proteins. Cadwell (1979), Popineau and Pineau (1987), and Popineau and Godon (1982) used HIC successfully to fractionate and to study surface hydrophobicity of gliadins. Chung

and Pomeranz (1979) used HIC in phenyl sepharose as the matrix to separate soluble glutenins and found that glutenins from low quality flours were less hydrophobic than those from good quality. Magnus and Khan (1992) found similar results when separating reduced and alkylated glutenins using phenyl sepharose.

Many studies have shown that a small number of HMW glutenin subunits are related to flour quality, especially mixing characteristics and bread volume (Payne et al 1979, Branlard and Dardevet 1985). On the other hand, this small number of polypeptides explains only a part of the variability of dough rheological characteristics and flour quality (Graybosch et al 1990). While the contribution from individual polypeptides is obviously important, it is the interactions of all proteins that determines gluten quality (Hoseney et al 1987, Primard et al 1991).

In this study, gliadins and acid-soluble glutenins were fractionated according to surface hydrophobicities using HIC in highly acetylated agarose. The small acetyl chains in the agarose matrix and the high density of substitution were used as an alternative to phenyl or larger alkyl chains of commercial products. The more hydrophobic fraction, called the surface hydrophobicity index, was related in a wide context and using a range of qualities in flour mainly from wheat cultivated in Mexico to specific dough rheological characteristics.

MATERIALS AND METHODS

Wheat

Twenty wheat samples (from 1997 and 1998), including commercial and experimental materials, from four quality groups were used: 1) strong gluten; 2) medium strong gluten; 3) weak gluten, and 4) tenacious gluten (as classified by the Norma Oficial Mexicana, NMX-ff-036-1996-SCFI). The samples included the cultivars, Rayon, Arivechi, Oasis, Opata, INIFAP, Tobarito, Cucurpe, Biavacora, and Tepoca. The cultivar Karl 92 (hard red winter wheat, crop 1996) was included. Wheat was milled in an experimental roller mill (Brabender Sr.).

Flour Quality

Flour protein content was determined by the micro-Kjeldahl method ($N \times 5.7$). Water absorption, dough development time, and stability were measured with a farinograph (Brabender 881043). Swelling index (G), resistance to deformation or tenacity (P), and work of deformation (W) of the dough were measured with a Chopin alveograph. Sedimentation test was also performed using Approved Methods (AACC 2000).

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Extraction of Gluten Proteins

Flour (2 g) was extracted with 10 mL of 0.7M acetic acid for 1 hr agitating every 30 min. Tubes were centrifuged at 10,000 × g for 15 min at 5°C with a refrigerated centrifuge (J2-M1, Beckman Instruments). Gliadins and acid-soluble glutenins (s-glutenins) were precipitated with 0.25M NaCl, procedure reported by Lew et al (1989). Gliadins were extracted from the pellet with 70% ethyl alcohol, and the residual proteins (acid-soluble glutenins) were solubilized in 0.7M acetic acid containing 50% propanol and 0.25% dithiothreitol (DTT).

The original flour pellet was extracted with 0.7M acetic acid containing 50% propanol and 0.25% DTT, based in the procedure reported by Kruger and Marchylo (1990). This last fraction was called acid-insoluble glutenins (i-glutenins). Protein content of protein solutions and chromatographic fractions was determined by the Bradford dye-binding procedure (Bradford 1976), calibrated for gluten proteins. To prepare gluten proteins for chromatography, gliadins and acid-soluble glutenins were dialyzed in 7 mM sodium acetate buffer (pH 3.6) for 12 hr. The residual flour pellet was saved, dried, and protein content determined with the micro-Kjeldahl method. The ratio between pellet and flour protein contents (% dry basis) was used as an estimator of insoluble polymeric protein (IPP), based in the work reported by Bean et al (1998). However, differences in the procedure of extraction were the presence of acetic acid and a reducing agent in the solution, in addition to the 50% propanol.

Hydrophobic Interaction Chromatography

Highly acetylated agarose was used as the hydrophobic matrix. This was synthesized using cross-linked agarose (CL-6B, Pharmacia, Uppsala, Sweden), and acetic anhydride with pyridine as the catalyst (Vazquez-Moreno et al 1992). Acetylated agarose matrix (1 mL) was used in a column with a 0.7 cm diameter. Chromatography was made using a controller system and peristaltic

pump (Bio-Rad Econo-System). The equilibrium buffer was 7 mM sodium acetate at pH 3.6. Two protein fractions (gliadins and acid-soluble glutenins) were applied onto the column in amounts of 2 mg (the capacity in the linear region of the isotherm was 8 mg/mL). The elution was in two steps. The first elution buffer was sodium acetate pH 3.6 containing 35% propanol, and the second was 0.015M Tris in 8M Urea. The flow rate was 0.5 mL/min. Buffer change was applied when fractions collected had a negligible protein content as estimated by the dye binding procedure (Bradford 1976). Recoveries were between 85 and 110%. The fraction eluted with 8M urea contained proteins that were more strongly bound to the hydrophobic matrix, and presumably had higher surface hydrophobicities. The proportion of protein eluted in the 8M urea fraction was called surface hydrophobicity index. The standard error of the proportion of protein eluted in the two fractions among repeated chromatographic runs was 2–3.5%.

Statistical Analysis

Descriptive statistical analysis was performed using the software Statistica (StatSoft, Inc.). Multiple regression, using the general linear model (Ott 1984) and the stepwise procedure was done using the SAS/STAT software (SAS Institute, Inc.). Additive effects of biochemical characteristics mainly from soluble gluten proteins with high surface hydrophobicity on dough rheological characteristics were studied.

RESULTS

Wheat Quality

The flours studied represented a wide range of qualities and exhibited a large variation in protein content, dough rheological characteristics, and sedimentation values (Table I).

TABLE III
Determination Coefficients for Models and Partial
for Farinograph Variables^a

Dependent Variable	Partial R ²	Model R ²	Prob > F
Stability			
IPP	0.45	0.45	0.0001
PROT	0.07	0.52	0.0271
HGLI	0.03	0.55	0.1096
Water absorption			
PROT	0.29	0.29	0.0003
SED	0.40	0.69	0.0001
HGLUS	0.07	0.77	0.0015
S-GLUT	0.04	0.81	0.0066
Development time			
IPP	0.11	0.11	0.0404
PROT	0.06	0.17	0.0978
SED	0.06	0.23	0.1068

^a IPP, insoluble polymeric protein (ratio between pellet protein and flour protein). PROT, flour protein content; HGLI, surface hydrophobicity index of gliadins; SED, sedimentation; HGLUS, surface hydrophobicity index of soluble glutenins; S-GLUT, acid-soluble glutenins extracted.

TABLE I
Mean Values and Variation of Flour Quality Parameters of Wheat

	Mean	Min	Max	SD
Protein content (%) ^a	12.24	09.54	15.52	1.24
Sedimentation (mL)	29.61	20.40	44.97	7.92
IPP (%) ^b	0.35	0.29	0.42	0.03
Alveograph measurements (mm)				
Tenacity, <i>P</i>	85.55	53.90	130.24	22.88
Extensibility, <i>L</i>	88.73	52.40	149.50	28.64
Swelling index, <i>G</i>	20.83	16.40	27.75	3.57
P/G	4.56	2.00	7.90	1.78
Work of deformation, <i>W</i>	233.74	134.65	371.00	66.32
Farinograph measurements				
Absorption (%)	61.37	54.80	66.90	3.61
Development time (min)	6.14	1.30	10.70	2.31
Stability (min)	10.92	3.00	22.90	4.90

^a N × 5.7

^b Insoluble polymeric protein (ratio between pellet protein and flour protein).

TABLE II
Correlation Coefficients Between Dough Rheological Properties and Biochemical Characteristics of Gluten Proteins^{a,b}

	SED	GLIA	I-GLUT	S-GLUT	<i>P</i>	<i>G</i>	<i>P/G</i>	<i>W</i>	ABS	DT	ST
PROT	0.52**	0.28*		0.34*		0.40**		0.49**	0.50**	0.25*	0.26*
HGLI		0.40*							0.34*		
HGLUS		0.38*									
SED			-0.26*		-0.39*	0.75**	-0.45*				
GLIA			0.33*								
IPP								0.40**		0.32*	0.68**
S-GLUT									0.34*		

^a SED, sedimentation; GLIA, amount of extracted gliadins; I-GLUT, amount of extracted acid insoluble glutenins; S-GLUT, amount of extracted acid-soluble glutenins; *P*, tenacity; *G*, swelling index; *P/G*, symmetry of alveograph curve; *W*, dough strength; ABS, farinograph absorption; DT, development time; ST, stability; PROT, flour protein content; HGLI, hydrophobicity index of gliadins; HGLUS, hydrophobicity index of soluble glutenins; IPP, insoluble polymeric protein.

^b *, ** significant at *P* < 0.05 and 0.01, respectively.

Extracted Protein

The amount of acid-soluble glutenins (s-glutenins) extracted from the flours was less than that of acid insoluble glutenins (i-glutenins) and gliadins (Fig. 1). The largest variation observed among the flours of the cultivars studied was in the amount of i-glutenins.

Hydrophobic Interaction Chromatography

HIC chromatograms of gliadins and s-glutenins are shown in Fig. 2A and B. Two fractions were obtained; the first eluted with sodium acetate buffer, pH 3.6, containing 35% propanol, and the second one eluted with Tris buffer in 8M urea. The proportion of protein eluted in the 8M urea fraction was the surface hydrophobicity index for gliadins and acid-soluble glutenins. Wide variation in surface hydrophobicity indexes were observed for the proteins of the cultivars studied (Fig. 3). Fractionation of gliadins and glutenins by HIC shows the importance and variability of surface hydrophobicities in this work as well as in earlier studies (Popineau 1985). Soluble glutenins showed a wider range of surface hydrophobicity indexes than did gliadins. The median and distribution of values shows that those proteins, on average, contain a higher amount of hydrophobic proteins than gliadins.

Simple Correlations

Table II shows the correlation coefficients for the variables involved in this study. For the farinograph variables, flour protein content correlated significantly ($P < 0.05$) with dough development time ($R^2 = 0.25$), stability ($R^2 = 0.26$), and farinographic water absorption ($P < 0.01$, $R^2 = 0.50$). The sedimentation value correlated only with farinographic water absorption. Development time and stability correlated with the same variables: flour protein content and insoluble polymeric protein (IPP). The difference between them was the higher correlation coefficient for IPP and stability ($R^2 = 0.50$) than that of IPP and development time ($R^2 = 0.33$). The relative importance of the insoluble polymeric protein for dough stability and the importance of protein content defining mixing characteristics can be inferred from the analysis of this data. However, the proportion of the variance that could be explained is very low. An interesting result in regard to the farinograph variables is the negative correlation between farinograph stability and the amount of extracted gliadin ($P < 0.05$, $R^2 = -0.30$).

For dough rheological characteristics measured with the alveograph, flour protein content was also important. Dough strength (W) and swelling index (G) correlated significantly ($P < 0.01$) with flour protein content ($R^2 = 0.49$ and 0.40). Dough extensibility (L) correlated significantly ($P < 0.01$) with the sedimentation value ($P < 0.01$, $R^2 = 0.87$), but no correlation was observed between W and the sedimentation value. On the contrary, W correlated with IPP ($P < 0.01$, $R^2 = 0.52$). The only correlation observed for dough

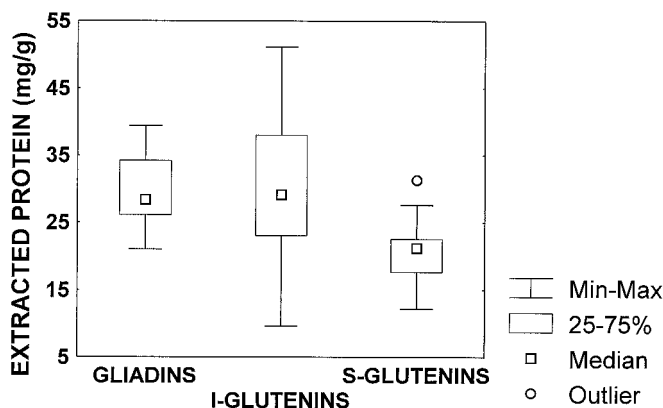


Fig. 1. Distribution of values (mg/g) of extracted gliadins, soluble glutenins (s-glutenins) and acid-insoluble glutenins (i-glutenins).

tenacity (P) was a negative one with sedimentation value ($P < 0.05$, $R^2 = -0.39$). Surface hydrophobicity index of gliadins correlated significantly with farinographic water absorption ($P < 0.05$, $R^2 = 0.34$). No simple correlations were observed between surface hydrophobicity index of soluble gluten proteins and dough rheological characteristics. The low simple correlation values for the variables where higher correlation coefficients were observed earlier suggested scattering of the data. The effect of the hydrophobic soluble proteins on dough rheological characteristics could have been not detected in such a set of diverse samples. Multiple regression, where combination of variables is taken into account to explain effects on a dependent variable, is a different way of analyzing the results.

Multiple Regression

Only 23% of the variability in dough development time could be explained with the variables and the set of wheat cultivars studied (Table III). Kinsella and Hale (1984) and Preston (1989) pointed out the importance of surface hydrophobicity of gluten proteins on hydration and mixing time. They explained the effect of simple neutral salts on dough development time as effect of accentuated hydrophobic interactions. In this study, the hydrophobic soluble gluten proteins fractionated by HIC did not affect development time. The most important factors defining development time were sedimentation value, insoluble polymeric protein, and flour protein content.

Protein content, sedimentation value, surface hydrophobicity index of s-glutenins (HGLUS), and the amount of s-glutenins extracted, explained 81% of the variability in water absorption (Table III).

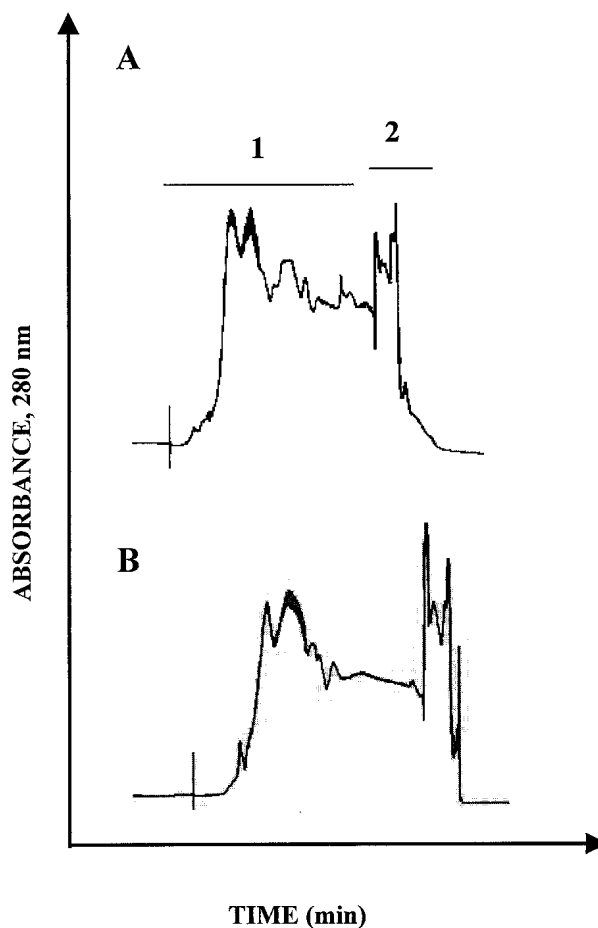


Fig. 2. Hydrophobic interaction chromatography elution profiles: A, gliadins from cultivar Oasis; B, acid-soluble glutenins from cultivar Karl; 1, fraction eluted with sodium acetate buffer pH 3.6 containing 35% propanol; 2, fraction eluted with 8M urea.

HGLUS explained 7% of this variability. Surface hydrophobicity index of gliadins (HGLI) also explained 1% of the variability. In multiple regression analysis, using the stepwise procedure, a 0.25% level of significance is recommended to avoid leaving important variables out of the model (Ott 1984). Because HGLI had a level of significance of 0.23%, this variable may be important in defining water absorption and might be more evident if a group of wheats with a smaller range in protein contents were used for the study.

Hydrophobic gluten proteins showed an opposite relationship to water absorption. For example, in Fig. 4A, HGLI shows a positive effect, while the effect of HGLUS is negative.

The variable that best explained dough farinographic stability (45%) was IPP (Table III). However, flour protein content and the surface hydrophobicity index of gliadins defined part of the farinographic stability variability (7 and 3%). In Fig. 4B, dough-mixing stability is greatly affected by IPP, whereas surface hydrophobicity index of gliadins has a negative effect on stability. It is interesting, that a destabilizing characteristic like hydrophobic gliadins positively affected water absorption. Findings agree with results reported earlier, where a strengthening and stabilizing effect such as the one expected by the hydrophobic interactions of glutenins is accompanied by decreased water absorption (Preston 1989).

Swelling Index

The variables that best explained swelling index (*G*) were the sedimentation value (76% of the variation), amount of the extracted i-glutenins, and surface hydrophobicity index of s-glutenins (HGLUS), (Table IV). These last two variables explained 3 and 2% of the variation, respectively. The multiple regression model explained 82% of the observed variability. Figure 5A shows the

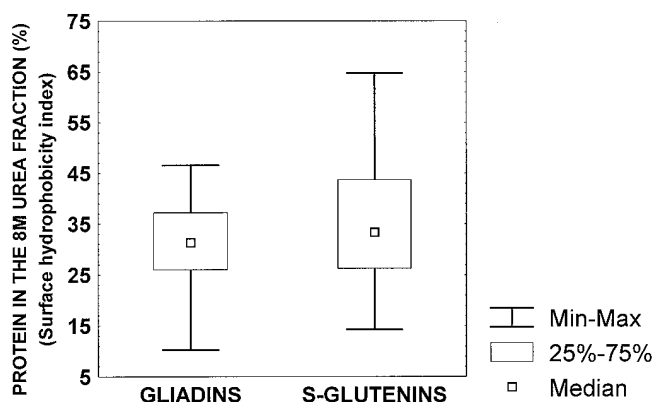


Fig. 3. Distribution of the estimated surface hydrophobicity indexes of gliadins and soluble glutenins.

TABLE IV
Determination Coefficient for the Model and Partial
for Alveograph Variables^a

Dependent Variable	Partial R^2	Model R^2	Prob > <i>F</i>
Work of deformation (<i>W</i>)			
PROT	0.27	0.27	0.0006
IPP	0.15	0.42	0.0033
HGLUS	0.06	0.49	0.0456
Swelling index (<i>G</i>)			
SED	0.76	0.76	0.0001
HGLUS	0.02	0.79	0.0374
I-GLUT	0.03	0.82	0.0236
Tenacity (<i>P</i>)			
SED	0.27	0.27	0.0006
PROT	0.17	0.45	0.0015
IPP	0.03	0.48	0.1605

^a IPP, insoluble polymeric protein (ratio between pellet protein and flour protein). PROT, flour protein content; HGLI, surface hydrophobicity index of gliadins; SED, sedimentation; HGLUS, surface hydrophobicity index of soluble glutenins; S-GLUT, acid-soluble glutenins extracted.

positive effect of sedimentation value and HGLUS. Preston (1984) observed increased extensibility (related to swelling index by a simple conversion) by the presence of high levels of a nonchaotropic salt and explained the effect by hydrophobic interactions between gluten proteins.

Dough Strength

Farinograph development time and alveograph tenacity (*P*) showed a relatively high ($R^2 = 0.73$) and very significant correlation ($P < 0.01$). These two variables were related and were defined by the same variables, but in different way. Tenacity was positively affected by IPP and negatively affected by the sedimentation value. On the other hand, the sedimentation value and IPP had a positive effect on dough development time.

The Zeleny sedimentation test provides a semiquantitative determination of the amount of glutenin macropolymer (Weegels et al 1996). Ciaffi et al (1996) found that the correlation between insoluble polymeric protein (left in the flour pellet after sonication) and *P* was better than the correlation between total polymeric protein and *P*. Our results are comparable to those obtained by Ciaffi (1996), even though the relationships were found in simple correlations that work, and we are using multiple regression and an indirect method (the sedimentation value) to index the polymeric protein. Both showed the relative importance of insoluble glutenin protein, defining *P* in regard to polymeric protein in flour.

Another way of estimating dough strength is using the alveograph parameter *W*, which is given by the area under the alveograph curve. Flour protein content, IPP, and surface hydrophobicity

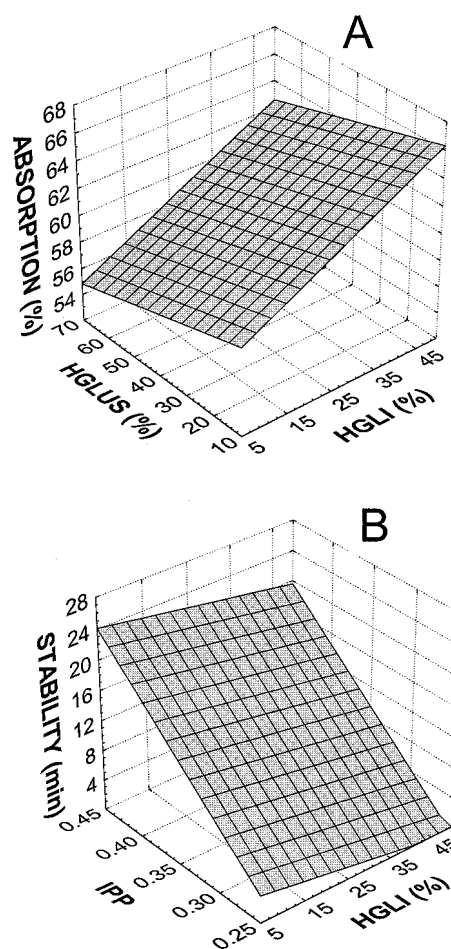


Fig. 4. A, Surface hydrophobicity index of gliadins (HGLI) and surface hydrophobicity index of soluble glutenins (HGLUS) on farinograph water absorption; B, insoluble polymeric protein (IPP) and surface hydrophobicity index of HGLI on dough farinograph stability.

DISCUSSION

index of s-glutenins (HGLUS) were the variables that best explained variation in W (Table IV). HGLUS explained 6% of this variation. Figure 5B shows the combined effect of remnant protein in pellet (IPP) and HGLUS on W . The higher W values were observed at the higher values of HGLUS. Both effects were observed earlier in separate studies. Preston (1984) found that hydrophobic interactions were important in defining W and Ciaffi et al (1996) found that insoluble polymeric protein correlated strongly with W . In Ciaffi et al (1996), the correlation coefficients were very high and found in simple correlation statistics. The extraction procedure used in the present work was partial reduction; Ciaffi (1996) used sonication. Both produced polymer breakage and both left the less accessible, entangled polymer. In general, we obtained low correlation values for the variables studied. On the contrary, Ciaffi (1996) obtained very high correlations between HP-SEC peaks and rheological characteristics. In this case, even with the differences mentioned, both works illustrate the relative importance of insoluble polymeric protein defining W .

Surface hydrophobicity of soluble glutenins is related to dough strength measured with the alveograph (Chung and Pomeranz 1979, Magnus and Khan 1992) where glutenins of better quality wheats were more hydrophobic. But results did not show relationships of hydrophobic soluble gluten proteins to mixing parameters. It is necessary to study the surface hydrophobicity of insoluble glutenin polymer and the aggregation properties resulting from the hydrophobic interactions and to relate them to dough mixing characteristics. However, it is improbable that HIC in the present conditions can be used for this purpose.

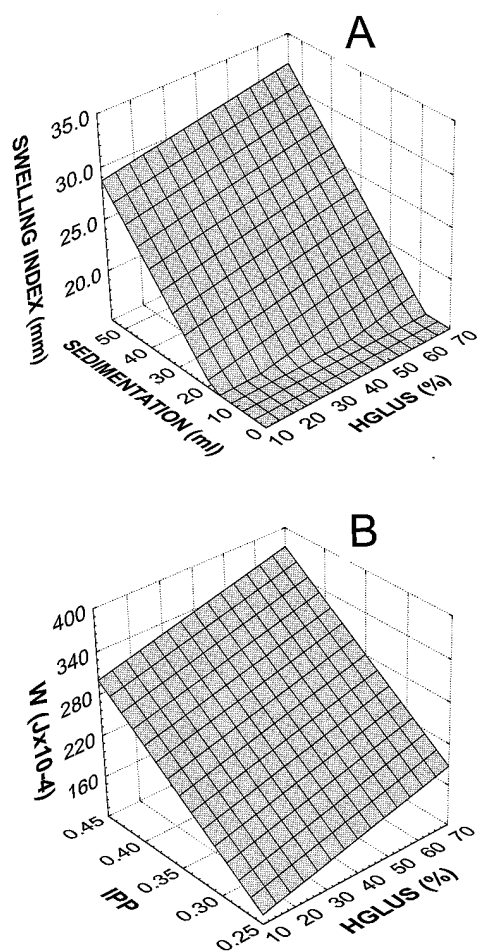


Fig. 5. A, Sedimentation value and surface hydrophobicity index of soluble glutenins (HGLUS) on swelling index (G); B, insoluble polymeric protein (IPP) and surface hydrophobicity index of gliadins (HGLI) on alveograph work of deformation (W).

HIC is appropriate to study hydrophobicity of gluten proteins because and it is a less denaturing procedure than reversed-phase HPLC (Popineau 1994). Adsorption to weakly hydrophobic stationary phases in HIC is due only to amino acids residues located in the surface (Hofstee 1976). In addition, the salt solutions used to induce adsorption to the hydrophobic matrix have a stabilizing effect (Arakawa and Nahri 1991). On the other hand, chromatographic conditions in RP-HPLC are denaturing. The high hydrophobicity of the stationary phases used for HPLC (generally C18) produces conformational changes. The high operation temperature used to separate gluten proteins by RP-HPLC (generally 70°C) (Bietz and Cobb 1985) is an additional denaturing factor. The adsorption in RP-HPLC is ruled by the hydrophobicity of extended chains (Popineau 1994). Hydrophobicity estimated by RP-HPLC relates to that calculated by amino acid composition, contrary to what happens with hydrophobicity calculated through HIC (Popineau 1994). During the HIC separation, elution produces denaturation when organic solvents are used, as is the case in this study.

Soluble glutenins were extracted with a reducing agent after the extraction with 0.7M acetic acid and precipitation with sodium chloride. After precipitation, part of the originally soluble glutenins became insoluble. Thus we included *n*-propanol and a reducing agent to solubilize the proteins of this fraction. Gliadins and acid-soluble glutenins were dialyzed against 7 mM sodium acetate buffer (pH 3.6) for chromatography. The equilibrium buffer, the same that was used to dialyze the protein fractions, contained the salt concentration needed only for a proper buffering action. To prevent protein precipitation, salt concentration was not increased. After removal of *n*-propanol and the reducing agent by dialysis, glutenin repolymerization occurred. SDS-PAGE without reducing agent of the dialyzed fractions showed undifferentiated smearing bands (data not shown).

Soluble gluten proteins were adsorbed by the matrix very easily, and it was not necessary to increase the ionic strength to induce protein adsorption. On the contrary, it was necessary to add $\leq 30\%$ propanol for the proteins to begin to elute (data not shown). Higher salt concentration would have produced protein aggregation. Proteins added to the HIC column were in solution; gliadins, monomers with no detectable aggregation, and soluble glutenins were relatively small polymers. Both acid-soluble glutenins and gliadins were soluble in the pH 3.6 acetate buffer.

Interactions of gluten proteins and other flour components are very complex. Proteins play a very important role defining dough rheological properties but they are not the only component. In addition, solubility and aggregation characteristics of the gluten proteins make difficult to mimic or reproduce in the laboratory the real conditions of a dough system. Due to the complexity of the system and to the ample genetic antecedents and diversity of growth environmental conditions of the samples studied, we cannot expect very high correlations. In fact, simple correlations did not show important relationship of surface hydrophobicity of soluble gluten proteins to dough rheological properties. Considering only the simple correlations, we can overlook the importance of surface hydrophobicity of soluble proteins defining dough rheology. It is necessary to look for another way of analyzing results. In multiple regression, considering a combination of the variables, only a small part of the variation that can be explained is important.

Hydrophobicity indexes were useful in explaining the relative importance of hydrophobic soluble gluten proteins in dough rheological properties. The equations would not be used for prediction because of the sample diversity. However, if an equation for prediction is needed, an appropriate set of samples should be analyzed.

CONCLUSIONS

HIC with highly acetylated agarose has shown that surface hydrophobicities of gluten protein varied widely. Soluble glutenins were

more hydrophobic and showed larger variability than gliadins. Simple correlations statistics did not show the relationship of surface hydrophobicity of soluble gluten proteins to dough rheological characteristics. Multiple linear regression with the selection variable procedure showed several relationships. Although the determination coefficients were relatively low, and surface hydrophobicity of soluble glutenins explained only a small part of the variation, the variables were included in the models with very high levels of significance.

Mixing time was not related to surface hydrophobicity of proteins measured with HIC. However, surface hydrophobicity of gliadins and soluble glutenins affect farinograph water absorption. Alveograph dough strength (*W*) and swelling index (*G*) were more likely to be affected by surface hydrophobicity of soluble gluten proteins.

Protein content and the sedimentation value defined large part of dough rheological properties. The insoluble polymeric protein (IPP) was related to dough tenacity, dough strength, and stability.

The hydrophobic fraction of soluble gluten proteins, being only a small part of the gluten proteins, explained a proportion of the variability of dough rheological properties.

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