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Hydrocolloid Interaction with Water, Protein, and Starch in Wheat Dough

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Interaction of hydrocolloids (xanthan gum, locust bean gum, guar gum, and high-methoxyl pectin) with macrocomponents of dough (water, starch, and protein) was evaluated by different techniques. ^{1}H spin−spin NMR relaxation assays were applied to study the mobility of the gluten−hydrocolloid−water matrix, and the amount of freezable water was determined by differential scanning calorimetry (DSC). Starch gelatinization parameters (*T*, enthalpy) were also analyzed by DSC. The influence of additives on the protein matrix was studied by Fourier transform (FT) Raman assays; analysis of the extracted gliadins and glutenins was performed by electrophoresis (SDS-PAGE). A significantly higher molecular mobility was found in matrices containing xanthan gum, whereas pectin led to the lowest molecular mobility. Freezable water showed a trend of increasing in the presence of hydrocolloids, particularly under conditions of water restriction. Starch gelatinization final temperature was decreased when hydrocolloids were added in the presence of enough water. In general, FT-Raman and SDS-PAGE indicated that hydrocolloid addition promoted a more disordered and labile network, particularly in the case of pectin addition. On the other hand, results obtained for dough with guar gum would indicate a good compatibility between this hydrocolloid and the gluten network.

KEYWORDS: Dough; xanthan gum; pectin; galactomannans; gluten; starch

INTRODUCTION

Hydrocolloids have deserved a particular attention in recent years as breadmaking improvers due to their effect on dough functionality, quality characteristics, and product preservation (*1*−*5*). These additives have the advantage of being extracted or derived from natural sources (*6*); in several cases they are considered to be GRAS (Generally Recognized As Safe). From a nutritional point of view, they can be regarded as fiber because they are only partially or not hydrolyzed by the enzymes in the human digestive tract.

Even though several papers have been published concerning the effect of hydrocolloids on dough behavior, less work has been done to elucidate the types of interaction that these additives can establish with the principal dough components. Bárcenas et al. (*7*) reported that hydrocolloids interacted with major components of wheat dough, affecting mainly the hydration properties of gluten and the gelatinization and retrogradation of starch. The effect of hydrocolloids varies depending on their chemical structure (*4*).

The hydrophilic character of gums is expected to modify water absorption when they are added to dough. In baking technology, this aspect has a strong impact because water absorption can influence bread quality as well as the cost of the baking process. In a previous work (*8*), the influence of different types of hydrocolloids (guar gum, xanthan gum, high-methoxyl pectin, and locust bean gum) on water absorption has been determined by different methods (farinograph, water-imbibing capacity, SDS test, and SRC sucrose test). Absorption values were particularly increased by xanthan and locust bean gum addition. Rheological changes were also observed, their magnitude depending on the level of hydrocolloid and water added. Thus, farinographic stability of dough was increased by guar gum and decreased by pectin. Texture profile analysis (TPA) showed that softer and less cohesive doughs were obtained in the presence of hydrocolloids. Textural attributes varied markedly when the level of water was higher.

NMR relaxation assays (T_2) can be applied for studying water mobility in dough and bread. Leung et al. (*9*) reported the mobility of water in doughs from different wheats; they found two distinct fractions: mobile water (with longer relaxation times) and immobile water (with shorter relaxation times). Also, they found that water mobilities of under- and overhydrated flour samples were different from those of the optimum doughs. More recent papers have also reported the results of NMR studies on wheat dough, at different stages of breadmaking. Esselink et al. (*10*) found that kneading for longer times rendered a gluten network with a threadlike structure with higher relaxation values. The highest mobility of this structure was attributed to the release of water due to the partial disruption of the gluten network. They also found that relaxation times were decreased (lower water mobility) after the molding step, involving gluten stretching and orientation, which led to a more ordered, less mobile network. Lopez-Da-Silva et al. (*11*) characterized dough from two wheat

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varieties differing in grain hardness and water absorption capacity. The dough obtained from the hardest variety exhibited a higher molecular mobility than the soft one. Engelsen et al. (*12*) applied NMR relaxation assays throughout the entire process from dough to bread, and they found that water mobility changed, particularly at the onset and offset of gelatinization and during the staling process of bread. However, even though studies on dough and bread are available, no characterization of water mobility in doughs containing hydrocolloids could be found.

With respect to hydrocolloid−starch interaction during breadmaking, the effect on starch gelatinization and retrogradation has been studied in different types of bread products, and particular attention has been paid to their antistaling effect (*5*).

Finally, the interaction of hydrocolloids with protein is a critical point because dough properties are closely related to the adequate formation of gluten network. A developed dough, having unique viscoelastic properties, is the result of protein hydration, unfolding, and orientation with complex reactions between sulfhydryl (S−H) and disulfide (S−S) bonds present in gluten (*13*, *14*). Because hydrocolloids are high molecular weight, hydrophilic molecules with variable structure (charged or not, linear or branched, and with or without chain flexibility), they should be expected to interfere (positively or negatively) in gluten development in a way related to their chemical structure. Several works have reported the dissimilar effect of hydrocolloids on dough rheological characteristics, but there is not abundant information about their influence on the structural aspects of gluten network. Ribotta et al. (*15*) found that some anionic hydrocolloids such as pectins and λ-carrageenans could form hydrophilic complexes with gluten proteins. Rosell and Foegeding (*16*) described the effect of modified celluloses (HPMC) on gluten properties.

Different techniques such as microscopy, calorimetry, Fourier transform (FT)-Raman, and NMR can give an approach to the structure and particularly the interaction among hydrocolloids and dough components. Other techniques such as electrophoresis are based on the isolation of the compound under study and involve a partial modification of the structure during the extraction process.

The objectives of this work were (a) to apply physical and chemical techniques to characterize the type of interaction between hydrocolloids and the principal dough components and (b) to compare the effect of different hydrocolloids on the main dough components.

MATERIALS AND METHODS

Materials. Medium-quality commercial flour (type 000, Código Alimentario Argentino) was provided by a local milling company (Molinos Campodónico, La Plata, Pcia de Buenos Aires). The characteristics of this flour were as follows: moisture, 14.9%; total protein content, 13.1% (wb); dry gluten, 9.7% (ratio wet gluten/dry gluten = 2.77); ash, 0.705%.

Commercial hydrocolloids (Saporiti S.A., Argentina) were used: xanthan gum (XG); locust bean gum (LBG) and guar gum (GG) (both galactomannans); and a highly methoxylated pectin (P). The mixture LBG+XG in equal proportions (1:1) was also assayed.

Dough Preparation. Percentage dough formulation was 100 g of flour, 2 g of NaCl, and 1.5 g of hydrocollloid.

For dough preparation two conditions of hydration were employed: (1) dough at constant hydration (amount of water corresponding to farinograph absorption of the flour without gums); or (2) dough at adapted hydration (amount of water adjusted for each blend, corresponding to each farinograph absorption value). Water absorption values from farinograph assays were 60.2% for the dough without hydrocolloid, 62.1% for dough with GG, 62% for dough with P, 66.4% for dough with XG, 66% for dough with LBG, and 65.5% for LBG+XG dough.

Samples were prepared in the kneader of a Brabender microfarinograph (capacity = 10 g flour) (Brabender, Duigsburg, Germany). Kneading time was adjusted for each sample by taking into account the farinographic development time [16.5 min for control dough, 18 min for GG dough, 15 min for P dough, 18.5 min for XG dough, 15 min for LBG dough, and 15.5 min for LBG+XG dough (*8*)].

After preparation, each dough was allowed to rest at room temperature for 10 min covered with a plastic film to avoid dehydration. For some assays fresh doughs were used, and in some cases (SDS-PAGE analysis), freeze-dried samples were required. Doughs were freeze-dried in a Heto-FD4 freeze-dryer (Heto-Holten, Denmark) and kept in hermetic plastic containers.

Gluten Preparation. For gluten isolation from doughs prepared with hydrocolloids a Glutomatic apparatus was used (Perten Instruments, USA). Dough with hydrocolloids requires different development times to reach the optimum consistency; for this reason the usual protocol for gluten determination (*17*) was modified. Ten grams of fresh dough prepared at adapted hydration was kneaded for 60 s and then washed for 5 min with a NaCl solution at 25 °C. The obtained gluten was freeze-dried in an Heto-FD4 freeze-dryer for further analysis in FT-Raman.

NMR Relaxation Assays. The molecular mobility of different doughs was analyzed by relaxation assays with RMN Bruker Minispec equipment (Bruker, USA). For these assays doughs were prepared under the two conditions described above (at constant hydration and at adapted hydration). A portion of fresh dough was put into the glass tube (10 mm diameter) up to 3 cm height, and the tube was then closed to avoid dehydration. ^{1}H spin−spin relaxation times (T_2) were measured using the Carr−Purcel−Meiboon−Gill pulse sequence. Assays were performed in duplicate.

DSC Assays: Freezable Water and Glass Transition Temperature. A Q100 calorimeter (TA Instruments, USA) was used to freeze samples and determine the amount of freezable water. Doughs were prepared under the two conditions (at constant hydration and at adapted hydration) described above. An aliquot of each fresh dough (10−14 mg) was weighed in an aluminum pan and hermetically sealed. An empty pan was used as reference in all runs. Samples were allowed to reach equilibrium inside the equipment cell at 20 °C for 5 min. Afterward, samples were frozen at a rate of 2 °C min^{-1} and tempered at −40 °C for 1 h. Samples were then heated at 2 °C min^{-1} to 20 °C to obtain ice fusion endotherms. From the area of the thermograms, enthalpies involved in the transition were determined, and the amount of ice was calculated by means of the ice-melting constant. Freezable water was obtained from the ratio of the amount of frozen water to the total water of each sample. Total water was determined by dehydration at 105 °C. The glass transition temperature was also measured as the temperature at which an endothermal step in heat flow was detected. Assays were performed in duplicate.

DSC Assays: Starch Gelatinization. Fresh doughs were prepared under optimum farinograph conditions for each mixture (farinograph absorption and development time), and an aliquot of each sample (10−14 mg) was exactly weighed in a DSC pan, then hermetically sealed and subjected to a heating program in a Q100 calorimeter. An empty pan was used as reference. Heating was performed from 20 to 130 °C at a rate of 10 °C min^{-1}. From thermograms, onset, peak, and final temperatures (T_o, TPI, TPII, and T_f) were obtained as well as gelatinization enthalpies (J g^{-1}). Assays were performed using four replicates.

FT-Raman Spectroscopy. Freeze-dried gluten samples were pulverized in a vibrating mill (ball mill Retsch, model MM-200) for 2 h, using agate sample holders and balls.

Raman spectra were collected on a Bruker IFS 113 FT-IR spectrophotometer (Bruker Optics, Germany) provided with the NIR Raman attachment equipped with an Nd:YAG laser at 1064 nm laser. Frequency calibration of the instrument was done using the sulfur line at 217 cm^{-1}. Spectra were recorded at room temperature with a laser power of 500 mW and spectral resolution of 6 cm^{-1}. Each spectrum was obtained after collecting and averaging 1000 scans to obtain high signal-to-noise ratio spectra. FT-Raman spectra were plotted as intensity (arbitrary units) against Raman shift in wavenumber (cm^{-1}). All spectra were vector normalized in the whole range (4000−500 cm^{-1}). The plotting, processing, normalization, manipulation, and evaluation of spectra were carried out through OPUS software (Bruker Optics, Germany). Band intensities were calculated after a linear baseline correction performed with the integration method developed within OPUS software. The intensity values obtained for the tyrosine doublet were calculated relative to the local baseline of each peak (830 and 850 cm^{-1}). Band assignment of the major vibrational motions of the side chains or the

peptide backbone was based on comparison to Raman data reported in the literature (*18*−*21*). All analyses were performed in three independent experiments, and the results of these replicates were reported on average. It was not possible to obtain a good Raman spectrum using the gluten−LBG−XG system, and for this reason the obtained data are not included under Discussion.

Separation and Identification of Protein Subunits by SDS-PAGE. Proteins were extracted from freeze-dried dough in a sequential form. For glutenin extraction two methods were utilized: (1) under dissociative conditions, from Singh et al. (*22*) as modified upon Nieto-Taladriz et al. (*23*), and (2) under acidic, nondissociative conditions (*24*).

For the first method, three solutions were used for protein extraction: 50% (v/v) 1-propanol (solution A); 50% (v/v) 1-propanol containing 0.08 M Tris-HCl buffer (pH 8.0) (solution B); and a buffer containing 0.08 M Tris-HCl buffer (pH 8.0), 2% (w/v) SDS, 40% (w/v) glycerol, and 0.02% (w/v) Bromophenol Blue (solution C).

Gliadins (20 mg) were extracted from dough with 0.5 mL of solution A, heated at 65 °C for 30 min with stirring every 10 min, and centrifuged for 2 min at 9300*g*. Supernatants were collected and dried at 60 °C overnight.

The precipitate was washed twice with solution A, disregarding the supernatants, and was used for glutenin extraction. Solution B with dithiothreitol (DTT) [1% (w/v), mixed immediately before use] was added (0.1 mL) to the former residue, incubated for 30 min at 65 °C, and centrifuged for 5 min at 15600*g*. Without discarding the supernatant, 0.1 mL of solution B containing fresh 4-vinylpyridine (1.4% v/v) was added, and samples were incubated at 65 °C for 15 min and centrifuged at 15600*g* for 2 min. Supernatants were transferred to another microtube containing 0.1 mL of solution C. Samples were incubated at 65 °C for 15 min and centrifuged at 15600*g*. Supernatants were used for glutenin characterization by SDS-PAGE.

Protein extraction under nondissociative conditions (method 2) was performed by a sequential procedure using dough (20 g) with 100 mL of the following solvents: (1) 0.15 M NaCl for albumins; (2) 50 mM Tris-HCl (pH 7.8) + 100 mM KCl + 5 mM EDTA for globulins; (3) 50% 1-propanol for gliadins; and (4) 0.1 M acetic acid for glutenins. Each step was carried out with permanent vortexing for 30 min, followed by centrifugation (1000*g* for 10 min). After each extraction, pellets were washed with the respective solvents, discarding the resulting supernatants. Gliadin and glutenin fractions were freeze-dried.

For electrophoresis runs, quantities from 6.0 to 8.0 mg of each protein sample were suspended into buffer solution (1.6−2.0 mL) composed of 0.37 M Trisma base, 25% w/v glycerol, 4% w/v SDS, and 0.1% w/v bromophenol blue. Two kinds of acrylamide gels were prepared (9 and 12%). Runs were performed in a Mini-PROTEAN 3 (Bio-Rad Laboratories, USA). The running buffer was composed of 0.192 M glycine, 0.025 M Trisma base, and 0.1% SDS (pH 8.3). Gels were stained for 24 h by an aqueous coloring solution containing acetic acid (16%), methanol (40%), and Coomassie Blue R (2%). The same solution without the colorant was used for discoloring the gels.

Low molecular mass standards (Amersham, GE, USA) of 97 kDa (phosphorylase *b*), 66 kDa (albumin), 45 kDa (ovalbumin), 30 kDa (carbonic anhydrase), 20.1 kDa (trypsin inhibitor), and 14.4 kDa (α-lactalbumin) were used.

Statistical Analysis. Statistical analysis was carried out using Systat 10.2 software (Systat Inc. 2002). ANOVA was performed to evaluate the effect of hydrocolloid addition and the Tukey test for comparison of means (95% confidence level). For comparison of two samples, the *t* test for comparison of means was applied at a confidence level of 95%.

RESULTS AND DISCUSSION

Effect of Hydrocolloids on Molecular Mobility and Water Binding. ^{1}H NMR relaxation assays have been extensively used for studying molecular mobility in different types of food systems (*25*−*28*). Nuclei are excited with pulses for a few miliseconds and when the pulse stops, nuclei return to basal state, emitting a signal. Typical relaxation plots can be fitted with equations having one, two, or more exponential terms, where the different relaxation times of each term (T_{21}, T_{22}, T_{23}, etc.) can be associated with different populations of molecules, having different mobilities. Species with shorter relaxation times are less mobile (solid-like state) than those with longer relaxation times (liquid-like state). The spin echo signal at $t = 0$ is proportional to the number of hydrogen nuclei of each species.

Table 1. Water Absorption, Molecular Mobility, and Freezable Water of Dough[a]

	adapted hydration			constant hydration		
sample	*A* (%)	T_2(ms)	freezable water (%)	*A* (%)	T_2 (ms)	freezable water (%)
C	60.2	12.9 bc	59.4 bc	60.2	12.9 a	59.4 c
XG	66.4	14.5 a	61.2 ab		13.0 a	62.0 b
GG	62.1	12.4 cd	58.1 c		12.4 ab	64.9 a
P	62.0	11.5 d	59.3 bc		11.6 b	60.1 c
LBG	66.0	13.8 ab	60.3 b		12.7 a	62.7 ab
LBG+XG	65.5	14.5 a	63.0 a		13.4 a	62.6 b

[a] Different letters within the same column indicate that values are significantly different ($p < 0.05$). Assays were performed in four replicates.

In the present work, molecules could not be differentiated in populations having different mobilities. Thus, the decay curves were fitted to an exponential model according to

$$I = A\, e^{(-t/T_2)}$$

where *I* represents the proton signal intensity, proportional to mobile water fraction in the sample, *t* is time, T_2 is a constant (the relaxation time), and *A* is the signal intensity of protons.

Leung et al. (*25*) investigated different systems based on corn starch, pectin, casein, and sodium alginate. Two different populations (with distinct mobility) were found only for corn starch; the other systems exhibited a monoexponential decay. These authors attributed this fact to the difficulty of detecting a multiexponential relaxation decay depending on the system under study. If the exchange rate between phases (bound water−mobile water) is fast compared with the relaxation rate, or if one phase is present as a small portion, or one of the relaxation times is very short, or both relaxation times are very similar, the instrument accuracy could be insufficient to detect the multiphase behavior. Thus, Leung et al. (*9*) reported a double-exponential decay for dough and assigned two different mobile fractions of water, although other authors (*11*) have also applied a simple exponential decay for modeling NMR relaxation curves in dough.

Values obtained for T_2 are shown in **Table 1**, ranging from 11.5 to 14.5 ms. A significant effect of hydrocolloid addition was found by ANOVA ($p < 0.05$). In the case of doughs at adapted hydration, it was observed that relaxation time significantly increased, with respect to control, when XG and LBG+XG were added. No significant differences were observed, compared to control when GG or LBG was added. A significant decrease in relaxation time was induced by P, indicating a lower molecular mobility.

When doughs with hydrocolloids were prepared at constant hydration (adding the farinographic amount of water corresponding to control sample), significantly ($p < 0.05$) lower relaxation times were obtained for XG, LBG, and LBG+XG samples when compared with the respective sample at adapted hydration. Besides, no significant differences were observed in relaxation times with respect to control except for P samples, which exhibited a significantly lower value.

Lusse and Arnold (*29*) studied the relaxation phenomenon in polysaccharide−aqueous systems, and they attributed the observed differences in the relaxation rate ($1/T_2$) of water in the bound state to reorientational processes with respect to the binding site and the mobility of the polymer backbone. Solutions with a stiff polymer backbone exhibited larger relaxation rates (lower relaxation times,

so lower mobility) than those of flexible polymers. Lopes-da-Silva et al. (*11*) reported different mobilities for protein/water matrices in doughs from different flours, stating that differences in mobility corresponded to differences in network rigidity.

This suggests that the final conformation and, particularly, flexibility of the combined gluten−hydrocolloid−water matrix would finally determine the degree of water binding. Therefore, if the final protein spatial conformation is modified by hydrocolloid presence, it will render different matrices with distinct rigidity/flexibility and ability to bind water.

A parameter usually used for the evaluation of the degree of water binding is the amount of freezable water (*28*) with respect to total water (%). Total water percentage for fresh dough ranged from 45 to 50% for samples at adapted hydration and from 46 to 48% for samples at constant hydration. To obtain frozen doughs with the maximum concentration of solids in the unfrozen matrix, tempering was performed at temperatures nearly below the T_g value. Control samples exhibited an onset glass transition temperature (T_g^o) of −34.4 °C and a final glass transition temperature (T_g^f) of −32.1 °C. The addition of hydrocolloids did not significantly affect these temperatures with respect to control in most cases (data not shown).

Percentage values for freezable water are shown in **Table 1** for doughs at adapted hydration and at constant hydration. ANOVA analysis indicated that there was a significant effect of the hydrocolloid addition ($p < 0.05$). For samples at adapted hydration, the percentage amount of freezable water was significantly increased only in the case of LBG+XG, although XG samples also showed high values (but not significantly different from control). These two samples exhibited the highest relaxation times (highest mobility). For the other samples (GG, P, and LBG), the availability of water for the freezing process was similar in all cases.

When doughs with hydrocolloids are prepared at constant hydration, restriction in water availability for freezing would be expected due to the strong hydrophilic character of these molecules. However, the percentage of freezable water was always significantly higher than the value obtained for the control sample, with the exception of P samples. Freezable water evidenced a trend toward higher values in comparison to samples without water restriction. This fact seems contradictory in relation to the lowest molecular mobility observed in samples prepared with constant water. However, mobility seems to reflect the degree of stiffness of the network. Campos et al. (*13*) reported that undeveloped doughs portrayed stiffer dough structure. Thus, when water restriction during dough development is imposed, a more rigid conformation of the gluten−hydrocolloid−water matrix could be expected. At the same time, these matrices could bind water in a less tight way, leading to more freezable water (%).

Effect of Hydrocolloids on Starch Gelatinization. Starch gelatinization has been thoroughly studied; it can be defined as a solvent- and heat-induced melting of starch crystallites, restricted by kinetic limitations (*30*). The first step for this nonequilibrium transition is water diffusion that plasticizes the amorphous regions of starch granules preceding the melting of crystalline zones. Afterward, swelling of granules continues due to hydration of the disordered polymers (*31*, *32*). Therefore, a reduction in water availability as a result of hydrocolloid addition should be reflected in gelatinization parameters.

Characteristic temperatures and enthalpies corresponding to starch gelatinization in the presence of hydrocolloids in dough at adapted hydration are shown in **Table 2**. The endotherms appear unfolded in two peaks (data not shown), a fact that is characteristic of starch systems with water restriction (*33*). Hydrocolloid addition markedly affected gelatinization enthalpies ($p < 0.05$).

Table 2. Gelatinization Temperatures of Doughs Made at Adapted Hydration[a]

sample	T_o (°C)	TPI (°C)	TPII (°C)	T_f (°C)	ΔH (J g^{-1})
C	60.79	69.95	87.66 a	98.35 a	5.08 ab
XG	61.30	69.32	85.61 ab	95.53 bc	3.90 b
GG	60.44	69.90	87.37 a	97.53 ab	4.48 ab
P	60.52	70.29	87.12 ab	95.80 bc	4.06 ab
LBG	59.83	68.78	84.32 b	95.33 bc	5.42 a
LBG+XG	60.24	69.46	84.94 ab	94.63 c	5.40 a

[a] Different letters within the same column indicate that values are significantly different ($p < 0.05$). Assays were performed in four replicates.

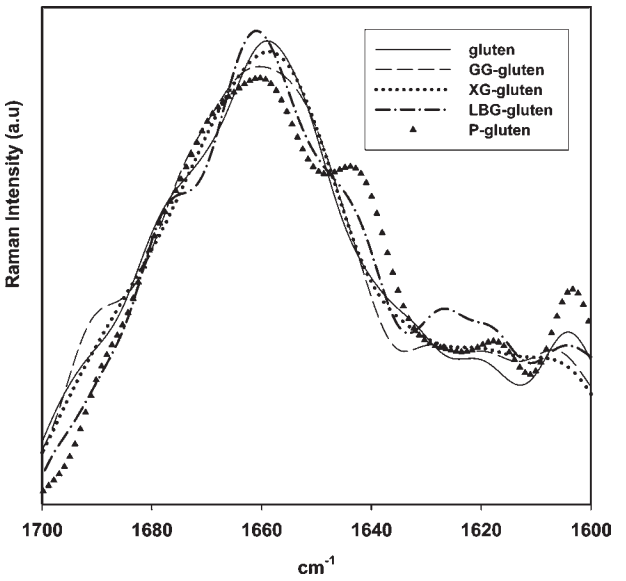

Figure 1. FT-Raman amide I bands of different gluten samples.

Significant differences were found between dough with XG and dough with LBG or LBG+XG, but samples with hydrocolloids were not significantly different from control samples. T_o and TPI were not significantly affected by hydrocolloid addition ($p > 0.05$). However, second peak temperature (TPII) and, particularly, the final temperature (T_f) were significantly affected ($p < 0.05$). Because enough water was added, there was not observed a shift of T_f to higher values due to water restriction in the presence of hydrocolloids (*34*). On the contrary, a significant shift to lower temperatures was shown with respect to control, with the exception of GG samples. A lower T_f indicates that water is more available for the gelatinization process, a fact coincident with the highest molecular mobility observed for the dough matrix in samples with hydrocolloids.

Effect of Hydrocolloids on Protein Structure. *FT-Raman. Amide I Band.* Raman bands corresponding to amides I and III can be used to characterize protein backbone conformation. Proteins that have α-helix contents show an amide I band centered around 1650−1660 cm^{-1} and other peaks at 1681, 1674, 1639, 1630, and 1619 cm^{-1} corresponding to β-antiparallel, β-turn, random coil, solvated helical structure, and β-sheet, respectively (*18*, *35*). **Figure 1** shows the amide I region in typical Raman spectra obtained for gluten and gluten−hydrocolloid samples. As can be seen, amide I is centered at 1657 cm^{-1}, this result being consistent with previously reported data (*36*), thus indicating that the secondary structure of gluten is dominated by α-helix conformation. Union of hydrocolloids to food proteins affects electrostatic and hydrophobic interactions, leading to conformational changes.

An increase of intensity of the band assigned to the α-helix conformation of LBG-gluten was observed, whereas a decrease of

intensity of this band was evident for GG−gluten, P−gluten, and XG−gluten (**Figure 1**). A higher intensity of this band in comparison with the corresponding band in control gluten is typically related with an increased folding of the protein, suggesting conformational changes that lead to a more ordered structure (*35*). In LBG−gluten systems, the change in the α-helix band was accompanied by a decrease in intensity of the bands assigned to β-antiparallel (1681 cm^{-1}) and β-turn (1674 cm^{-1}) and by an increase of β-sheet (1619 cm^{-1}) and solvated helical (1630 cm^{-1}) structures. On the contrary, lower intensity of the band assigned to the α-helix conformation for GG−gluten (minor changes), P−-gluten, and XG−gluten systems, with respect to control, was related to a certain degree of unfolding of the protein. In this case, there was an increment in the intensity of the bands that are associated with disordered structures and β conformations: (i) for the GG−gluten system, there was a slight increase in the intensity of the bands related to β-antiparallel, β-turn, and β-sheet components; (ii) for the P−gluten system, there was an increase in the intensity of the bands related to random coil (a pronounced change), solvated helix, and β-sheet components; (iii) for the XG−gluten system, there was an increase in the intensity of the bands related to β-turn, solvated helix, and β-sheet components.

A certain trend seems to relate freezable water to FT-Raman results. More freezable water in systems with hydrocolloids (XG and LBG, **Table 1**, adapted hydration) seems to be related to higher intensity of α-helix band and, in general, a more ordered gluten structure. On the other hand, for P and GG doughs, with less mobility and freezable water, a more disordered structure is suggested by the lesser intensity of the α-helix band.

Side-Chain Vibration Analysis. (*a*) *Tryptophan Modes*. The tryptophan band (760 cm^{-1}) has been proposed to be used as an indicator of strength of H-bonding and hydrophobicity of the environment of the indole ring. The addition of hydrocolloids caused strong changes in the normalized intensity of the 760 cm^{-1} band. **Figure 2** shows that XG was the hydrocolloid that led to the major decrease in this band, followed by P and GG. These results suggest that the residue comes from a buried hydrophobic microenvironment and contributes to the formation of a more disordered structure. Dissimilar behavior was observed for the intensity of this band in the LBG system; a great increase in its intensity was obtained, suggesting an increment in the "buriedness" of the tryptophan residues in the protein after the addition of the LBG.

(*b*) *Tyrosine Doublet*. Hydrocolloids produced dissimilar and significant changes in gluten samples. Changes indicated that the microenvironments of tyrosyl groups were greatly altered by the interaction of the additive with the gluten protein. The ratio of the tyrosyl doublet around 850 and 830 cm^{-1} ($I_{850/830}$) is known as a good indicator of the hydrogen bonding of the phenolic hydroxyl group. A decrease of the $I_{850/830}$ ratio was reported to reflect an increase in buriedness, suggesting possible involvement of tyrosyl residues in intermolecular or intramolecular interactions. When tyrosine residues are exposed, the 850 cm^{-1} band becomes more intense than the 830 cm^{-1} band. These changes can be observed in **Figure 2**. In GG−gluten and GX−gluten systems, there was a decrease of the $I_{850/830}$ ratio in comparison with control gluten. This behavior is likely to be related with the chemical structure of the additives, particularly to the availability or not of the OH groups to the interaction with the tyrosine residues of the protein. Probably, tyrosine residues would be buried and do not interact with these hydrocolloids. For P−gluten and LBG−gluten, a small and a great increase, respectively, of the $I_{850/830}$ ratio was observed.

(*c*) *Disulfide Region*. The disulfide stretching vibration (500−550 cm^{-1}) is commonly determined to evaluate conformational changes, because the disulfide bridge is very important in maintaining a particular tertiary structure of a protein. The

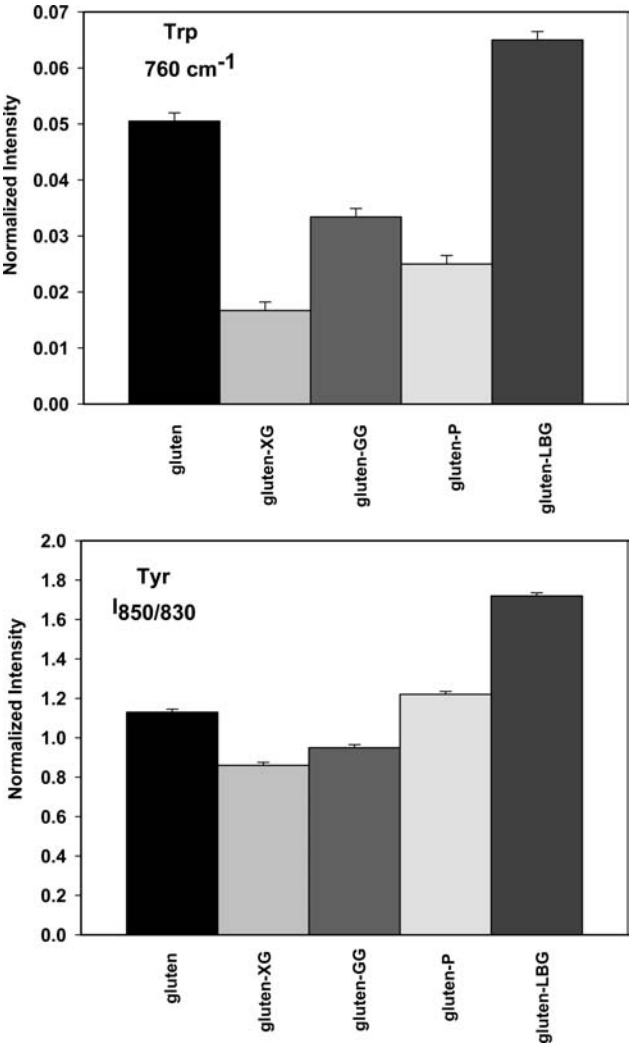

Figure 2. FT-Raman normalized intensity of tryptophan band (760 cm^{-1}) and intensity ratio of tyrosine bands ($I_{850/830}$) of gluten samples. Bars on columns indicate standard deviation.

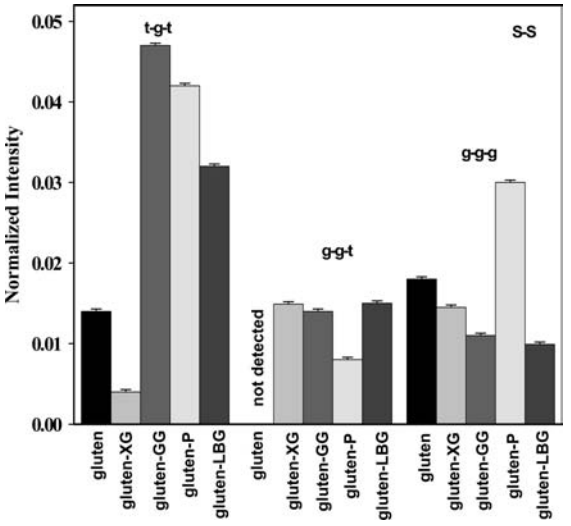

Figure 3. FT-Raman normalized intensity of the Raman S−S stretching vibrations in the 470−550 cm^{-1} region. Bars on columns indicate standard deviation.

symmetrical stretching vibration of the S−S bond is influenced by the conformation of the C atoms in the disulfide bridge. The

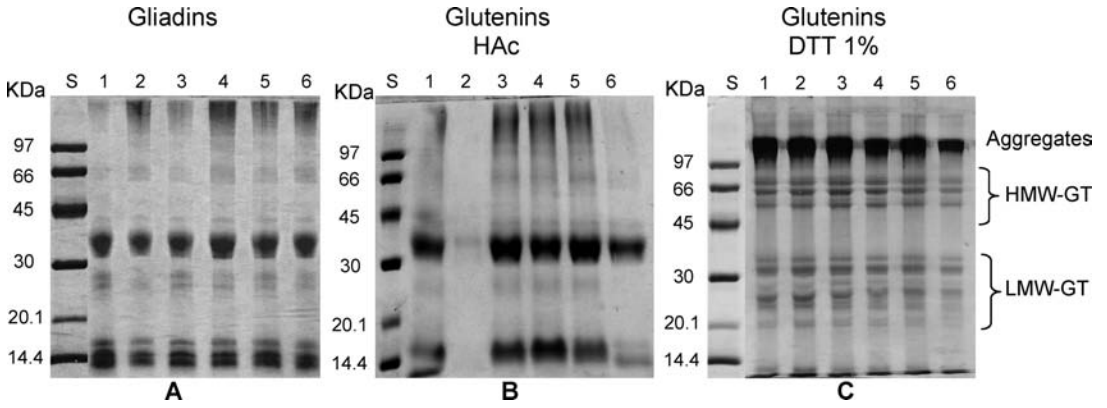

Figure 4. SDS-PAGE electrophoretic patterns of gliadins (**A**), glutenins extracted in acetic acid (**B**), and glutenins extracted in dissociative conditions (**C**) of dough. Lanes: control dough (1); dough with XG (2); GG (3); P (4); LBG (5); LBG+XG (6); S, molecular weight standard.

vibration at 510 cm^{-1} can be assigned to the gauche−gauche−gauche (g-g-g) conformation, the band at 525 cm^{-1} to the gauche−gauche−trans rotamer (g-g-t), and the vibration at 540 cm^{-1} as the band that belongs to the trans−gauche−trans conformation (t-g-t). In control gluten the bands associated with these vibrational modes are located at 534 cm^{-1} (t-g-t rotamer) and at 515 cm^{-1} (g-g-g rotamer), respectively. Modifications of intensity in these bands are shown in **Figure 3**. The g-g-g conformation was the most intense band in control gluten. In samples with hydrocolloids, several alterations were detected. Except for P−gluten, the g-g-g conformation was decreased in its relative intensity. The g-g-t conformation was absent in control gluten, but it appeared in hydrocolloid systems, with the lowest signal intensity in P−gluten systems. The t-g-t rotamer was strongly increased in its relative intensity except for XG−gluten. These results might suggest that conformation of disulfide bonds was strongly altered after the addition of hydrocolloids. In the most ordered system (LBG−gluten), the relationship corresponding to the t-g-t, g-g-t, and g-g-g conformations band intensities were in a ratio expressed by integers (*18*, *37*). For P−gluten a pronounced change in the proportion of disulfide conformations was observed.

Protein Composition. Gliadin and glutenin subunits can be separated by SDS-PAGE (*38*). **Figure 4A** shows the electrophoretic profiles for the gliadins extracted from control dough and dough prepared in the presence of hydrocolloids. With the exception of GG dough, high molecular mass proteins (> 97 kDa) were extracted from doughs in the presence of gums, indicating the existence of soluble aggregates. These aggregates would be formed by gliadins linked through noncovalent interactions (*14*). Within the range of 30 and 45 kDa, a defined group of bands was observed in all samples, corresponding to α-, β-, and γ-gliadins. Below 20.1 kDa, several intense bands corresponding to low molecular mass peptides were observed. No differences in the profile of gliadins were detected among samples. With respect to the assignment of these bands, several authors (*39*−*41*) have reported the existence of gliadins with molecular masses lower than 30 kDa.

The profile of glutenins extracted with acetic acid is shown in **Figure 4B**. Low molecular weight glutenins (LMW-GT) were more extractable than those of high molecular weight (HMW-GT). It is evident that a minor quantity of glutenins was extracted from dough prepared with XG alone or mixed with LBG. These results suggest that, in comparison with the other hydrocolloids, a more entangled gluten network was formed in the presence of xanthan gum. In addition, in a previous work (*8*) it was found that one of the most elastic networks had been obtained in the presence of xanthan gum. On the other hand, LMW-GT were more labile in the presence of GG, P, and LBG.

When glutenins were extracted with a reducing agent such as DTT, both kinds of subunits, LMW-GT and HMW-GT, were observed in protein profiles (**Figure 4C**). No differences in the HMW-GT profiles were evidenced, although in the LBG+XG sample, a lesser proportion of these proteins and aggregates was detected. From electrophoresis results, it can be concluded that the presence of gums during the gluten network formation can affect the lability of certain protein subunits. Thus, different proteins seem to stabilize the matrix. Differences in gluten network are also reflected in the distinct farinographic stability found for doughs with hydrocolloids (*8*).

In conclusion, even in the presence of enough water and after optimum development time, doughs with hydrocolloids were affected in their gluten network conformation. In most cases, protein is more unfolded and the conformation of disulfide bonds is different from dough without hydrocolloids as demonstrated by FT-Raman results. In general, more subunits could be extracted from the matrix in all cases, particularly from the sample of dough with pectin. This fact indicates greater lability of certain components of the protein network, particularly of gliadins, that are not bound through covalent unions.

The particular matrix (gluten−hydrocolloid−water) can be more or less rigid, and this is reflected in its molecular mobility. A significantly higher mobility was found in XG containing matrices, probably indicating a more flexible network. On the other hand, pectin led to a more disordered gluten conformation, but the obtained network would seem rather rigid in accordance with the lesser stability of this dough as reported in previous studies.

In general, hydrocolloid addition promoted a more disordered and labile network, the degree of rigidity and water-binding capacity being dependent on the type of hydrocolloid added. With regard to pectin, the interaction with protein led to a labile, less mobile matrix, whereas in the guar-added dough, results would globally indicate a good compatibility between this hydrocolloid and the gluten network.

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