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ATR-FT/IR Study on the Interactions between Gliadins and Dextrin and Their Effects on Protein Secondary Structure

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The effects of heat treatment and dextrin addition on the secondary structure of gliadins were investigated by means of attenuated total reflection Fourier transform infrared spectroscopy (ATR-FT/IR). Gliadins and gliadin/dextrin mixtures (before and after thermal treatment) were prepared as a dried protein film on the ATR-FT/IR zinc selenide cell plate and equilibrated at a water activity (a_w) of 0.06. The results show that gliadins undergo conformational changes upon thermal treatment both in the absence and in the presence of dextrin. In particular, in the thermally treated gliadins, the decrease of the band at around 1651 cm^{-1} and the increase of the bands at around 1628 and 1690 cm^{-1} suggest a loss of α-helix structure and a higher content of protein aggregates. The same trend was observed in the presence of dextrin. Concerning the interactions between gliadins and dextrin, gliadin/dextrin mixtures show variations in the amide I region compared to native gliadins (e.g., an increase of the band at 1645 cm^{-1} and the absence of the band at around 1668 cm^{-1}) that might be due to hydrogen bond formation between gliadins and dextrin. It was also found that the spectrum of gliadin/dextrin mixtures was less affected by the hydration state than that of native gliadins, as observed from the differential spectra obtained by subtraction of the spectrum obtained at $a_w = 0.06$ (driest condition tested) from the spectrum of the sample equilibrated at $a_w = 0.84$. This could be due to the fact that C═O and N−H groups of gliadins are engaged to form hydrogen bonds with the hydroxyl groups of dextrin, and so they are not perturbed by the presence of water molecules. Finally, water activity effects on the secondary structure of gliadins are also discussed.

KEYWORDS: Heat treatment; water activity; attenuated total reflection Fourier transform infrared spectroscopy; protein−polysaccharide interactions; hydrogen bonds

INTRODUCTION

The prolamins are the storage proteins of cereal seeds. In the wheat seed the prolamins, namely, gliadins, are a family of heterogeneous polypeptides of molecular mass between 30000 and 75000 Da. Gliadins are monomeric and disulfide-bonded proteins that are formed by a nonrepetitive domain rich in α-helix structure and by a heterogeneous repetitive domain rich in β-reverse turns (*1*). These proteins are of relevant industrial interest as revealed by the numerous patents that have been developed in recent years by the food and nonfood industries. In particular, zein and gliadins were used with polysaccharides to encapsulate microcomponents (fats, enzymes, pesticides, small molecules for antifungal and cancer immunotherapy use). Even though the secondary structure of gliadins has been extensively studied (*2*−*5*), little is known about the interactions between this class of protein and polysaccharides, and in only a few cases have conformational studies of gliadins been conducted in the presence of macromolecules (e.g., lipids, polysaccharides, nonstarch polysaccharides) (*6*−*8*). Besides this, it is important to note that the peculiar sequence (rich in prolines and glutamines) and structure of gliadins have been suggested to be responsible for their toxicity in people affected by coeliac disease (*1*). Therefore, a better understanding of the structure of gliadins mixed with macromolecules commonly employed in the food industry and of the interactions existing between them should provide useful indications that might lead to the preparation of nontoxic foods for people affected by nutritional diseases. In the study herein described we have investigated crude gliadin samples because from the application point of view these are the forms that are actually used for industrial applications.

We have previously reported some evidence suggesting an interaction between gliadins and polysaccharides such as β-dextrin and starch (*9, 10*). In these experiments, we observed that the rate of the hydrolysis of the polysaccharides, carried out by amyloglucosidase (used as enzyme probe), decreased in the presence of gliadins. Because of this we hypothesized the presence of gliadin/polysaccharide interactions that hinder the access of the polysaccharide to the catalytic active site of the

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enzyme. In the present work we hoped to gain more information on the effects of dextrin on the secondary structure of gliadins (molecular mass of 31000 Da) and on the type of the interactions formed between the protein and the polysaccharide. To this end, infrared spectroscopy is a very powerful technique because it allows the analysis of the secondary structure of proteins and of their hydrogen-bonding pattern (*11*, *12*), even in the presence of other macromolecules (*13*−*15*). In particular by ATR-FT/IR it is possible to analyze the protein sample as a film formed directly on the ZnSe plate of the attenuated total reflection Fourier transform infrared spectroscopy (ATR-FT/IR) cell without using any plasticizer or salt (e.g., potassium bromide) that could interfere with the interaction between the protein and dextrin. Dextrin (a short polymer of glucose, molecular mass of 3000 Da, produced by maize amylopectin) was chosen because it is present in flour or it is added to raw food materials to improve their technological processing.

MATERIALS AND METHODS

Materials. Gliadin [crude, from wheat, a mixture of α-, *β*-, *γ*-, and *ω*-gliadins (*3*)] and dextrin (type III from corn) were purchased from Sigma and used without any further purification. All other reagents were of analytical grade.

Sample Preparation and FT/IR Measurements. Native gliadins and dextrin were used by suspending them in water. The sample of gliadins mixed with dextrin (gliadins + DEX) was obtained by mixing the two components (ratio of 1:1, w/w) with 60% of water for the dough formation, at 25 °C. Then it was freeze-dried, and the lyophilized powder was sieved at 60 mesh and stored at 4 °C. The sample of gliadins mixed with dextrin and thermally treated (gliadins + DEXt) was identically prepared except that it was closed in a vial and heated in a thermostatic oven at 100 °C for 30 min before being freeze-dried. The thermally treated gliadins sample after mixing the proteins with 60% of water was prepared analogously to gliadins + DEXt.

In all cases the sample was prepared for the ATR-FT/IR analysis by injecting the protein suspension (10 mg of gliadins or gliadin/dextrin mixture in 300 μL of distilled water) into the ATR-FT/IR cell and draining water by dry nitrogen. A homogeneous thin layer of gliadins or gliadin/dextrin mixtures was obtained at the solid state that, for convenience, we call film. The film was equilibrated to the desired water activity (a_w), at 25 °C, by piping the cell to a sealed vessel containing a saturated salt solution of known a_w (LiBr for $a_w = 0.06$ and KCl for $a_w = 0.84$) (*16*), until the absorbance of the sample remained constant.

ATR-FT/IR spectra were recorded on an FT/IR-600 spectrophotometer (Jasco) using an ATR-FT/IR cell, the internal reflection element of which was a ZnSe plate, with an aperture angle of 45°, yielding 17 internal reflections. Spectra were recorded as the average of 256 scans at 2 cm^{-1} resolution, using the empty cell (equilibrated at the same a_w value of the sample) as blank. During measurements the cell compartment was flushed with dry nitrogen. In all cases sample analyses were performed at least twice.

Data Treatment. Spectra were Fourier self-deconvoluted by the Jasco software (application Spectra Analysis) using a full-width of the deconvolution filter of 30 cm^{-1}. Next, the relative contribution of each band component of the amide I band was determined by curve fitting of the deconvoluted spectra following the procedure of the GRAMS/32 program. To start the fitting, parameters including maximum number of peaks, sensitivity, shape of peaks, full width at half-height (fwhh), and baseline function were set. We operated with a fwhh value of 16 cm^{-1} and a linear baseline between two points that are far from the position of the extreme underlying peaks considered in computing areas. Fitting was automatically performed by iterative adjustment of data until χ^2, a statistical parameter that indicates the "goodness-of-fitting", was $0 < \chi^2 < 0.18$. Because the Fourier deconvoluted spectra were reproducible (they had always the same bands of **Figure 2**), the curve fitting analysis (**Figure 3**) was performed for only one set of samples. The quantitative analysis of protein structure from Fourier deconvoluted FT/IR spectra was not carried out to quantify the absolute content of various secondary structure components, but aimed only to verify if heating or dextrin addition causes changes in the secondary structure of the same protein.

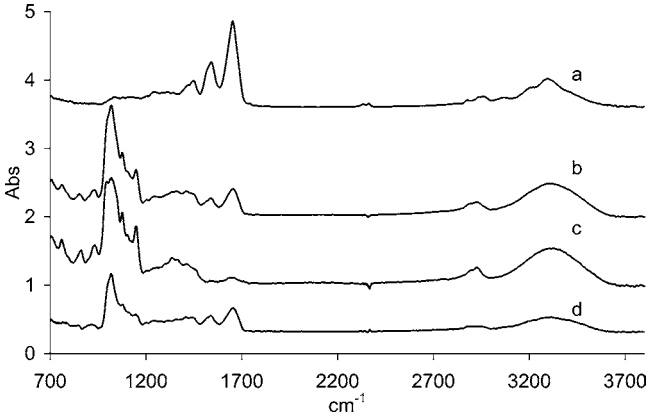

Figure 1. Original FT/IR (ATR) spectra of films at $a_w = 0.06$: (**a**) native gliadins; (**b**) gliadins + dextrin (ratio 1:1, w/w); (**c**) dextrin; (**d**) spectrum obtained from spectrum **b** minus 0.5 × spectrum **c**. Spectra are offset along the *Y*-axis for clarity.

RESULTS

FT/IR Spectra and Band Assignment. Spectra **a**−**c** of **Figure 1** display the original spectra obtained at $a_w = 0.06$ of a film of gliadins in the absence (**Figure 1a**) and presence (**Figure 1b**) of dextrin and the spectrum of dextrin (**Figure 1c**). Both gliadins and dextrin show bands at higher wavenumbers in the 3000−3600 cm^{-1} region. These were ascribed to the O−H stretching vibrations in the case of dextrin and to the O−H and N−H stretchings in the case of gliadins. Analogously, they show a common group of bands in the 2800−3000 cm^{-1} region that are due to C−H vibrations. More specific bands of dextrin fall in the 950−1185 cm^{-1} region, which might be attributed to C−O and C−C stretchings but also to deformational vibrations of the CCH, COH, and HCO bonds (*17*−*19*). Instead, the broad bands in 1180−1500 cm^{-1} region could be due to C−H bending of CH_2 and to O−H bending (*17*). It is important to note that all of these intense bands do not overlap with the bands observed for gliadins in the 1600−1700 cm^{-1} region (amide I) and in the 1500−1600 cm^{-1} region (amide II), which are due to C═O stretching and N−H bending, respectively. We focused on these two bands to monitor the secondary structure and the pattern of hydrogen bonds of gliadins in the various conditions tested. In particular, the amide I band is considered to be the most reliable indicator for the secondary structure analysis of proteins (*12*, *20*, *21*). It is composed of several overlapping components that can be assigned to different secondary structure conformations. Moreover, shifts of amide I and II maxima can be used to monitor the formation of hydrogen bonds between the C═O and N−H of the protein amide bonds and water molecules (*22*−*24*).

In the spectrum of dextrin (**Figure 1c**) can be noted a small band in the region from 1600 to 1700 cm^{-1}. Because this band could cause a misestimation of the peak maximum and intensity of the amide I band of gliadins in gliadin/dextrin mixtures, we subtracted from the spectrum of gliadins in the presence of dextrin (**Figure 1b**) the spectrum of dextrin alone (**Figure 1c**) according to the equation

$$\text{spectrum d} = \text{spectrum b} - (\gamma \times \text{spectrum c})$$

The resulting spectrum is shown in **Figure 1d**.

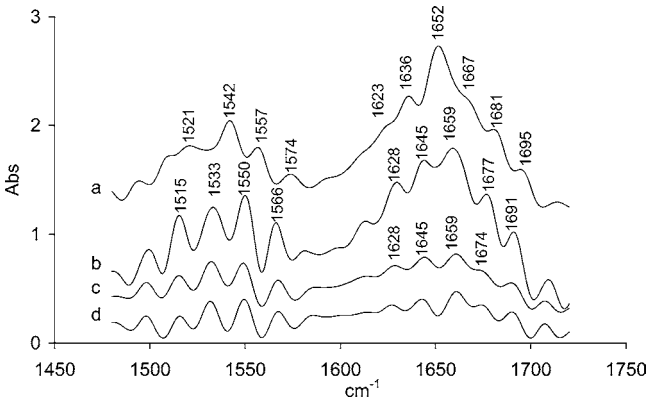

Figure 2. Fourier-deconvoluted FT/IR (ATR) spectra at $a_w = 0.06$ of (**a**) native gliadins, (**b**) gliadins thermally treated, (**c**) gliadins + DEX, and (**d**) gliadins + DEXt. Spectra are offset along the *Y*-axis for clarity.

Spectrum c (spectrum of dextrin) was multiplied by a factor γ in order to avoid an over- or underestimation of the absorbance that has to be subtracted from spectrum b. It was not possible to neglect the use of this factor because in ATR-FT/IR spectra the intensity of absorbance depends on several elements, including, among others, the thickness of the film and its refractive index (*25*), which might be different for gliadins and dextrin or mixtures of them. The value of γ was chosen by imposing the resulting spectrum d as similar as possible to that of the spectrum of gliadins without dextrin. To quantify the overall similarity between the two spectra, the correlation coefficient *r*, calculated using the equation

$$r = \Sigma^N x_i y_i / (\Sigma x_i^2 \Sigma y_i^2)^{0.5}$$

was used (*14*). x_i and y_i represent the spectral absorbance values of the reference (gliadins without dextrin) and sample (gliadin/dextrin mixture subtracted of dextrin) spectra at the *i*th frequency position. For identical spectra, a value of 1.0 will be returned. We found for both gliadins + DEX and gliadins + DEXt the highest correlation coefficient (for the 1200−1750 cm^{-1} range *r* was 0.99 and 0.80, respectively) when $\gamma = 0.5$. An alternative method taken into account to determine γ was to subtract dextrin from gliadin/dextrin mixtures until the intense band of dextrin at around 1024 cm^{-1} completely disappeared in the differential spectrum (e.g., obtaining a flat line). However, this approach did not produce satisfactory results, because the resulting protein spectrum was quite different from that of native gliadins, especially in the regions of 1200−1500 and 2800−3700 cm^{-1}. Therefore, the use of the correlation coefficient as criterion to evaluate how to subtract the spectrum of dextrin from that of gliadin/dextrin mixtures appeared to be the most appropriate one and was adopted in this study.

Amide I Band and Secondary Structure Analysis: Effect of Heat Treatment on Native Gliadins. To verify the positions of the components forming the amide I and amide II bands, the Fourier self-deconvolution method was applied to the original spectra of gliadins prepared in the absence or in the presence of dextrin and equilibrated at the same water activity value ($a_w = 0.06$) (**Figure 2**). Next, to have an estimation of the area of each of the components of the amide I band, the curve fitting of the Fourier deconvoluted amide I band was achieved (**Figures 3** and **4**) (*20*). Note that by the curve-fitting analysis the peak maxima were slightly shifted compared to those that were only Fourier deconvoluted and new bands had to be included to have $0 < \chi^2 < 0.18$. However, this did not interfere with the secondary structure assignment, because it was done by referring to ranges of wavenumbers rather than to a specific frequency.

To investigate dextrin and temperature effects, the study of native gliadins in the absence of polysaccharide was necessary as a control. We found that the spectral position of the components of the amide I in native gliadins (**Figure 2a**) is in good agreement with that reported by Wellner et al. (*2*) and by Mangavel et al. (*4*). The Fourier deconvoluted spectra of native gliadins (**Figure 2a**) show several differences with respect to the thermally treated ones (**Figure 2b**), which indicate that the protein undergoes conformational modifications upon heat

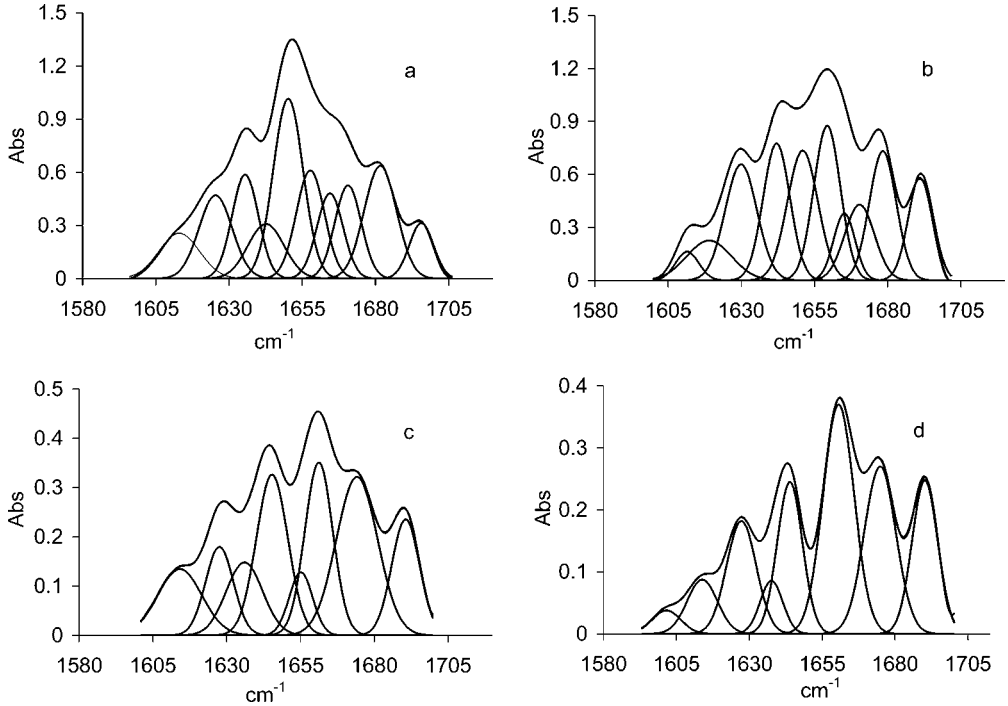

Figure 3. Fourier-deconvoluted FT/IR (ATR) spectra in the amide I region at $a_w = 0.06$ and reconstituted spectra after curve fitting. The reconstituted spectrum was drawn as the sum of fitted Gaussian bands. For description of samples a–d see the caption of **Figure 2**.

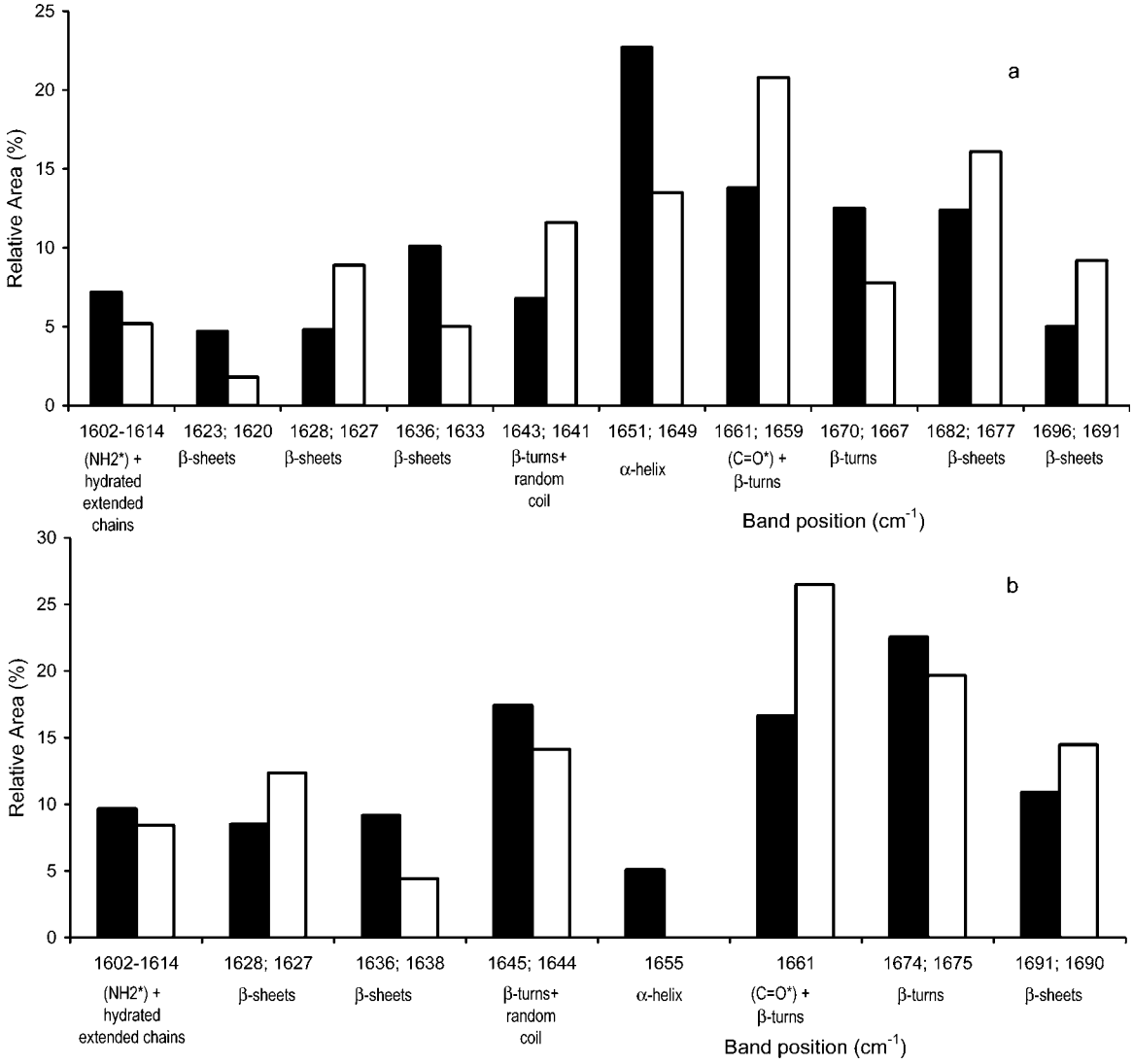

Figure 4. Peak position and relative areas of the bands fitted to the Fourier-deconvoluted spectra of gliadins (**a**, black bars), gliadins thermally treated (**a**, white bars), gliadins + DEX (**b**, black bars), and gliadins + DEXt (**b**, white bars). Each band was assigned to the component of the secondary structure according to previous data.

treatment. It can be observed that the amide I band of the thermally treated gliadins does not show the maximum at 1652 cm^{-1} (present in native gliadins), but has two maxima at 1659 and 1645 cm^{-1}. Further differences are the absence of the maxima at around 1681 and 1636 cm^{-1} and of the shoulder at 1623 cm^{-1} in the thermally treated sample. Instead, the latter one shows two maxima at 1677 and 1628 cm^{-1}.

By the curve-fitting analysis it was found that the component in the amide I band at 1651 cm^{-1} has the highest relative area in native gliadins (**Figure 4a**). Although this component might also contain some contributions due to random coil conformation (*4*, *26*) and to the glutamine side chain (*6*, *12*, *20*, *21*, *27*−*30*), it is conventionally assigned to the α-helix. Tatham et al. (*1*) suggested for gliadins α-helix structures in their nonrepetitive domains.

The two bands present in native gliadins (**Figure 3a**,**b** and **4a**) at 1643 cm^{-1} (evident only by the curve-fitting analysis) and at 1670 cm^{-1} (or at 1667 cm^{-1} in thermally treated gliadins) can be assigned to the stretching of the carbonyls of the *β*-turns, in particular, to the internally hydrogen-bonded (acceptor C═O of the strong 1←4 intramolecular hydrogen bond) and to the nonbonded ones, respectively (*2*, *11*). However, the band at 1643 cm^{-1} could also be due to aperiodic or random coil conformation (*6*, *11*, *12*), in agreement with the result that the relative area of the band at 1641 cm^{-1} in thermally treated gliadins is higher (relative area of 11.6% instead of 6.8%) than for native gliadins (**Figure 4a**). In fact, it is reasonable that the thermal treatment of gliadins could cause an increase of the unordered structures (*31*). On the contrary, the band at 1667 cm^{-1} observed in the thermally treated sample has a relative area of 7.8% that is lower than in native gliadins (peak maximum at 1670 cm^{-1} and relative area of 12.5%), verifying that the fraction of the non-hydrogen-bonded carbonyls of *β*-turns decreased upon thermal treatment.

More puzzling was the attribution of the band at around 1660 cm^{-1}, which is significantly more intense for thermally treated gliadins than for native gliadins (relative area of 20.8% instead of 13.8%). A large contribution to this band might be due to the C═O stretching of the glutamine side chain. Moreover, Wellner et al. (*2*) have shown that poly-L-glutamine and *ω*-gliadins as well have a broad band with the maximum at around 1658 cm^{-1}, particularly evident for the sample analyzed in dry conditions. It was suggested by the same authors that the broadening of this band could be due to the presence of a wide range of conformations originated from the distortion of the native secondary structure upon formation of intra- and

intermolecular hydrogen bonds between glutamine side chains and backbone peptide groups. An increment of such hydrogen bonds might also be present in thermally treated gliadins, causing the enhancement of the band at 1659 cm^{-1} that we observed (**Figure 4a**). In fact, it is well documented that proteins undergo aggregation and precipitation upon thermal treatment, a condition that could favor intra- and intermolecular formation of hydrogen bonds (*32*).

The bands at around 1628, 1636, and 1696 cm^{-1} in native gliadins are highly characteristic of β-sheet structures presenting differences in hydrogen bonds between β-strands. In particular, we found that both bands at 1628 and 1696 cm^{-1} doubled their intensity in the thermally treated sample (**Figure 4a**).

The components between 1600 and 1623 cm^{-1} were assigned to hydrated β-sheets structures (*4*) and extended hydrated chains (*30*). Moreover, the NH_2 scissoring of the glutamine side chains also contributes to the band with maximum between 1600 and 1614 cm^{-1}.

The two bands observed at around 1682 cm^{-1}, in the case of native gliadins, and at around 1677 cm^{-1} for thermally treated gliadins (**Figures 3a**,**c** and **4a**) have not been definitively assigned. For example, Mizutani et al. (*6*) assigned the bands at 1682 and 1673 cm^{-1} to β-sheets and β-turns, respectively, in the case of zein analyzed as solid powder. On the contrary, Yang et al. (*33*) assigned the same FT/IR bands to β-turns and β-strands in myoglobin, cytochrome *c*, and β-lactoglobulin. These papers show the uncertainty in the assignment of these components of the amide I band. Nevertheless, according to Mizutani et al. (*6*), we tentatively assigned the band at 1682 cm^{-1} to β-sheets and that at 1674 cm^{-1} to β-turns (this latter band can be observed in the spectra obtained with gliadin/dextrin mixtures, which are discussed in the next section). Indeed, the band with maximum at 1677 cm^{-1} (slightly shifted toward higher wavenumbers, with respect to that at 1674 cm^{-1}) in the thermally treated sample could be due to the broadening of the signal in the region between 1680 and 1695 cm^{-1} typically observed for intermolecular hydrogen-bonded β-sheets.

Dextrin Addition. The self-deconvoluted FT/IR spectra of gliadins + DEX and that of gliadins + DEXt are shown in **Figure 2c**,**d**, respectively. Both spectra show a very similar shape, and they are quite different from those of gliadins without additive, especially from that of native gliadins. The comparison of the relative areas (**Figure 4**) obtained by curve fitting shows that the band at 1655 cm^{-1} in gliadins + DEX is much smaller than that of native gliadins at 1652 cm^{-1}, being 5.1 and 22.7%, respectively. Furthermore, it is important to note that in the case of gliadins + DEXt no contributions were present in the spectrum from 1644 to 1661 cm^{-1}. Analogously to what was observed passing from gliadins to thermally treated gliadins, these data suggest that the band at 1655 cm^{-1} in the case of gliadins + DEX is due to α-helix structures, which are lost in gliadins + DEXt because of the thermal treatment.

With regard to the marked reduction of the intensity of the band between 1651 and 1655 cm^{-1} of gliadins + DEX (**Figure 4b**) compared to native gliadins (**Figure 4a**), it is worth noting that it might depend on a different hydrogen bond pattern of the protein caused by dextrin and that it is not necessarily related to changes of secondary structures. In this context it is worth noting that a general trend observed for amide I is that C═O stretch vibrations of hydrogen-bonded carbonyls appear at lower wavenumbers than those of free carbonyls (*22*, *34*).

The more intense bands at around 1627 and 1690 cm^{-1} in gliadins + DEXt than in gliadins + DEX (**Figure 4b**) could be indicative of a higher content of intermolecular hydrogen-bonded antiparallel β-sheets. Moreover, as in the absence of dextrin (**Figure 4a**), the band at 1661 cm^{-1} in gliadins + DEXt is higher than that observed in gliadins + DEX (**Figure 4b**). These data show that dextrin does not have effects on the groups that contribute to this band, which could be in favor of its assignment to the C═O of those glutamine side chains which form intra- and intermolecular hydrogen bonds with the backbone peptide groups. Such interactions should not be perturbed by dextrin that might not have access to the buried parts of the molecule of gliadins because of steric hindrance.

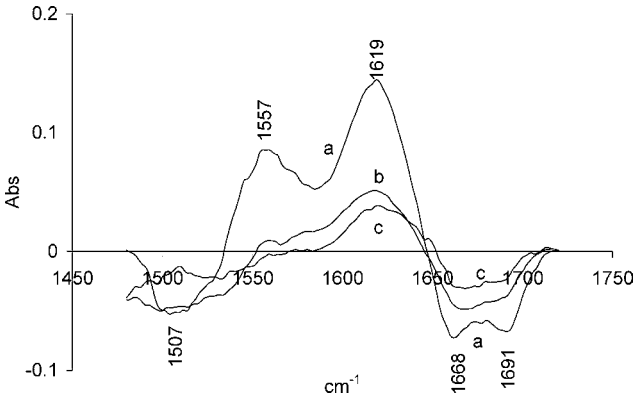

Figure 5. Differential spectra of (**a**) native gliadins, (**b**) gliadins + DEX, and (**c**) gliadins + DEXt obtained by subtracting from the spectrum at $a_w = 0.84$ the spectrum at $a_w = 0.06$.

The band at 1674 cm^{-1} in gliadins mixed with dextrin instead of that at around 1682 cm^{-1} present in native gliadins might suggest that dextrin causes a decrease of intermolecular hydrogen-bonded β-sheets in gliadins.

Water Activity Effects. To have more indications on the nature of the interactions between gliadins and dextrin, the water activity effect on the FT/IR (ATR) spectra of native gliadins and gliadins/dextrin mixtures was investigated.

There are numerous reports on the comparison of the secondary structure of protein analyzed by FT/IR at different states of hydration (*13*−*15*). Nevertheless, a different approach to the study of the water uptake of protein has been proposed by Maréchal and co-workers, who analyzed the variations of the protein spectrum upon protein hydration (*22*, *23*). The method consists of analyzing differential spectra obtained by subtracting the spectrum of the protein in the driest conditions from those in more hydrated states. The differential spectra which are obtained show minima and maxima that correspond, respectively, to the decrement of the non-hydrogen-bonded and to the increment of the hydrogen-bonded C═O and N−H groups, which establish hydrogen bonds with water molecules. We adopted this approach to monitor the variations in the amide I and amide II regions upon hydration of gliadins and gliadin/dextrin mixtures, obtaining a roughly quantitative indication on the fraction of C═O and N−H groups of gliadins that pass from a non-hydrogen-bonded to a hydrogen-bonded status. To this end the differential spectra of native gliadins (**Figure 5a**) and gliadin/dextrin mixtures (**Figure 5b**,**c**) were obtained by subtracting from the spectrum at $a_w = 0.84$ that at $a_w = 0.06$. It can be observed that native gliadins (**Figure 5a**) show a broad minimum with two main components at 1691 and 1668 cm^{-1}, a maximum at 1619 cm^{-1} in the amide I region, and a maximum/minimum in the amide II region at 1557/1507 cm^{-1}. In agreement with the assignment of Grdadolnik and Maréchal both of these differential bands are indicative of hydrogen-bond formation of native gliadins with water molecules (*22*, *24*). In particular, in native gliadins (**Figure 5a**), the minimum/

maximum in the amide I region suggests that a fraction of carbonyl groups accepting no hydrogen bonds (minimum at 1691 cm^{-1}) and carbonyls accepting one hydrogen bond from N−H amide group (minimum at 1668 cm^{-1}) at $a_w = 0.06$ form hydrogen bonds with water molecules (maximum at 1619 cm^{-1}) when hydrated at $a_w = 0.84$. Analogously, the maximum/minimum in the amide II region shows an increment of hydrogen-bonded N−H groups (maximum at 1557 cm^{-1}) and a corresponding decrease of free N−H groups (minimum at 1507 cm^{-1}). Unfortunately, due to the overlapping between the signal of gliadins and that of dextrin in the region at around 1400 cm^{-1} (where the FT/IR bands of free and hydrogen-bonded carboxyl groups typically fall), no reliable minima/maxima could be detected and univocally assigned to the carboxyl group of the protein. This hindered our evaluation of whether carboxyl groups of gliadins were rehydrated in the protein/dextrin mixtures.

Interestingly, if the differential spectrum of native gliadins is observed referring to the secondary structures of the protein, the maximum at around 1619 cm^{-1} (which corresponds to an increment of a component having maximum at around 1619 cm^{-1} in the amide I band) indicates a higher fraction of hydrogen-bonded carbonyls of β-sheets and of hydrated extended chains (*30*) as expected when passing from $a_w = 0.06$ to $a_w = 0.84$. Moreover, the minimum at 1668 cm^{-1} (**Figure 5a**) could be indicative of a decrease of non-hydrogen-bonded carbonyls of β-turns (*2*, *11*). Finally, the decrement of the bands at 1691 cm^{-1} might also suggest a decrease of the intermolecular hydrogen-bonded antiparallel β-sheets typical of aggregates. This latter observation agrees well with the hypothesis reported by Mizutani et al. (*6*), who suggested that the decrement of the band at around 1690 cm^{-1} upon protein hydration could be due to the fact that water molecules disrupt such intermolecular hydrogen bonds. In summary, all of these observations seem to verify that the hydration of native gliadins does affect their hydrogen-bonding pattern but without an evident effect on their secondary structures.

DISCUSSION

The results presented in this study show that the secondary structure of gliadins and gliadins + DEX is altered by heat treatment, which likely causes loss of α-helix structures, as generally observed with globular proteins (*31*) and as indicated by the strong decrease of the amide I band at around 1651 cm^{-1} passing from native (relative area = 22.7%) to thermally treated gliadins (peak maximum at 1649 cm^{-1} and relative area = 13.5%). In favor of this interpretation variations in the amide II region were also observed. In fact, the bands at 1542 cm^{-1} and at 1521 cm^{-1} (**Figure 2a**) that several authors have assigned to α-helix (*33*, *35*) are absent in the thermally treated gliadins (**Figure 2b**). Moreover, the increment of the bands at around 1627 and 1693 cm^{-1}, which well represent the splitting of the amide I absorption observed in the case of intermolecular hydrogen-bonded antiparallel β-sheets (*6*, *36*), confirms the presence of a higher fraction of protein aggregates in the thermally treated sample than in native gliadins. Dextrin does not prevent either conformational changes or protein aggregation of gliadins caused by the heat treatment.

Some evidence that gliadins interact with dextrin by means of hydrogen bonds has been provided by analyzing the FT/IR spectral position of the free and hydrogen-bonded C═O and N−H groups. We found that the total area of the bands from around 1645 to 1600 cm^{-1} in gliadins + DEX (total area = 44.8%) is higher than that measured for native gliadins in the same range of frequencies (total area = 33.6%). Such a difference suggests a higher fraction of hydrogen-bonded C═O in gliadins + DEX than in native gliadins. This hypothesis is based on the fact that the side-chain C═O groups of glutamines, abundant in gliadins, might form hydrogen bonds with the hydroxyl groups of dextrin, causing the increment of the relative area at lower wavenumbers. To this end it is important to note that the redoubling of the band at around 1645 cm^{-1} in gliadins + DEX with respect to that of native gliadins (**Figure 4**) and the absence of the band at around 1668 cm^{-1} in gliadin/dextrin mixtures both indicate that these latter ones might have a higher fraction of hydrogen-bonded carbonyl groups than native gliadins. In particular, the absence of the band at around 1668 cm^{-1} is indicative of a decrease of non-hydrogen-bonded carbonyls of β-turns. If these carbonyls become hydrogen bonded, for example, with the hydroxyl groups of dextrin, a shift of their absorption toward lower wavenumbers should be expected. Moreover, it has to be emphasized that because native gliadins and gliadins + DEX have been compared at the same water activity value (0.06), the presence of a higher area at lower wavenumbers in the case of gliadins + DEX should be due to the interaction of dextrin with gliadins. Concerning such interaction, it might be argued that, because of their high molecular mass (3000 Da) that causes steric hindrance, dextrin might not form hydrogen bonds with gliadins. However, it has to be noted that gliadins contain repetitive domains (rich in prolines and glutamines) which might form protein regions with repetitive secondary structure elements (e.g., β-turns organized in a β-spiral structure) (*37*). Such regions give rise to relatively long fragments with a polymer-like secondary structure, to which dextrin could approach and form hydrogen bonds. To this end it is important to note that gliadins in the absence and in the presence of dextrin show an intense band at around 1660 cm^{-1} (**Figures 2**−**4**), which has been assigned to secondary structure elements such as 3_{10}-helices (these are repeats of type III β-turns). [The assignment to 3_{10}-helix of the 1660−1662 cm^{-1} component band is based on studies of α-amino isobutyric acid peptides with 3_{10} geometry based on X-ray crystallographic data as reported by Kennedy et al. (*38*).]

In favor of interactions between gliadins and dextrin are also the differential spectra of gliadins + DEX and gliadins + DEXt (**Figure 5b**,**c**). In the presence of dextrin the less intense minimum/maximum in the amide I region and basically the complete absence of maximum/minimum in the amide II region strongly suggest that the hydration of gliadin/dextrin mixtures (at least up to the a_w value of 0.84) does not cause a strong variation of the protein hydrogen-bonding patterns of gliadins. This is in favor of the hypothesis that the C═O and N−H groups of gliadins form hydrogen bonds with the hydroxyl groups of dextrin. In fact, if the C═O and N−H groups of gliadins are engaged to form hydrogen bonds with the hydroxyl groups of the polysaccharide, they cannot form hydrogen bonds with water molecules and no spectral variations should be observed upon hydration of the gliadin/dextrin samples. This might also be confirmed by analyzing the differential spectrum reported in **Figure 1d**. It can be noted that gliadins + DEX subtracted of dextrin show a maximum/minimum (1017/951 cm^{-1}) in the region between 950 and 1150 cm^{-1}, where the C−O stretching and the O−H bending fall (*17*−*19*). This maximum/minimum might be due to an increase of hydrogen-bonded hydroxyl groups of dextrin. In fact, when the differential spectrum of dextrin at $a_w = 0.84$ minus dextrin at $a_w = 0.06$ is obtained, an analogous maximum/minimum is observed (data not shown). However, in the differential spectrum obtained from the

spectrum of gliadins + DEX minus the spectrum of dextrin (**Figure 1d**), the maximum/minimum should indicate an increase of the number of hydrogen-bonded hydroxyl groups of dextrin with gliadins and not a different hydration state of dextrin; indeed, the spectrum of gliadins + DEX and that of dextrin were obtained from samples both equilibrated at $a_w = 0.06$.

The fact that different kinds of gliadins were studied as a mixture instead of as singular components does not really affect the findings. In fact, both the modification of secondary structure (only a general variation of the conformation, e.g., loss of α-helix structures, has been taken into account) and the interaction between dextrin and gliadins (e.g., hydrogen-bond formation between the protein and the polysaccharide) should be a common phenomenon for all kinds of gliadins. To this end it should be noted that α-, *β*-, and *γ*-gliadins have very similar primary structures and are rich in α-helices in the nonrepetitive domains (*1*). Moreover, their repetitive domains have peptide motifs very similar to that of *ω*-gliadins (*3*).

In conclusion, the study conducted in this work supports our previous hypothesis of an interaction between dextrin and gliadins (*9*). Moreover, in agreement with Grant et al. (*39*) we found that, despite their low solubility in water, gliadins have hydrophilic character as evidenced by hydrogen-bond formation with water molecules and dextrin. Concerning this point it is important to note that in water gliadins form homogeneous suspensions (and not big aggregates) with a high surface that allows the interaction with dextrin. Finally, because dextrin seems to form hydrogen bonds with gliadins, they might be good candidates to prepare nontoxic food derivatives for coeliac patients. Indeed, the formation of complexes between dextrin and gliadins (and more in general wheat protein) could be an approach to be pursued to decrease the allergenic power of these classes of proteins.

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