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Article

Structural Properties of Globulin: A Critical Parameter for Sunflower Meal as Wood Panel Adhesives

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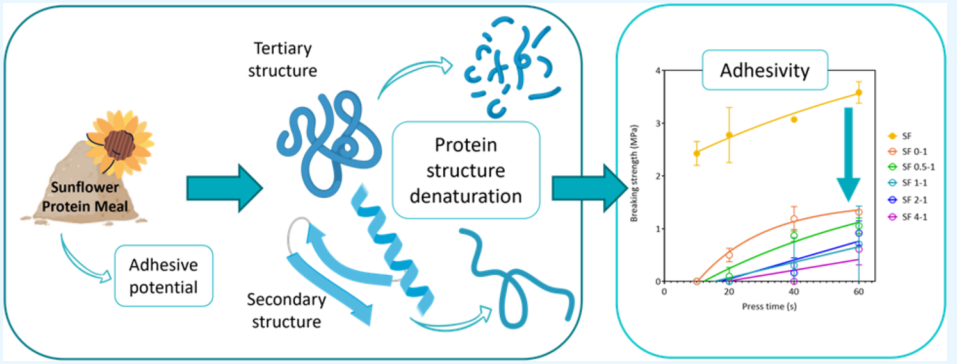

ABSTRACT: Alternative biobased adhesive formulations are currently being investigated to replace urea-formaldehyde (UF) as wood panel adhesives. In this regard, oilseed meals are valuable alternatives, as it is anticipated that the sticky potential of these meals is linked to their protein content. This work focuses on the protein parameters (primary and/or secondary structures) that could impact the adhesiveness of sunflower meals. The proteins contained in these meals were first separated from the other components and identified using electrophoresis. Oilseed meals contain several families of proteins: globulins, albumins, prolamins, and oleosins. Sunflower meal is mainly composed of globulin (53%) and albumin (45%). The protein structures have then been either oxidized with H_2O_2 (in the presence or not of NaOH) or physically treated by microwave (MW). The oxidation treatment cleaves the protein backbone and creates smaller peptides, while the MW process converts α-helices into random coils. The adhesive potential of these treated proteins was evaluated by using shear tests onto wood panels. The results demonstrate that the primary and secondary structures of globulins are key parameters toward the sunflower protein meal adhesivity.

1. INTRODUCTION

The wood adhesive industrial sector is a huge market with a panel production of 63.7 million m^3 in Europe, according to the *European Panel Federation*. This growth has led to an increase in the consumption of adhesives and more specifically of urea-formaldehyde (UF), which is widely used in the process of making wood panels.[1] UF adhesive has many advantages suitable for the wood industry, including fluidity at room temperature, good penetration into the wood, simplicity of use, and fast curing.[2,3] However, in 2004, formaldehyde was classified as a carcinogenic volatile organic compound (VOC) by the International Agency for Research on Cancer (IARC).[4] Since this warning, some standards have emerged to limit formaldehyde emission in the home. For example, in Europe, wood panels of class E1 must comply with the standard NF EN 717, which requires a concentration limit of formaldehyde in the air of 0.124 mg/m^3, or less than 8 mg of formaldehyde per 100 g of dry panels.[5] Biobased adhesive substitutes, such as plant-based adhesives, have been developed to conform to these new standards. Adhesives based on oilseed meals from cotton,[6] soybean,[7,8] and rapeseed[9] have already been described. It is accepted that the protein content of these oilseed meals is responsible for their adhesivity.[10] However, some studies have discussed the adhesive potential of globulin which is a major protein in soybean meal.[11,12] The globulin family is a 320−375 kDa protein with a hexameric structure and is composed of three subunits from 70 to 50 kDa. This protein is also highly conformed with β sheet barrels and some α helices.[13] Mo et al.[11] and Zhang and Hua[12] have shown that the adhesive properties of globulins could be linked to their structure, especially to the β sheet structure, to the amino acid functions (hydroxyl, amino, thiol), and to the protein chain length. These structural changes in proteins can also lead to a

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reduction in viscosity and an improvement in adhesive performance.[14,15]

Globulin is a main protein component of sunflower meal,[16,17] which is an available oilseed crop in Europe (14.4 million tons according to FAO), on par with rapeseed (14.3 million tons) and soybean meals (17.7 million tons). The wood adhesive potential of sunflower meals has been recently evaluated by *Evertree* company, which commercializes sunflower meal for formaldehyde-free wood adhesives, through the products *Green Boost* and *Green Ultimate*.[18]

The objective of this study is to further investigate the adhesive potential of sunflower meals and, in particular, to better understand the specific role of globulin in the adhesive activity. The purpose of this work is to examine the influence of the primary and secondary structural parameters of globulin on the adhesive properties of sunflower globulin extracts (**SF**). For that, the primary and secondary structures of the globulin were modified by oxidation and microwave (MV) irradiation. Usually, these treatments are used to modulate the extractability[19] and digestibility[20] of oilseed meal proteins. In this study, the primary and secondary structures obtained after treatments were characterized by electrophoresis, circular dichroism (CD), and Fourier transform infrared (FTIR) spectroscopy, to evaluate the structural changes and the impact on adhesive features. The adhesive potential of such modified **SF** samples was then evaluated using shear tests onto wood samples and compared to UF and soybean protein adhesive standards.

2. MATERIALS AND METHODS

2.1. Protein Extract and Treatments. *2.1.1. Sunflower Protein Extraction.* Crushed sunflower meal supplied by *Avril* (France) was used to get sunflower globulin extract (**SF**). The protein was extracted following the isoelectric point (pI) precipitation.[21,22] Sunflower meal was dispersed in an alkaline solution (NaOH 3 M) and then incubated for 1 h at 42 °C under stirring. The suspension was centrifuged for 20 min at 10,000*g* and 20 °C. The supernatant was kept, and the pellet was once more dispersed with the alkaline solution, incubated, and centrifuged. All of the supernatants were combined, and the pH was adjusted at 4.5 with HCl 2 M. To recover all of the extracted proteins, they were centrifuged for 15 min at 10,000*g* and 20 °C. The protein pellet was recovered and washed few times with distilled water until a neutral pH. Finally, the sunflower meal globulin extract (**SF**) was lyophilized to obtain a protein powder.

The soybean proteins used as controls in this study underwent the same extraction treatment as that for the sunflower proteins.

2.1.2. SF Treatments. **SF** was treated by oxidation using hydrogen peroxide (H_2O_2). The following protocol was based on the Mehats et al. procedure (2015).[23] A volume of distilled water (mass equal to 20 times the mass of **SF** previously weighed) was deposited in a balloon, with 1 wt % NaOH and 0.4 wt % sodium metasilicate. The balloon was placed in an oil bath under stirring. When the solution temperature reached 60 °C, 4 vol % of aqueous solution of H_2O_2 (35 wt % from Acros Organics) was added with **SF**. After 1 h, the reaction was neutralized at pH 7 and then lyophilized.

SF was also irradiated with microwaves (MW). **SF** was dispersed at 20 wt % in distilled water. Different powers (50, 100, 200, and 300 W) and processing times (30 s, 1 min, 5 min, 15 min, and 30 min) were tested with a microwave reactor (Discover from CEM).

All of the **SF**-treated samples were named as follows (Table 1):

Table 1. Abbreviations of Treated SF Samples

	treatment	abbreviation
H_2O_2/NaOH	H_2O_2 0% NaOH 1%	SF 0−1
	H_2O_2 0.5% NaOH 1%	SF 0.5−1
	H_2O_2 1% NaOH 1%	SF 1−1
	H_2O_2 2% NaOH 1%	SF 2−1
	H_2O_2 4% NaOH 1%	SF 4−1
H_2O_2	H_2O_2 0.5%	SF 0.5
	H_2O_2 1%	SF 1
	H_2O_2 2%	SF 2
	H_2O_2 4%	SF 4
microwave (MW)	50 W 30 s	SF 50−30
	100 W 30 s	SF 100−30
	150 W 30 s	SF 150−30
	200 W 30 s	SF 200−30
	300 W 30 s	SF 300−30
	300 W 1 min	SF 300−1
	300 W 3 min	SF 300−3
	300 W 5 min	SF 300−5
	300 W 10 min	SF 300−10

2.2. Electrophoresis Analysis (SDS-PAGE). **SF** samples (10 mg/mL) were dissolved in Tris-HCl 0.1 M pH 8, SDS 5 wt/vol % buffer and centrifuged for 2 min at 2000*g* and 20 °C. The supernatant was combined with Laemmli 2× solution (Biorad). The samples were heated at 90 °C for 3 min and cooled at room temperature. Once the samples prepared, 10 μL was loaded on the electrophoresis gel SDS-PAGE (BioRad MiniPROTEAN TGX, untinted, gradient 4−20%). A molecular weight marker (BioRad Precision Plus Protein Unstained Standards) from 10 to 250 kDa was also loaded for each experiment. Samples deposited on the gel are migrated using BioRad MiniPROTEAN Tetra System apparatus at 300 mV, 25 mA/gel, and 50 min, with Tris/Glycine/SDS buffer (BioRad 10× TGS) as a migration solvent. The gels were washed and revealed with the BioRad GelDoc EZ Imager and analyzed with BioRad software.

2.3. Circular Dichroism (CD). Solutions of **SF** were prepared in distilled water at a concentration of 0.1 mg/mL. Data were collected at 20 °C using a Jasco J-815 spectrometer with a 10 mm path length quartz cell, a 200 nm/min scan rate, and a 2 nm bandwidth. The spectra were made in triplicate from 180 to 400 nm. The spectrum of water is used as a blank and subtracted for each spectrum to achieve corrected analysis. The data were analyzed with Spectra Manager. The CD spectra presented in the manuscript do not show the absorbance spectrum; the full CD spectra are presented in the Supporting Information (SI).

2.4. Infrared Spectroscopy. Fourier Transform Infrared (FTIR) spectroscopic data were collected with a Vertex 70 spectrometer (Bruker). The dried samples were analyzed by ATR and triplicate spectra were obtained from 64 scans, with a resolution of 4 cm^{-1}, in the range 400−4000 cm^{-1}.

2.5. Shear Test. *2.5.1. Adhesive Preparation.* **SF** and treated **SF** were formulated with distilled water at 20 wt/wt %. The formulations were stirred for 8 h at room temperature. 30 mg of the adhesive formulation was applied to a surface of 325

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mm^2 on oak veneer. The 1.5 mm thick veneers used were cut from the fiber into 100 mm × 25 mm test pieces (Figure 1),

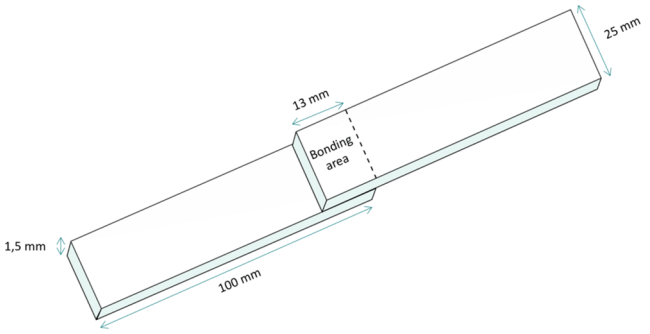

Figure 1. Wood veneer shear test specimen assembly procedure.

before the adhesive application. The adhesive was applied to one of the two veneers used per sample. Once the adhesive was applied, between three and five samples were placed between sheets of aluminum foil, on a manual press (Darragon) at 110 °C, 10 bar, and a range of time between 10 and 60 s. The samples were then placed in an oven for 24 h, at 25 °C and 23% humidity.

2.5.2. Shear Test. The traction bench Synergie 400 (MTS) with a 2 kN detector and 1 mm/min traction speed was used to determine the adhesive strength. The data collected correspond to the maximal strength measured at sample breakage.

3. RESULTS AND DISCUSSION

The objective of the study is to evaluate the globulin parameters responsible for the adhesive properties of sunflower meal. For that, the primary and secondary structures of sunflower globulin extract (**SF**) were modified thanks to two types of treatment. The first one is chemical with two variations. **SF** was either treated with H_2O_2 and NaOH or with H_2O_2 only without NaOH. The second type of treatment is physical, with the use of microwave irradiations (MW) on **SF**. The effects of these treatments on the **SF** primary structure were characterized by electrophoresis (SDS-PAGE). Then the impact of the treatments on the secondary structure was studied by circular dichroism (CD) and infrared (FTIR). Finally, the influence of these treatments on the **SF** adhesivity was evaluated by shear tests and compared with the adhesive profiles of urea-formaldehyde (UF) and soybean meal proteins.

3.1. Electrophoresis Analysis. Electrophoresis analysis is an analytical technique used in biochemical research that determines the proteins present in a mixture. The conditions of this analysis separate the different subunits constituting a protein with respect to their molecular weight, allowing them to be identified.

The electrophoresis pattern of **SF** treated with H_2O_2 and NaOH is presented in Figure 2a, and the electrophoresis pattern of **SF** only treated with H_2O_2 is presented in Figure 2b. For these two treatments, H_2O_2 was used at different concentrations: 0.5, 1, 2, and 4 vol %.

The **SF** globulin subunits are visible at 60 and 50 kDa[16,24] (Figure 2). The oxidation treatment of **SF** at different H_2O_2 concentrations and in the presence of NaOH revealed spots between 25 and 10 kDa (Figure 2a). The molecular weight of **SF** proteins decreases with increasing H_2O_2 concentration (Figure 2a), indicating that H_2O_2 could cleave the peptide bonds, thus leading to smaller peptides (between 25 and 10 kDa). However, the presence of NaOH to facilitate protein solubilization may also cause the cleavage of peptide bonds in addition to the denaturation of the protein structure, as frequently discussed in the literature.[25] The control treatment containing 1 wt % NaOH, without H_2O_2, produced bands, ranging in size from 25 to 10 kDa, which clearly confirms that soda cleaves the proteins (Figure 2a).

In order to confirm that H_2O_2 alone could alter the protein structure, some H_2O_2 treatments were performed without NaOH (Figure 2b).

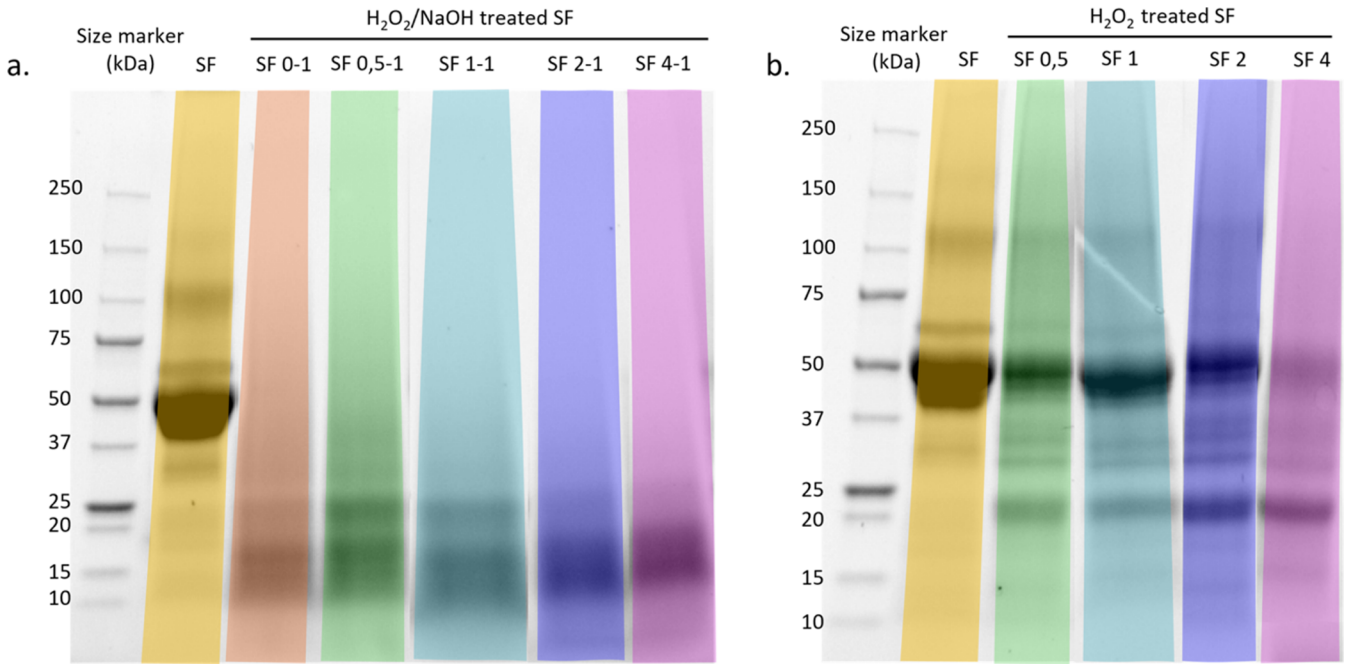

Figure 2. SDS-PAGE electrophoresis characterization of **SF** treated with different concentrations of H_2O_2 with (a) and without (b) NaOH.

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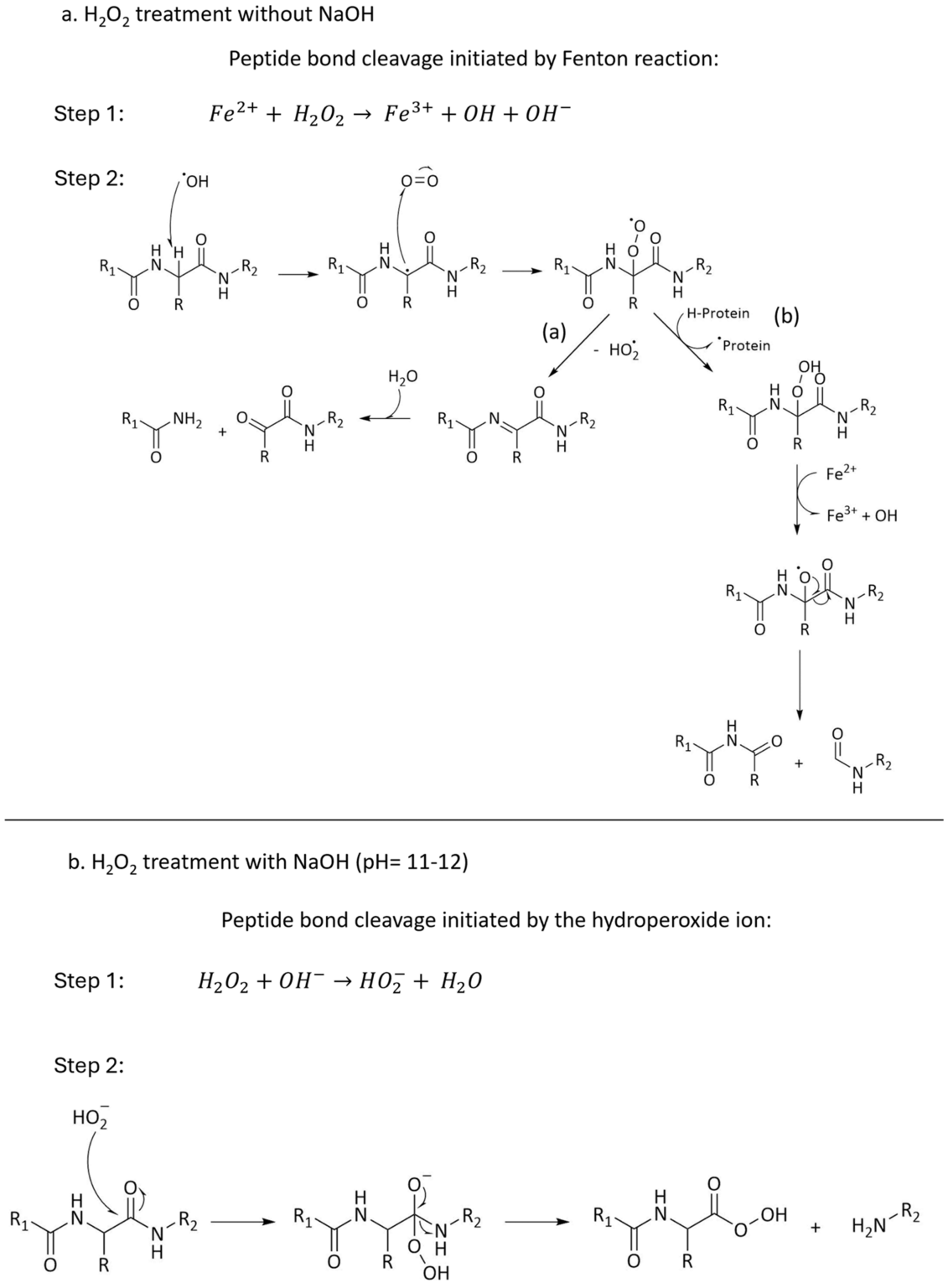

Figure 3. Proposed mechanisms for H_2O_2 peptide bond cleavage are based on several mechanisms. Adapted with permission from Papuc, C.; Goran, G. V.; Predescu, C. N.; Nicorescu, V. Mechanisms of oxidative processes in meat and toxicity induced by postprandial degradation products: a review. *Comp Rev Food Sci FoodSafe* ***2017****, 16 (1), 96−123. doi: 10.1111/1541-4337.12241. Copyright 2017 Comp Rev Food Sci Food Safe.*

Once **SF** samples were treated with 0.5 vol % H_2O_2, three new bands were observed on the electrophoretic profile; two of these bands are about 30 kDa and one at 20 kDa (Figure 2b). Moreover, the band at 20 kDa becomes stronger and the one at 50 kDa weaker, as the concentration of H_2O_2 rises. These results demonstrate that H_2O_2 could cleave proteins up to a molecular weight of 20 kDa while the H_2O_2/NaOH treatment generates peptides with a molecular weight of 10−15 kDa.

The effect of protein cleavage by H_2O_2 treatment was also observed by El-Kadiri et al., on rapeseed meal proteins.[19] The

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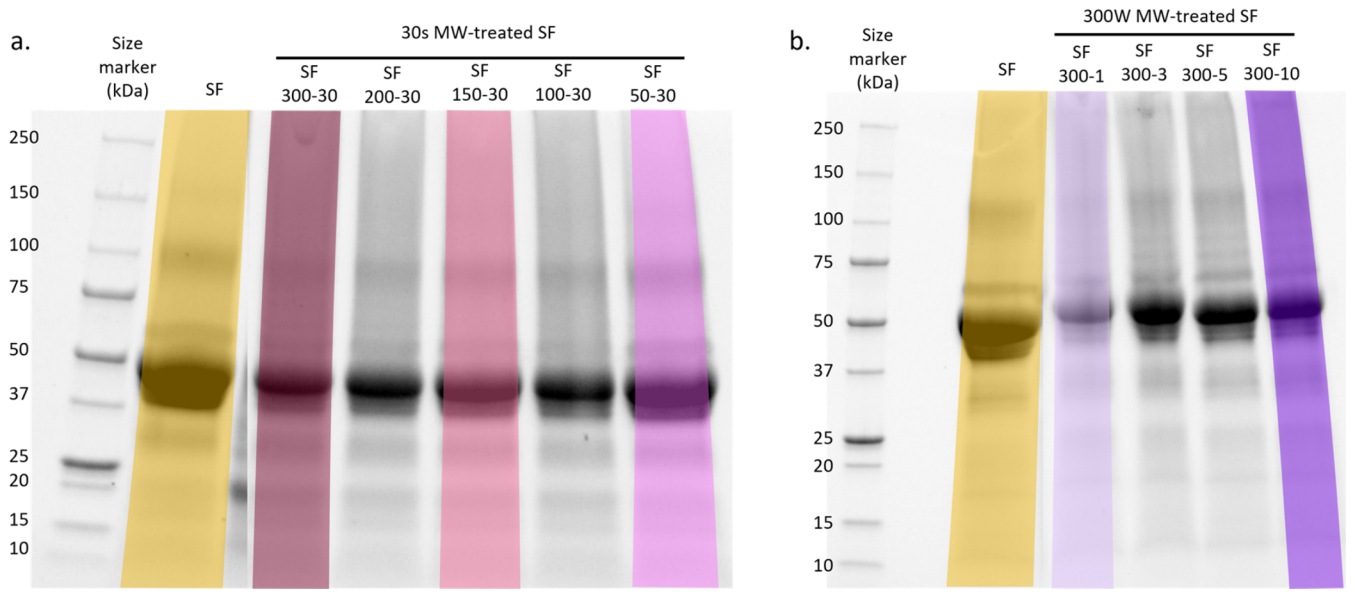

Figure 4. SDS-PAGE electrophoresis characterization of MWs treated **SF** samples, at a constant time of 30 s (a) and at a constant power of 300 W (b).

hydrogen peroxide activity could involve several mechanisms. In this study, peptide bond cleavage is linked to the presence of H_2O_2 and enhanced by the presence of NaOH.

Some studies identify few action modes of H_2O_2 on peptide bond.[26−30] First, the Fenton reaction is emphasized, due to its ability to induce the production of hydroxyl radicals[31] (Figure 3a, Step 1). Meal in the presence of H_2O_2 could initiate this reaction because oilseed meal contains iron residues.[32]

The hydroxyl radicals ($^{\bullet}OH$) could react with amino acids.[30] Stadtman and Levine[30] state that $^{\bullet}OH$ is capable of transferring an α hydrogen from the peptide bond, resulting in the creation of a radical with a carbon center and possibly a cleavage of the peptide bond (Figure 3a, Step 2a). The peroxyl radical form of the protein can also trap hydrogen from a nearby protein to create a hydroperoxylated protein (Figure 3a, Step 2b). If iron is still present in the system, a redox process could take place with the hydroperoxylated protein to produce an alkoxy radical (Figure 3a, Step 2b). Finally, the rearrangement could lead to the rupture of the peptide bond (Figure 3a, Step 2b).[26−28]

As described earlier, this phenomenon of peptide bond breaking is amplified by the presence of NaOH (Figure 2). The pH increases to 11−12 under these conditions, making the Fenton reaction less favorable to produce radical species. Therefore, at high pH values, hydroperoxide ion (HO_2^-) is the active species of H_2O_2 (Figure 3b, Step 1).[33,34] Because of its strong nucleophilicity, the hydroperoxide ion has the potential to cause protein cleavage as proposed in the reaction scheme shown in Figure 3b, Step 2.

In addition, these alkaline conditions also uncoil the proteins and increase the protein solubility in the reaction system. Peptide bond accessibility to H_2O_2 would increase, accelerating protein cleavage. These hypotheses could explain the observed reduction of protein molecular weight on electrophoresis after H_2O_2 treatment.

Then **SF** treated with microwave (MW) was characterized by electrophoresis (Figure 4). **SF** samples were exposed for 30 s, 1 min, 3 min, 5 min, and 10 min, at different powers (50, 100, 150, 200, and 300 W).

SF proteins were distinguished by the bands at 100, 50, 30, and 20 kDa, corresponding to their subunits[16,24] (Figure 4a). Whatever the power used, the visible bands for **SF MW** match those of the unirradiated **SF** (Figure 4a). This result suggests that protein cleavage would not be induced by applying microwave radiation for 30 s (Figure 4a).

The **SF** samples were irradiated at the highest power (300 W) and at different times (1, 3, 5, and 10 min) (Figure 4b). Again, the irradiated sample showed no difference compared to that of untreated samples. Therefore, the MW treatment time could not induce molecular weight changes on **SF**.

For the **SF MW**, it could be concluded that the application of microwave does not cause any alterations to the primary structure of **SF** proteins. Similar results concerning microwave irradiation on oilseed proteins—such as those from soy and rapeseed—as well as gluten, have been documented in the literature.[20,35−37] Their electrophoretic analyses also revealed unchanged molecular weight. However, after treatment at 100 W, Xiang et al. showed, by FTIR analysis, protein secondary structure modifications. An increase in the fraction of α helices and β turns, against a decrease in β-sheets and random coil was observed.[37] The authors proposed that microwaves only affect proteins at the secondary structural level, which could create isopeptide bonds or disulfide bridges.[20,36,37]

Based on these findings, the analyses of the secondary structure of **SF** and **SF** treated chemically and physically have been done by circular dichroism (CD) and infrared spectroscopy (FTIR).

3.2. Secondary Structure Analysis by CD and FTIR.

3.2.1. CD Analysis. Reference CD spectra of secondary structures (α-helices, β-sheets, β-turns, and irregular structures) were recorded to interpret CD analyses.[38−40] The typical spectrum of α-helices shows two minimum peaks at 222 and 208 nm and a maximum peak at 193 nm. Antiparallel β-sheets have a minimum peak at 218 nm and a maximum peak at 195 nm. Finally, proteins with random structure exhibit low ellipticity, reaching a maximum at 210 nm and a minimum at 195 nm.[38,40]

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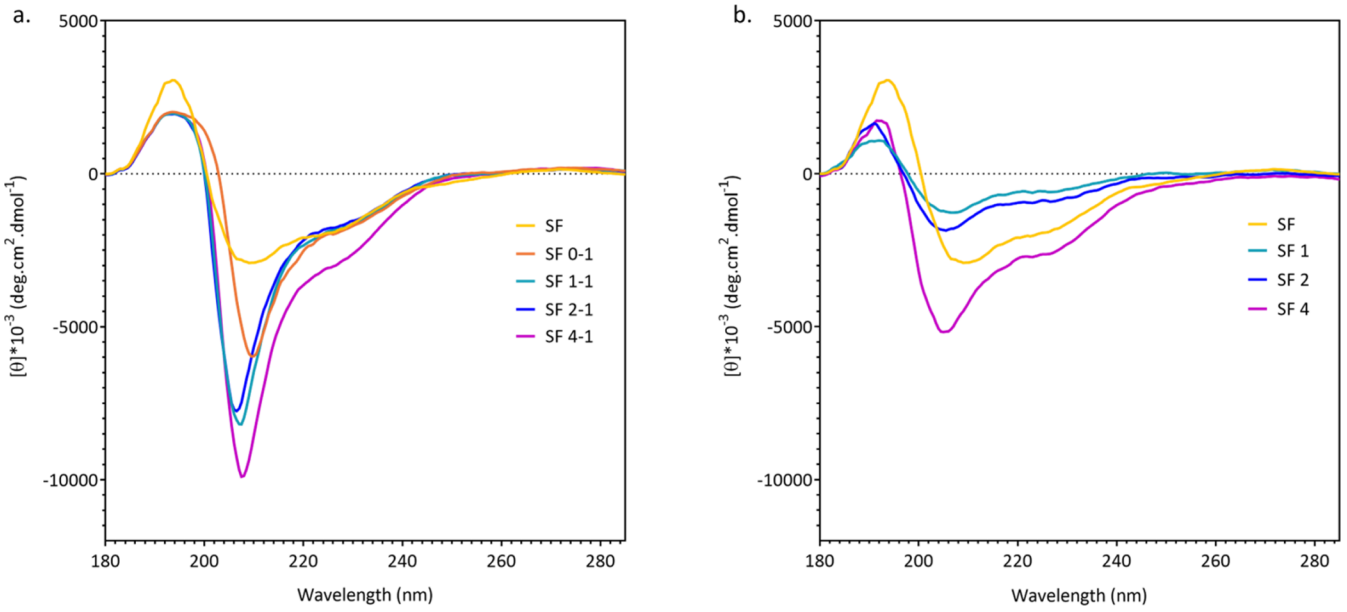

Figure 5. Circular dichroism characterization of **SF** and **SF** samples treated with H_2O_2 in the presence of NaOH (a) and treated with H_2O_2 in the absence of NaOH (b). The full CD spectra are shown in the Supporting Information (Figure S1).

The CD spectra recorded for **SF**, **SF** H_2O_2/NaOH, and **SF** H_2O_2 are gathered in Figure 5a.

The **SF** spectrum showed a high peak at 193 nm and two minimum peaks at 225 and 209 nm (Figure 5a) in agreement with a structure mostly made up of α-helices. Nevertheless, the low definition observed between the two minor peaks must be associated with the existence of β-sheets. The spectrum pattern found for **SF** is comparable to the one found in the structural analysis of sunflower globulin by González-Pérez et al.[41]

Figure 5a presents the CD spectra of **SF** treated with H_2O_2 and NaOH. The **SF 0−1** spectrum showed that the lowest peak at 209 nm has become deeper. The **SF 1−1**, **SF 2−1**, and **SF 4−1** show the same structural spectra, with a deep peak at 209 nm. With an increase in H_2O_2 concentration, this minimal peak becomes more intense (Figure 5a).

The structural profiles of **SF** samples that were exclusively exposed to H_2O_2 were also examined (Figure 5b). The **SF 1** and **SF 2** samples exhibit a peak at 205 nm that is less intense than that at 209 nm of **SF**. However, **SF 4** reveals two distinct minimal peaks, at 227 and 205 nm, which are more intense (Figure 5). This structural pattern is similar to that in Figure 5a.

Consistent with electrophoresis analysis (Figure 2), these investigations have shown that H_2O_2/NaOH treatments could produce structural changes in the **SF** secondary structures. This spectral pattern has also been documented in the literature, displaying a peak at 209 nm deeper than that at 225 nm.[42,43] Marullo et al.[43] have shown a link between the peptide size and secondary structure modifications. In fact, the smallest peptides have revealed random structures combined with some α-helices, and the α-helices fraction increases with the peptide chain length.[43] According to the authors, H_2O_2 treatments induce a modification of protein α helices toward random coils.

In addition, the H_2O_2 treatment without NaOH also showed a peak at 205 nm that was deeper than that at 225 nm for high H_2O_2 concentrations (Figure 5b, **SF 4**). These results show that H_2O_2 alone could also create protein structural modifications to random structures.[42,43] Moreover, **SF 4** has shown the intensification of this peak at 205 nm (Figure 5b), which is linked to the cleavage observed in the electrophoresis analysis of the **SF 4** sample (Figure 2b).

Therefore, an increase of H_2O_2 concentration amplified the structural alterations induced by the H_2O_2/NaOH oxidative treatment, leading to a random coil.

The CD spectra of **SF** samples treated with microwaves (MW) are presented in Figure 6. **SF 50−30** exhibits a minimum peak at 230 nm and a maximum one at 203 nm (Figure 6). This spectral pattern has a low intensity and is difficult to analyze. Its spectral pattern differs from typical CD spectra from the literature and does not correspond to any

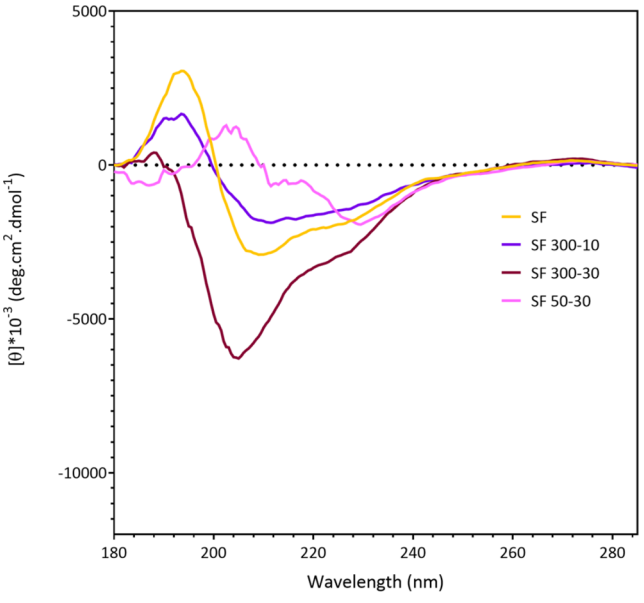

Figure 6. Circular dichroism secondary structure analysis of **SF** and microwave-treated **SF** samples. The full CD spectra are shown in the Supporting Information (Figure S2).

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known structural combination. We anticipate that solubilization issues can explain this structural profile.

SF 300−30 exhibits a distinct profile, with only two minimal peaks at 227 and 204 nm (Figure 6). This spectrum pattern has similarities with the ones of **SF H_2O_2/NaOH** samples, wherein α-helices are denaturated toward random coil. **SF 300−10** has a minimum peak at 210 nm, a maximum one at 193 nm and a loss of the minimum peak at 225 nm (Figure 6), in agreement with a random structural profile. Consequently, the MW treatment time would accelerate the **SF** secondary structural modifications.

These microwave treatments produce structural changes toward random coils without altering the primary structure of the **SF** proteins; the degree of these changes varies according to the power and time of the MW treatment.

3.2.2. FTIR Analysis. FTIR analyses were performed to characterize sunflower protein extract (**SF**), SF treated with H_2O_2/NaOH (**SF1−1**), and SF only treated with H_2O_2 (**SF 1**) (Figure 7). The absorption bands of amides I (from 1700 to 1600 cm^{-1}) and II (from 1600 to 1500 cm^{-1}) were specially analyzed. These bands are associated with the stretching of the C═O bond of the amide groups (amide I) and with the bending of the N−H ones (amide II). Amide III (from 1300 to 1200 cm^{-1}) is characteristic of the stretching vibrations of the N−H bonds in the plane and of the C−N bond. These bands are then representative of α-helices, β-sheets, and random coils.[44−46]

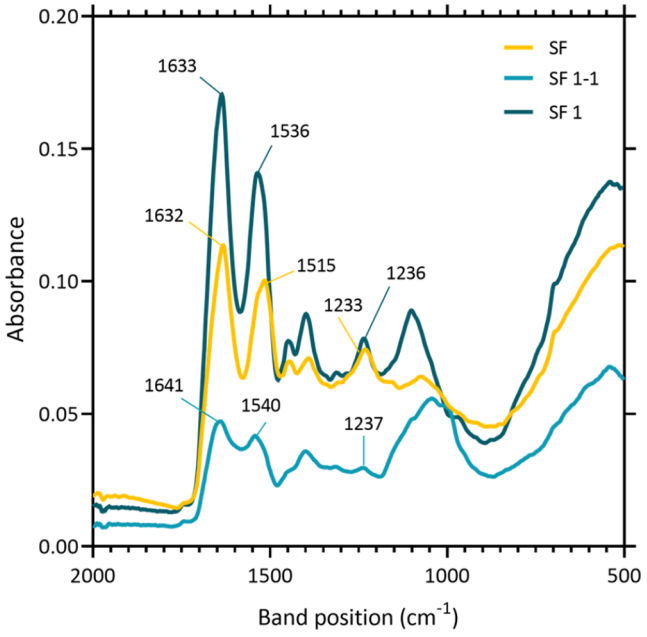

Figure 7. FTIR characterization of **SF** and H_2O_2-treated **SF** samples.

Bands at 1632 cm^{-1} for amides I, at 1515 cm^{-1} for amides II and at 1233 cm^{-1} for amides III were visible on the FTIR spectra of **SF** (Figure 7). These results confirm the presence of β-sheets in **SF** proteins, which are barely visible on the CD spectrum (Figure 5). The FTIR spectrum of **SF 1** exhibits bands at 1641, 1544, and 1237 cm^{-1}, which are consistent with β-sheets signature. The FTIR spectrum of **SF 1−1** displays bands at 1641 cm^{-1} for amides I and at 1237 cm^{-1} for amides III, in agreement with the presence of β-sheets.

These FTIR spectra have shown the β sheets conservation of SF treated with H_2O_2/NaOH and SF only treated with H_2O_2. Meanwhile, circular dichroism (CD) analysis indicates that such treatments promote a modification of α-helices toward random coils (Figure 5). The combination of FTIR and CD studies permits one to show that H_2O_2/NaOH and H_2O_2 treatments have modified the α helices into random coils but have not damaged the β sheets structures of **SF**.

SF 50−30 (MW treatment) (Figure 8) exhibits a band at 1625 cm^{-1} for amides I, at 1533 cm^{-1} for amides II, and at 1237 cm^{-1} for amides III. These bands would correspond to a structure close to the one of the **SF** reference, mainly composed of β-sheets (Figure 8), suggesting that MW treatment could not affect β-sheets.

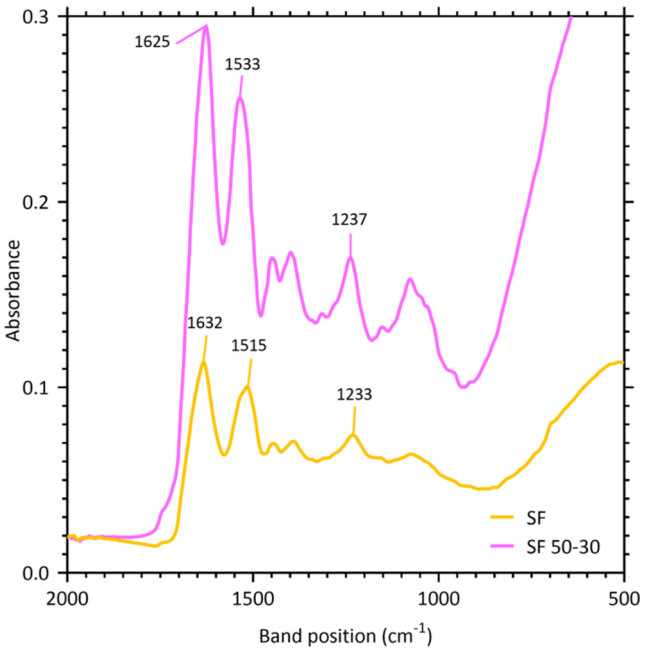

Figure 8. Analysis of secondary structures by FTIR, **SF**, and microwave-treated **SF** samples.

Electrophoretic characterizations have suggested that microwave treatments have no effect on the primary structure of **SF** proteins. On the other hand, microwave treatments would preferentially change α-helices toward random coil, according to structural investigations by CD (Figure 6). Finally, FTIR characterization has shown no β-sheet damage (Figure 8).

All of these structural analyses (electrophoresis, CD, and FTIR), permit the evaluation of the influence of H_2O_2/NaOH, H_2O_2, and MW treatments on the primary and secondary structures of **SF**. H_2O_2/NaOH and H_2O_2 treatments are responsible for cleaving **SF** peptide bonds, yielding smaller peptides. These same treatments have also shown the modification of α helices into random coils while preserving the β sheets. The MW treatments conserved the primary structure and β sheets secondary structures of **SF**, but nevertheless damaged the α helices into random coils.

For the last part, the adhesive properties of **SF**, **SF H_2O_2/NaOH**, **SF H_2O_2**, and **SF MW** were investigated to evaluate the structural modifications on adhesivity properties.

3.3. Adhesive Behavior Analysis by Shear Strength. Shear tests were performed on sunflower globulin extract (**SF**) and SF that were chemically and physically treated. For each type of treatment, three wood samples have been made and evaluated by shear test.

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First, the **SF** adhesive properties have been compared to the adhesive profiles of urea-formaldehyde (UF) and soybean meal protein (Figure 9). UF has displayed a breaking force of 1.3 MPa for 10 s of pressing, rising to around 3 MPa for 30−60 s of pressing.

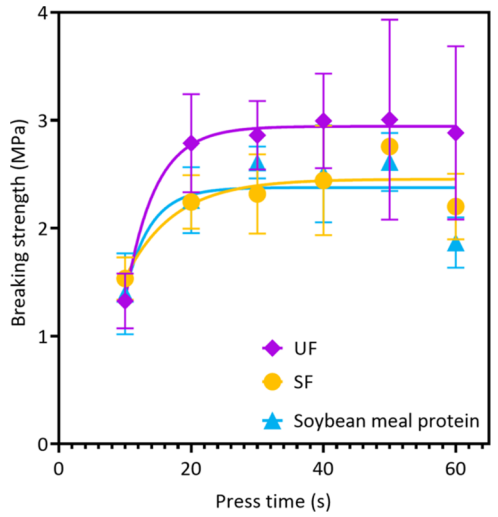

Figure 9. Shear tests of UF, SF, and soybean meal protein.

Soybean meal protein has shown a breaking strength at 1.4 MPa for 10 s of pressing. Like the UF adhesive profile, the breaking force of the soybean meal protein rose to a plateau around 2.4 MPa for 30−60 s of pressing. Then, the **SF** has shown a breaking strength at 1.5 MPa for 10 s of pressing and has reached a plateau around 2.4 MPa from 30 to 60 s of pressing (Figure 9). These results show that **SF** and soybean meal protein have the same adhesive potential. Moreover, these two protein extracts revealed adhesive performances that were close to those of the UF, indicating that the protein sunflower meal may be competitive with the synthetic chemical adhesive.

The shear test has then been used to analyze the adhesive capabilities of **SF H_2O_2/NaOH**, **SF H_2O_2**, and **SF MW** in order to assess the influence of protein primary and secondary structure on the SF adhesive potential (Figures 10 and 11).

The **SF** extract displayed a breaking force of 2.4 MPa for 10 s of pressing, rising to 3.6 MPa for 60 s of pressing (Figure 10).

After 10 s of pressing, no resistance was observed for **SF** samples treated with H_2O_2 and 1 wt % NaOH, but, as the press time increased, the fracture strength rose (Figure 10a). The highest breaking force value for the **SF H_2O_2/NaOH** samples was only 1.3 MPa at 60 s of pressing—less than the 3.6 MPa for **SF** for the same time. In addition, the **SF H_2O_2/NaOH** samples breaking force decreased with H_2O_2 content. At 60 s of pressing, **SF 0−1, SF 0.5−1, SF 1−1, SF 2−1**, and **SF 4−1** reached 1.3, 1, 0.7, 0.9, and 0.6 MPa, respectively (Figure 10a). The decline in adhesive performance for the **SF H_2O_2/NaOH** samples indicates that H_2O_2/NaOH treatment has degraded **SF** adhesive performances.

The adhesive performances of **SF** samples treated with various H_2O_2 without NaOH, were then characterized following the same tests (Figure 10b).

All of the adhesive performances of **SF H_2O_2** samples have increased with the press time. **SF 1** and **SF 2** achieved 2.9 MPa, while **SF 4** reached 1.9 MPa after 60 s of pressing (Figure 10b). These results indicate that adhesive performances of samples treated just with H_2O_2 were still below the **SF** reference values (3.6 MPa for 60 s). Moreover, the adhesive properties of **SF H_2O_2** samples decreased with an increase in the H_2O_2 concentration increase.

These shear tests of **SF H_2O_2/NaOH** and **SF H_2O_2**, have revealed a decrease of **SF** adhesive performances, which is linked to the increase of H_2O_2 concentration. This loss of adhesive performance could be compared with the structure analysis made by electrophoresis, CD, and FTIR. In fact, these characterizations have shown that the H_2O_2/NaOH and H_2O_2 treatments cleaved the peptide bond of **SF** and degraded the α helices into random coils. These structural alterations appear to be detrimental to adhesive performance.

Therefore, shear tests were performed on **SF MW** (Figure 11). **SF**, **SF 50−30**, **SF 150−30**, and **SF 300−30** breaking forces (Figure 11a) were compared with those of **SF 300−1** and **SF 300−10** (Figure 11b).

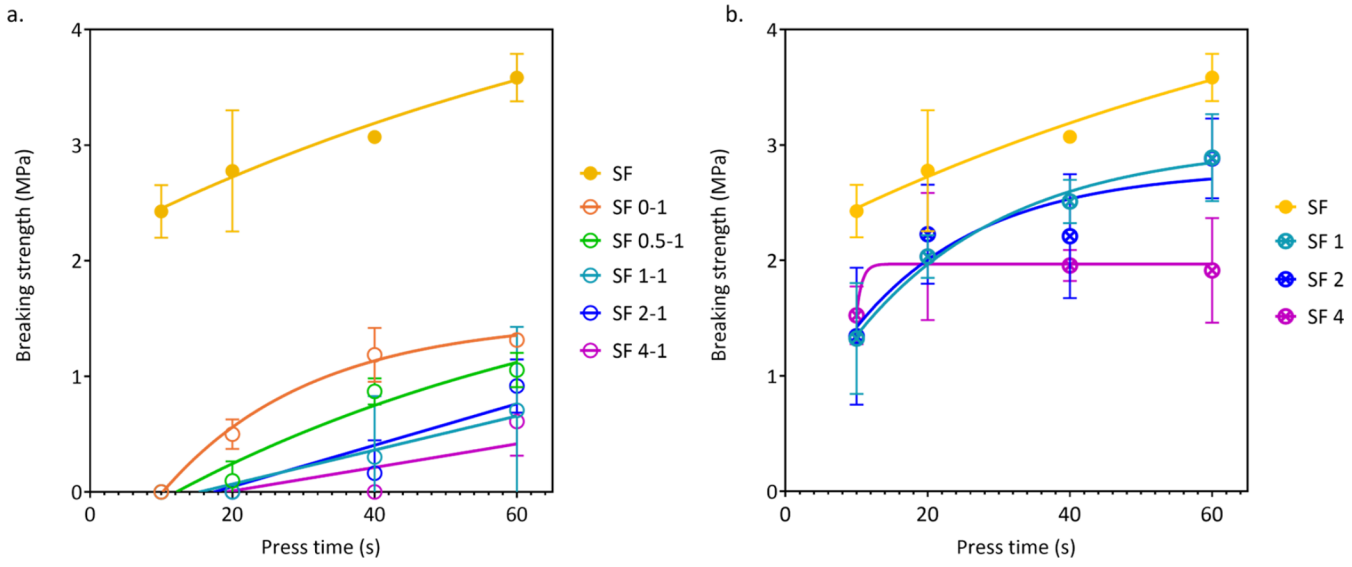

Figure 10. Shear tests of **SF** and **SF**-treated with H_2O_2, with NaOH (a) and without NaOH (b).

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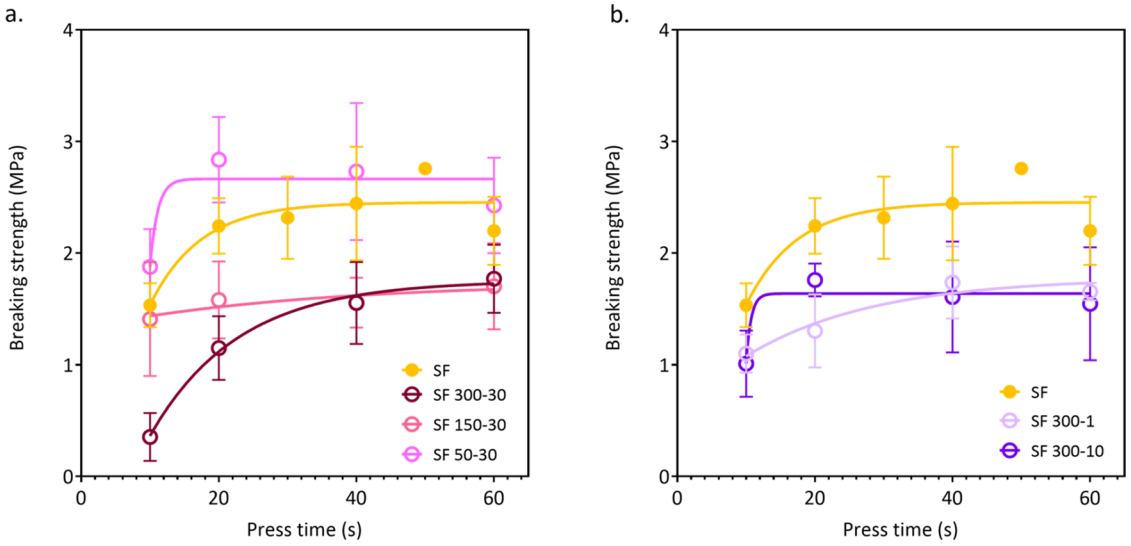

Figure 11. Shear tests of **SF** and **MW-treated SF** samples for 50, 150, and 300 W, at 30 s (a) and at 300 W at 1 and 10 min (b).

SF has shown a 1.5 MPa breaking strength after 10 s of pressing, and after 40 s of pressing, this strength raised a plateau of 2.4 MPa (Figure 11).

The **SF 50−30** exhibits a breaking strength of 1.9 MPa at 10 s of press, and after 20 s, this strength increased to reach its maximum value at 2.7 MPa. This adhesive profile of **SF 50−30** looked similar to that of **SF** (Figure 11a), indicating that MW treatment, in these conditions (50 W and 30 s), had no effect on **SF** adhesive ability.

SF 150−30 and **SF 300−30** have shown a breaking force increasing with press time. **SF 150−30** exhibits a breaking force of 1.4 MPa at 10 s of press and reached a maximum of 1.7 MPa for 60 s of press time. The tensile strength of the **SF 300−30** was about 0.4 MPa at 10 s of press and reached a maximum of 1.8 MPa at 60 s of press (Figure 11a). **SF 150−30** and **SF 300−30** exhibited comparable adhesive performance profiles (1.7 and 1.8 MPa, respectively, at 60 s), but showed lower adhesive performances than the **SF** standard (2.2 MPa at 60 s) (Figure 11a).

The MW treatments at a constant power (300 W) and at different times (1 and 10 min) were also examined by a shear test (Figure 11b). The breaking strength of **SF 300−1** starts at 1.1 MPa at 10 s of pressing and increases with press time until it reaches 1.7 MPa for 60 s of press. **SF 300−10** has demonstrated a breaking strength of 1 MPa at 10 s. After 20 s of pressing, this sample reached a maximum breaking force of about 1.6 MPa (Figure 11b). The **SF 300−1** and **SF 300−10** adhesive performance profiles were similar and comparable to those of **SF 150−30** and **SF 300−30** (Figure 11). These results indicate that microwave treatments have a limited impact on the adhesive performance of **SF**. In fact, at 60 s of pressing, **SF 150−30**, **SF 300−30**, **SF 300−1**, and **SF 300−10** reached 1.7, 1.8, 1.6, and 1.5 MPa, respectively. Beyond 150 W and 30 s of treatment, the adhesive performances were similar.

The limited impact of MW on the **SF** adhesive performances can be compared to the protein structural modifications made by this treatment. The structural analysis by electrophoresis, circular dichroism, and FTIR revealed that MW treatments damaged only the α helices secondary structure, while preserving the β sheets and the primary structures. Thus, MW affects only secondary protein structures, which could explain the limited decrease of the adhesive performances of SF MW.

4. CONCLUSIONS

This study highlighted the structural globulin parameters involved in the adhesive character of the oilseed meals. Sunflower globulin extract (**SF**) was treated chemically and physically through microwave irradiation, to induce structural modifications and analyze their adhesive properties. The protein structure modifications were characterized by electrophoresis, circular dichroism (CD), and infrared (FTIR) and shear tests.

Oxidation denaturation by hydrogen peroxide treatment, with and without soda, induced a change in the primary structure of **SF** proteins by reducing their size. This treatment also led to secondary structure modifications. The α helices would be modified into random coils, while the β sheets were conserved. These primary and secondary structural changes have resulted in the loss of adhesive performance of **SF** proteins.

The microwave denaturation technique did not induce any primary structural modifications of **SF** proteins. In fact, no change in molecular weight was observed. However, CD analyses revealed secondary structure alterations. As the treatment intensity and time increase, α helices degenerate into random coils. Adhesive characterization methods revealed a loss of performance for all treatments except for **SF 50−30**, which has the same performance as the untreated **SF**. Indeed, the FTIR analysis revealed no change in β sheets level. The impact of MW treatment on secondary structures might then be investigated further using solid NMR or Raman spectroscopy.

These chemical and physical treatments have demonstrated that the primary and secondary protein structures affect their adhesive ability. The adhesive capability of globulins is diminished when their primary structures are fully broken down. Adhesive efficacy may also decrease due to secondary structural damage, including α helix changes to a random coil. These findings confirm that sunflower meal adhesivity is dependent on the structural characteristics of globulin. In the following of this study, it will be of interest to evaluate the adhesive properties of other protein families found in oilseed

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meals. These extraction and structural characterization methods can be transposed to other meals. Finally, the exclusive use of meal proteins would be too costly for the wood panel industry, even if new methods make it possible to obtain protein-enriched meal. To develop biobased adhesives, it is therefore necessary to use the meal in its entirety. To do this, the other ligneous compounds and polysaccharides need to be more reactive in order to adhere to the wood.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c04944.

Circular dichroism characterization of SF and SF samples treated with H_2O_2 with NaOH and H_2O_2 without NaOH and absorbance of all samples in dotted line (Figure S1); circular dichroism secondary structure analysis of SF and microwave-treated SF samples in solid line and absorbance of all samples in dotted line (Figure S2) (PDF)

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Notes

The authors declare no competing financial interest.

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