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Rheological behavior and bonding performance of an alkaline soy protein suspension

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ABSTRACT

The goal of this work is to study the rheological properties of based-modified soy protein concentrate (SPC) adhesives and the relationship between viscoelastic properties and bonding performance. Chemical modification of SPC with sodium hydroxide was made to evaluate the effect of alkali on the viscoelastic proper ties. Viscosity and solubility depends directly on the 3D structure and the isoelectric point (pI) of the protein. Results show that viscosity is strongly pH dependent due to the protein unfolding. Solubility profiles exhibit the typical U-shaped curve, being higher on either side of the isoelectric point. Fourier transformed infrared analysis was used to analyze Amide I (1720–1600 cm^{-1}) and Amide III (1400–1200 cm^{-1}) band patterns which reflect the different secondary structures in proteins. The intensity of the band at 1250 cm^{-1} increases with respect to that at 1235 cm^{-1} for higher pH values. This could be associated with the destruction, at least partially, of the β-sheet structure. Bonding performance was measured in dry conditions and the wetting properties were analyzed by scanning electron microscopy. The bonding performance improves when the SPC is stabilized at pH 12 due to the protein unfolding, revealing a strong interaction between the secondary structure and the wood surface. As part of an ongoing project it was concluded that alkali modification is a suitable procedure to modify a protein suspension, improving application conditions and mechanical properties of bioadhesives of a semistructural type.

1. Introduction

Petroleum-based adhesives like urea–formaldehyde (UF) are widely used in plywood, composite wood panels and furniture because of high adhesion strength and low cost. This adhesive has replaced historic based protein products such as casein or blood [1], which were displaced from the market.

However, highly toxic formaldehyde is emitted during the production and post-production processes. It is important to note that formaldehyde was declared a carcinogen by the World Health Organization (WHO) in 2004. Besides, the future shortage of petrochemical-based products supposes a rise in the relative price and lack of availability, leading to an increase in the development of green products from inexpensive and renewable resources. A potential solution to this problem is the use of soy based products which are renewable, non-toxic and environment friendly materials [2]. Soybean is an abundant raw material in the South American region commonly used for the production of oil and starch with different protein amount. Argentina is the third largest producer of soybeans after the United States and Brazil due to the favorable weather conditions of the region [3]. Soybean meal can be processed to concentrate, and furthermore, isolate the protein and obtain a more concentrated product. Soy protein concentrate (SPC) contains approximately 60% of protein. Although soy protein isolate (SPI) contains nearly 90% of the protein, it has been decided to work with the SPC as it is cheaper than SPI but maintains an acceptable bonding performance. Nishinari et al. studied the main components of soybean proteins [4]. They studied a mixture of various proteins, and the main ingredients are classified into four protein categories according to their sedimentation coefficients 2S, 7S, 11S and 15S. Among these four, 7S (β-conglycinin) and 11S (glycinin) represent more than 80 wt%, and the ratio 7S/11S has been reported to be about 0.5–1.3 depending on the varieties [5]. 11S globulin is a hexamer, made up of five different subunits, each of which consists of an acidic subunit A (acidic isoelectric point) and a basic subunit B (basic isoelectric point), linked by a disulfide bond.

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Bonding strength of protein plant adhesives must be improved to achieve the performance of conventional wood adhesives. Proteins are formed by a combination of different amino acids. Each amino acid contains functional groups attached to the polypeptide backbone of the biopolymer. The denaturation of the protein by chemical modification exposes the functional groups; such as OH, COOH, NH_2, and SH; being available to interact with the wood and improve the bonding strength [6].

Several researchers have employed different physical and chemical methods to improve the bond strength and water resistance of the soybean protein. Their studies report on the successful use of heat treatment and addition of different denaturation agents, e.g. alkali, urea, guanidine hydrochloride; and different anionic and cationic surfactants, as a means to unfold the protein structure and make it more available for interaction and reaction. Zhong et al. reported that proteins are completely denatured after treatment with a 6 M solution of guanidine hydrochloride. The same authors also reported that the maximum strength for wood joints was achieved with 1 M guanidine hydrochloride solution. More recently, Santoni et al. [7] modified soy protein in acid (pH 3) and basic (pH 10) media to evaluate the solubility and the mechanical properties as a wood adhesive. From Fourier transformed infrared (FTIR) analysis, they found that variations in the acidity level did not change the conformation of all proteins, as evidenced by the invariance in the position of the amide I band at 1630 cm^{-1}. In addition, IR spectra evidenced the effect of pH on the protein structure; an increase in pH increases the unfolding of the protein. Nordqvist et al. [8] compared the bonding performance of soy protein isolate and wheat gluten when a sodium hydroxide solution (0.1 M) was used as a denaturation agent. Recently, the same author expanded the characterization to acidic media [9] and evaluated adhesion measurements at the nanoscale using atomic force microscopy.

Rheology has become an important and useful technique to study the properties of viscoelastic materials. This method allows analyzing the internal structure of the adhesive that will be affected by chemical phenomena, such as interactions due to weak intermolecular forces; and physical phenomena, such as entanglement and coiling of the polymer chains. The study of the internal structure of viscoelastic materials allows us to understand the behavior during processing conditions, i.e. such as mixing and applying. Furthermore, viscosity is an essential adhesive's property affecting the wetting parameter and, therefore, modifying their performance. The operating viscosity limits of soybean glues are very large ranging from 500 to 75,000 cP depending upon the application and the nature of the materials to be glued. A viscosity of 500–5000 cP is needed for gluing materials which are highly absorbing such as paper, soft board and dried wood aggregates, 5000–25,000 cP for most wood laminating purposes (both cold or hot press) and over 50,000 cP for mastic consistency wood laminating operations [10]. A less viscous adhesive is preferable as it is easier to produce and to apply.

The main goal of this work was to study the rheological properties of base-modified soy protein concentrate suspensions and simultaneously explore the relationship between viscoelastic properties and bonding performance. This work is part of a wider research plan aimed to modify vegetable proteins by means of additives to improve their water resistance, thus to obtain high performance adhesives of a semistructural type.

Rheological studies were carried out with different pH values to analyze suspensions' microstructure and FTIR analysis was made to study the denaturation process. Solubility profiles of the SPC in water were made to determine the relationship between viscosity and the isoelectric point. Bonding performance was measured in dry conditions meanwhile the wetting properties were analyzed by scanning electron microscopy (SEM) to study the relationship between viscoelastic properties and bonding performance.

Table 1
Designation code of the samples.

Sample	pH
SPC-8	8
SPC-9	9
SPC-10	10
SPC-11	11
SPC-12	12
SPC-13	13

2. Experimental

2.1. Materials

Soy protein concentrate (SPC) Alpha DK was kindly donated by Tecnoalimenti S.A. Sodium hydroxide (NaOH) was purchased from Merck. The TEGO Foamex 1488 was used as antifoam, provided by Clariant Argentina S.A.

2.2. Preparation of SPC adhesives

All samples were formulated by dispersing 20 g of SPC in 154 g of de-ionized water in an industrial stirrer at 300 RPM for two hours. Degree of denaturation was achieved by adding different amounts of NaOH to adjust the pH ranging from 8 to 13. A few drops of a diluted antifoam solution were added and then a vacuum was applied for air elimination before analysis or application. Table 1 summarized the identification of each sample.

2.3. Characterization

Viscosity was measured at 25 °C over a shear rate range of 10–250 s^{-1} using a rotational test on an oscillatory rheometer (Physica MCR301, Anton Paar GmbH) equipped with concentric-cone geometry (CC27). Viscoelastic properties were evaluated through an amplitude sweep test at 10 s^{-1} over a strain range of 0.01–100% using an oscillatory test equipped with cone–plate geometry (CP50) at 25 °C. The viscosity of each suspension was measured 6 h after preparation. In order to obtain a representative value, the test was replicated five times for each sample and the average values are reported.

Solubility profiles of the protein were determined over the pH range 8–13. SPC samples were suspended in de-ionized water at 1 g/100 g protein concentration and stirred for 1 h at room temperature, readjusting the pH if necessary. The dispersions were then centrifuged at 4600 RPM for 30 min at room temperature. Crude protein content in the supernatant was determined using a spectrophotometer (Shimadzu UV-1800) by the Biuret method. This procedure was repeated three times with each sample and the average values are reported. Solubility was expressed as the percentage of original protein present in the supernatant and the solubility of SPC at pH 7 was determined as standard.

The effect of protein modification was studied through FTIR (ATR) using a Thermo Scientific Nicolet 6700 spectrometer. Each spectrum was collected by accumulating 64 scans at a resolution of 6 cm^{-1}. Samples were freeze dried before FTIR analysis.

A tensile testing machine (INSTRON 4467) with a cross-head speed of 2.54 mm/min was used to evaluate the bonding strength. The test was replicated 10 times for each sample. The adhesive was manually applied on two pieces of hardwood (*Balfourodendron riedelianum*), over an area of 5.0×5.0 cm^2. They were then heat pressed together at 0.27 MPa for 20 min at 70 °C. After that, the samples were left to stabilize for 48 h in room temperature conditions.

After the mechanical test, the bonding surfaces were analyzed with a scanning electron microscope (QUANTA 250 FEG) without any surface treatment required. The voltage, magnification and the segment value in microns are indicated in each micrograph.

3. Results and discussion

3.1. Rheological behavior

Fig. 1 presents the viscosity values as a function of shear rate. The viscosity of all the samples decreases as a consequence of the breaking of Van der Waals forces between the proteins' chains due to applied deformation. During particleboard preparation, the application of adhesive is by spray which is characterized by a shear rate of approximately 100 s^{-1}. The higher viscosity value for this shear rate corresponds to SPC-10, followed by SP-11; SPC-9 and SPC-12 present a similar viscosity value and the lowest values correspond to SPC-8 and SPC-13.

Viscosity and solubility depend directly on the 3D structure and the isoelectric point (pI) of the protein. At the pI, the solubility decreases until a minimum value at which point the viscosity reaches the highest value. Fig. 2 shows the solubility profile versus pH, which exhibits the typical U-shaped feature. The solubility presents the lowest value for SPC-11, it increases at pH 12 and 13 due to the production of soluble peptides and increased number of exposed ionizable amino and carboxyl groups during hydrolysis [11].

The effect of the pH can also be analyzed by fitting the viscosity curves to the Ostwald I method (power-law):

$$\tau = c\,\dot{\gamma}^{p}$$

where τ is the shear stress (Pa), $\dot{\gamma}$ is the shear rate (s^{-1}), c is the flow consistency index (Pa s) and p is the flow behavior index (dimensionless). Table 2 presents c and p values for all samples. Based on these parameters, all specimens are classified as pseudoplastic fluids ($c > 0$ and $0 < p < 1$). This means that the viscosity of the material is dependent on the degree of the shear load (shear rate) and the flow curve shows a decreasing curve slope, i.e. viscosity decreases with increasing load.

Shear thinning behavior can be explained by the entanglement model: at rest, each single macromolecule can be found in the state of the lowest energy consumption and the backbone of the protein tends to entangle many times with neighboring macromolecules. During the shear process, the molecules are more or less oriented in the shear direction. In doing this, the molecules disentangle to a certain extent, which lowers their flow resistance. Lin and Gunasekaran [12] studied the rheological behavior of protein adhesives; the relative low viscosity at high shear rates makes the adhesive easy to mix and pour, and high viscosities at low shear rates gives good suspension properties. The change of pH of the solution alters the structural conformation of the protein, unrolling it and allowing weak intermolecular interactions (hydrogen bonds, disulfide bonds, etc.) [13]. These changes can be analyzed by observing the viscoelastic properties, i.e. through frequency sweep and strain sweep experiments. Fig. 3 presents the amplitude sweep test for all samples. SPC-8, SPC-9 and SPC-13 present the limit of the linear viscoelastic region (LVE) at low strain values (lower than 1%) while for SPC-10 and SPC-12 the LVE is approximately 3% of the strain, and is approximately 20% for SPC-11. This behavior could be explained through the modification of the protein induced by high pH values. The LVE of SPC-8 and SPC-9 decay at a relatively low strain, indicating that the protein is

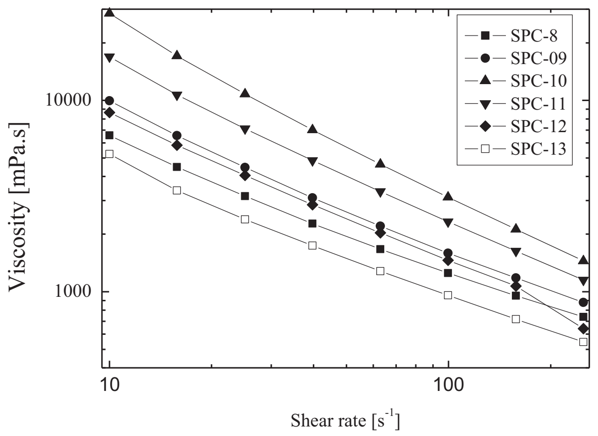

Fig. 1. Effect of shear rate on the viscosity of the formulated adhesives.

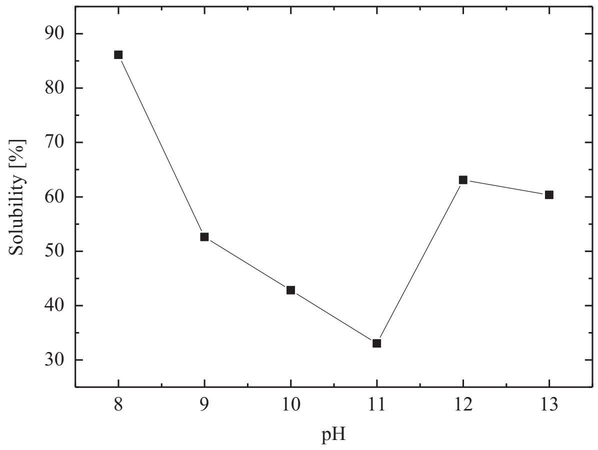

Fig. 2. Solubility profile of SPC as a dependence of pH.

Table 2
Power-law index values of all adhesives.

Sample	c	P
SPC-8	23.20	0.31
SPC-9	51.17	0.22
SPC-10	105.70	0.17
SPC-11	216.32	0.08
SPC-12	51.88	0.25
SPC-13	28.93	0.32

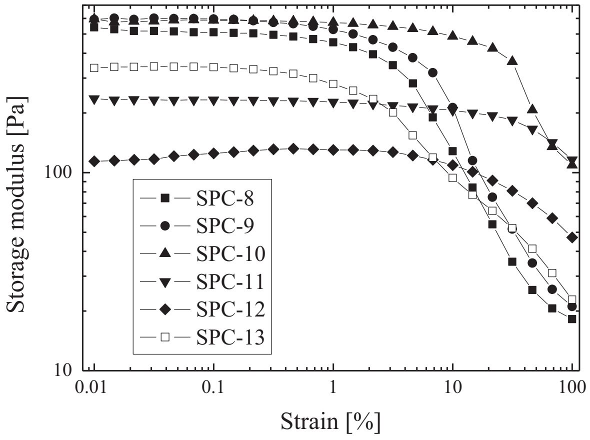

Fig. 3. Amplitude sweep test and G' values as a dependence of pH.

partially unrolled. The LVE of SPC-11 decreases at higher strain values which indicate the appearance of non-covalent interactions producing a highly elastic structure. LVE decays drastically for SPC-13 due to the high alkali concentration that starts to hydrolyze the protein, losing the elastic structure.

Fig. 4 shows tan δ values as a dependence of strain for all samples. SPC-8 and SPC-9 partially maintains the secondary structure, therefore the backbone of the protein chain is relatively folded. SPC-13 reaches the flow point (tan δ=1) at low strain values due to the appearance of shortened chains. The increase of loss modulus (G") represents the deformation energy spent by the sample during the process of changing the material's structure. SPC-10 and SPC-11 reach the flow point at high strain values due to the unfolding of the protein and the interactions between the functional groups of the side chains, generating a gel-like structure. Table 3 presents the flow point values as a dependence of the pH. SPC-12 shows a desirable viscoelastic behavior, consequently the adhesive will tend to flow in application conditions. Nevertheless it will recover and regain its structure at rest, adhering to the surface and holding the bond between the two pieces of wood

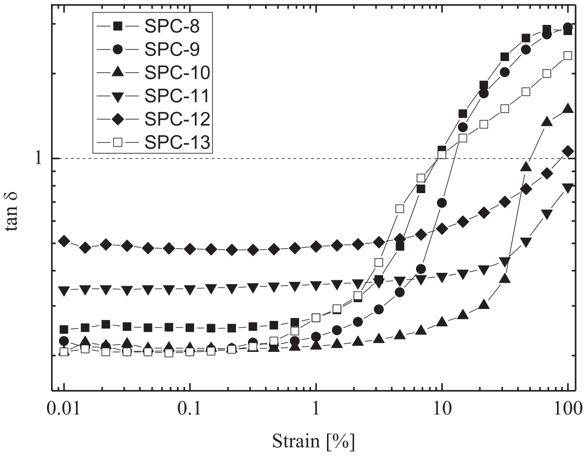

Fig. 4. tan δ Values for all samples in the ranging strain from 0.1% to 100%.

Table 3
Flow point values as a dependence of the pH.

Sample	Flow point (Pa)
SPC-8	18.17
SPC-9	26.96
SPC-10	134.5
SPC-11	194.5
SPC-12	63.29
SPC-13	12.99

FTIR is a useful tool to understand the denaturation process [14]. The vibrational spectrums at medium IR range present Amide I (1720–1600 cm^{-1}) and Amide III (1400–1200 cm^{-1}) band patterns which reflect the different secondary structures in proteins [15]. The sensitivity of infrared absorptions peaks change according to hydrogen bonding, dipole–dipole interactions, and peptide backbone geometry, indicating secondary structure modification. Changes in the FTIR spectra suggested changes in the secondary structure [16]. Although the Amide I region has been the most studied and used in the qualitative and quantitative analysis of secondary structures [17], the Amide III region has also been used to analyze protein conformational changes and may have advantages over the Amide I area for lyophilized samples [18]. The Amide III absorbs in the 1400–1200 cm^{-1} range due to N–H bending, C–N stretching with small contributions of C–O bending and C–C stretching. In polypeptides, the composition of Amide III mode is more complex, since it depends on the structure of the side chains and because N–H bending contributes to several modes in the 1400–1200 cm^{-1} region [19]. The following correspondence exists between the location of bands and different secondary structures: α-helix (1330–1320 cm^{-1}), β-sheet (1240–1220 cm^{-1}), unordered structure (1280–1260 cm^{-1}) and extended chain structure (1250–1240 cm^{-1}); a decrease of these bands suggest a significant change in the secondary structure [20].

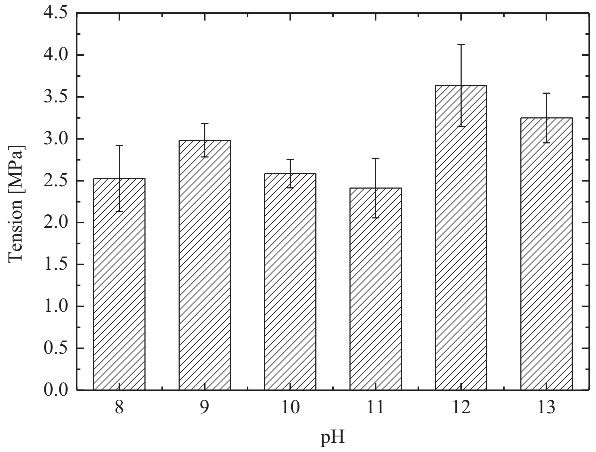

Fig. 6. Bonding performance for all SPC suspensions.

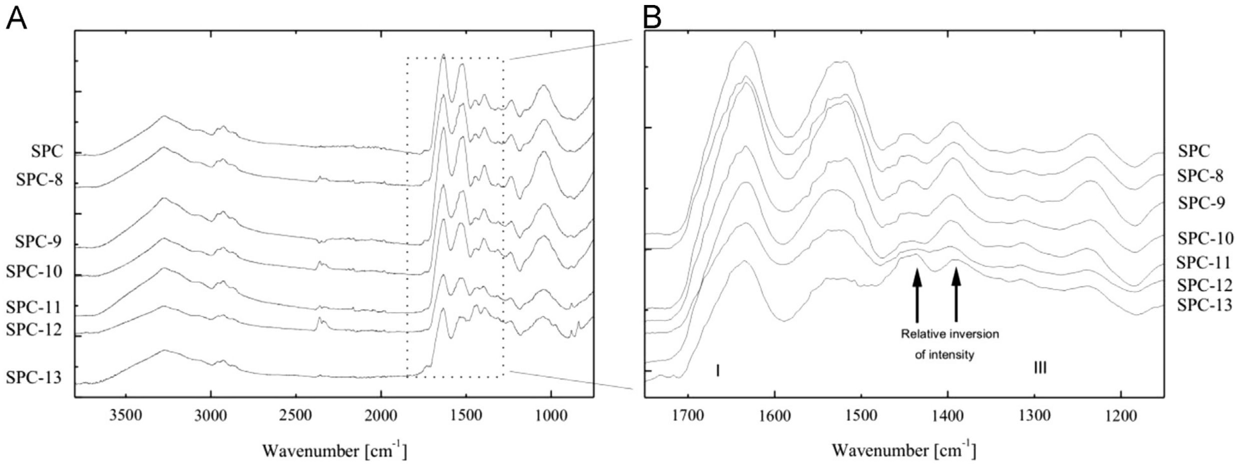

Fig. 5. (a) ATR-FTIR spectra of SPC suspensions for different pH, (b) details of the amide III and amide I region.

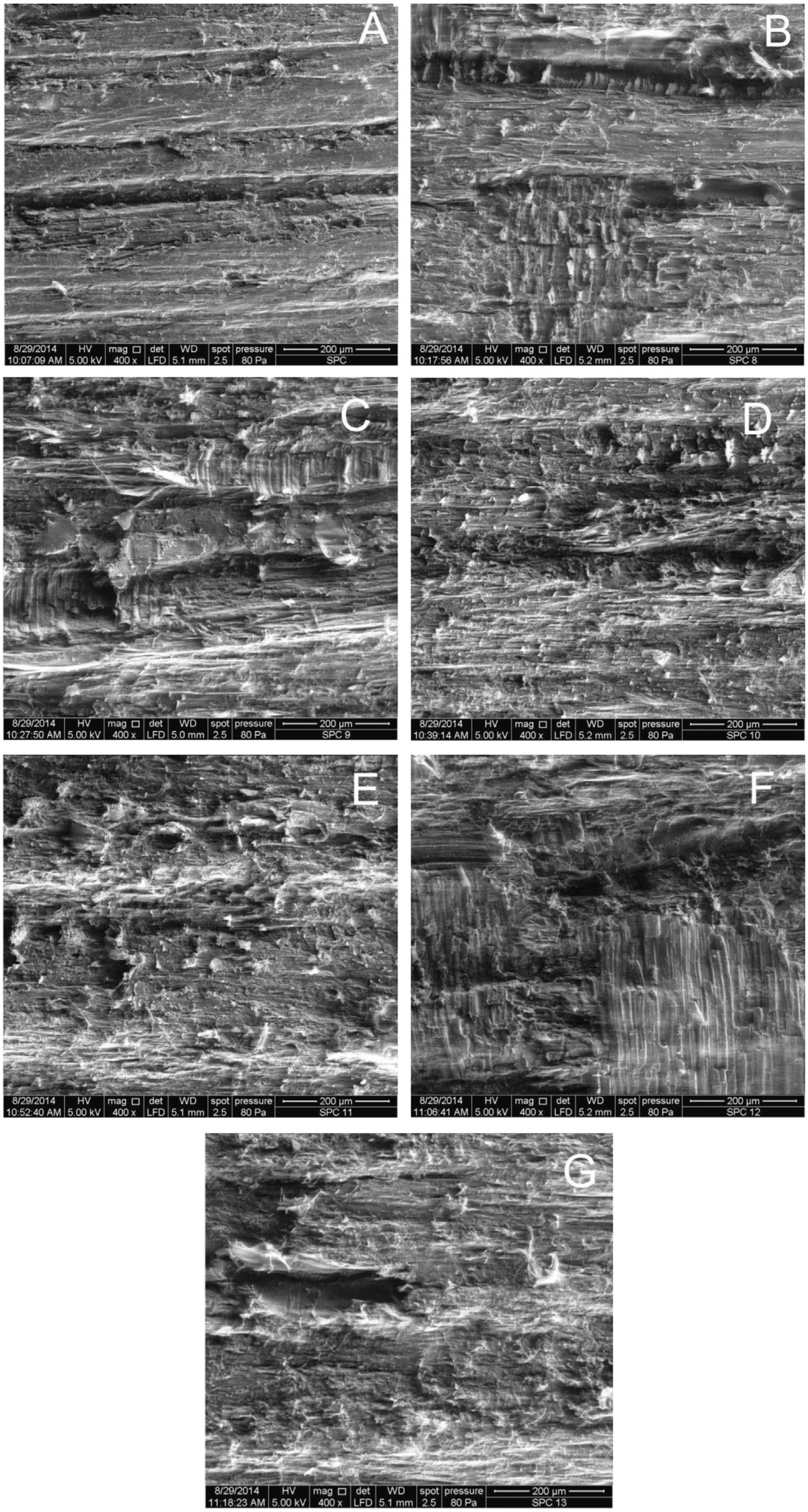

Fig. 7. Crio-SEM pictures of wood surface after adhesion test. (a) Reference (without treatment), (b) pH 8, (c) pH 9, (d) pH 10, (e) pH 11, (f) pH 12, and (g) pH 13.

Fig. 5 shows infrared spectra and the full details of the Amide III region where there are variations in the band pattern according to the different evaluated conditions of pH. The spectrum of the SPC-8 sample shows a dominant band at 1235 cm^{-1}, corresponding to the β-sheet structure area and its lower intensity bands corresponding to the unordered structure, extended chain structure and α-helix. By increasing pH value, the ratio of intensities between the different zones is changed remarkably, the intensity of the major absorptions at 1250 cm^{-1} increase with respect to the band at 1235 cm^{-1}, which would correspond to destruction, at least partially, of the β-sheet structure. Shifts are also observed in the band positions and widening thereof, suggesting structural changes compatible with a process of protein denaturation. While the above analysis is essentially qualitative, the spectra indicates an intensification of this behavior for pH higher than 9. Moreover, SPC-12 and SPC-13 present significant changes in the bands at 1430 and 1530 cm^{-1} associated with the stretching vibrations of the carboxylate ion that not only involve a conformational change but also the effect of the partial hydrolysis.

3.2. *Bonding performance*

Bonding shear strength of SPC adhesives of different pH values are shown in Fig. 6. This can be grouped in three categories. The first one include pH 8, 10 and 11 whose bonding strength values were 2.52 ± 0.31 MPa, 2.58 ± 0.16 MPa and 2.41 ± 0.35 MPa, respectively. These samples are the weakest group. The second category includes pH 9 and 13 whose bonding strength values were 2.98 ± 0.19 MPa and 3.24 ± 0.29 MPa, respectively. These specimens perform at an intermediate level. The last category includes pH 12 with 3.63 ± 0.38 MPa and is considered as the strongest performing group. These results can be explained taking into account the structural characterization described above. SPC-8 and SPC-9 present a low denaturation degree, which means that most of the functional groups are unavailable due to the native conformation of the protein which tends to fold and impedes the interactions between adhesive and substrate [21]. SPC-11 presents very high viscosity and flow point values which impedes the application on to the wood surface leading to the lowest bond strength. SPC-12 and SPC-13 display the best bonding properties because the protein is fully unfolded and permits the functional groups of the side chain to establish interaction with the substrate by van der Waals forces, hydrogen bonds, disulfide bonds and more. However, for SPC-13, the low viscosity shows that the degree of hydrolysis of the proteins which produces the shortening of the backbone therefore leading to excessive and undesirable water absorption and decreasing bonding properties.

Adhesive penetration is generally believed to have a strong influence on bonding mechanical performance. Adequate penetration provides a substantial interphase that promotes interaction, perhaps reaction, and also mechanical interlocking. On the other hand, excessive penetration could lead to a “starved” bond-line having poor performance [22]. The main components of wood are cellulose, hemicellulose, and lignin. Hemicellulose is the fraction most accessible to water and is reported to be the main site of interactions between wood and water [23]. Fig. 7 shows the fractured surface of the samples after the adhesion test. For comparison, Fig. 7a presents a wood surface free of adhesive and shows a regular oriented structure. Most of the adhesives showed cohesive failure (i.e. break in the glue line – indicating weak joint). SPC 12 presents a partial perpendicular orientation pattern which corresponds to the rupture of the substrate (i.e. break in wood substrate – good joint), which could indicate a slight improvement in bond strength.

4. Conclusion

The rheological behavior and the bonding performance at dry conditions of based modified SPC suspensions were studied. The adhesive viscoelastic properties are strongly pH dependant and viscosity of the suspension can be easily adjusted to allow a proper adhesive–wood interaction. SPC-12 achieved the better bonding performance and it will be selected for further testing, such as bonding performance in wet conditions, by the implementation of different protein modification techniques. As part of an ongoing project it was concluded that alkali modification is a suitable procedure to modify a protein suspension to improve the mechanical properties of bioadhesives of a semistructural type.

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