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PII: S0143-7496(23)00150-1

DOI: <https://doi.org/10.1016/j.ijadhadh.2023.103470>

Reference: JAAD 103470

To appear in: *International Journal of Adhesion and Adhesives*

Received Date: 18 August 2022

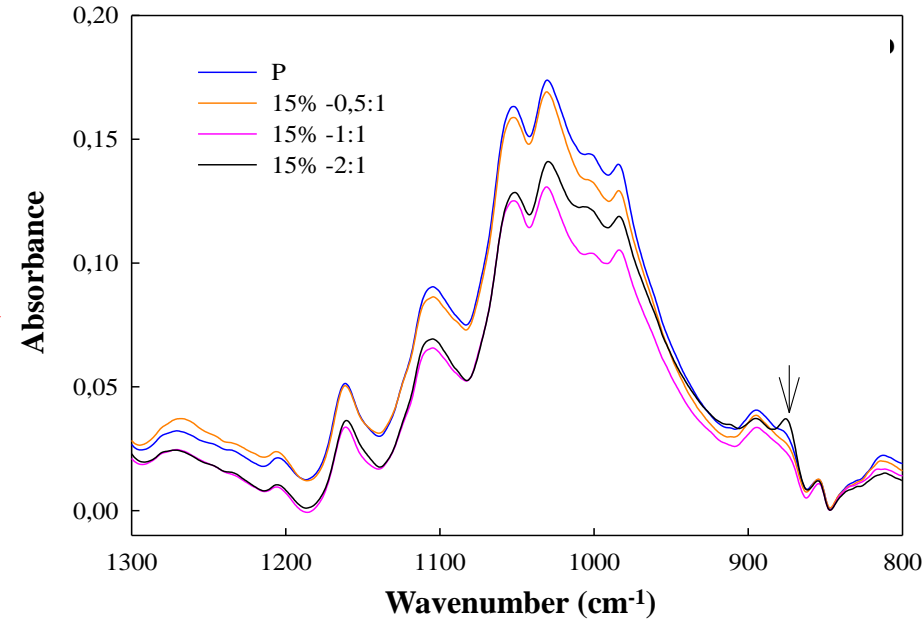
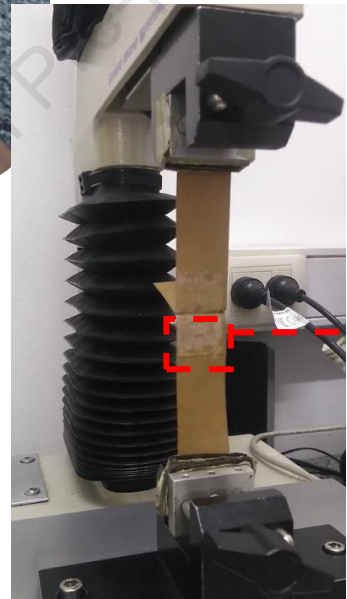
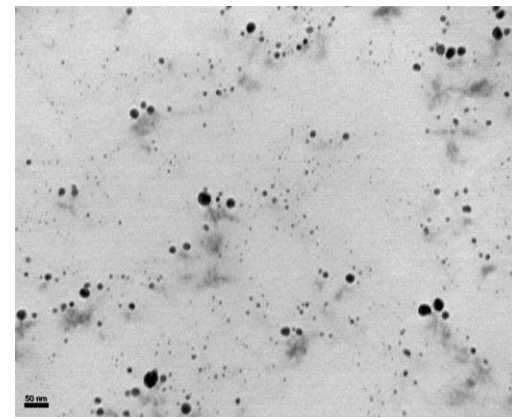
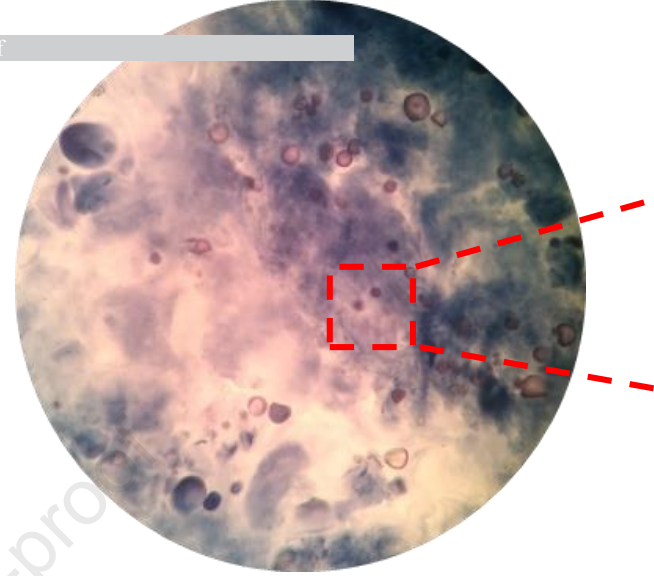
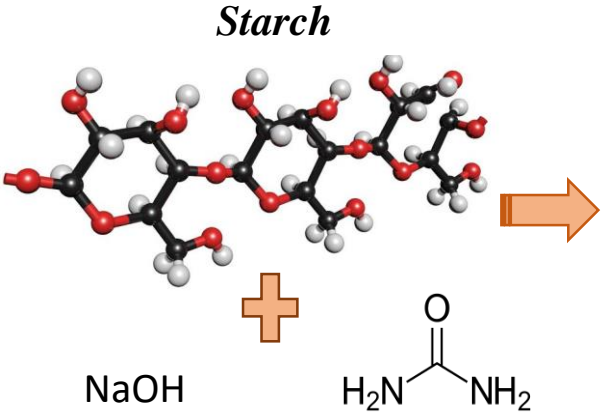
Revised Date: 10 July 2023

Accepted Date: 17 July 2023

Please cite this article as: Monroy Y, Cabezas D, Rivero S, García MariA, Structural analysis and adhesive capacity of cassava starch modified with NaOH:urea mixtures, *International Journal of Adhesion and Adhesives* (2023), doi: <https://doi.org/10.1016/j.ijadhadh.2023.103470>.

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Structural analysis and adhesive capacity of cassava starch modified with NaOH:urea mixtures

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Abstract

In this research the interactions and structural changes induced by NaOH:urea mixtures on cassava starch suspensions as well as their adhesive capacity were studied. Cold gelatinization of 10-20% w/w cassava suspensions was induced by adding different NaOH:urea mixtures (0.5-2:1). Chemical modification of cassava starch was analyzed through optical microscopy as well as SEC-HPLC and size particle determinations, ATR-FTIR spectral and chemometric analysis were also carried out.

Cassava starch treatment with NaOH:urea mixtures generated structural disorganization, induced cold gelatinization and promoted the hydrolysis of the polymeric chains. SEC-HPLC and ATR-FTIR results showed that the modifications induced in the adhesive formulations depend both on the starch content and the NaOH:urea ratio. All tested formulations exhibited adhesive capacity, being effective for Kraft paper substrate bonding. Thus, this simple and low-cost procedure was effective to chemically modify cassava starch allowing to obtain derivatives with tailor-made adhesive properties.

Keywords: cassava starch, chemical modification, structural analysis, adhesive capacity.

1. INTRODUCTION

Cassava starch is a polysaccharide in high demand due to its low-cost, renewable nature and biodegradability [1]. This biopolymer can be used for adhesive applications, especially for paper and wood surfaces, with distinctive characteristics over petroleum-based polymers [2]. Wang et al. [3] have reported the increasing use of cassava starch in the production of paper, textiles, adhesives, cardboard, and other industrial products, due to its versatility and availability. In addition, the global adhesives and sealants market is growing rapidly and is expected to be worth USD 85.8 billion by 2026 [4]. Environmental aspects such as pollution from petroleum spills, global warming, and the depletion of fossil fuels have guided the researchers to develop 'green' formulations as alternatives to petrochemical adhesive components [5].

However, in order to meet the specific requirements demanded by the technological progress in the adhesive's field, structural modifications of starch are performed. To this end, different treatments are carried out to improve properties such as consistency, temperature or pH stability, gelatinization conditions and dispersibility to adapt them to specific adhesive applications [6]. In this sense, chemical modification by the addition of acid or alkali solutions can be performed using uncomplicated steps with simple instruments to obtain starch derivatives that find application in a wide variety of industrial products [1]. Nasiri et al. [7] have reported that the alkaline treatment with NaOH, in addition to cold hydrolysis and gelatinization of the starch, exhibits oxidative properties that allow obtaining a product with more adhesive character. In the same way, the incorporation of urea to the starch suspension also induces its gelatinization at room temperature, a process called cold gelatinization and retard the retrogradation during storage [8].

To the best of our knowledge the operating mechanism has not been addressed in-depth, even less the effect of the combined action of NaOH and urea, and the structural modifications that these components cause. By virtue of these findings, this research aimed to study the size particle by SLS as well as size exclusion chromatography by SEC-HPLC of cassava starch adhesives obtained with NaOH:urea

mixtures; to elucidate the interactions on modified cassava starch suspensions through ATR-FTIR spectral analysis and chemometric tools. The study of the modifications from a structural point of view will contribute to the knowledge of the chemical structure-performance relationship by studying their mechanical properties and thus their applicability as adhesives.

2. Materials and methods

2.1 Materials

Cassava (*Manihot esculenta* Crantz) starch was provided by the Montecarlo Mixed Agricultural Cooperative (Misiones, Argentina). NaOH and urea of analytical grade were supplied by Cicarelli (Argentina) and Biopack (Argentina), respectively.

2.2 Chemical modification of cassava starch

Composite aqueous solutions based on 4% w/w urea (Biopack, Argentina) and NaOH (Cicarelli, Argentina) in the range of 2 to 8% w/w to obtain different NaOH:urea ratios were prepared and stored for 24 h at 0°C. From these solutions, the cold gelatinization of cassava starch in the range of 10 to 20% w/w was induced (Table 1). These starch contents were established by setting the maximum concentration that is capable of being prepared in conventional thermal gelatinization as the lower limit (10%), and the concentration at which it was possible to prepare this system in cold, as the upper one (20%). The suspensions were stirred for 30 min with a rod to promote the starch gelatinization. Finally, the samples were subjected to a freezing cycle at -18°C for 24 h and subsequent thawing with agitation at room temperature for 30 min. This procedure was adapted from Zhang et al. [9]. Likewise, formulations were characterized after seven days of storage at room temperature to study the behavior of the adhesive and its stability depending on the preparation time.

Samples intrinsic viscosity was determined following the procedure of Nie et al. [10] to estimate the hydrodynamic volume of starch derivatives by using a Cannon-Fenske Opaque (Cannon Instrument Company, State College, USA) capillary viscometer. The reported values are the average of six replicates.

Table 1. Adhesive formulations: composition and nomenclature used.

Starch (g)	NaOH:urea mixture (g)			Nomenclature
	0.5:1	1:1	2:1	
10	90	-	-	10% - 0.5:1
10	-	90	-	10% - 1:1
10	-	-	90	10% - 2:1
15	85	-	-	15% - 0.5:1
15	-	85	-	15% - 1:1
15	-	-	85	15% - 2:1
20	80	-	-	20% - 0.5:1
20	-	80	-	20% - 1:1
20	-	-	80	20% - 2:1

A Leica SMLB microscope equipped with polarized light (Heerbrugg, Germany) was used to study the cold gelatinization process of starch. An *in situ* gelatinization experiment was designed before its observation under the microscope, which consisted of placing powdered starch with a drop of different NaOH:urea ratios and Lugol's indicator.

2.3 Particle-size distribution

The hydrodynamic diameters of the particles present in the freshly and stored (room temperature, for 7 days) formulations were determined by the static light scattering (SLS) technique using a Malvern Mastersizer 2000E particulate analyzer (Worcestershire, UK). Measurements were performed in quadruplicate at room temperature (20°C). The refractive indices used were 1.33 and 1.52 for water and starch, respectively [11]. Samples were analyzed at least in triplicate. The polydispersity index (PI) was determined.

2.4 Molecular weight determination

Molecular weight was determined by High Performance Liquid Chromatography (HPLC) using an OH-AK SB-805HQ gel permeation column (SHODEX, Japan) equipped with a refractive index detector, model 410 (Waters Milford, USA). The formulations were diluted with milliQ water, vortexed (Arcano 2002, China) and sonicated for 5 min in an ultrasonic equipment Branson 2510E-DTH (Branson Ultrasonics Corporation, Danbury, USA). Before injection, all samples were filtered through 0.45 μm pore size microfilters (Millipore, São Paulo, Brazil). Samples were eluted at room temperature using deionized water at a flow rate of 1 mL min⁻¹. ALO-2770 dextran polymers (Phenomenex, Torrance, Ca, USA) of molecular weight within the range of 97,000 to 3,800,000 Da were used as calibration standards. Samples were analyzed at least in duplicate. On the other hand, the molecular weight of the formulations stored for 7 days at room conditions was also determined. Once the peaks were identified, the contribution percentage was calculated as the ratio between the peak's partial area and the corresponding total area.

2.5 ATR-FTIR and chemometric analysis

The spectra were recorded by Fourier transform infrared spectroscopy (FTIR) using a Nicolet iS10 Thermo Scientific equipment (Madison, USA). Films of each formulation obtained by drying in an oven at 37°C were placed on the diamond crystal accessory to obtain FTIR spectra combined with attenuated total reflectance (ATR). Measurements were obtained as the average of 32 scans in the spectral region between 4000 and 400 cm⁻¹, with a resolution of 4 cm⁻¹.

A principal component analysis (PCA) was performed from the processing of the spectral bands within the region of 1200-900 cm⁻¹ (the fingerprint of carbohydrates) to elucidate the interactions between the different components present in the samples, as well as to analyze, from the structural point of view, the structural modifications produced by the different NaOH:urea ratios.

2.6 Applicability of formulations as adhesives

The consistency of the formulations and the changes experienced with storage time were studied using the back extrusion technique. Tests were carried out in a TA-XT2i texturometer (Stable Micro Systems, Surrey, UK) at a speed of 1 mm s⁻¹ to determine the consistency parameters (firmness, consistency,

cohesiveness, and viscosity index), at a controlled room temperature of 25°C. The back extrusion rig P/75 probe with a diameter of 40 mm was submerged at a distance of 10 mm, and a cylinder containing the sample with an internal diameter of 50 mm was used. A commercial adhesive (Voligoma™ with a solid content of 15%) was used for comparative purposes.

The adhesive formulations were used to bond Kraft paper substrates to study the strength of bonding or adhesion, and to evaluate their potential applications in the packaging area. In this sense, Ortiz-Fernandez et al. [12] have explained that the peeling technique allows estimating the efficacy of the adhesive bond (adherent/adhesive/adherent). The formulations were applied by forming a thin and uniform adhesive film of approximately 0.1 to 0.2 g on the paper strips with the aid of a glass rod.

Peel strength tensile tests, called peel T, were performed on a TA.XT2i – Stable Micro Systems (Stable Micro Systems, Surrey, UK) texture tester using A/TG tensile grips at a test speed of 10 mm s⁻¹. Kraft paper specimens (12 cm length × 2.5 cm width × 170 μm thickness) were used following the geometry established in the ASTM F904 standard, being the bonding area of 50 mm × 25 mm. The curves of force (N) as a function of deformation (mm) were automatically recorded by using the Texture Expert Exceed software). At least 15 specimens were tested for each sample.

2.7 Statistical analysis

All experiments were performed at least in duplicates, with individually prepared cassava starch treated samples as replicated experimental units. The InfoStat Software (Version 2009, InfoStat Group, Agricultural Sciences College, National University of Cordoba, Argentina) was used. Analysis of variance (ANOVA) and comparison of means with the Fisher's least significant difference (LSD) test were conducted, at a significance level $p=0.05$.

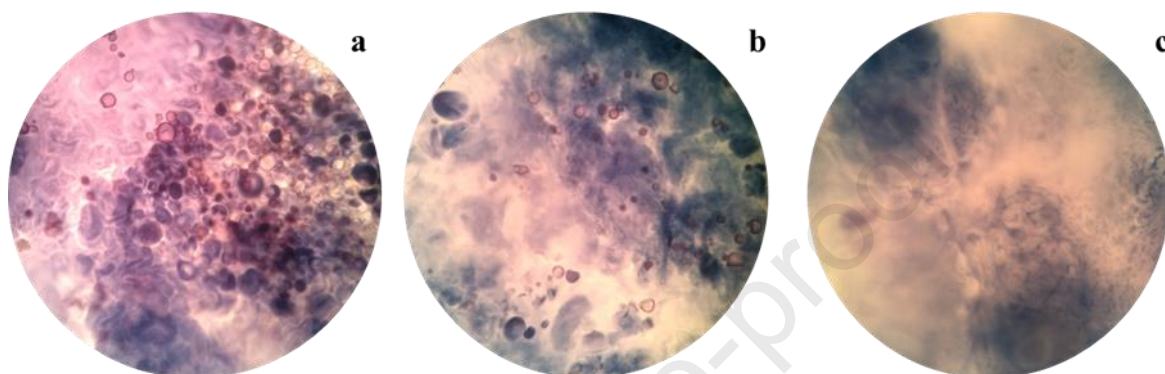
3. Results and discussion

3.1 Structural modifications

During the cold gelatinization of starch in the presence of NaOH, not only the swelling of the starch granules occurs irreversibly, but also the hydrolysis and leaching of the polymeric component chains,

as evidenced by optical microscopy (Figure S1). Likewise, Hu et al. [13] reported that NaOH induces electrostatic forces between the chains of the starch constituents, allowing the polymer dissolution.

Figure S1. Images obtained by optical microscopy of adhesive samples prepared at 10% w/w of cassava starch at different NaOH:urea ratios: **a)** 0.5:1, **b)** 1:1 and **c)** 2:1. The magnification used was 40 \times .



When this gelatinized starch suspension freezes, the solvent first crystallizes while the solute concentrates in the remaining liquid phase. Then, the ice crystal growth provokes a disruption of the polymer-polymer interactions facilitating the progress of alkali hydrolysis once the sample is thawed and stirred. In this sense, Zhang et al. [9] emphasized that after this procedure (freezing, thawing, and stirring), the size of the starch particles decreased because of the disaggregation of the monomeric units that conformed the starch clusters. The urea presence in the formulations prevented the self-association of the remaining chains of hydrolysis, limiting the retrogradation process and promoting the stability of the samples. This fact could be supported by the absence of syneresis in the stored adhesives. In summary, the presence of NaOH causes the breaking of inter and intramolecular hydrogen bonds in the chains of starch constituents.

All formulations regardless of the solvent mixture composition based on NaOH:urea induced starch gelatinization. Visually, starch-based samples became viscous and transparent, intrinsic viscosity being an appropriate and straightforward measurement to evidence the modifications involved in this process. In this sense, the formulation prepared with 15% starch and 1:1 NaOH:urea ratio presented an intrinsic viscosity of 112 mL g⁻¹ before the freezing stage. After the freeze-thaw cycle, the intrinsic viscosity was 187 mL g⁻¹, showing an increase of 67%, which could be related to a higher hydrodynamic volume due

to the aggregation of the constituent polymeric chains of starch. When the samples were stored for 7 days at room conditions, they registered an intrinsic viscosity of 60 mL g^{-1} (68% lower than the freeze-thawed samples), providing that the hydrolyzing action of the NaOH remained still active during the storage.

From a microscopic point of view, low concentrations of NaOH caused partial erosion of the starch granules surface (arrow in Figure S1a). With increasing of alkali concentration (up to 4% w/w) a greater coloration using Lugol's iodine solution was observed (arrow in Figure S1b) due to amylose leaching. At higher ratios, there is a complete absence of granular structures (arrow in Figure S1c), therefore the indicator is not capable of intercalate into the helix of the starch components and, furthermore, it undergoes a dismutation at the pH of the formulation. The NaOH:urea solvent system was effective as a precursor to induce starch gelatinization. These results were in agreed with those reported by Hu et al. [13]. The most plausible hypothesis to explain this fact is a synergistic effect of urea and NaOH in solution, causing swelling with concomitant starch gelatinization [13,14].

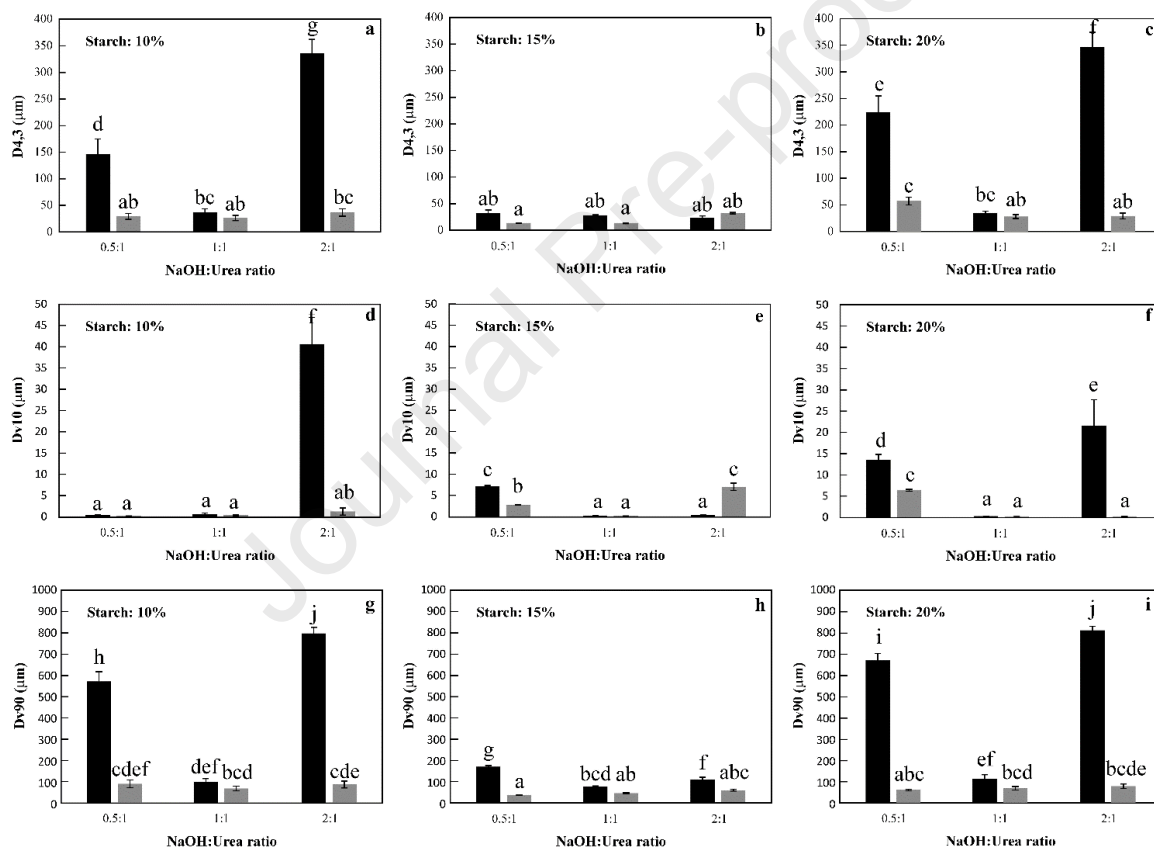
3.1.1 Particle-size distribution

Regardless of the NaOH:urea mixture and the starch concentration, all the formulations studied presented a high degree of polydispersity. In this sense, up to three populations of particles could be observed in the different formulations: the particles between $0.1\text{-}1 \mu\text{m}$ are associated with fragments of very short chains or clusters of NPs generated by an intense hydrolysis process on the starch chains, the size between $10\text{-}100 \mu\text{m}$ would be fragments with an intermediate hydrodynamic volume and the population centered in the $100\text{-}1000 \mu\text{m}$ range would be related to the formation of aggregates. To analyze these distributions, Figure 1 shows the mean diameter $D_{4,3}$, the D_{v10} percentile (10th percentile of the cumulative volume distribution), and the D_{v90} percentile (90th percentile of the cumulative volume distribution).

At the lowest concentration of starch, for a NaOH:urea ratios of 0.5:1 and 1:1, low D_{v10} values associated with very short chain particles were obtained (Figure 1d). The presence of NaOH in starch suspensions add ions into the system that modify the hydration properties of starch. OH- groups can promote chain scissions of starch constituents, disrupting both inter- and intramolecular hydrogen bonds

[15]. Similarly, Nasiri et al. [7] highlighted that the addition of NaOH accelerates the rupture of the hydrogen bonds between the constituent polymeric chains of starch.

Figure 1. Measured parameters for particle size distribution of starch-based adhesive formulations using different cassava starch concentrations (10-20%) and NaOH:urea ratios (0.5:1- 2:1): De Brouckere mean diameter (D4,3) (a-c), Dv10 percentile (d-f) and Dv90 percentile (g-i). Mean values \pm standard deviation are presented. Different letters within the same parameter indicate significant differences ($p < 0.05$). Fresh samples (black bars) and samples stored for 7 days (grey bars).



On the other hand, these systems differed in the Dv90 values (Figure 1g), where the presence of large structures was observed when the lowest NaOH:urea ratio was used. This behavior could be attributed to two factors, the presence of aggregates of structures generated during hydrolysis, and the low NaOH content, which is not enough to partially hydrolyze the amylopectin chains. On the other hand, when the NaOH content increases (2:1), the system presents D4,3 values greater than 300 µm, with the

absence of particles smaller than 10 μm ($D_{v10}>40\text{mm}$) and with structures of large hydrodynamic volume ($D_{v90}>750\text{mm}$). Momenpoor et al. [16] correlate this fact with the presence of a higher concentration of starch and consequently with a possible aggregation of the polymer chains.

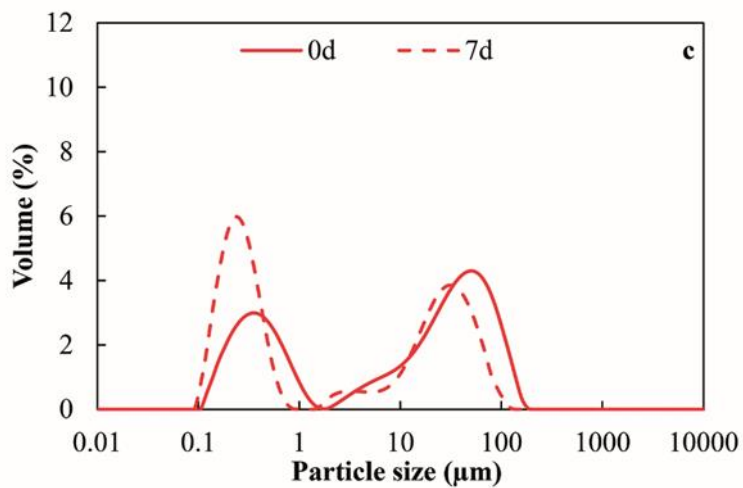
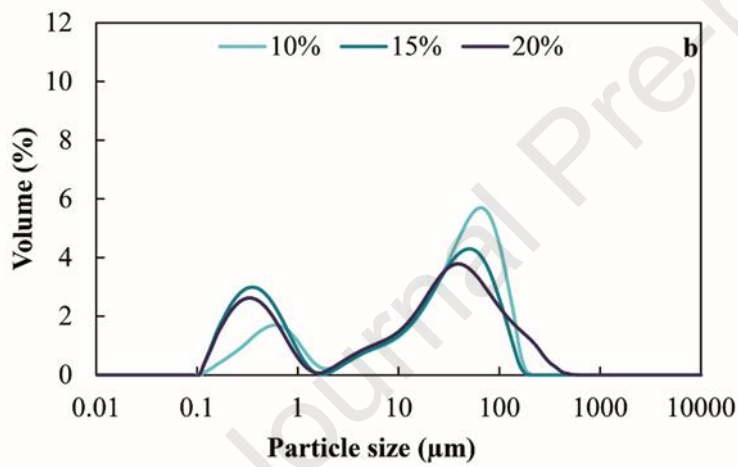
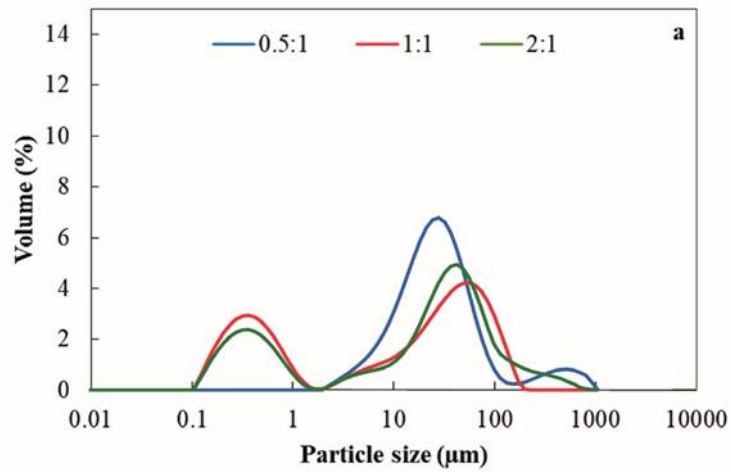
At higher starch concentrations (15% and 20%) a similar behavior of the formulations was noted, observing the presence of an NP cluster using at an intermediate concentration of NaOH (NaOH:urea 1:1) (Figure 1e,f) and an increase in the content of particles larger than 100 μm , related to a deficient hydrolysis process and/or to the formation of aggregates, mainly in the extreme NaOH contents (0.5:1, 2:1) (Figure 1h,i). As an example, Figure 2a shows the effect of increasing the NaOH ratio on the particle size distribution for the formulations prepared with 15% of starch.

Formulations with NaOH:urea 1:1, particularly those containing 15% starch (Figure 1b,e,h), allowed obtaining adhesive solutions with the smallest particle sizes and with a low proportion of particles larger than 100 μm (Figure 2b). These results allow inferring a formulation with an optimal concentration of NaOH to ensure complete hydrolysis of the starch and avoid the formation of aggregates from the structures generated during the alkaline treatment.

After 7 days of the adhesive solution preparation, a higher contribution of the populations of smaller particles, in the range of 0.1-1 μm , was evidenced (Figure 1d-f). Han and Lim [17] explained that in a neutral aqueous solution, the amylose chains exist as an interrupted helix, but upon the addition of alkali, the hydrogen bonds that stabilize the helix are progressively broken and the amylose macromolecules adopt a more random conformation. Furthermore, with the addition of a higher concentration of alkali, a progressive increase in the negative charge of the polymer chains promotes charge repulsion between them, avoiding the presence of aggregates [18]. The effect of storage in starch-based adhesive formulations at 15% w/w cassava starch with NaOH:urea 1:1 is shown in Figure 2c.

Figure 2. Particle size distribution, expressed as volume showing the influence of: **a)** different NaOH:urea ratios on cassava starch adhesives at 15% w/w; **b)** starch concentration (% w/w) at NaOH:urea 1:1 ratio; and **c)** the storage in starch-based adhesive formulations prepared at 15% w/w.

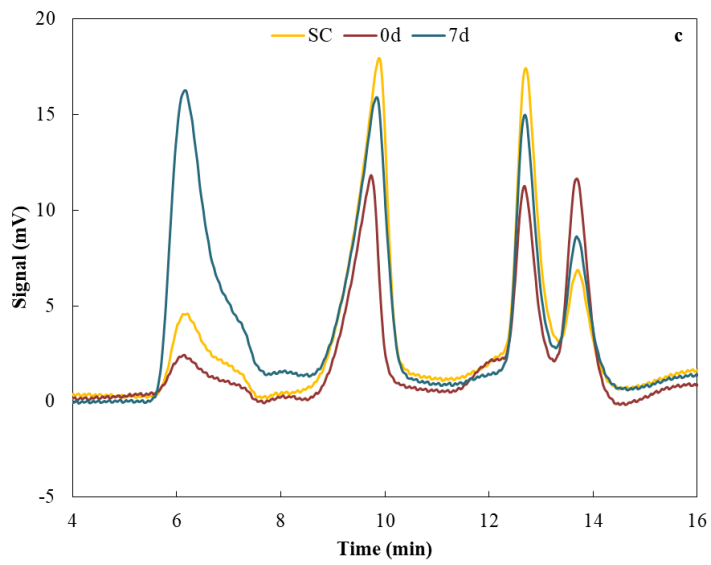
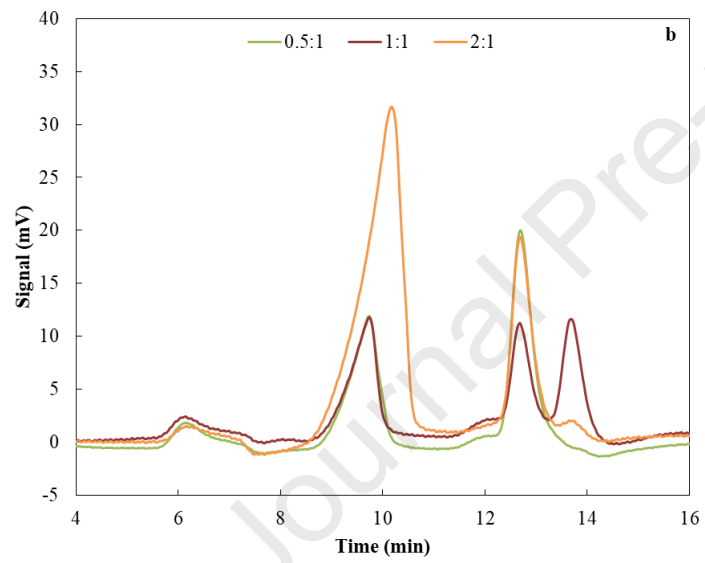
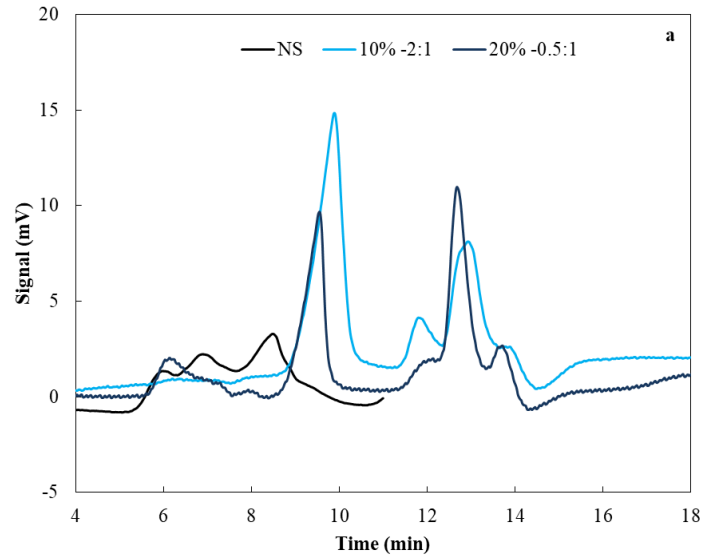
Solid lines represent samples of fresh adhesives (0d), and dotted/dashed lines represent samples stored for 7 days at room temperature (7d).



3.1.2 Molecular weight by SEC-HPLC

On the other hand, to analyze the degree of hydrolysis due to the NaOH:urea ratio as well as the influence of storage conditions, SEC-HPLC was performed. From the chromatograms, the molecular weights of the sample constituents were estimated, and the relative contribution of the peaks was calculated considering the percentage ratio of the areas. The chromatogram of native cassava starch (NS) exhibited a peak at 5.8 min and two main elution peaks corresponding to the amylopectin (6.9 min) and amylose (8.5 min) chains, respectively. Finally, the run ended at 12.5 min, which corresponds to the elution volume of solutes that do not enter the pores or interact with the chromatography resin or “void volume” (Figure 3). The molecular weights obtained from the calibration curve for amylose (1.1×10^6 Da) and amylopectin (6.5×10^7 Da) were within the range of those reported by Lemos et al. [19] who studied the composition of cassava starch. The first peak (5.8 min) assigned to a molecular weight of 9.9×10^8 Da could be attributed to molecular association compounds and/or chains with a high degree of branching. Charoenkul et al. [20] reported the existence of extra-long chains of amylopectin in cassava starch, which would support the proposed hypothesis.

Figure 3. Chromatograms obtained by SEC-HPLC of: **a)** native starch (NS), starch-based adhesive formulations at 10% w/w and a ratio of 2:1 and 20% w/w and at a ratio of 0.5:1, **b)** effect of the NaOH:urea ratio on starch-based adhesive formulations at 15% w/w and **c)** effect of freezing and storage time (nomenclature used 0d: fresh samples, 7d: samples stored 7 days and WF: adhesive without freezing).



Likewise, several authors have emphasized that the molecular weight distribution of the amylose and amylopectin chains depends on the plant cultivar, age, harvest season and agroecological conditions of the crop, among other factors, [21].

According to Simsek et al. [22], each amylose chain contains approximately 200 to 700 glucose residues, corresponding with molecular weights of 32,400 to 113,400 D, respectively. In addition, more than 80% of starch is composed of amylopectin, which is a much larger molecule with a molecular mass ranging from 10^6 to 10^8 Da. As already mentioned, amylopectin is a highly branched polysaccharide composed of approximately 95% (1 → 4) - α -linkage and 5% (1 → 6) - α -linkages.

The hydrolyzing action on the starch components was demonstrated (Figure 3) with the addition of NaOH:urea. The highest concentration of starch and the lowest concentration of alkali (20% -0.5:1) led to chain aggregation. As a result, a fraction with MW of 4.6×10^8 Da was observed eluted at the 6.1 min, associated with the presence of fragments from amylopectin hydrolysis. This behavior can be explained considering the restrictions of the solvent diffusion caused by the high viscosity of the system. In addition, low molecular weight fractions resulting from hydrolysis were detected (Table 2). To complete the analysis, the system presenting conditions opposite to those previously described, that is, the lowest concentration of starch and the highest concentration of alkali (10% -2:1), where the hydrolyzing effect of alkali on amylose and short chains corresponding to the branches of amylopectin was observed. The adhesive formulations presented fractions with higher retention times, with a molecular weight of 5.4×10^4 Da corresponding to short chains of about 300 glucose units and the glucose elution at 197 Da.

Table 2. Molecular weight determination by SEC-HPLC of starch polymer fractions: Percent contribution of the peaks and molecular weights.

Samples	Retention time (min)	% Contribution	Molecular weight (Da)
SN	5.83	26.86	9.9×10^8
	6.9	28.38	6.6×10^7
	8.5	44.41	1.1×10^6
10% -2:1	5.9	9.88	8.3×10^8
	9.7	44.80	5.1×10^4
	11.9	5.54	197
	12.9	36.15	-*
20% -0.5:1	6.1	14.64	4.6×10^8
	9.5	30.88	7.8×10^4
	12.7	40.29	-*
	13.7	11.29	-*
15% -0.5:1	6.15	9.89	4.4×10^8
	9.7	37.26	4.9×10^4
	12.2	4.30	-*
	12.7	45.07	-*
15% -1:1	7.2	10.67	3.3×10^7
	9.9	36.91	2.7×10^4
	12.7	26	-*
	13.6	18.91	-*
15% -2:1	6.2	6.7	3.6×10^8
	10.2	62.49	1.3×10^4
	12.7	22.30	-*
	13.8	5.55	-*
15% -1:1 7d	6.1	38.13	4.4×10^8
	9.8	29.08	3.6×10^4
	12.7	19.09	-*
	13.7	10.88	-*

15% -1:1 WF	6.2	13.98	3.9×10^8
	9.9	41.32	3.3×10^4
	12.7	29.17	-*
	13.7	10.94	-*

*Although the peaks on the chromatogram are resolved and discriminable, they are outside the reported gel fractionation range for the used column.

Figure 3b shows the molecular weight of formulations containing 15% w/w of starch treated with the different NaOH:urea ratios. At low NaOH concentration, three peaks were observed at times of 6.1; 9.7 and 12.7 min, which experienced a shift at higher times compared to the addition of a higher concentration of alkali. This fact reveals the hydrolyzing effect of the alkali-based solvent. In addition, an increase in the contribution of the peak at 9.7 min was observed, which may be related to the aggregation of short chains induced by the hydrolysis.

After 7 days of preparation (Figure 3c), the starch retrogradation phenomenon occurs, observing that the peak intensities at a lower volume ($\sim t_r = 6$ min), from which an aggregation of hydrolyzed chains corresponds to a molecular weight of 4.4×10^8 Da. Similarly, although the addition of urea has been reported to affect retrogradation by minimizing chains association [14], the presence of short chains and the storage conditions would favor this process.

During the freezing, the formation and growth of ice crystals causes structural damage that is associated with an increase in the proportion of short chains that are eluted at longer times. Wang et al. [14] pointed out that during storage in a cold environment or when subjected to freeze/thaw cycles, the association between the chains increases to form a gel, thus losing its flow properties.

3.1.3 ATR-FTIR spectral and chemometric analysis

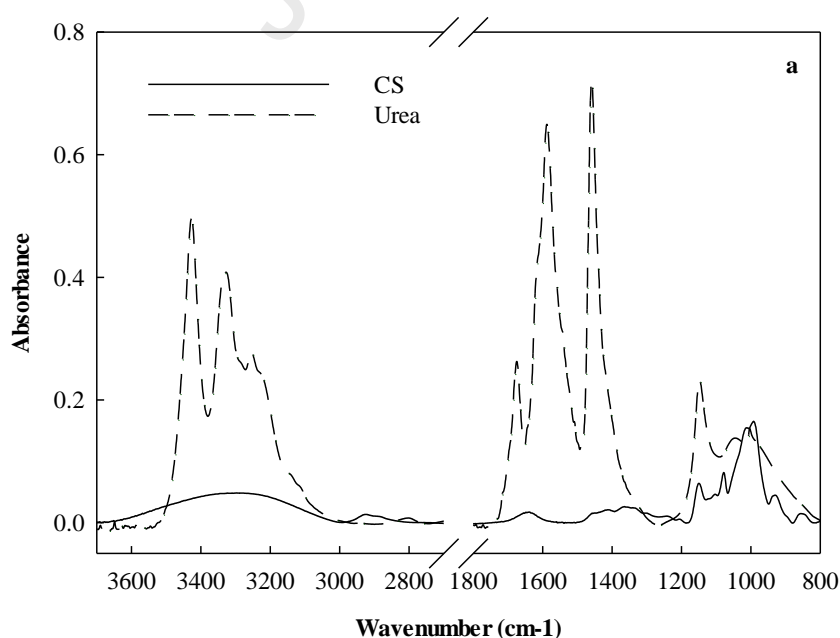
ATR-FTIR spectra for pristine components are shown in Figure 4a. Concerning urea ATR-FTIR spectrum, the characteristic peaks can be recognized as two main spectral regions: $3700\text{-}3000\text{ cm}^{-1}$ and $1700\text{-}1300\text{ cm}^{-1}$ [23]. The absorption peaks at about 1623 cm^{-1} and 3459 cm^{-1} were attributed to the bending vibration and stretching vibration of NH_2 group of urea, while the absorption at 3400 cm^{-1} were assigned to OH stretching vibration of starch. The peak of OH stretching vibration gradually became

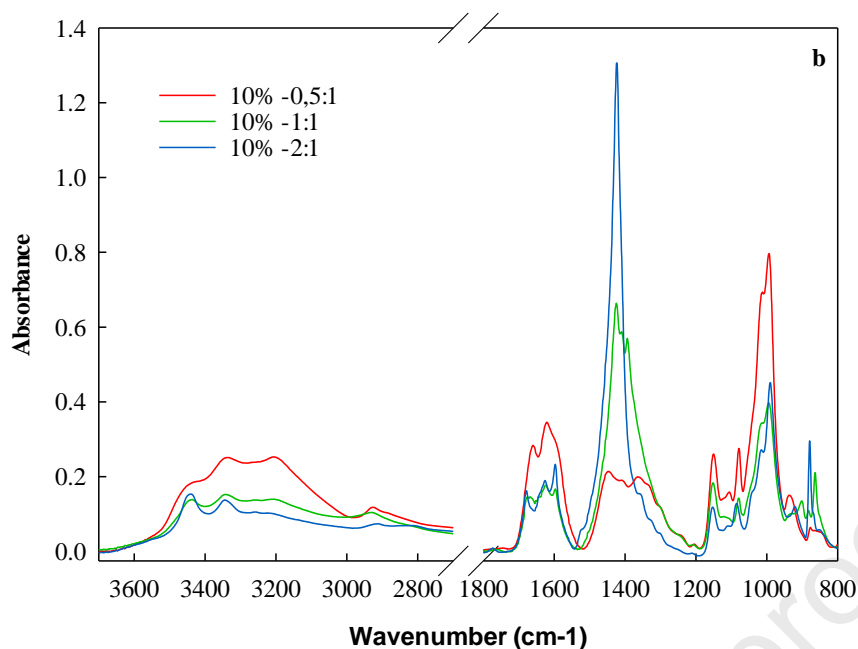
less distinct and overlapped with that of the -NH_2 group stretching vibration. It demonstrated that urea molecules were physically absorbed on starch surfaces through hydrogen bonding.

On the other hand, the spectrum of native cassava starch showed a broad band between $3700\text{-}3000\text{ cm}^{-1}$ corresponding to the stretching vibrations of the hydroxyl groups, another peak at 1650 cm^{-1} associated with the bending of the OH bond in water molecules with moderately strong hydrogen bonds and the characteristic bands of COC stretching vibrations and COH bending vibrations in pyranose glycosidic rings at $1200\text{-}900\text{ cm}^{-1}$ [24] (Figure 4).

The peaks located near 2925 cm^{-1} and 2881 cm^{-1} correspond to symmetric and asymmetric stretching vibration peaks of -CH_2 and -CH , respectively [15]. Formulations containing 10% of starch showed that with increasing NaOH concentration increased, the peaks at 3435 cm^{-1} and 3340 cm^{-1} were intensified due to the hydrolysis of the starch chains and consequently to the greater exposure of the -OH groups of the polymer chains. With increasing NaOH content, both the bending and stretching vibration peaks of the -NH_2 group shifted to lower wavenumbers. These results indicated the interaction between the -NH_2 groups of urea and the -OH groups of starch (Figure 4).

Figure 4. Spectral region $3600\text{-}800\text{ cm}^{-1}$ from the ATR-FTIR analysis of **a)** cassava starch (CS) and urea, **b)** the adhesives with 10% w/w of cassava starch at the different NaOH:urea ratios used.





The peak at 1022 cm^{-1} is associated with the amorphous region of starch and the absorbance at 1045 cm^{-1} is relative to the crystalline region of starch. The ratio of absorbances $1045/1022$ is associated with the ordered degree of starch and the proportion of amorphous to ordered in the polymer. The ratio $1022/995$ reflects the ratio of amorphous to ordered in the structure of starch [25].

The formulations prepared with 10% w/w of starch registered a higher ratio $1022/995$ as the concentration of sodium hydroxide increased, as a result of the hydrolysis of the starch components, thus increasing the ratio of the amorphous to ordered region in starch (Table inset in Figure 5b). This can be attributed to the fact that the shorter side chains of the starch were effectively hydrolyzed. However, when the NaOH:urea ratio was 2:1, a significant decrease in this ratio was observed, which could be associated with excessive hydrolysis. These results were consistent with the changes observed in the region ascribed to the stretching vibrations of the hydroxyl groups between adjacent chains from starch hydrolysis. These findings suggested that the ordered structure of starch changed because of hydrolysis, indicating that the treatment has a significant effect on starch disorganization and subsequent molecular rearrangement [25]. Consequently, starch retrogradation was promoted, which was also reflected by the increase in the value of the $1045/1022$ ratio, which is related to the reorganization of the crystalline structure by rearrangement of short chains (Table inset in Figure 5a).

Similar behaviors were observed for formulations prepared with 15 and 20% of starch. On the other hand, for samples formulated with NaOH:urea 2:1, a significant decrease in the 1045/1022 ratio was observed with increasing starch concentration in the formulation.

Yang & Hui [15] reported that the NaOH concentration affects the absorbance ratio 1045/1022. At low NaOH concentration, molecular chain rearrangements are mainly promoted, reflected in an increase in the 1045/1022 ratio. On the contrary, at NaOH in excess, the value of the ratio 1045/1022 registers a decrease (Table inset in Figure 5a). The authors also suggest that the rearrangement of polymer chains played a secondary role and NaOH destroyed the ordering of starch constituents by alkaline hydrolysis, oxidation, and crosslinking.

From a chemical point of view, the developed adhesive formulations are highly complex systems. Therefore, their analysis requires the use of statistical methods for interpretation (chemometric analysis). PCA transforms spectral data, using orthogonal transformation, into variables with no linear correlation, called Principal Components (PCs). This transformation is defined so that the first PC has the largest possible variance. Each subsequent component has the largest possible variance, subject to the constraint that it is orthogonal to the preceding components. This has the advantage of eliminating multicollinearity in the use of the PCA results in a dependency analysis [26]. In this way, PCA allowed the grouping of samples according to their spectral similarities.

PCA in the fingerprint region $1200-900\text{cm}^{-1}$ was performed to establish a relationship between the studied factors (starch concentration, NaOH:urea ratio and storage). Figure 5 shows the two-dimensional plots (Bi-plots) used to visualize and group the relationships between the samples, through variance.

Figure 5. Spectral region $1200-900\text{ cm}^{-1}$ and biplots obtained from the ATR-FTIR analysis of the adhesives. Effect of: **a)** NaOH:urea ratio at 15% w/w of starch, **b)** starch concentration at 1:1 NaOH:urea ratio and **c)** storage conditions for samples prepared with 15%-1:1 sample (nomenclature used, NS: native starch, 7d: samples stored 7 days).

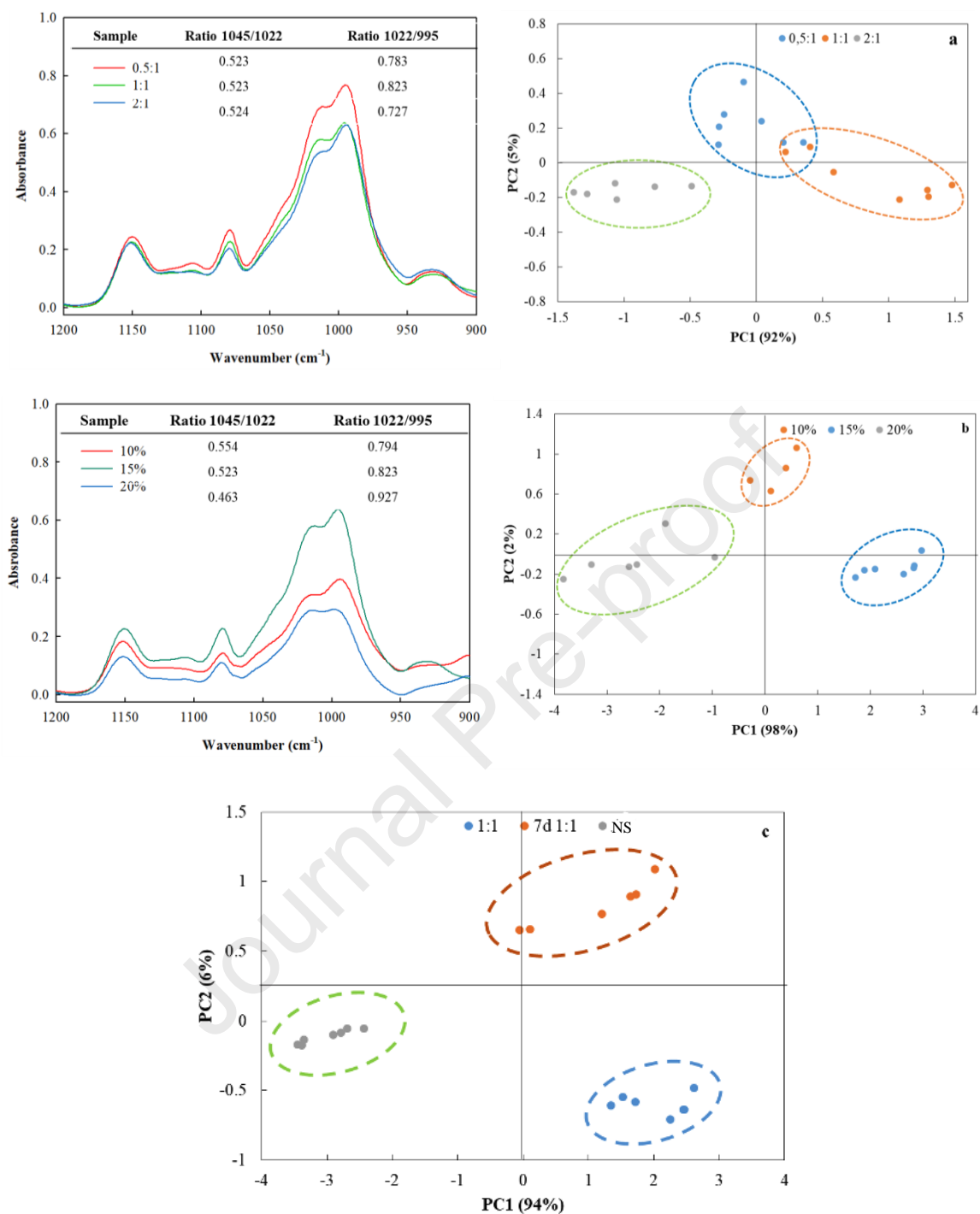


Figure 5a shows the difference between the different groups of adhesives, more precisely between the clusters belonging to the formulations with different NaOH:urea ratios at a fixed starch concentration (15% w/w). When comparing the ATR-FTIR absorption spectra of the adhesives in the spectral region between 1200-900 cm^{-1} , the separation into three groups was observed, confirming the structural modifications induced by the alkali:urea mixtures. The PCA analysis allowed explaining the total

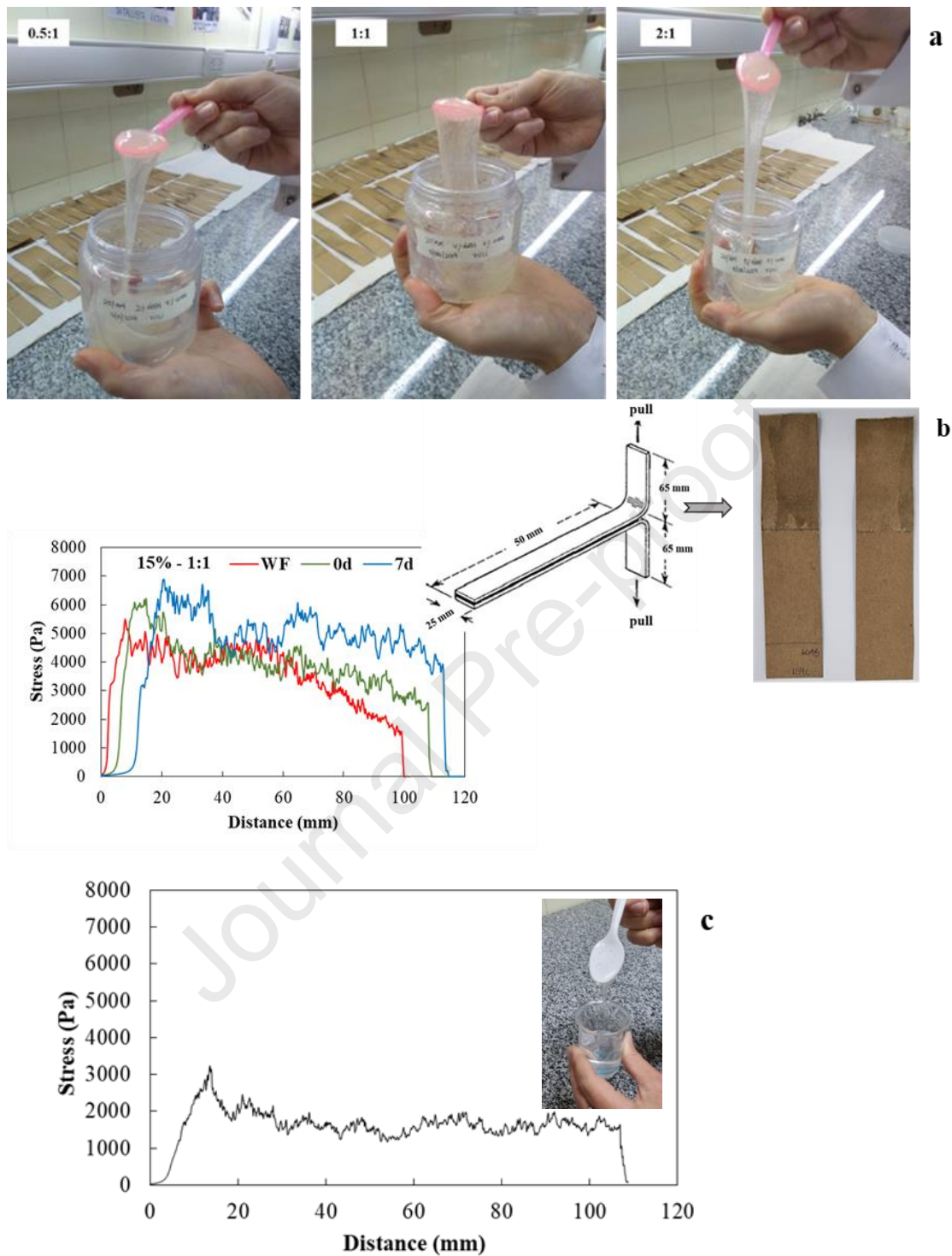
variance through two components, PC1 (92%) and PC2 (6%). Bearing in mind the complexity of the system, the assignment of the main component (PC1) is not simple. It could be attributed to the interrelationship of the variables studied (starch concentration and alkali:urea ratio). The dispersibility of the data would be given by the system. A separation between the formulations was observed along the axis of PC1. However, all the samples prepared with 15% starch and a 2:1 ratio were located to the left of the PC1 axis. In contrast, the formulations with a 1:1 NaOH:urea ratio were located to the right of the PC1 axis. On the other hand, formulations prepared with a 0.5:1 ratio were located on both sides of the axis. The high percentage of variance explained by PC1 (92%) indicated the accuracy of discriminating the ATR-FTIR spectra of the different adhesives.

The PCA chemometric analysis technique confirmed the interactions established between the components of the systems and the structural changes induced by the treatment due to the separation of the samples into clusters. These findings corroborated the results obtained by SEC-HPLC and the particle size analysis.

3.2 Applicability of formulations as adhesives

The visual appearance of starch-based adhesives is shown in Figure 6, which illustrates the effect of formulation on adhesive viscosity. As expected, as the starch concentration increased, the adhesive became more consistent, making it more difficult to prepare. In the case of starch-based systems, 20% was the maximum concentration that was plausible to prepare operationally. In this sense, Momenpoor et al. [16] explained that higher concentrations of starch lead to the formation of a highly viscous solution that causes a greater restriction to the diffusion of the solvent. Likewise, Table 3 shows the parameters obtained from the back extrusion test for the formulation based on 15% fresh cassava starch and after 7 days of processing, stored at room temperature.

Figure 6. a) Photographs of starch-based adhesives with different NaOH:urea ratios. Mechanical adhesion test profiles of: b) Kraft paper samples bonded with adhesive formulations prepared with 15% starch and 1:1 NaOH:urea ratio evaluated under different conditions. Nomenclature used 0d: fresh samples, 7d: samples stored for 7 days and WF: adhesive without freezing, c) Kraft paper samples bonded with commercial adhesive.



The NaOH:urea ratio affected the consistency parameters of the freshly prepared starch-based adhesive. Thus, the firmness and consistency of the adhesive varied with the alkali concentration, a significant increase ($p < 0.05$) was recorded for the 1:1 ratio while it decreased for the NaOH:urea 2:1 ratio, again

suggesting that the hydrolysis of the polymer chains of the starch components occurred. Similar trends were observed for cohesiveness and viscosity index (Table 3).

Furthermore, the more fluidized adhesives were those formulated with the highest concentration of NaOH (2:1 NaOH:urea ratio). Similar results were reported by Manjaree et al. [27] who explained that a simultaneous increase in the degree of hydrolysis was observed with the concentration of alkali. In this sense, cassava starch as a base for the preparation of adhesive formulations is characterized by functional properties, such as the high paste viscosity, the paste clarity, and the freeze-thaw stability, which are advantageous to the adhesive industry. A similar explanation has been proposed by Olomo [28].

Table 3. Parameters of the back extrusion curve for starch-based adhesive formulations at 15%, fresh (0d) and storage (7d).

Samples	Firmness (g)	Consistency (g s)	Cohesiveness (g)	Viscosity index (lg s)	
Commercial adhesive	48.5 ± 0.3	422.5 ± 6.7	27.7 ± 0.5	294.3 ± 1.3	
0d	0.5:1	98.9 ± 1.9 ^b	805.4 ± 11.2 ^a	52.3 ± 0.01 ^a	543.4 ± 13.3 ^a
	1:1	142.7 ± 4.4 ^c	1041.9 ± 12.2 ^b	54.1 ± 3.8 ^a	588.7 ± 31.1 ^b
	2:1	88.9 ± 5.7 ^a	756.4 ± 45.4 ^a	52.3 ± 3.9 ^a	527.7 ± 28.1 ^a
7d	0.5:1	0.261 ± 0.005 ^b	2.29 ± 0.06 ^b	0.158 ± 0.001 ^b	1.27 ± 0.01 ^b
	1:1	0.227 ± 0.004 ^a	2.11 ± 0.01 ^a	0.146 ± 0.001 ^a	1.07 ± 0.01 ^a
	2:1	0.258 ± 0.005 ^b	2.32 ± 0.08 ^b	0.166 ± 0.004 ^c	1.29 ± 0.06 ^b

*Mean values ± standard deviations are presented. Different letters in the same column indicate significant differences ($p < 0.05$). 0d: fresh samples, 7d: samples stored 7 days.

On the other hand, there was a significant decrease ($p < 0.05$) in the parameters when evaluating the effect of storage time. It can be inferred that the alkaline solution continued its hydrolyzing action. These results correlate with the visual observation of the starch adhesives (Figure 6) and the intrinsic viscosity measurements, where both the effect of the NaOH:urea ratio and the storage time were observed in the consistency of the adhesive formulations. When the visual appearance of the adhesives

was examined as a function of time, a greater clarity of the formulations and a decrease in their consistency during storage was observed (Table 3).

All the formulations tested exhibited adhesive capacity and were effective in bonding Kraft paper substrates (Figure 6). Taking into account that the effectiveness of the adhesive bond depends on several factors, such as intermolecular forces of the adhesive, wettability of the adhesive in the adherent, types of chemical bonds and functional groups [12], mechanical tests were performed. For this purpose, the bond strength of a commercial adhesive (Figure 6c) was recorded to compare it with the adhesive formulations obtained by chemical modification.

The effect of the freeze-thaw cycle and the stability of the adhesive formulation were also studied. The adhesive obtained without the freezing step exhibited a stress value of 2766 ± 254 Pa, while the formulation prepared with the freeze-thaw cycle registered a significant increase ($p < 0.05$), reaching an average of 3724 ± 498 Pa. In the light of the results, it could be inferred that the freeze-thaw cycle constitutes a strategy that improves the adhesive properties of the formulations offering greater versatility to them. This adhesive showed a significant increase of 66% in the peel strength value compared to commercial formulation (Figure 6).

Also, the adhesive formulation without the freezing step maintained a slightly constant peel strength value throughout the test. This could be explained by a 50% cohesive and 50% adhesive failure, respectively, breaking of the bond with the substrate and fracture of the adhesive, respectively. While the formulation with the freeze-thaw cycle presented a characteristic profile of a 100% cohesive failure, a profile characterized by a maximum effort to initiate delamination (Figure 6).

Although the adhesive systems became fluidized during the storage (Table 3), they exhibited good adhesion and consequently maintained their mechanical performance (Figure 6).

4. Conclusions

The treatment of cassava starch with NaOH:urea mixtures caused structural disorganization at room temperature and induced cold gelatinization of the starch, resulting in a sustainable process from an economic point of view.

Through the proposed alkaline treatment, it was possible to formulate adhesives with high starch content, which results impossible when thermal gelatinization of starch is carried out in the absence of chemical agents. This is a promising strategy considering its scalability and the economic viability of the process.

The particle size of the adhesive formulations varied with both the starch concentration and the NaOH:urea solution ratio. Formulations with NaOH:urea 1:1, especially those containing 15% starch allowed the production of adhesive solutions with the smallest particle sizes. Thus, depending on the adhesive starch content of the adhesive, the optimal NaOH:urea ratio arises from the compromise between the condition that ensures the complete hydrolysis of the starch and the one that prevents the formation of aggregates from the structures generated. In this sense, SEC-HPLC, and chemometric studies based on ATR-FTIR spectral analysis highlighted that the modifications depend on both the starch content and the NaOH:urea ratio used in the adhesive mixtures.

The developed formulations exhibited adequate adhesive properties for application to a cellulosic substrate. The NaOH:urea ratio influenced the consistency parameters of the starch-based adhesives, the formulations showed good bonding capacity and consequently maintained their mechanical performance.

Despite the hydrolyzing effect of the NaOH:urea mixtures was evidenced during storage, the adhesive capacity to the Kraft paper substrate was maintained. Thus, the chemical treatment was a simple and low-cost procedure to chemically modify cassava starch to obtain derivatives with tailor-made adhesive properties.

Acknowledgements

Authors acknowledge the financial support of Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT, Projects: PICT 2019-2827 and PICT 2019-03380, CONICET and University of La Plata (UNLP). Authors wish to thank Dra. María Fernanda Hamet (CIDCA).

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Figure 1. Measured parameters for particle size distribution of starch-based adhesive formulations using different starch concentrations (10-20%) and NaOH:urea ratios (0.5:1- 2:1): De Brouckere mean diameter (D_{4,3}) (a-c), Dv10 percentile (d-f) and Dv90 percentile (g-i). Mean values \pm standard deviation are presented. Different letters within in the same parameter indicate significant differences ($p < 0.05$). Fresh samples (black bars) and samples stored for 7 days (grey bars).

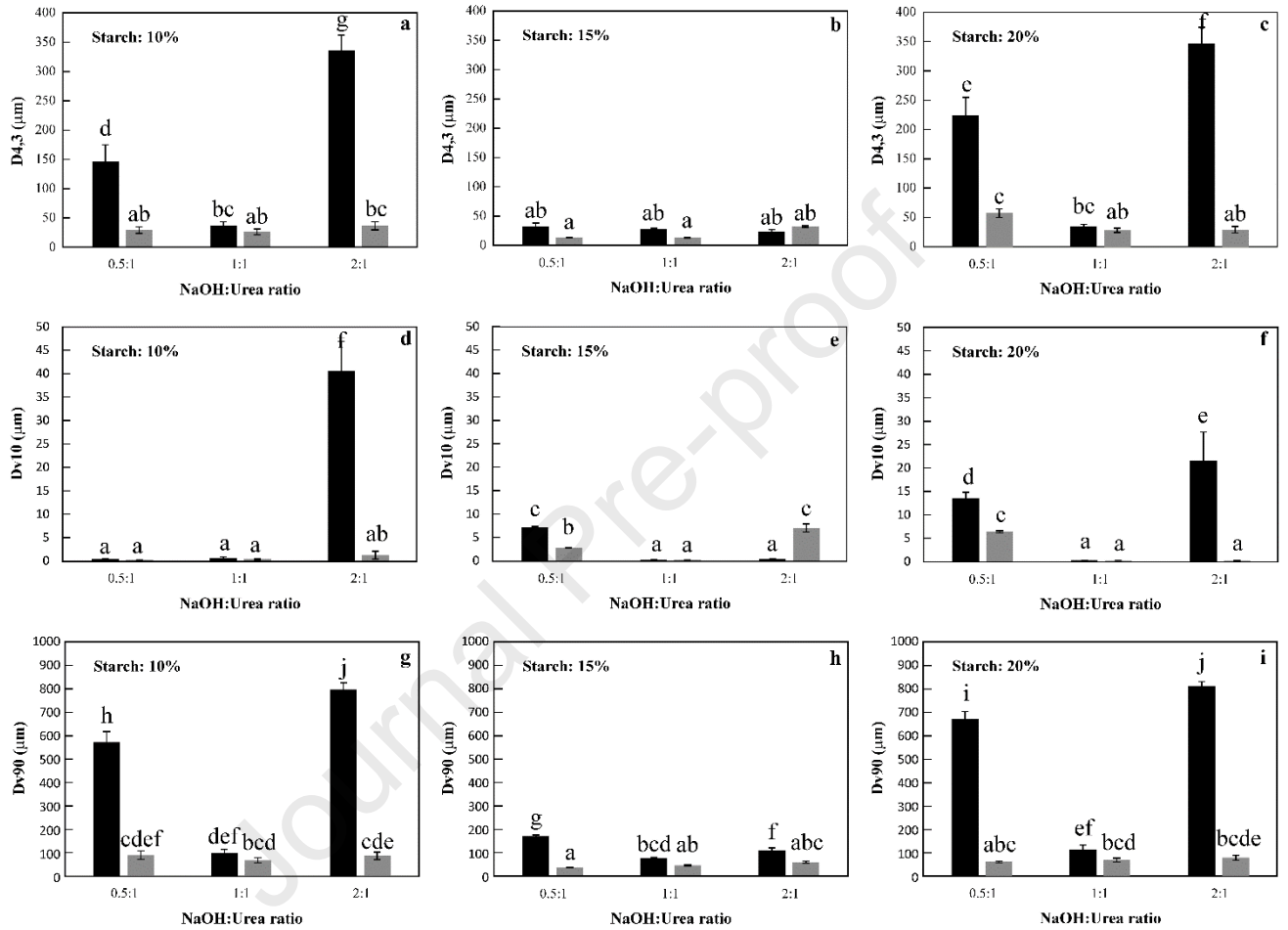


Figure 2. Particle size distribution, expressed as volume showing the influence of: **a)** different NaOH:urea ratios on cassava starch adhesives at 15% w/w; **b)** influence starch concentration (% w/w) at NaOH:urea 1:1 ratio; and **c)** the storage in starch-based adhesive formulations prepared at 15% w/w. Solid lines represent samples of fresh adhesives (0d), and dotted/dashed lines represent samples stored for 7 days at room temperature (7d).

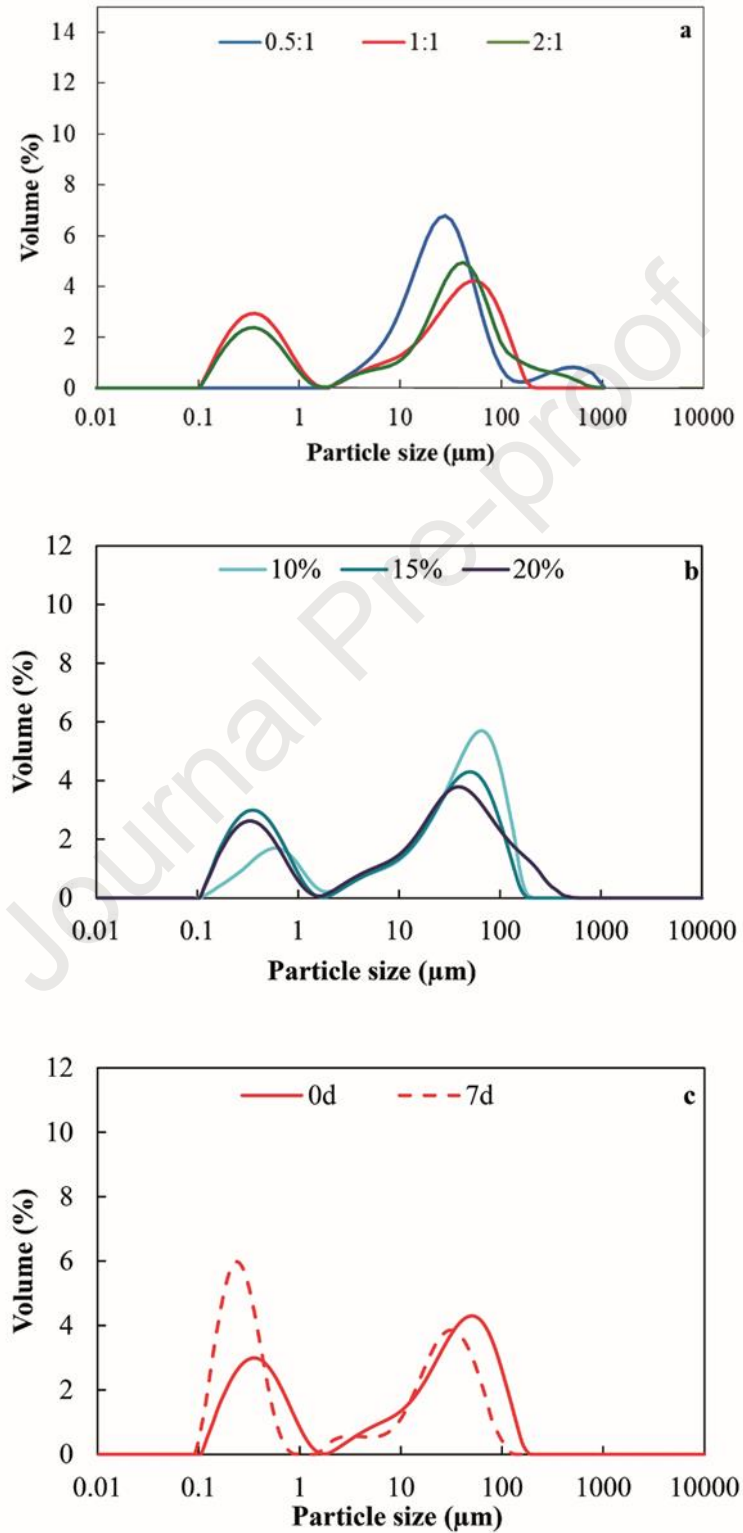
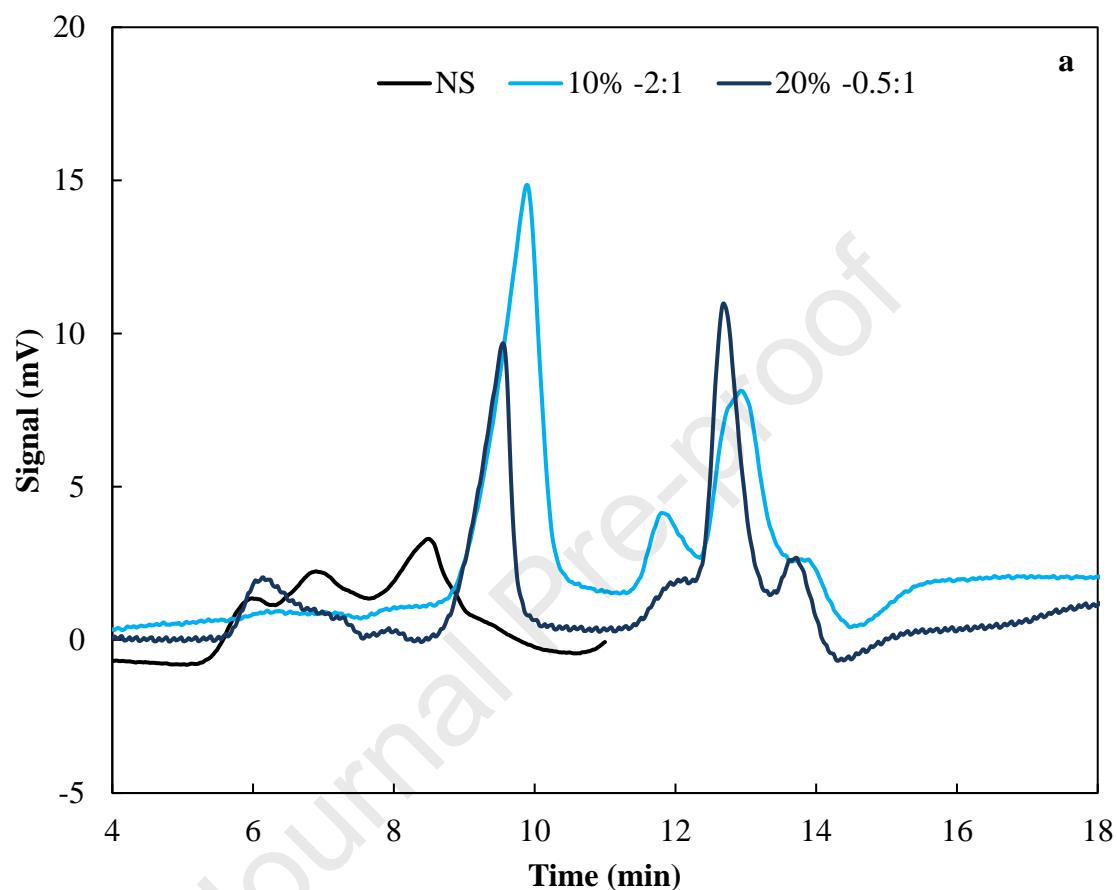


Figure 3. Chromatograms obtained by SEC-HPLC of: **a)** native starch (NS), starch-based adhesive formulations at 10% w/w and a ratio of 2:1 and 20% w/w and at a ratio of 0.5:1, **b)** effect of the NaOH:urea ratio on starch-based adhesive formulations at 15% w/w and **c)** effect of freezing and storage time (nomenclature used 0d: fresh samples, 7d: samples stored 7 days and WF: adhesive without freezing).



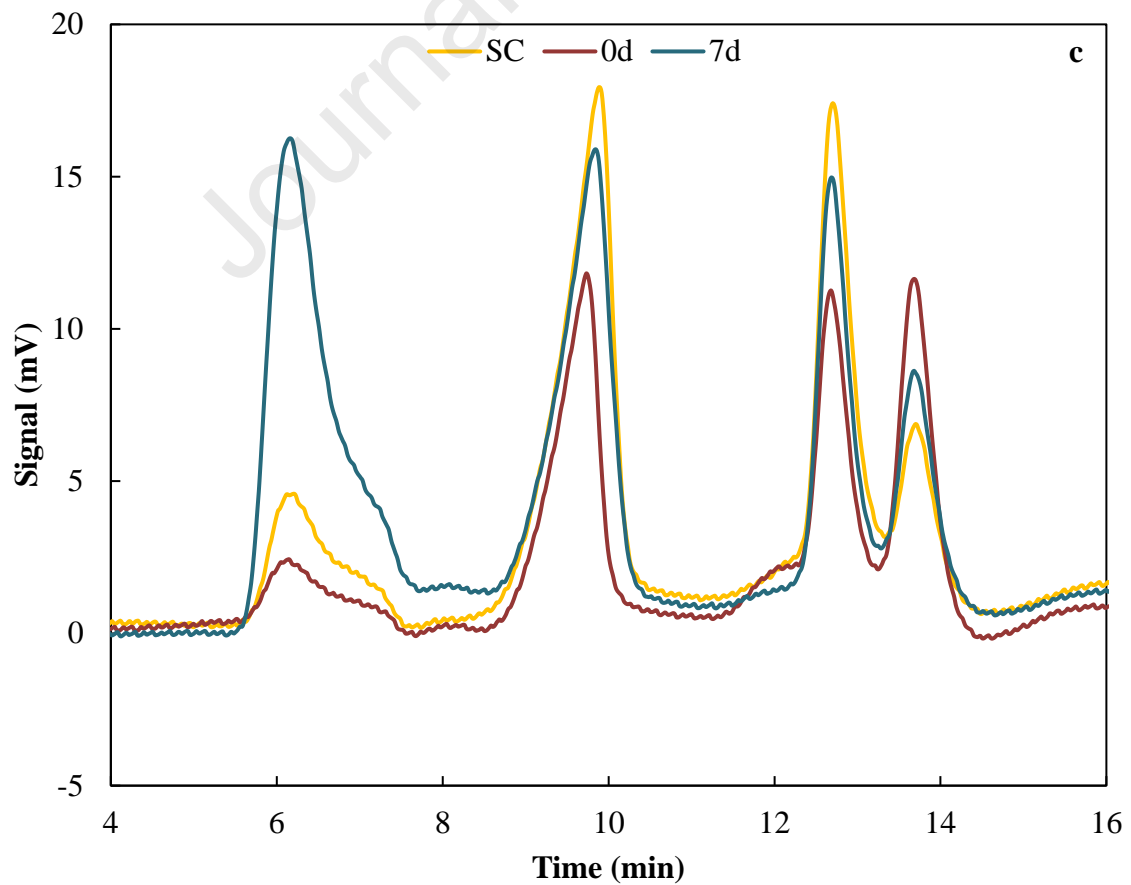
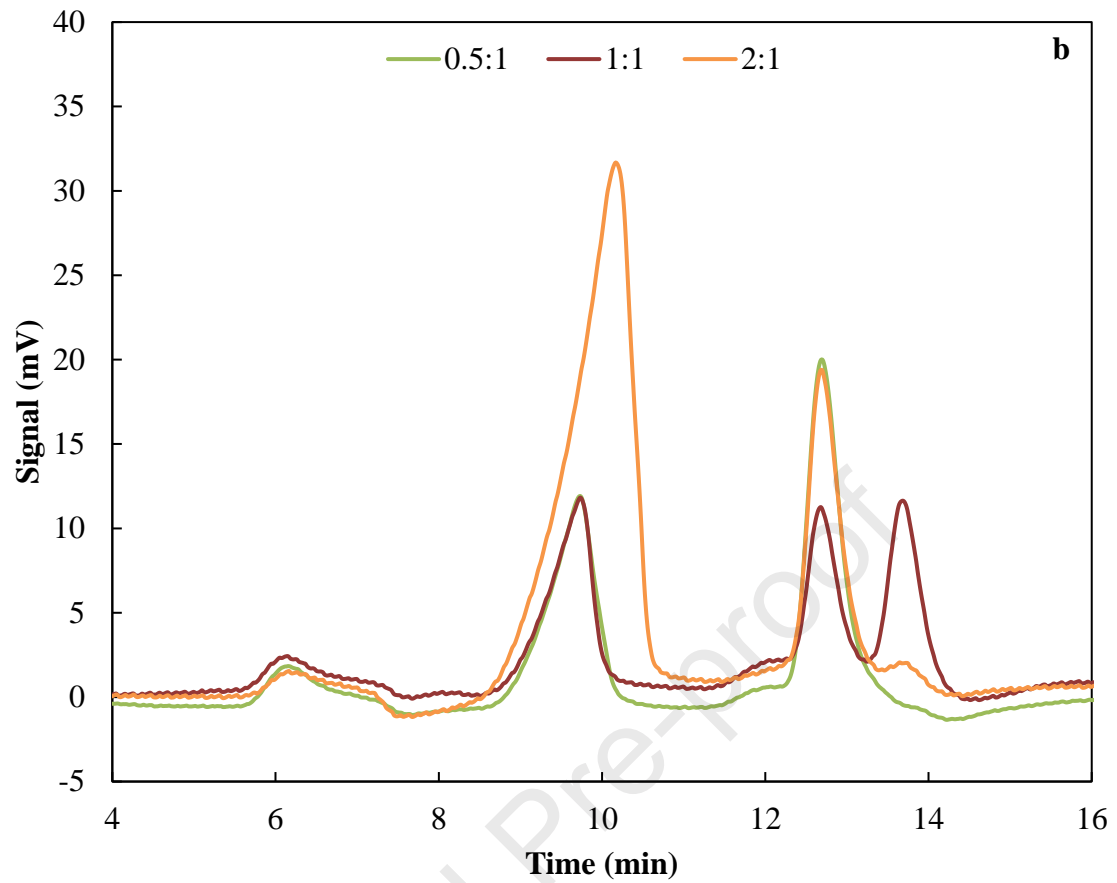


Figure 4. Spectral region 3600-800 cm^{-1} from the ATR-FTIR analysis of **a)** cassava starch (CS) and urea, **b)** the adhesives with 10% w/w of cassava starch in the different NaOH:urea ratios.

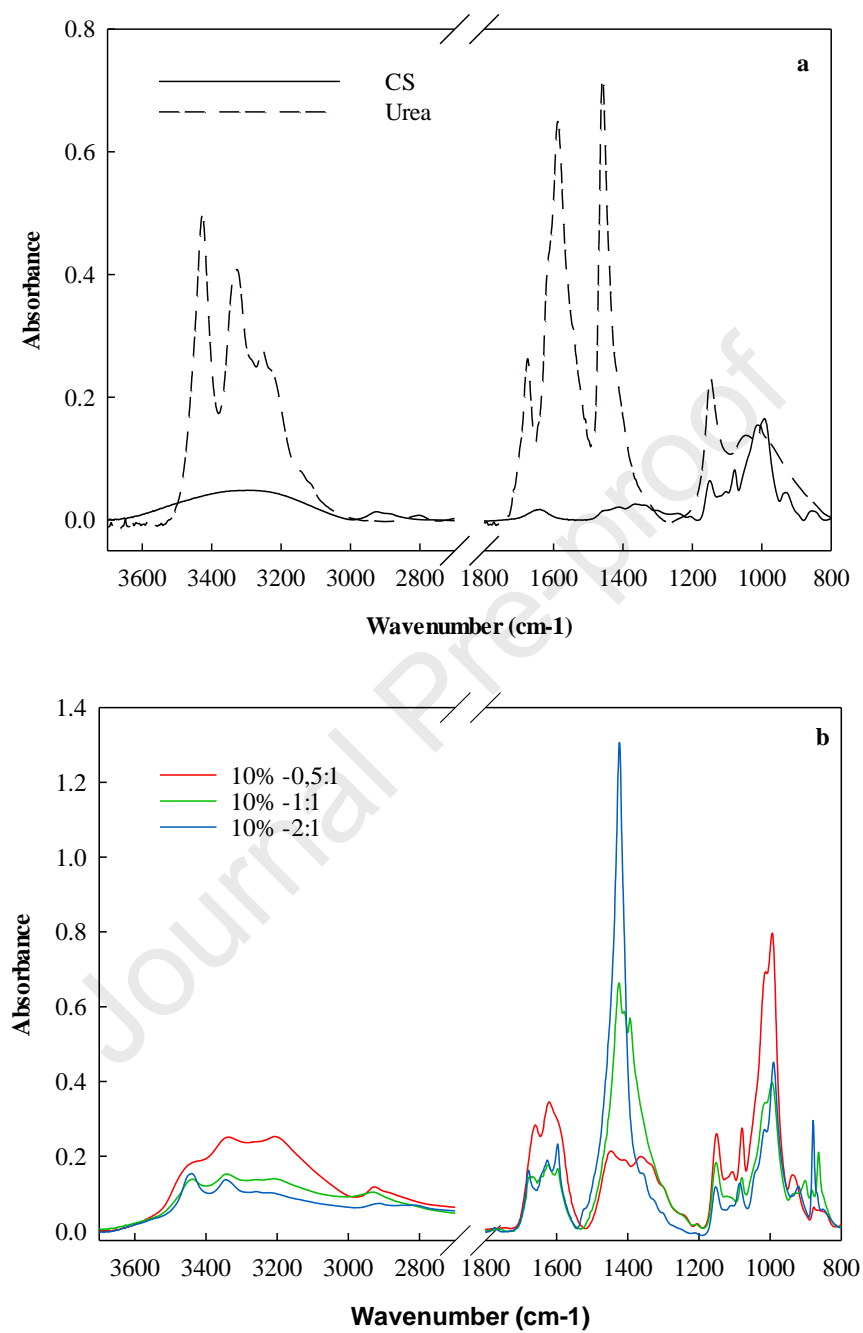


Figure 5. Spectral region 1200-900 cm^{-1} and biplots obtained from the ATR-FTIR analysis of the adhesives. Effect of: **a)** NaOH:urea ratio at 15% w/w of starch, **b)** starch concentration at 1:1 NaOH:urea ratio and **c)** storage conditions for samples prepared with 15%-1:1 sample. (nomenclature used, NS: native starch, 7d: samples stored 7 days).

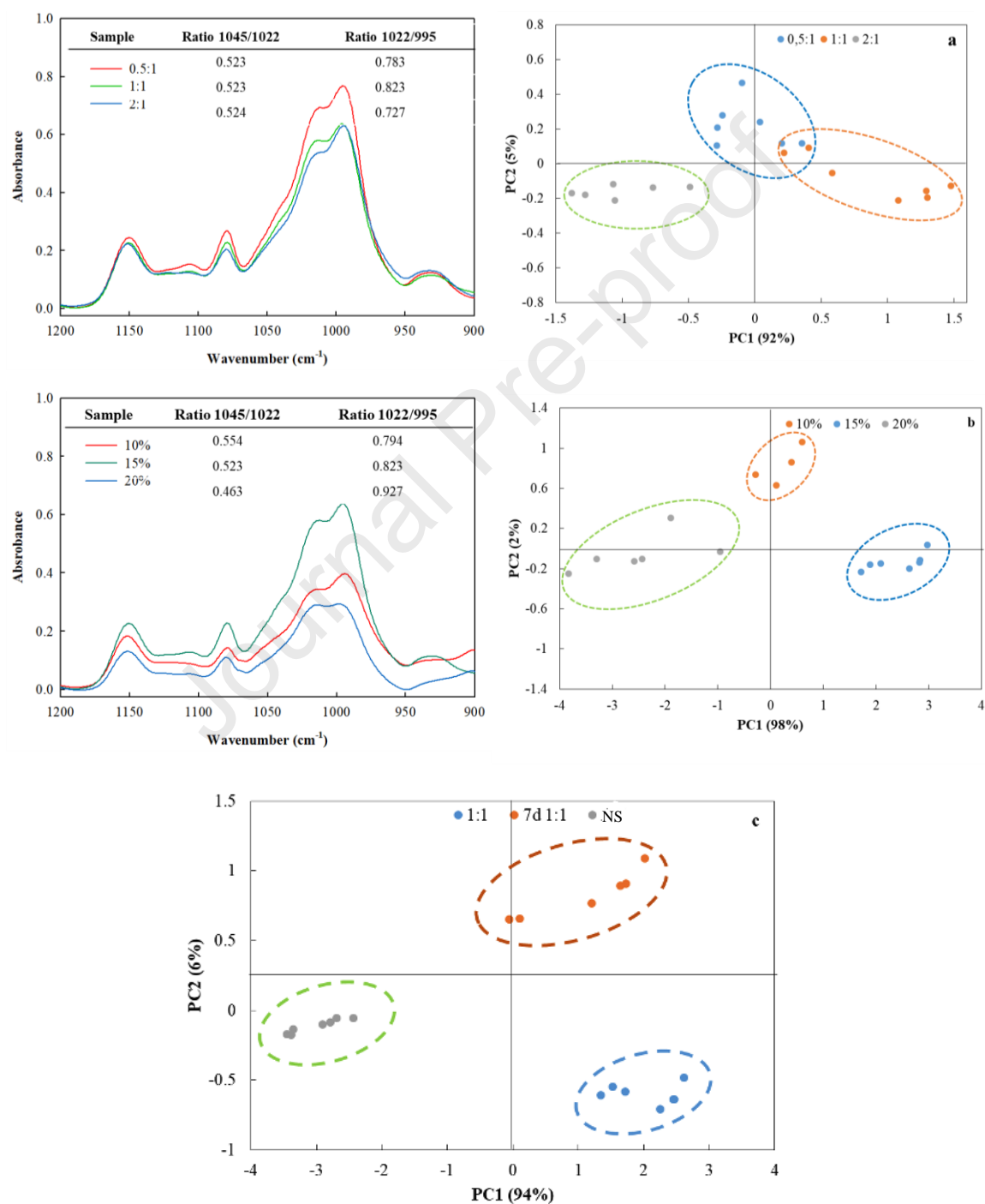
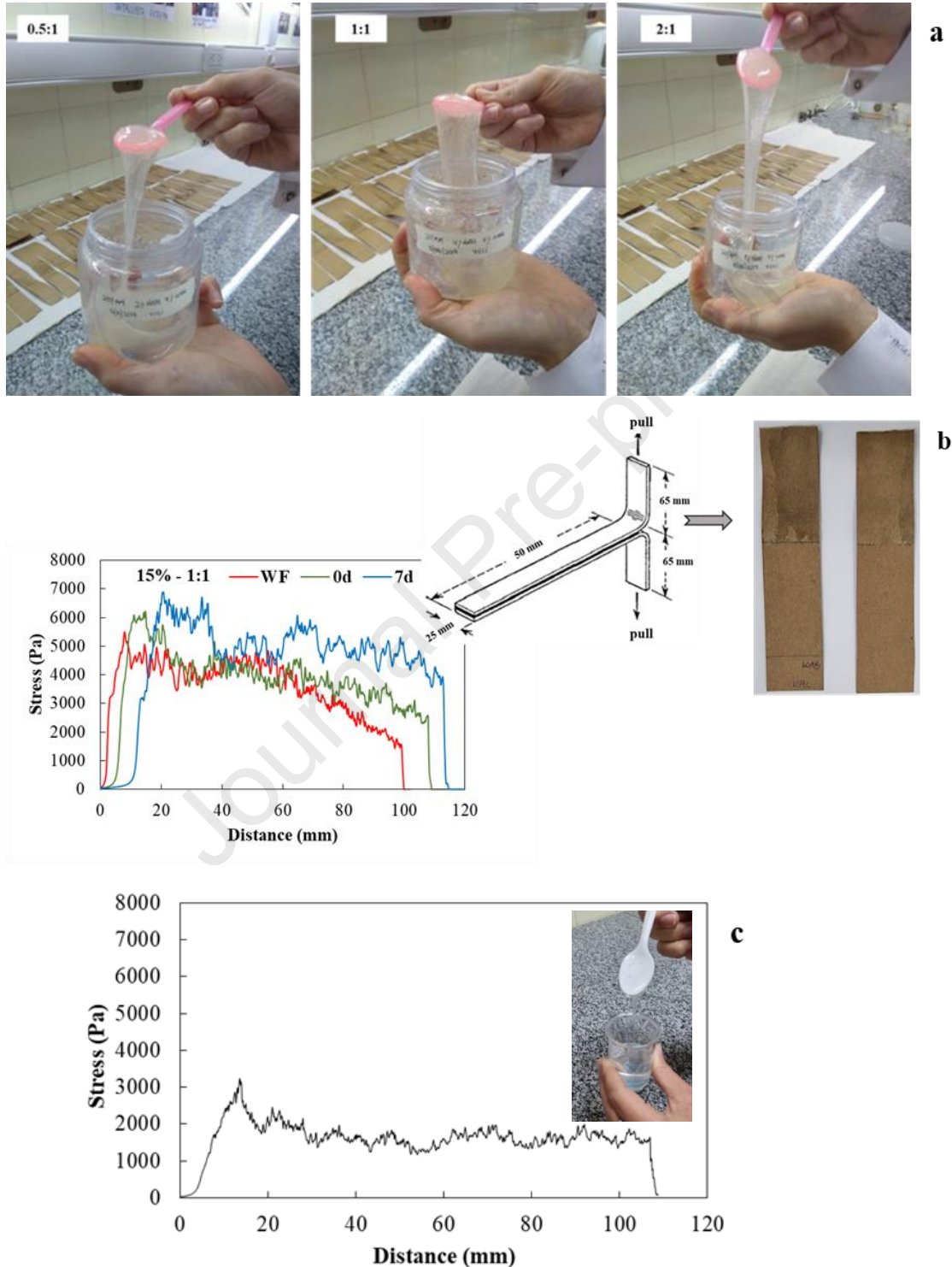


Figure 6. a) Photographs of starch-based adhesives with different NaOH:urea ratios. Mechanical adhesion test profiles of: b) Mechanical adhesion test profiles of Kraft paper samples bonded with adhesive formulations prepared with 15% w/w starch and 1:1 NaOH:urea ratio evaluated under different conditions. Nomenclature used 0d: fresh samples, 7d: samples stored for 7 days and WF: adhesive without freezing, c) Kraft paper samples bonded with commercial adhesive.



Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Sandra Rivero reports financial support was provided by ANPCyT. Sandra Rivero reports a relationship with ANPCyT that includes: funding grants.

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