Chitosan-genipin paper coating – an alternative approach for active packaging materials

Final Report

Action number: FP1405 - ActInPak

Start date: 5th March

End date: 8th April

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Summary

The use of genipin as a crosslinking agent for chitosan-based films revealed to be a suitable approach to improve not only the mechanical performance and acidic stability of these materials, but also to potentiate their active profile, namely their oxygen scavenging properties.

During this STSM it was studied the ability of using, for the first time, chitosan-genipin formulations on paper coating processes.

Experimental procedures and Results

1st Week:

The first paper coating experiments were carried out using 1% wt. of chitosan prepared in 1% wt. of acetic acid solution in a blade coater machined equipped with an infrared section (Endupap, France) (**Figure 1**).

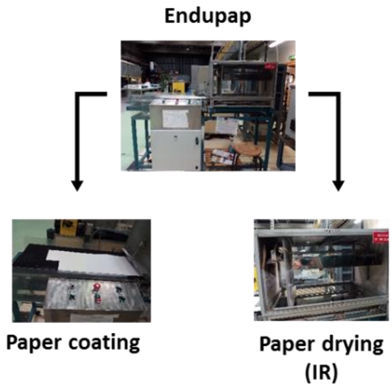

Figure 1: Blade coater equipped with an infrared section.

Prior to the paper coating, the chitosan solution viscosity was measured using a Brookfield viscometer (**Figure 2**). Comparing with the refence values (449 cP), the chitosan solution presented a lower viscosity profile (350 cP).

Figure 2: Brookfield viscometer.

Afterwards, a printing paper was used on the trial tests aiming understanding the suitability of using 1% wt. of chitosan on paper coating as well as to choose the most proper Mayer bar rugosity and coating speed. Four different Mayer bar rugosities (0; 0.2; 0.5; and, 0.7 mm) and three distinct coating speeds (1; 5; and 10 cm.s^{-1}) were tested. The most efficient and homogeneous coating was achieved using a 0.5 mm Mayer bar at 5 cm.s^{-1}.

2nd Week:

✓ Chitosan-genipin crosslinking

Chitosan-genipin paper coating was studied. Herein, 0.05% (w:v) of genipin pre-dissolved in ethanol was added to the 1% wt. of chitosan solution, as previously described for the chitosan-genipin films production.[1, 2] After 1 h of reaction time at RT, a small portion of chitosan-genipin was taken out and used on paper coating while the remaining chitosan-genipin solution was maintained under stirring for 24 h. In addition, aiming to increase the crosslinking degree, another small portion of the chitosan-genipin solution was exposed to 50 ºC for 2 h.

Figure 3 shows the viscosity behavior of each obtained solution. As expected, during the crosslinking processing time, chitosan changes from a yellowish to a greenish solution with concomitant increasing of the solution viscosity. After 24 h, at RT, a strong chitosan-genipin gel was formed. By increasing the temperature, the crosslinking reaction was speed it up and a gel was achieved sooner than the 24 h.

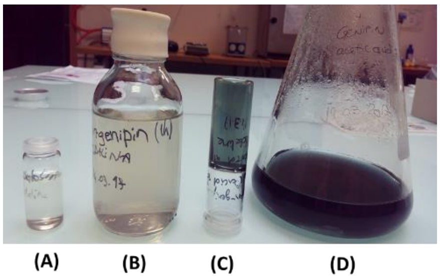

Figure 3: Evolution of the chitosan-genipin crosslinking: (**A**) Chitosan solution; (**B**) Chitosan-genipin solution after 1 h stirring at RT; (**C and D**) Jellified chitosan-genipin solutions after (**C**) 1 h stirring at RT followed by 2 h stirring at 50 ºC, and (**D**) 24 h stirring at RT.

As for paper coating it cannot be used a gel, different crosslinking times (30; 60; 90; and, 120 min) at 50 ºC were studied aiming understanding the maximum crosslinking degree that can be obtained in a viscous chitosan-genipin solution, avoiding the gel formation. **Figure 4** demonstrated that during the heating time, the solution viscosity strongly increased, yielding a gel after only 60 min of reaction. After 90 min, the chitosan-genipin gel became darker which corresponds to an increasing of the crosslinking degree. At this stage, once a strong gel was already formed which is not intended to the paper coating, the experiment was stopped.

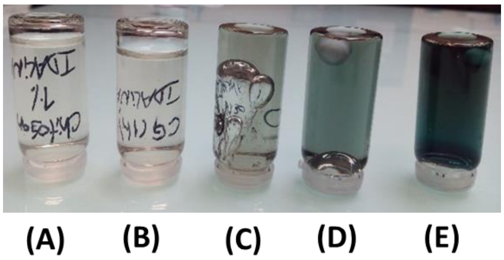

Figure 4: Evolution of the chitosan-genipin crosslinking: (**A**) Chitosan solution; (**B**) Chitosan-genipin solution after 1h stirring at RT; (**C**) Chitosan-genipin solution after 1h stirring at RT followed by 30 min stirring at 50 ºC; (**D**) Chitosan-genipin after 1h stirring at RT followed by 60 min stirring at 50 ºC; (**D**) Chitosan-genipin after 1h stirring at RT followed by 90 min stirring at 50 ºC.

3rd to 5th Week:

✓ Rheology of chitosan-genipin solutions

As a complementary methodology, some rheological experiments were performed using the modular compact MCR 302 rheometer (Anton Paar, France), presented on **Figure 5**, aiming to adjust the best experimental conditions for chitosan-genipin paper coating. Herein, the viscosity of both 1 wt.% of chitosan and 1 wt.% chitosan containing 0.05 wt.% of genipin solutions was measured at different shear rates at 20 ºC. Furthermore, chitosan-genipin solution was also submitted to the viscosity measurements at 50 ºC **(Figure 6)**.

Figure 5: Modular compact MCR 302 rheometer supplied by Anton Paar.

Figure 6A shows the characteristic shear thinning of 1% wt. of chitosan solution, i. e., a decreasing of viscosity the increasing of the shear rate. When genipin was immediately added into the chitosan solution, a decreasing of the chitosan solution viscosity was observed even when the temperature was raised up to 50 ºC for 5 min of reaction (**Figure 6A and Figure 6B**). However, by increasing the duration time of the experiment for 250 min, maintaining the application of various shear rates during measurement, it was possible to confirm the high viscosity increase of the chitosan-genipin solution at 50 ºC (**Figure 6C and Figure 6D**), which corroborates with the experimental results previously illustrated (**Figure 4**). On **Figure 6D** it is possible to observe a decreasing of the chitosan-genipin viscosity until approximately 30 min followed by an increasing that stops after around 90 min of reaction. Next, the viscosity values decreased due to the solvent evaporation and posterior film production.

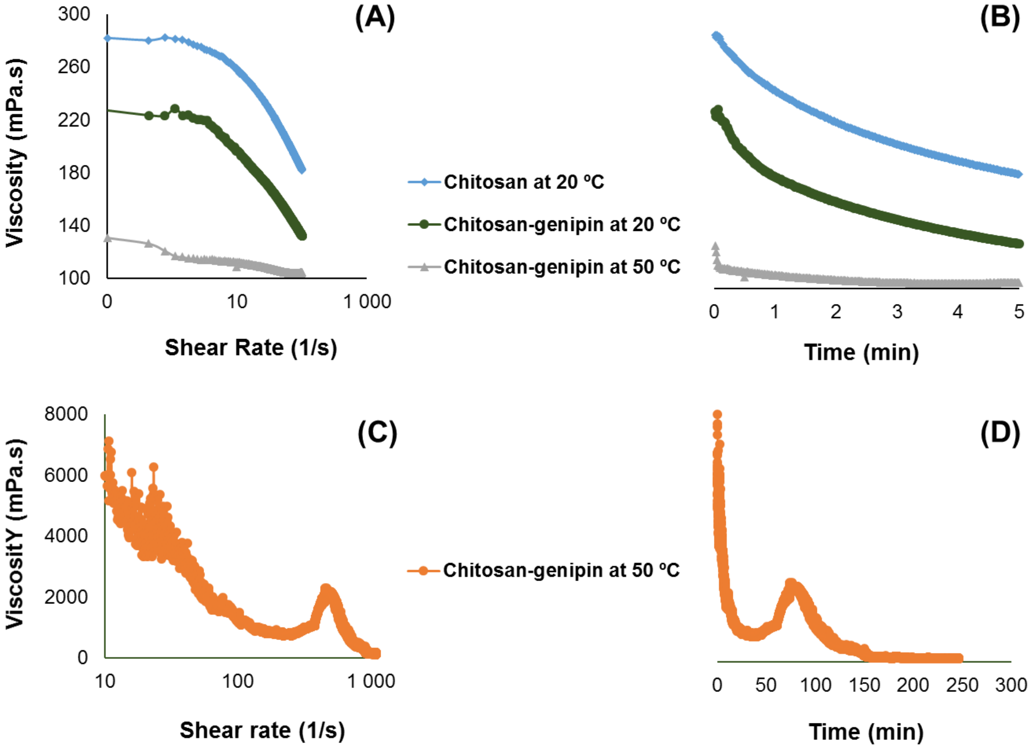

Figure 6: Viscosity profile of (**A and B**) chitosan solution at 20 ºC; chitosan-genipin solution at 20 ºC; and, chitosan-genipin solution at 50 ºC, obtained using different share rates for <u>5</u> min; and, (**C and D**) chitosan-genipin solution at 50 ºC, obtained using different share rates for <u>250</u> min.

Posteriorly, the viscosity measurements of chitosan-genipin crosslinking using fixed shear rates (10; 100; and, 1000 s^{-1}) and different temperatures (40; 50; and, 60 ºC) were tested. **Figure 7** shows that a well-defined viscosity profile was achieved by increasing the shear rate from 10 **(Figure 7A)** to 1000 s^{-1} **(Figure 7C)**. As expected, **Figure 7D** evidences that the chitosan-genipin solution viscosity is directly related to the processing temperature. As higher is the temperature applied, higher will be solution viscosity and, therefore, high crosslinking degree can be achieved. This measurement corroborates with the experimental observation previously cited **(Figure 4)**, i. e., the use of 50 ºC during 30 min on the chitosan-genipin crosslinking step prior to the coating process is a suitable condition to increase the crosslinking degree without compromise the solution manipulation on coating.

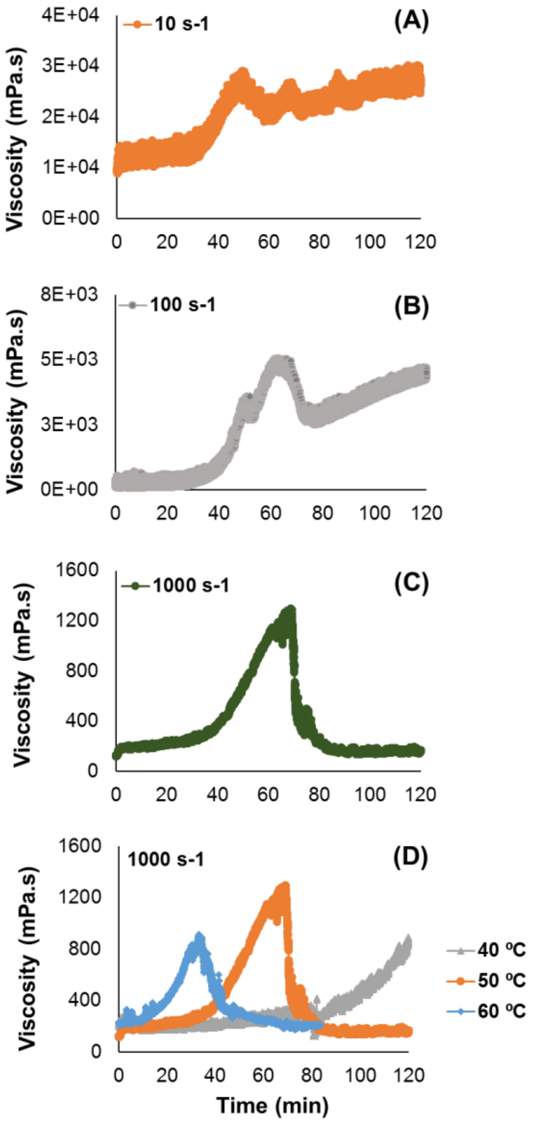

Figure 7: Viscosity profile of chitosan-genipin formulation at **(A)** 50 ºC with 10 s^{-1} of shear rate; **(B)** 50 ºC with 100 s-^{1} of shear rate; **(C)** 50 ºC with 1000 s^{-1} of shear rate; and, **(D)** 40; 50; and, 60 ºC with 1000 s^{-1} of shear rate.

Additional measurements using 10.000 s^{-1} of shear rate on chitosan-genipin formulations at 60 ºC were carried out. As result, the small peaks evidenced on **Figure 7D** were well designed (**Figure 8**). Further assays using 10.000 s^{-1} shear rate, at 40 and 50 ºC should also be performed.

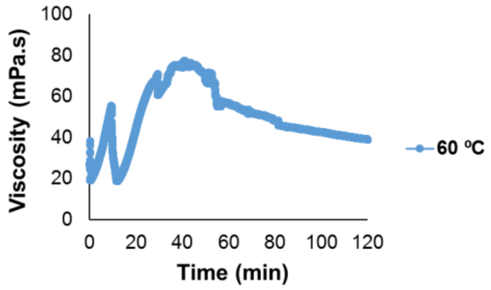

Figure 8: Viscosity of chitosan-genipin solution at 60 ºC for 120 min with a 10.000 s^{-1} of shear rate.

✓ Paper coating

One-sided paper coating processes were performed using with a bar-coating process (Endupap, France). Three different formulations were studied on vegetal and non-calendared sized paper:

- (i) 1% wt. of chitosan (C);
- (ii) 1% wt. of chitosan containing 0.05% (w:v) of genipin (CG);
- (iii) 1% wt. of chitosan containing 0.05% (w:v) of genipin mixed with 12.2% wt. of cellulose nanocrystal (CNC) in a 1:1 C:CNC ratio (CGCNC).

This last one aimed to increase the solid content of the formulation once only 1% wt. of chitosan was being used. Prior the coating, the genipin crosslinking occurred at 30 min at 50 ºC in agreement of the previous results achieved (**Figure 4**). During coating, the drying step was tested at both IR 1000 and 2500 W. Afterwards, each coated paper, pre-conditioned at a temperature of 23 ºC and relative humidity of 50% at least for 24 h, was characterized.

✓ Weight and thickness

Figure 9 shows the basic and coated weight gain as well as the thickness for each sample were determined by measuring the weight of at least 10 samples of 1 x 1 cm^2 on a digital scale (Mettler Toledo, ± 0.0001 g of precision). The thickness of each sample was measured using an Adamel Lhomargy micrometer (± 0.001 mm precision).

For non-calendared paper, the samples weight increased with the coating number of each performed coating. The most pronounced weight increasing was observed when 6 coatings of CGCNC, dried at 2500 W, were applied on the paper surface. On the other hand, for vegetal paper, despite of the 6 coatings of CG and CGCNC dried at 2500 W highly increased the paper weight, to the other coatings, the weight gain was not directly related to the coating number. For instance, from the 1st to the posterior CG coatings, a weight loss was verified. Perhaps, due to the vegetal paper structure, it can be explained by the previous CG coating removal during the various CG layers spreading onto the paper surface. Further assays must be performed to clarify this behavior.

Regarding the paper thickness changes, comparing with the non-coated samples, the highest thickness increasing was evidenced, for both non-calendared and vegetal papers, when 6 coatings of CGCNC dried at 2500 W. To the other performed coatings, the samples thickness was not meaningfully affected.

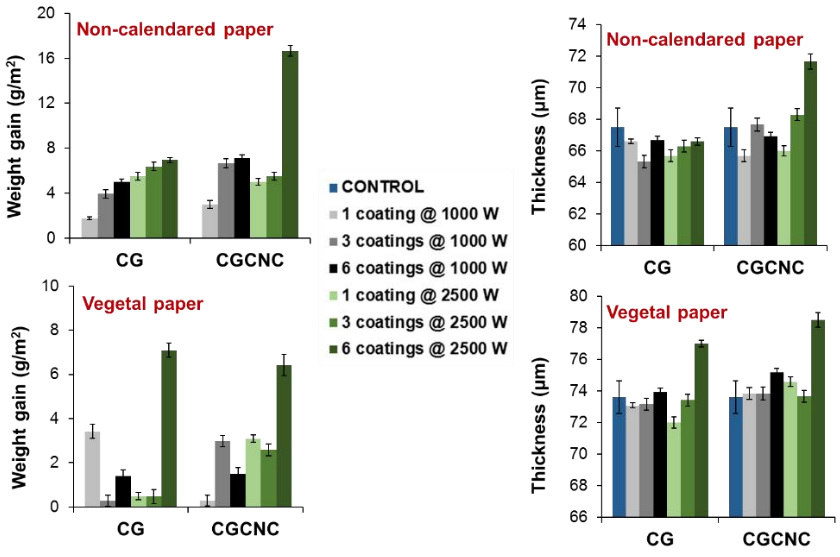

Figure 9: Weight gain and thickness of both non-calendared and vegetal coated papers. Chitosan-genipin (CG) and chitosan-genipin-CNC (CGCNC) were the coatings tested.

✓ Oxygen permeability

Oxygen permeability was monitored at 23 ºC and relative humidity of 0% by the equal pressure method using the M8001 oxygen permeation analyser (Systech ILLINOIS, United Kingdom) presented on **Figure 10**.

The oxygen transmission rates of both non-calendared and vegetal papers, with or without coating, were higher than the apparatus limit (400 cm^3 m^{-2} day^{-1} kPa-1). Therefore, the oxygen permeability profile of each sample was not measurable.

Figure 10: M8001 oxygen permeation analyser acquired on Systech ILLINOIS.

✓ Colorimetry

The color change of each coated and non-coated paper was assessed by tristimulus colorimetry (CIELab) from the entire visible spectrum (380-780 nm) using a SpectroEye portable spectrophotometer (X-rite, France), demonstrated on **Figure 11**.

Figure 11: SpectroEye portable spectrophotometer

For the calculations, Illuminant D65 and 10° observer were used. CIELab parameters, namely *a** (red/green), *b** (yellow/blue), and *L** (luminosity) components were determined. The results were expressed by the mean of 5 measurements performed at each sample.

Table 1 shows that the chitosan coating of both non-calendared and vegetal papers interfered with their own luminosity parameter(*L**), acquiring a slight yellowish coloration after 6 chitosan coatings dried at IR 2500 W, as observed by the highest *b** value increasing. In addition, as expected, both chitosan-genipin and chitosan-genipin-CNC coatings reduced the *a** value of both tested papers due to the presence of the greenish crosslinked chitosan-genipin network. This effect was more pronounced on non-calendared than on vegetal paper, especially when more than 1 chitosan-genipin or chitosan-genipin-CNC coatings were applied and dried at IR 2500 W.

Table 1: CIElab parameters for both non-calendared and vegetal papers, with and without chitosan, chitosan-genipin and chitosan-genipin-NCC coatings

Paper sample	Coating	IR power (W)	*L**	*a**	*b**
Non-calendared	0	----------	92.802	-0.012	-0.418
	1 x Chitosan	1000	92.690	-0.052	-0.344
		2500	92.954	-0.036	-0.434
	3 x Chitosan	1000	92.770	-0.008	-0.450
		2500	92.840	-0.058	-0.460
	6 x Chitosan	1000	92.938	-0.048	-0.006

		2500	92.557	-0.338	0.948
	1 x Chitosan-genipin	1000	92.566	-0.098	-0.384
		2500	92.874	-0.122	-0.344
	3 x Chitosan-genipin	1000	92.348	-0.326	0.350
		2500	92.294	-0.580	0.610
	6 x Chitosan-genipin	1000	91.260	-1.170	2.428
		2500	87.592	-2.732	6.856
	1 x Chitosan-genipin-CNC	1000	92.712	-0.052	-0.570
		2500	92.800	-0.114	-0.316
	3 x Chitosan-genipin-CNC	1000	92.696	-0.170	-0.164
		2500	91.368	-0.786	3.230
	6 x Chitosan-genipin-CNC	1000	92.670	-0.274	0.316
		2500	89.113	-1.660	6.358
Vegetal	0	----------	91.036	0.436	-2.566
	1 x Chitosan	1000	91.008	0.440	-2.392
		2500	91.118	0.466	-2.394
	3 x Chitosan	1000	91.372	0.508	-2.890
		2500	90.952	0.488	-2.786
	6 x Chitosan	1000	91.376	0.442	-2.442
		2500	89.297	0.262	-2.020
	1 x Chitosan-genipin	1000	90.840	0.484	-2.592
		2500	91.024	0.354	-2.412
	3 x Chitosan-genipin	1000	90.443	0.207	-1.838
		2500	90.456	-0.300	-0.730
	6 x Chitosan-genipin	1000	89.872	-0.430	-0.300
		2500	87.604	-1.282	1.836
	1 x Chitosan-genipin-CNC	1000	91.044	0.410	-2.408
		2500	91.198	0.404	-2.424
	3 x Chitosan-genipin-CNC	1000	90.676	0.297	-2.384
		2500	90.556	0.116	-1.610
	6 x Chitosan-genipin-CNC	1000	90.732	0.128	-1.668
		2500	88.318	-0.535	1.086

Figure 12 shows the color change of non-calendared paper with 6 coatings of chitosan-genipin and 6 coatings of chitosan-genipin-CNC, dried with IR 2500 W, the experimental conditions that presented the lowest *a** values, as described on **Table 1**.

CONTROL 6 x Chitosan-Genipin 6 x Chitosan-Genipin-CNC

Figure 12: Macroscopic effect of 6 chitosan-genipin and chitosan-genipin-CNC coatings on non-calendared paper.

✓ Water vapor permeability

According with the available laboratorial material, only one sample of some performed coatings were selected for the water vapor permeability measurements. The experiments followed the official standard T-448 method that provides the gravimetric determination of the water vapor transmission rate (WVTR) of sheet materials at 23 °C, with an atmosphere of 50% RH on one side and a desiccant on the other. Anhydrous sodium chloride was the desiccant agent used. Successive weights of the samples were performed to the nearest 0.001 g until constant rate of weight gain was attained. The water vapor transmission rate (WVTR) calculation followed the next equation:

$$\text{WVTR, g/m}^2\text{.day} = 24x/Ay$$

where,

x = gain in grams for the time period y, (during constant rate of gain period)

y = time in hours for the gain of x,

A = exposed area of specimen, m^2.

The quantities x and y can be derived conveniently from a plot of weight gain in grams *vs*. elapsed time in hours.

Due to the limited number of test dishes as well as to the low available number of coated samples, only one measurement of a few coatings was tested. **Table 2** shows the samples tested for each vegetal and non-calendared coated papers.

Table 2: Vegetal and non-calendared paper samples used on WVTR measurements

Vegetal paper	Non-calendared paper
Control: non-coated sample **1C2500**: 1 x chitosan coating dried at IR 2500 W **6C2500**: 6 x chitosan coating dried at IR 2500 W **1CG2500**: 1 x chitosan-genipin coating dried at IR 2500W **6CG2500**: 6 x chitosan-genipin coating dried at IR 2500 W **1CGCNC2500**: 1 x chitosan-genipin-CNC coating dried at IR 2500 W **6CGCNC**: 6 x chitosan-genipin-CNC coating dried at IR 2500 W	**Control:** non-coated sample **6C2500**: 6 x chitosan coating dried at IR 2500 W **6CG2500**: 6 x chitosan-genipin coating dried at IR 2500 W **1CGCNC2500**: 1 x chitosan-genipin-CNC coating dried at IR 2500 W **6CGCNC**: 6 x chitosan-genipin-CNC coating dried at IR 2500 W

Figure 13 shows that, as expected, for both vegetable and non-calendared paper, chitosan coating reduced the WVTR, even when only one coating layer was performed. On the other hand, for chitosan-genipin and chitosan-genipin-CNC coatings, the WVTR reduction was only verified when more than one coating layers were applied. Only one coating layer of chitosan-genipin or chitosan-genipin-CNC seems do not contribute to the WVTR decreasing for both paper samples. 6C2500 and 6CG2500 were the most promising approaches to reduce the WVTR of vegetal paper while 6CGCNC2500 was the most efficient on non-calendared paper. As major conclusion, the genipin crosslinking did not prejudice the chitosan WVRT effect and, according to the paper sample, increase the solid content of the formulation can be a solution to reduce the WVTR. It must be noted that the results obtained, especially for vegetal paper, may be influenced by the well performed coatings.

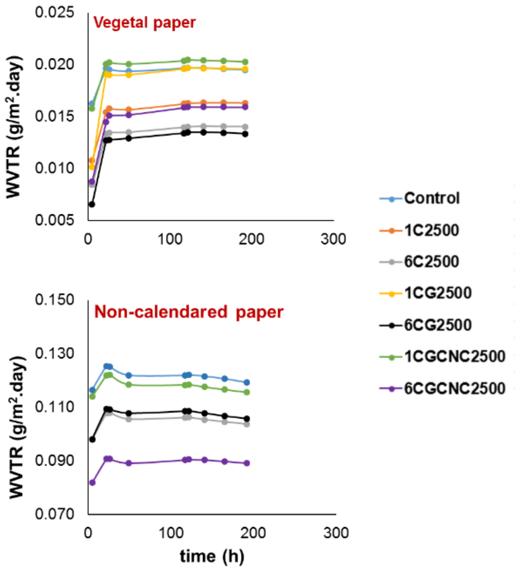

Figure 13: Water vapor transmission rate (WVTR) of chitosan (C), chitosan-genipin (CG) and chitosan-genipin-CNC (CGCNC) coatings on vegetal and non-calendared papers measured during 8 days at 23 ºC with a controlled relative humidity (50% RH). 1 and 6 coating layers were tested. Each coating was dried at 2500 W infra-red power.

✓ Acidic Stability

Samples (1 cm^2) of each vegetal and non-calendared coated paper were immersed in acidic aqueous solution (pH 3.5), at RT for 7 days. Non-coated samples were used as control. The acidic stability will be evaluated by the weight difference determination.

The moisture of each sample was determined at the beginning of the acidic stability assay. Pre-weighted 1 cm^2 samples were dried at 105 ºC for 24 h and further cooled down in a desiccator containing silica gel. The moisture percentage was calculated by means of the weight difference before and after the drying process. Each humidity and acidic stability assay was performed in triplicate.

Figure 14 shows that, for both vegetal and non-calendared paper, 6 coatings of chitosan-genipin reduced the samples acid solubility, especially when the drying process was carried out at 2500 W infra-red power. Nevertheless, the vegetable paper coated 6 times with chitosan-genipin-CNC also seems to resist to acidic aqueous conditions which was not observed on the equivalent coating for non-calendared paper. Further assays must be performed to clarify this behavior.

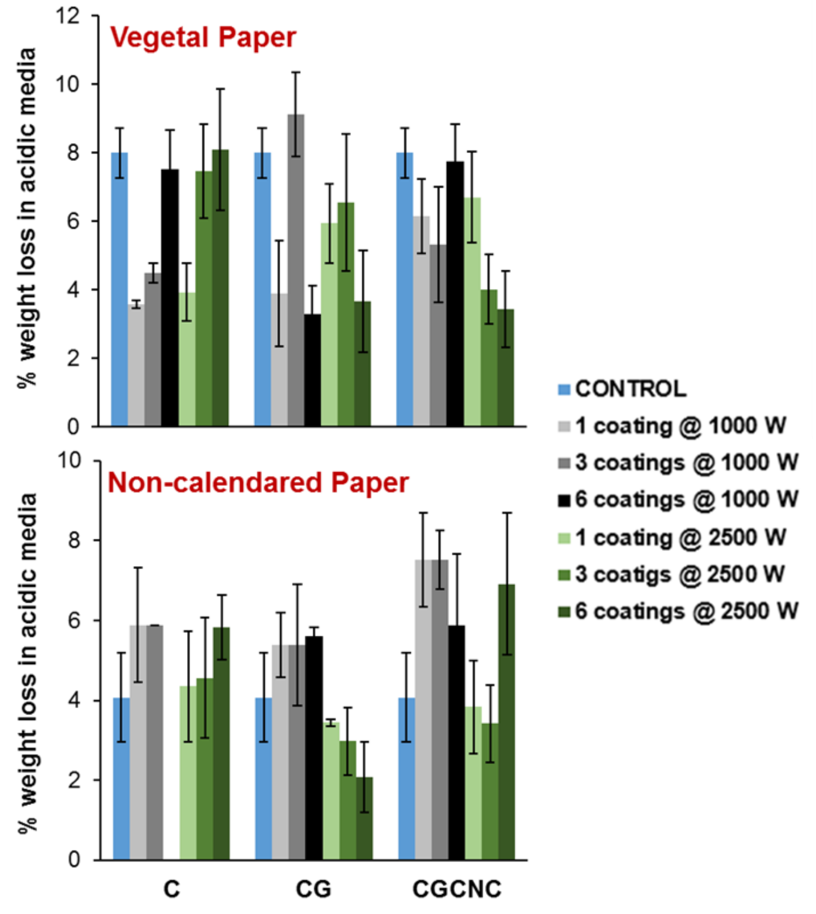

Figure 14: % weight loss in acidic conditions of both non-calendared and vegetal papers, with and without the chitosan, chitosan-genipin and chitosan-genipin-CNC coatings, dried at IR 1000 and 2500 W.

✓ Grease permeability

Due to the low available amount of coated papers resulted from the previous characterization tests that included destructive samples, only non-calendared coated papers were used for the grease permeability determination.

Firstly, the standard T-507 method that consists in the painting of the samples surface using a colored commercial oil was tested on non-calendared paper. As this sample already has a surface coated with some polymeric matrix suitable for printing processes, this procedure was not efficient for the grease permeability evaluation.

Alternatively, the grease penetration into the sample was measured with red turpentine oil, following the standard T-454 method. Herein, samples of coated and non-coated paper with 4 cm^2 were used; 5 g of sand was placed onto the samples' surface; and, 1,1 mL of the red turpentine oil was deposited on the sand. The examination of the oil penetration was conducted every 15 s for the first minutes, then once a minute during 5 min, and finally after 10 minutes.

Figure 14 shows that the 6 layers of chitosan or chitosan-genipin slightly increased the red turpentine oil resistance. The incorporation of CNC on the chitosan-genipin formulation seems to prejudice the grease resistance.

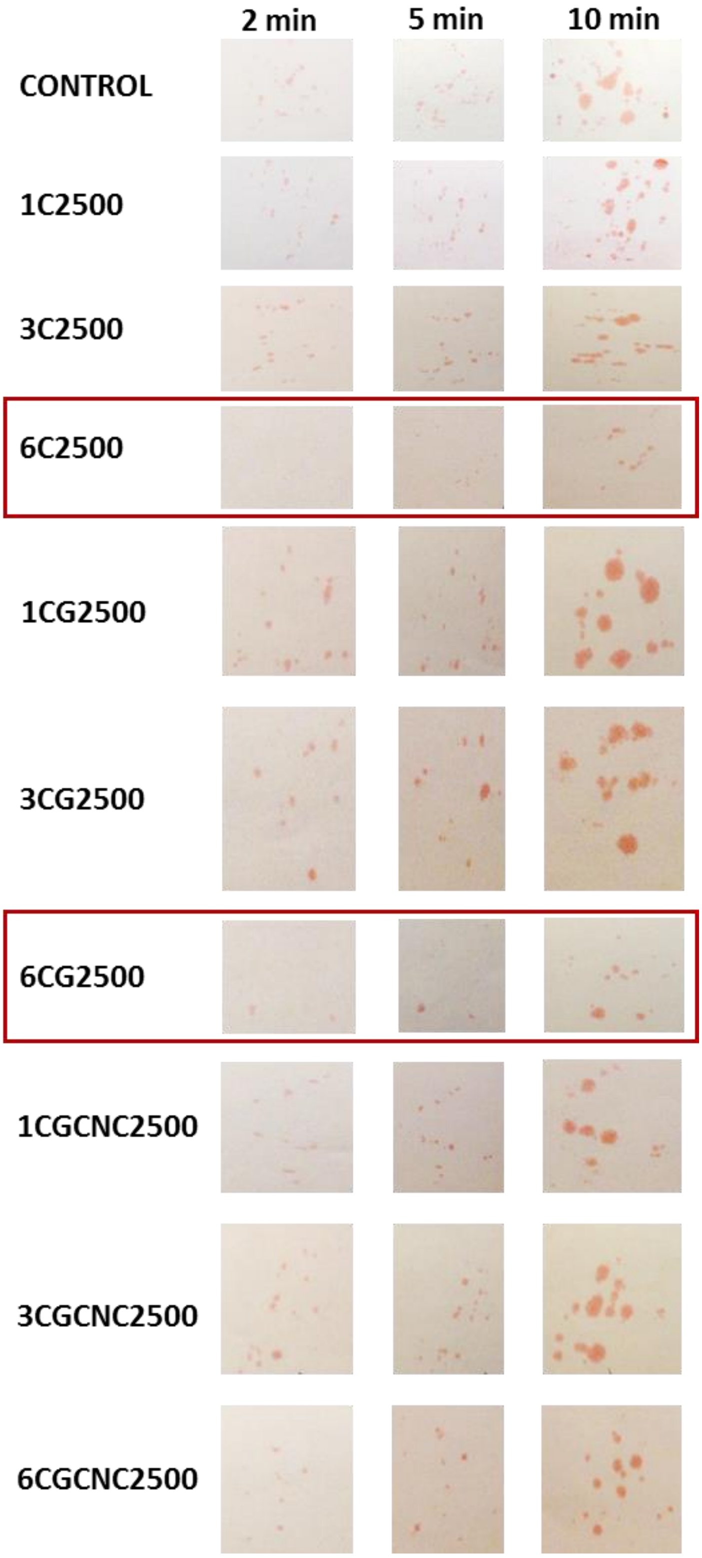

Figure 15: Pictures of “report paper” after testing the grease resistance of coated papers using with turpentine oil principle. C = Chitosan; CG = Chitosan-genipin; CGCNC = Chitosan-genipin-cellulose nanocrystals. 1; 3; and 6 coatings of each formulation were tested. Samples were dried at 2500 W.

✓ Air permeability

The air permeance tests were carried out with the system of Mariotte vase (**Figure 15**), following the ISO 5636. A sample area of 10 cm^2 and a vacuum of 2.5 kPa were used. Only one measurement of chitosan-genipin (CG) and chitosan-genipin-CNC (CGCNC) coated non-calendared paper was performed due to the lack of samples. The two faces, UP (for coating) and DOWN (for printing), of the paper samples were tested.

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Figure 16: Mariotte vase.

Figure 16 shows that both chitosan-genipin and chitosan-genipin-CNC coatings reduced the air permeability of non-calendared paper.

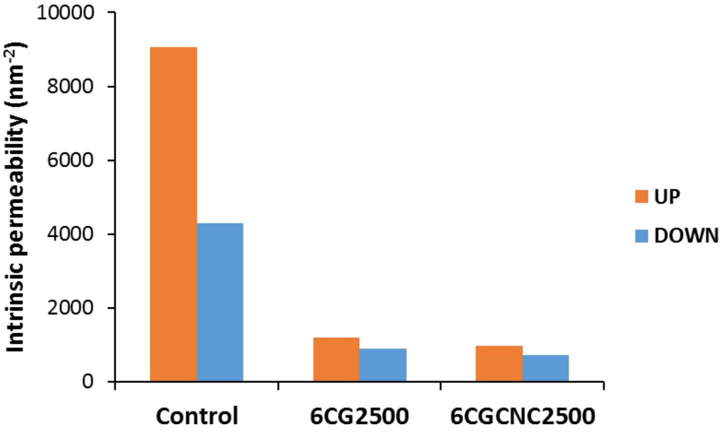

Figure 17: Intrinsic permeability of non-coated (Control) and coated chitosan-genipin (6CG2500) and chitosan-genipin-CNC (6CGCNC2500) paper. Six coating layers were performed and dried with 2500 W infra-red power. Both UP (for coating) and DOWN (for printing) faces of each sample were tested.

- ✓ **Mechanical properties**

Tensile strength, Young’s modulus and elongation at rupture were determined using the Instron 5965 apparatus (**Figure 17**). Chitosan-genipin and chitosan-genipin-CNC coatings applied on non-calendared paper and dried at 2500 W were tested. Samples of 15 cm x 10 cm were cut. Five replicates of each coated were measured. These tested were performed under a conditioned atmosphere (23 ºC, at 50% RH).

Figure 18: Instron 5965 apparatus used to measure the mechanical influence of each tested coating on paper samples.

Table 4 shows that, in general, each tested coating led to a slight decrease of the Young’s modulus profile with concomitant increasing of the tensile strength and elongation at rupture values. Therefore, chitosan-genipin based coatings seems to improve the paper traction resistance and its flexibility which can be advantageous for packaging applications.

Table 3: Percentage of tensile strength, Young’s modulus and elongation at rupture of coated non-calendared paper. Non coated samples were used a control

	Coatings number	**Tensile strength**	**Young's modulus**	**Elongation at rupture**
Chitosan-genipin @ 2500 W	1	-8.71 ± 0.13	-18.06 ± 0.19	15.99 ± 0.67
	3	-1.81 ± 0.21	-19.95 ± 0.36	19.19 ± 0.59
	6	**6.67 ± 0.22**	**-10.92 ± 0.54**	**10.48 ± 0.99**
Chitosan-genipin-CNC @ 2500 W	1	6.73 ± 0.07	-5.41 ± 0.24	-2.08 ± 0.65
	3	7.07 ± 0.04	-11.19 ± 0.45	11.36 ± 0.34
	6	**7.69 ± 0.08**	**-18.86 ± 0.58**	**14.77 ± 0.64**

Conclusions

- ✓ In general, the number of coatings as well as the IR drying power influenced the coated paper properties.
- ✓ Chitosan-genipin and chitosan-genipin-CNC increased the weight of both non-calendared and vegetal paper. When 6 coating layers were employed, the papers thickness considerably increased.
- ✓ Chitosan-genipin and chitosan-genipin-CNC coated papers presented a greenish coloration resulted from the chitosan-genipin crosslinking.
- ✓ Chitosan-genipin based coating revealed to be a proper approach to reduce the water vapor and air permeability of paper and simultaneously to increase its grease resistance.
- ✓ Chitosan-genipin and chitosan-genipin-CNC coatings improved the mechanical properties of non-calendared paper.
- ✓ Chitosan-genipin coatings presented lower weight loss when exposed to acidic conditions than the other tested coatings which is a good indication that this coating fits with food packaging requirements.

Future experiments

Antioxidant properties, surface hydrophobicity and even some morphological characterization of the coated papers must be carried out to complement the described results.

Note that some characterization techniques were only applied to a certain group of samples due to the lack of time and the available samples. It was not a priority do a high quantity of coated papers, but to test different processing conditions. Therefore, according to the best results achieved during this journey it must be considered to reproduce the experimental work and characterize each material following the standard methods.

Further, some pilot scale experiments should also be considered.

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