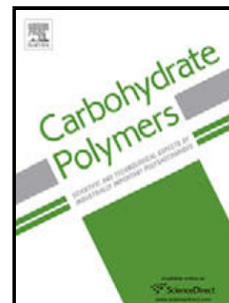


Journal Pre-proof

Relevance of genipin networking on rheological, physical, and mechanical properties of starch-based formulations



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Relevance of genipin networking on rheological, physical, and mechanical properties of starch-based formulations

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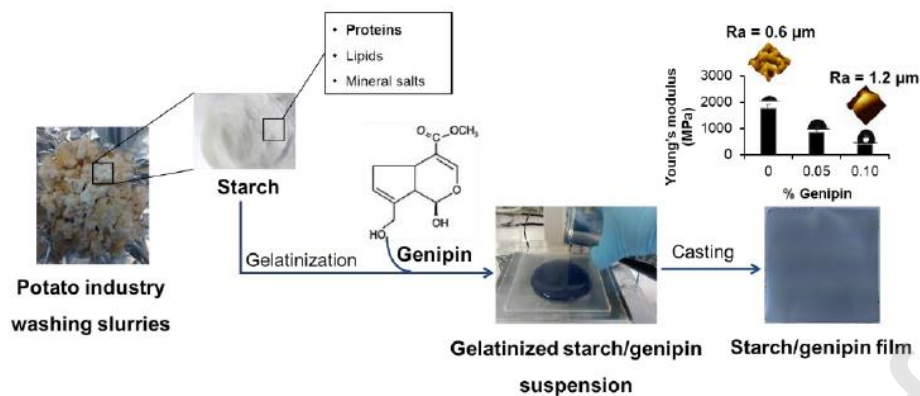
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Graphical Abstract



Highlights

- Starch-rich food industry byproducts are used to prepare films with genipin.
- Starch protein-genipin networks give rise to bluish starch films.
- Genipin increases the surface roughness and hydrophobicity of starch films.
- Genipin improves the starch films stretchability.

Abstract

The small amount of proteins in starch-rich food industry byproducts can be an advantage to crosslink with genipin and tailor the performance of biobased films. In this work, genipin was combined with non-purified starch recovered from industrial potato washing slurries and used for films production. Starch recovered from potato washing slurries contained 0.75% protein, 2 times higher than starch directly obtained from potato and 6 times higher than the commercial one. Starch protein-genipin networks were formed with 0.05% and 0.10% genipin, gelatinized at 75 °C and 95 °C in presence of 30% glycerol. Bluish colored films were obtained in all conditions, with the higher surface roughness (R_a , 1.22 μm), stretchability (elongation, 31%), and hydrophobicity (water contact angle, 127°) for 0.10% genipin and starch gelatinized at 75 °C. Therefore, starch-rich byproducts, when combined with genipin, are promising for surpassing the starch-based films hydrophilicity and mechanical fragilities while providing light barrier properties.

Keywords: food byproducts, polysaccharide-based films, crosslinking, hydrophobicity, stretchability

1. Introduction

Polysaccharides could play a key role in decreasing the fossil fuels dependence for plastics production and, subsequently, in the environment preservation. Unlike petroleum-based raw materials, these compounds prompt a lower green-house effect during their transformation. Besides, polysaccharides are biodegradable i.e. can be degraded through the action of living organisms, thus avoiding their exponential accumulation in landfills (Babu, O'Connor, & Seeram, 2013; Bastioli, 2001). Polysaccharides and their derivatives have been widely explored to develop bioplastic materials (Cyras, Tolosa Zenklusen, & Vazquez, 2006; A. R. V. Ferreira, Alves, & Coelho, 2016; Jiménez, Fabra, Talens, & Chiralt, 2015). In 2018–2019, the production capacity of polysaccharide-based plastics, particularly starch-based blends, reached 18% of bioplastics global production capacity (European Bioplastics, 2019). The granular structure of starch, composed by amylose and amylopectin that have a linear (1,4)-linked α -D-glucan chain branched with (1,6)-linked α -D-glucans, is of growing interest within the plastic industry due to its thermoplastic properties, great film-forming ability, and high abundance in nature as a renewable resource (Jiménez, Fabra, Talens, & Chiralt, 2012; Sagnelli et al., 2016). The basis of starch-based bioplastics production relies on the disruption of hydrogen bonding of gelatinized starch suspensions in the presence of plasticizers, giving rise to thermoplastic materials (Liu, Xie, Yu, Chen, & Li, 2009; Schirmer, Jekle, & Becker, 2015). Nevertheless, starch-based materials have restricted physicochemical and mechanical properties as moisture sensitivity, brittleness, and poor barrier properties, which limit their practical application (Mekonnen, Mussone, Khalil, & Bressler, 2013). Blending starch with other polymers as natural

polymers (cellulose-derivatives, chitosan, alginate, gelatin, collagen, whey/soy proteins, and lipids), synthetic polymers (polylactic acid, poly- ϵ -caprolactone, polyvinyl alcohol, polybutylene succinate, polyethylene, polystyrene, polyacrylic acid, polyurethane, and ethylene-vinyl acetate), and inorganic compounds (lithium perchlorate, silica, clay, silver, and talc) allows to minimize these drawbacks, enhancing the materials stiffness and moisture tolerance (Tabasum et al., 2019). However, the non-biodegradability of some incorporated substrates, poor compatibility with starch chains, limited water tolerance, and high-production cost remain as obstacles.

Crosslinking reactions have been proposed for improving polysaccharide-based materials performance (Persin et al., 2011). Hence, using natural crosslinking agents that do not interfere with the biodegradability and innocuity of biobased matrices is a challenge. Genipin, a biocompatible iridoid compound that reacts with amine groups through nucleophilic substitution to form heterocyclic amines (Mi, Shyu, & Peng, 2005), has shown to be a suitable bifunctional crosslinking agent that improves chitosan-based films and hydrogels performance, prompting strong intermolecular associations through covalent bonding (Ferreira, Nunes, Castro, Ferreira, & Coimbra, 2014; Gonçalves et al., 2017; Meena, Prasad, & Siddhanta, 2009; Mu, Zhang, Lin, & Li, 2013; Muzzarelli, 2009; Nunes et al., 2016; Nunes et al., 2013; Nunes et al., 2015). Despite the absence of amine groups in starch polymeric chain, it can be hypothesized that genipin may crosslink with starch granule proteins, providing similar effects to those observed for chitosan films. To verify this hypothesis, in this work, the protein content of non-purified starch recovered from potato industry byproducts, a raw material that revealed to have film-forming ability

even after submitted to industrial processing (Gonçalves et al., 2020), and the feasibility of blending it with genipin to produce starch-based films with improved properties was studied. The influence of genipin dosage and gelatinization temperature conditions on rheology of gelatinized starch-based suspensions, as well as on optical, surface morphology, hydrophobicity, water solubility, and mechanical properties of the films was evaluated.

2. Materials and Methods

Potato washing slurries containing water (56%), starch (33%), and small potato slices (11%), described in Gonçalves et al. (2020), were provided by A Saloinha, Lda., a Portuguese potato chips company. Mineral oil and purified commercial potato starch were supplied by Sigma-Aldrich (Germany), and glycerol (99.5%) was purchased from Scharlau (Spain). Genipin ($\geq 98\%$ purity) was acquired from Challenge Bioproducts Co. (Taiwan, China). All the chemical reagents have an analytical grade and, therefore, were used without any further purification.

2.1. Recovery of starch from industrial potato washing slurries

After freeze-drying, the small potato slices existing in potato washing slurries were removed by sieving using a 125 μm stainless-steel sieve (Gonçalves et al., 2020). The recovered starch was stored in a desiccator containing silica gel until further application.

2.2. Protein content of recovered potato starch

Nitrogen content of starch recovered from 4 independent batches of potato washing slurries was determined by elemental analysis using a Truspec 630-200-200 equipment, setting the analysis parameters for: sample size micro (up to 10 mg), combustion furnace temperature of 1075 °C, afterburner temperature of 850 °C and nitrogen detection method by thermal conductivity. Purified commercial potato starch was used for comparison. The protein content was calculated using the nitrogen content correlation factor mostly used for potato proteins (6.25) (Van Gelder, 1981).

2.3. Production of gelatinized starch/genipin-based formulations

After pre-drying at 105 °C during overnight and cooling down at room temperature in a desiccator containing silica gel, starch suspensions (4% w/V) prepared in distilled water containing glycerol (30% w/w of dry starch weight) were gelatinized during 30 min under stirring, at 2 different temperatures: 75 °C and 95 °C. After vacuum filtration using a porous glass filter (G1), genipin was incorporated into gelatinized starch-based suspensions at 2 different final concentrations, 0.05% and 0.10% w/V, and stirred during 1 h at the corresponding gelatinization temperature. Each genipin solution was prepared in distilled water and heated at 75 °C immediately prior of its addition into the starch-based formulations. Formulations produced without genipin were used as control.

2.4. Rheology of gelatinized starch/genipin-based suspensions

As a strategy to predict the influence of genipin on mechanical performance of starch-based films, rheological experiments were carried out on starch-based

suspensions gelatinized at 75 °C and 95 °C without genipin (control) and with 0.10% genipin, the highest genipin dosage used in this work. After preparing each formulation following the experimental conditions referred in section 2.3, the samples were cooled down to room temperature (≈ 25 °C) for 90 min. The influence of genipin in rheological behavior of starch-based formulations was studied using a controlled stress rheometer (Kinexus, Malvern), equipped with a cone–plate geometry (cone diameter 40 mm and angle 4°, CP 4/40 SR0195 SS). During the experiment, the solvent evaporation was prevented by a thin layer of low viscosity mineral oil spread on the exposed surface of the sample. Storage modulus (G') and loss modulus (G'') were recorded using a constant shear strain (0.05) in a frequency range from 0.010 to 10 Hz. The flow behavior of the samples was also analyzed by steady shear flow tests performed within a shear range from 0.1 s^{-1} to 100 s^{-1} for 5 min. Both assays were carried out at 25 °C.

2.5. Preparation of starch/genipin-based films

Starch- and starch/genipin-based films were prepared through solvent casting technique. After the formulations' preparation (section 2.3), each one was degassed, transferred into plexiglass plates of 144 cm^2 with 3 mm deep, and the solvent was evaporated in an air ventilated oven at 25 °C overnight. The films were stabilized at room temperature under controlled humidity atmosphere (54% RH) for at least 5 days until further use.

2.6. Films characterization

The visual aspect of each developed film was photographed in triplicate using a digital camera and the most representative images were used.

The visible light barrier properties of each developed film were monitored through spectrophotometry using a UV-VIS JASCO V-560 spectrophotometer by adapting the methodology described by Ortega, Giannuzzi, Arce, & García (2017). Films were cut in strips with 4 cm x 1 cm and the corresponding absorbance spectra were obtained between 380 to 780 nm wavelength. Measurements were made in triplicate and averaged.

Traction tests were performed to study the films mechanical resistance and elastic/plastic properties; water contact angle measurements were carried out to determine the films surface wettability; weight loss of samples immersed in aqueous solution was assessed to explore the films solubility; and atomic force microscopy (AFM) was used to evaluate the films topography. The detailed conditions are described in Gonçalves et al. (2020).

The statistical analysis of all the obtained data was calculated using the *t*-student test with a significance level of 95%.

3. Results and Discussion

3.1. Protein content of starch recovered from potato washing slurries

Element analysis revealed that starch recovered from potato washing slurries was composed by $0.753\% \pm 0.057$ of protein. This value was 2 times higher than the 0.30% usually existent in starch directly obtained from potato (Alvani, Qi, Tester, & Snape, 2011). In turn, purified commercial potato starch contained $0.134\% \pm 0.016$

of protein. Therefore, starch recovered from potato washing slurries contained a protein content at least 6 times higher than the commercial one. Raw potato tubers are constituted by 2% protein (Dourado et al., 2019). Therefore, the high protein content of starch obtained from potato washing slurries is related to the protein released from potato flesh or juice during the overall industrial processing and starch granule proteins that remained accumulated in the wasted washing slurries. These proteins may crosslink with genipin and change the rheological properties of gelatinized starch-based suspensions.

3.2. Rheology of gelatinized starch/genipin-based suspensions

The influence of 0.10% genipin on rheological properties of gelatinized starch-based suspensions heated at 75 °C and 95 °C during 30 min was studied (**Fig. 1**).

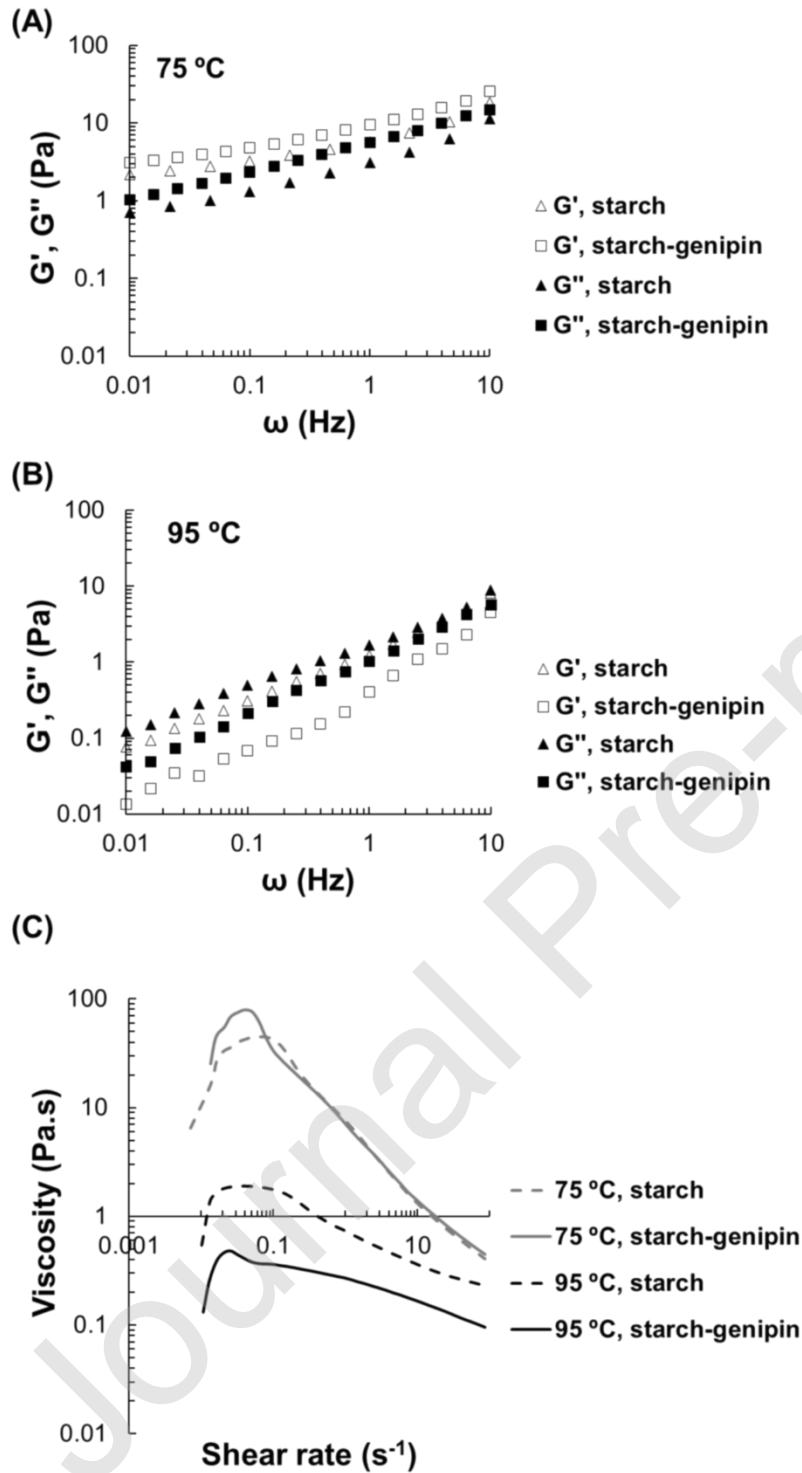


Fig. 1 – Influence of processing temperature (75 °C and 95 °C) and genipin (0.10% w/V) on rheology of gelatinized starch-based suspensions. **(A)** Elastic modulus (G'), **(B)** viscous modulus (G''), and **(C)** viscosity changes of gelatinized starch-based suspensions.

At 75 °C (**Fig. 1A**), the elastic modulus (G') of gelatinized starch without genipin was superior to its viscous modulus (G''). In the presence of genipin, this behavior was maintained, although with an increase of both G' and G'' values, when compared to the gelatinized starch without genipin. At 95 °C (**Fig. 1B**), an opposite effect was observed, i.e. the gelatinized starch without genipin presented a G' value lower than G'' . The addition of genipin decreased both G' and G'' values. Regarding the gelatinized starch viscosity data, starch suspensions gelatinized at 75 °C exhibited higher viscosity than the ones heated at 95 °C (**Fig. 1C**). For shear rates below 0.1 s^{-1} , the general flow behavior showed a shear thickening. This phenomenon may be related to the interactions among intact or partially damaged starch granules remaining in the gelatinized suspensions (Conde-Petit, Nuessli, Handschin, & Escher, 1998), which are enhanced under low shear range. Consequently, a shear-thinning behavior occurs, thus decreasing the apparent viscosity of gelatinized starch-based suspensions with the shear rate increase. In the presence of genipin, for shear rates lower than 0.1 s^{-1} , a minor viscosity increase was observed in starch-based suspensions gelatinized at 75 °C, but no significant differences were observed for higher shear rates, when comparing with the suspensions gelatinized at 95 °C. Therefore, according to the temperature at which the starch-based suspensions are gelatinized, genipin can act differently. When the dispersions are heated at 75 °C, the proteins present in intact or partially destroyed starch granules hardly react with genipin, while the proteins available in the starch matrix could form intermolecular linkages with genipin, promoting the G' , G'' , and viscosity increase. In turn, when the gelatinization occurs at 95 °C, the higher temperature leads to the starch granules disruption, rendering available all their

content. The higher number of proteins available and the acceleration of genipin reaction with amine groups could promote intramolecular crosslinking, contributing for the G' , G'' , and apparent viscosity decrease. Each one of these effects might be related with the genipin ability to crosslink with proteins available in the gelatinized starch-based suspension, as well as with the amount of intact or partially damaged starch granules. Indeed, as higher the temperature at which the starch-based suspensions were subjected, higher the damaged starch granules amount (Lund & Lorenz, 1984), and thus higher will be the starch granule proteins available for reacting with genipin. Consequently, a high amount of starch protein-genipin networks are formed. These structures, when entrapped in the polymeric matrix, can avoid the hydrogen bonding between both amylose and amylopectin chains released during gelatinization, conferring mobility to the polymeric system, and therefore decreasing the gelatinized system viscosity. On the contrary, as lower the gelatinization temperatures are, higher will be the number of intact or partially disrupted starch granules in the gelatinized starch-based suspensions (Conde-Petit et al., 1998) and lower will be the amount of starch granule proteins available to react with genipin. Hereinafter, lower amount of starch granule protein-genipin networks will be formed. Instead of avoiding the hydrogen bonding between amylose and amylopectin chains, these structures will promote the interaction between the intact or partially disrupted starch granules, thus contributing for a more structured system.

3.3. Characterization of starch/genipin-based films

3.3.1. Light barrier properties

The incorporation of genipin conferred a bluish coloration to starch-based films, originating a 600 nm absorbance peak when spectrophotometrically observed, thus conferring light barrier properties to the films (**Fig. 2**). The blue colour intensity was directly related to genipin amount and gelatinization temperature used in the films' formation. Thus, the increase of genipin concentration from 0.05% to 0.10% and reaction temperature from 75 °C to 95 °C gave rise to dark blue films. Genipin itself is a colourless compound. However, in the presence of primary amines, it spontaneously reacts through nucleophilic attack with the olefinic carbon, producing water-soluble bluish pigments due to the formation of conjugated double bonds (Muzzarelli, 2009; Neri-Numa, Pessoa, Paulino, & Pastore, 2017). Thus, the bluish coloration of starch/genipin-based films derived from the network promoted between starch proteins and genipin. The protein-genipin interaction is deeply dependent on many experimental parameters such as temperature and genipin-to-amino source ratio (Neri-Numa et al., 2017). An increase in the reaction temperature and genipin concentrations potentiates the protein-genipin interactions, thus leading to a gradual deepening of the blue coloration (Bi et al., 2011). These phenomena justify the dark blue colorations of films produced at the highest gelatinization temperature (95 °C) and genipin amount (0.10%) used in this work. The light barrier property was not evidenced when films were prepared with starch/genipin-based suspensions gelatinized at 75 °C using a purified commercial starch, which had 6 times less protein than the protein present in the starch used in this work (**Fig. S1**).

This highlights the relevance of proteins present in starch and the genipin-to-amino source ratio as promoters of the protein-genipin network.

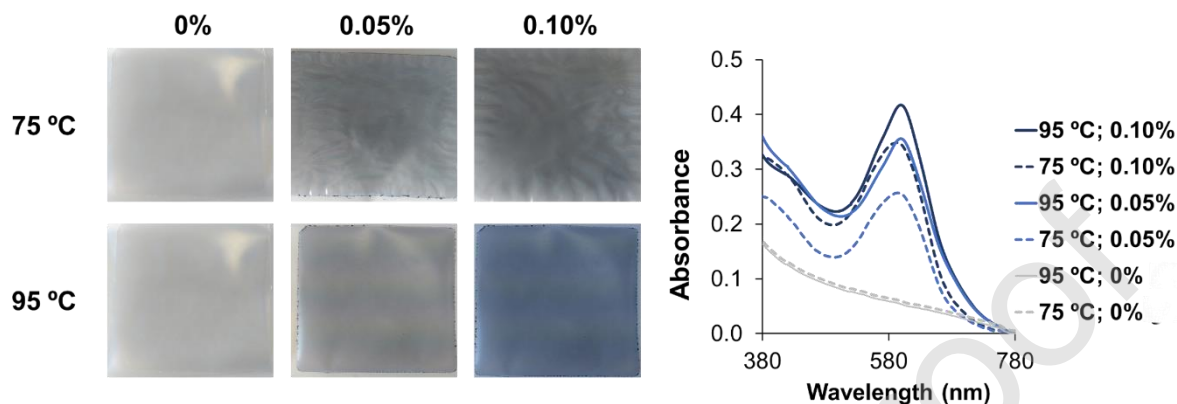


Fig. 2 – Photographs of the films taken with a digital camera and UV-visible spectra of starch-based films gelatinized at 75 °C (– –) and 95 °C (—) with different genipin concentrations (0%, 0.05%, and 0.10% w/V).

3.3.2. Mechanical Properties

When 0.05% and 0.10% genipin were incorporated into starch-based formulations gelatinized either at 75 °C or 95 °C, no changes in films thickness were observed, keeping the averaged 70 μm (**Fig. 3A**). However, the mechanical performance of starch-based films was influenced by both genipin concentrations and gelatinization temperatures. At 75 °C, 0.05% and 0.10% genipin significantly decreased the films tensile strength from 15 MPa down to 11 MPa and 6 MPa, respectively (**Fig. 3B**), meaning that starch/genipin-based films withstood a lower traction force till break than the pristine samples. In addition, 0.05% and 0.10% genipin decreased the Young's modulus from 1757 MPa down to 855 MPa and 403 MPa, respectively (**Fig. 3C**), thus promoting film's elasticity. The films elongation changed in an opposite way, increasing from 8% up to 18% and 31% when 0.05% and 0.10% genipin were added (**Fig. 3D**). Thus, the starch-based films with genipin

were able to hold a longer distance till break than pristine films. This tendency was similar for films developed using a gelatinization temperature of 95 °C and with 0.05% genipin. However, the incorporation of 0.10% genipin provided films with lower traction resistance, elasticity, and stretchability than films containing 0.05% genipin, with properties like pristine films.

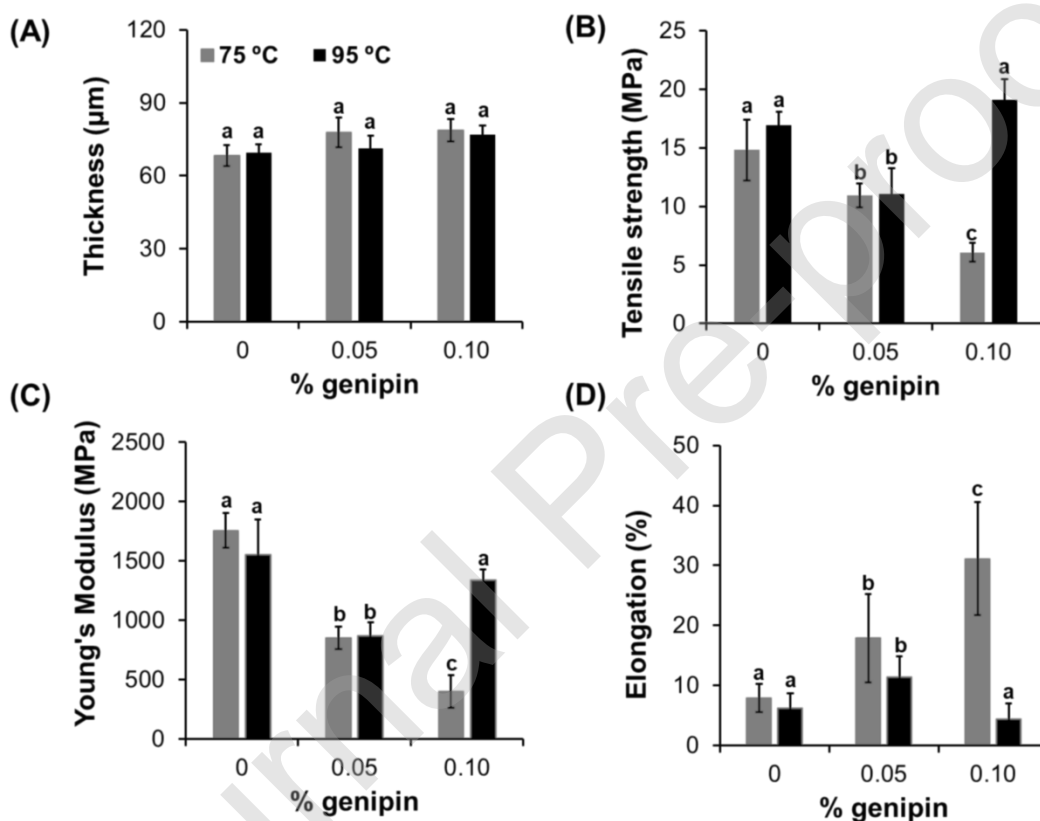


Fig. 3 – Mechanical properties of starch-based films containing different genipin concentrations (0, 0.05, and 0.10% w/V). (A) Thickness, (B) tensile strength, (C) Young's modulus, and (D) elongation at break. Different letters for each sample represent values that are significantly ($p < 0.05$) different.







Although genipin is known as a natural agent that reinforces the mechanical strength and stiffness of materials (Bi et al., 2011; Muzzarelli, 2009; Nunes et al., 2013), in this work, an opposite effect was observed, i.e. genipin brought flexibility to starch-based materials. This effect was more accentuated when the starch

gelatinization was carried out at 75 °C than at 95 °C. This flexibility can be related with three main aspects: (i) genipin is not reacting with starch, the main polymeric chain of the films, but with minor components that are in the matrix, the starch proteins; (ii) the low level of starch granules disruption that can directly influence the starch protein-genipin networks formation; and, (iii) the genipin that remained free in the matrix due to the low amount of amine groups available in the formulation and act as a plasticizer. The flexibility limitation observed for films whose starch was gelatinized at 95 °C may be caused by the pronounced formation of starch protein-genipin networks that by interacting with amylose and amylopectin chains can form a more cohesive structure, instead of promoting mobility to the films. These mechanical changes are in accordance with the influence of genipin and gelatinization temperature on rheological properties of gelatinized starch-based formulations (**Fig. 1**), where G' was higher than G'' for 75 °C, predicting an elastic solid material, and the opposite was observed at 95 °C, predicting more rigid films. Furthermore, the genipin-to-amino source ratio and, therefore, the protein content in starch also affected the mechanical performance of starch/genipin-based films. When prepared with starch/genipin-based suspensions gelatinized at 75 °C using commercial potato starch, the films elasticity and stretchability promoted by genipin incorporation was only verified on formulations containing 0.10% genipin (**Fig. S2**). Here, both starch protein-genipin networks and the remnant free genipin may contribute to films flexibility.

3.3.3. Wettability and water solubility

Table 1 shows the influence of genipin and gelatinization temperature on surface wettability of starch-based films when a drop of ultrapure water was dispensed onto the samples surface. The incorporation of 0.05% and 0.10% genipin into starch-based suspensions gelatinized at 75 °C increased the films surface water contact angle from 67° to 99° and 116°, respectively. When gelatinized at 95 °C, an increase of the water contact angle from 47° to 120° and 127° was observed for films containing 0.05% and 0.10% genipin, respectively. Clearly, the presence of genipin led to starch-based films with hydrophobic surfaces (hydrophobicity benchmark > 90°) (Law, 2014). This water tolerance increase was also evidenced on films prepared with starch/genipin-based suspensions gelatinized at 75 °C using commercial potato starch (**Table S1**). Such behaviour can be explained by the formed starch protein-genipin networks which may be more hydrophobic than the starch polymeric network and, when entrapped between the polymeric chains, may hinder their hydroxyl groups, avoiding their hydrogen bonding with water molecules.

Table 1 – Water contact angle values of starch-based films gelatinized at different temperatures (75 °C and 95 °C) and with different genipin concentrations (0, 0.05, and 0.10% w/V). Different letters for each sample represent values that are significantly ($p < 0.05$) different

% Genipin	Gelatinization temperature	
	75 °C	95 °C
0	 67 ± 2^a	 47 ± 2^d
0.05	 99 ± 5^b	 120 ± 2^e
0.10	 116 ± 7^c	 127 ± 3^e

Despite the films surface hydrophobicity promoted by genipin, none of them were resistant to water conditions (**Fig. 4**).

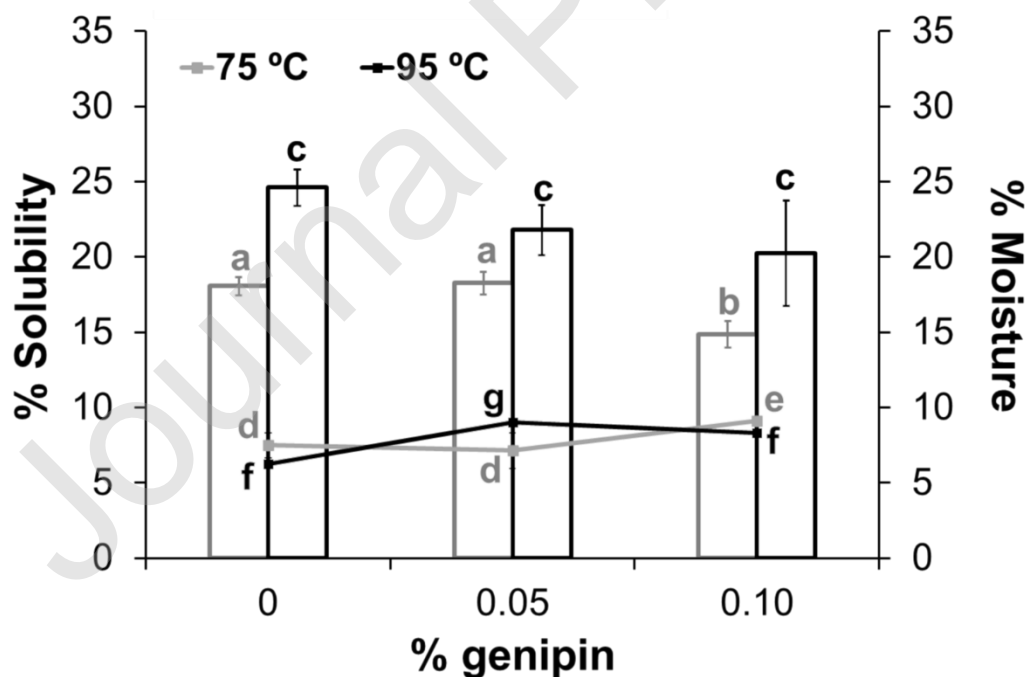


Fig. 4 – Solubility (columns) and moisture content (lines) of starch-based films containing different genipin concentrations (0; 0.05; and 0.10% w/V). Different letters for each sample represent values that are significantly ($p < 0.05$) different.

When compared to control samples, only the films gelatinized at 75 °C and containing 0.10% of genipin showed a significant decrease in their weight loss after being immersed in aqueous medium (pH = 6.5) for 7 days. At 95 °C, the films weight loss was similar for each genipin dosages tested and, therefore, the starch protein-genipin networks formed were not enough to provide water resistance to the starch-based films. The observed water solubility can be related with the glycerol amount that did not interrelate with starch molecules and starch protein-genipin networks. Due to its hydrophilic character, glycerol readily migrates into the aqueous medium (Farahnaky, Saberi, & Majzoubi, 2013). At 75 °C, the presence of starch protein-genipin structures in combination with intact or partially disrupted starch granules may form a tridimensional network within the starch matrix, thus promoting a cohesive structure. Consequently, it may prevent the films swelling, avoiding glycerol migration into the surrounding aqueous medium, leading to an upgrade of films resistance to solubilization (Reddy & Yang, 2010). On the other hand, at 95 °C the highest level of starch granules disruption avoided the cohesive structure, thus facilitating glycerol mobility within the starch matrix and its consequent release into the aqueous environment. These results corroborate with the moisture content increase observed for films containing 0.1% genipin, whose gelatinization was carried out at 75 °C and the non-significant moisture content alteration for films with the same genipin amount whose gelatinization was carried out at 95 °C. Furthermore, the content of protein in starch also affected the films water solubility and moisture content profiles. When prepared with starch/genipin-based suspensions gelatinized at 75 °C using starch with protein content 6 times lower that

the starch used in this work, genipin did not promote significant changes in the films water solubility and moisture content, even for the highest genipin dosage studied (**Fig. S3**), meaning that the starch protein-genipin networks formed did not interfere with the cohesive structure of pristine starch-based materials.

3.3.4. Surface topography

Fig. 5 shows the influence of starch gelatinization temperature and genipin dosage on surface topography of starch-based films. When gelatinized at 75 °C, starch gave rise to films with a hill-valley-structure with peak height around 1.10 μm and a roughness (R_a) value of 0.57 μm . Raising the gelatinization temperature to 95 °C decreased the peaks height and R_a values of starch-based films to 0.87 μm and 0.13 μm , respectively, thus becoming smooth. The disruption degree of granular envelopes resulted from dissolution of amylose and amylopectin upon gelatinization is potentiated by the processing temperature (Mathew, Brahmakumar, & Abraham, 2006). Therefore, at 75 °C, starch granules that remain intact and withered can contribute for the films hill-valley-structure, while at 95 °C, the high disruption degree of these granular envelopes justifies the films surface smoothness. On the other hand, the incorporation of genipin increased both the film peaks height and R_a , when compared with the pristine films, without depending on the starch gelatinization temperature. Nevertheless, the starch/genipin-based films produced at 95 °C were always smoother than the ones whose gelatinization was carried out at 75 °C. The lower hydrogen bonding between either starch granular envelopes or amylose and amylopectin chains caused by the formed starch protein-genipin networks may justify these topographic changes.

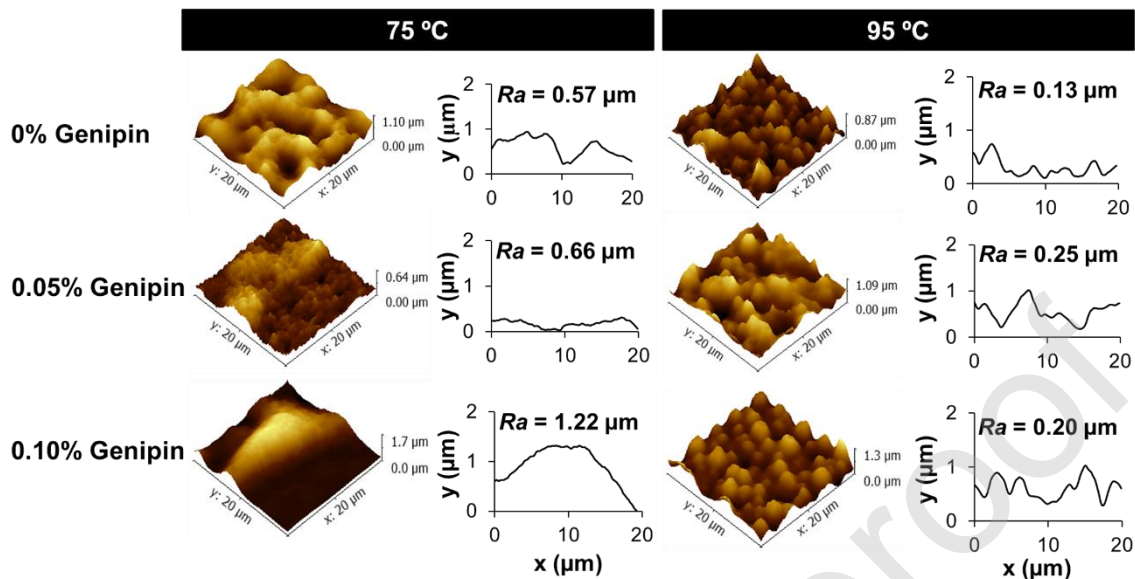


Fig. 5 – 3D AFM topographic images of starch-based films gelatinized at 75 °C and 95 °C with different genipin dosages (0, 0.05, and 0.10% w/V).

4. Conclusion

Starch recovered from potato washing slurries revealed to have a higher protein content than starch directly extracted from potato and purified commercial potato starch, thus offering a high portion of amine groups available to form a network with genipin. A bluish coloration is developed during the starch/genipin-based films production. The rheological properties of gelatinized starch-based suspensions and the films mechanical performance depend on gelatinization temperature and genipin amount due to the presence of intact or partially disrupted starch granules and inter- and/or intramolecular bonds established. Moreover, starch/genipin-based films showed a rough and hydrophobic surface, thus contributing for diminishing their sensitivity to humidification. Therefore, starch protein-genipin networking reveals to be relevant for enhancing starch-based films physical and mechanical properties.

This study also highlights the advantage of using raw starch-rich food industry byproducts for polysaccharide-based films development.

Credit author statement

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Figure captions

Fig. 1 – Influence of processing temperature (75 °C and 95 °C) and genipin (0.10% w/V) on rheology of gelatinized starch-based suspensions. **(A)** Elastic modulus (G'), **(B)** viscous modulus (G''), and **(C)** viscosity changes of gelatinized starch-based suspensions.

Fig. 2 – Photographs of the films taken with a digital camera and UV-visible spectra of starch-based films gelatinized at 75 °C and 95 °C with different genipin concentrations (0%, 0.05%, and 0.10% w/V).







Fig. 3 – 3D AFM topographic images of starch-based films gelatinized at 75 °C and 95 °C with different genipin dosages (0, 0.05, and 0.10% w/V).

Fig. 4 – Mechanical properties of starch-based films containing different genipin concentrations (0, 0.05, and 0.10% w/V). **(A)** Thickness, **(B)** tensile strength, **(C)** Young's modulus, and **(D)** elongation at break. Different letters for each sample represent values that are significantly ($p < 0.05$) different.

Fig. 5 – Solubility (columns) and moisture content (lines) of starch-based films containing different genipin concentrations (0; 0.05; and 0.10% w/V). Different letters for each sample represent values that are significantly ($p < 0.05$) different.

Tables

Table 1 – Water contact angle values of starch-based films gelatinized at different temperatures (75 °C and 95 °C) and with different genipin concentrations (0, 0.05, and 0.10% w/V). Different letters for each sample represent values that are significantly ($p < 0.05$) different

% Genipin	Gelatinization temperature	
	75 °C	95 °C
0	 67 ± 2^a	 47 ± 2^d
0.05	 99 ± 5^b	 120 ± 2^e
0.10	 116 ± 7^c	 127 ± 3^e