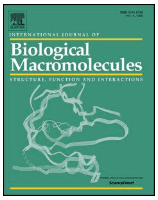
International Journal of Biological Macromolecules 329 (2025) 147804

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac



Combined effects of starch dry heat treatment (DHT) and chitosan blending on materials produced by extrusion

Thomás Corrêa Vianna [a,c], Bruna de Oliveira Gomes [a,b], Sylvain Foret [a], Patrick Perré [a], Osvaldo H. Campanella [a,c], Brahim Mazian [a], Pedro E.D. Augusto [a,*]

[a] *Université Paris-Saclay, CentraleSupélec, Laboratoire de Génie des Procédés et Matériaux, Centre Européen de Biotechnologie et de Bioéconomie (CEBB), 3 rue des Rouges Terres, 51110, Pomacle, France*

[b] *"Luiz de Queiroz" College of Agriculture (ESALQ), University of São Paulo (USP), Piracicaba, SP, Brazil*

[c] *Department of Food Science and Technology, Ohio State University, Columbus, OH, United States of America*

ARTICLE INFO

Keywords:
Bio-based materials
Starch
Chitosan
Blend
Dry heat treatment
Extrusion

ABSTRACT

Starch is a biodegradable polysaccharide with potential application as bio-based plastic. However, its utilization is limited due to poor mechanical properties and high-water affinity. This study evaluated strategies to address these limitations: (1) starch modification through dry heat treatment (DHT); (2) blending starch with chitosan; and (3) combining DHT-modified starch with chitosan. Unlike previous reports describing a two-step extrusion process for producing chitosan/starch materials, this study developed a single-step extrusion process, based on two feed streams - a dry (wheat starch and chitosan – up to 45 %) and a wet (water, glycerol and acetic acid) mixture. Experiments were conducted to determine the minimum amount of acetic acid required to solubilize chitosan, as well as the maximum chitosan concentration capable of forming a homogeneous blend with the starches. Results showed DHT enhanced tensile strength (TS) by 25 % and Young's modulus (YM) by 110 %, while reducing elongation at break (EB) by 25 % and water uptake by 40 %. Blending native starch with chitosan enhanced water-stability (reducing water uptake by 69 %) and improved YM by 370 %, but reduced TS and EB by 15 and 80 %, respectively. Combining DHT-modified starch with chitosan did not provide additional advantages compared to blending native-starch. These findings demonstrate DHT-modified starch is promising for enhancing mechanical properties and water stability, while blending native starch with chitosan enhances water stability, highlighting their potential as packaging.

1. Introduction

The concept of bioeconomy involves the industrial use of renewable resources, being the production of bio-based materials one sustainable alternative to substitute petroleum-based products [1,2]. Bio-based materials have a wide range of applications including food, and pharmaceutical packaging (especially biodegradable materials), biomedical devices, with potential impact in waste management, agriculture, and environmental sustainability [3].

Among the biobased polymers, starch stands out as one of the most promising for developing novel bio-based materials, due to its abundance, renewability, biodegradability, edibility, and low cost [4]. Although some starch-based materials are already being commercialized, challenges remain in their production and performance, particularly regarding their limited mechanical properties and high water affinity [5,6]. To overcome these drawbacks, two main strategies have been proposed in the literature: (1) starch modification [7–13], and (2) blending starch with other polymers [14–18]. However, the potential benefits of combining these two approaches remain underexplored.

Among starch modification methods, dry heat treatment (DHT) has received significant attention due to its simplicity and safety [19,20]. This physical modification of starch serves as an alternative to chemical approaches, offering advantages in cost, processing time, and reduced chemical usage [21]. DHT modifies the molecular size distribution of starch by partially cleaving the glycosidic linkages, which leads to a new intermolecular packing that enhances the material properties [22–25]. For instance, these structural changes have improved the mechanical properties of starch films derived from cassava, corn and millet when produced by casting [7,10,12].

In the context of starch-based blends, chitosan emerges as a bio-

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[*] Corresponding author.
E-mail address: pedro.augusto@centralesupelec.fr (P.E.D. Augusto).

https://doi.org/10.1016/j.ijbiomac.2025.147804
Received 29 July 2025; Received in revised form 6 September 2025; Accepted 18 September 2025

based, biodegradable, biocompatible and versatile polymer, with greater strength and lower hydrophilicity than starch [26,27]. Chitosan is a polysaccharide composed of *N*-acetyl D-glucosamine and D-glucosamine units, commercially obtained through the partial deacetylation of chitin, which is the second-most abundant polysaccharide on Earth after cellulose [28]. Chitin is primarily found in marine invertebrates and insects, as a major constituent of the exoskeleton, and it is often discarded as a waste [29,30].

Although previous research has demonstrated that incorporating chitosan can enhance the performance of starch-based materials [31–33], literature presents contradictory findings, with no clear consensus on the effects of chitosan on starch materials. For instance, Mendes et al. produced films with 5 % and 10 % (*w*/w) chitosan, reporting a decrease in tensile strength (TS) and Young modulus (YM), alongside an increase in elongation at break (EB) [34]. Conversely, Pelissari et al. observed no differences in TS nor in EB when producing films containing 2.5 % and 5 % chitosan, although YM was reduced [35]. Dang & Yoksan found that films containing 1.45 % chitosan exhibited higher TS and YM, but lower EB [32]. In contrast, Rodriguez Llanos et al., working with films containing 25 % and 50 % chitosan, reported decreases in TS, EB, and YM [36]. Additionally, all these studies required a two-step production process, involving the formation of a paste before extrusion. This extra processing step complicates industrial scalability. However, a simplified approach that incorporates the native forms of starch and chitosan in a single-step process has yet to be evaluated.

Finally, although materials have already been produced by blending native starches with chitosan, the use of modified starches still needs to be explored. To the best of our knowledge, this is the first time DHT-modified starch and chitosan are combined. Moreover, it is hypothesized that DHT modification would enhance the interaction between starch and chitosan, as the partial depolymerization caused by DHT increases the availability of functional groups, facilitating the formation of bonds and, thus, improving material performance.

Furthermore, this work aims not only to develop novel materials, but also to clarify the effects of chitosan on native starch across a higher range of concentrations. Moreover, to the best of our knowledge, this is also the first time in which starch and chitosan blends are produced using a single-step extrusion process, introducing innovations in both the use of materials and processing techniques. Therefore, this study explores three strategies to enhance starch-based materials: (1) starch modification by DHT; (2) chitosan blending with native starch across a large range of concentrations, and (3) combining DHT-modified starch with chitosan.

2. Materials and methods

This work combined two approaches to enhance the performance of starch-based materials: physical starch modification (dry heating treatment - DHT) and blending the native or modified starch with chitosan. Fig. 1 summarizes the workflow conducted. Initially, wheat starch was modified by DHT, following the parameters of a previous work [23]. Next, the materials were produced using a single-step extrusion process, employing native or modified starches blended with chitosan - resulting in a total of 12 treatments, and covering a range of polymer proportions unexplored in the literature. Subsequently, the materials were characterized in terms of their structure and composition, mechanical properties, and interaction with liquid water.

2.1. Raw materials

The materials used in the study include wheat starch (11 % of moisture, 0.3 % ash, 0.3 % protein, kindly donated by ADM Starch, Bazancourt, France); and chitosan (99 % purity, 9 % of moisture, Jiangsu Aoxin Biotechnology Co., Ltd., China) with a deacetylation degree ≥90.0 %. Glycerol (98 % purity, Fisher Scientific) and acetic acid (99.5 % purity, Sigma-Aldrich) were used as plasticizer and solvent agent for chitosan, respectively.

2.2. Materials processing

2.2.1. Starch modification through dry heat treatment (DHT)

Starch modification was conducted following the methodology reported by [23]. Sixty grams (60 g) of wheat starch were placed into

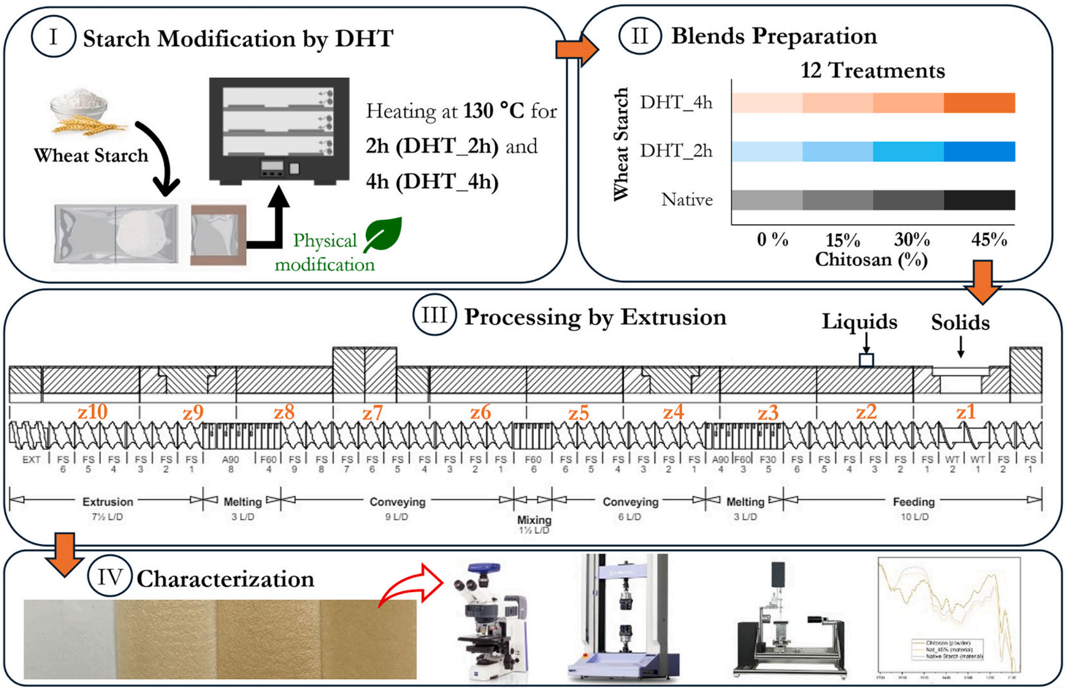

Fig. 1. Illustrative scheme of methodology. Step I – modification of wheat starch by DHT; Step II – Preparation of starch/chitosan blends; Step III – Processing the blends by extrusion; Step IV – Characterization.

aluminum foil envelops (30 × 30 cm) to be heated at 130 °C for 2 or 4 h in a hot-air convective oven (Memmert, IN 260, Germany). Subsequently, the samples were cooled at room temperature, ground and sieved (<250 μm). The dry heated starch for 2 h (DHT_2h, with moisture content of 5.07 g/100 g after preparation) and 4 h (DHT_4h, with moisture content of 4.54 g/100 g after preparation) were packed and stored at room temperature for further analysis. A comprehensive characterization of the obtained starches, including granule characterization (morphology and size distribution), molecular characterization (pH, functional groups, molecular size distribution), and different properties (thermal properties, crystalline profile, pasting and hydrogel rheology and firmness) is presented by [23]. Briefly, the wheat starch modified by DHT at 130 °C for 2 or 4 h present an increase in granule size, partial molecular depolymerization and no formation of carbonyl nor carboxyl groups, with relevant impact on properties - lower peak apparent viscosity during pasting, higher structural strength at rest, higher resistance to external stresses, higher gel firmness, and lower hydrogel syneresis. The new structures and properties of DHT-modified starch reinforces the relevance of studying it in materials production, in special interacting with other biopolymers – as here proposed.

2.2.2. *Materials preparation by extrusion*

Native and modified starches with varying chitosan concentrations were processed using a twin-screw extruder (Haake Polylab Rheomex OS PTW16, D = 16 mm, L/D = 40) equipped with a gravimetric feeder. The screw configuration is illustrated in Fig. 1. This one-step protocol differs from two-step methods found in the literature, which involve a preliminary step to form a paste prior to extrusion [32,34–36].

In the present study both solids (starches and chitosan) were blended and fed together in the extruder's zone 1, while the liquid phase (a solution of glycerol and acetic acid into water) were fed in zone 2 (Fig. 1).

Preliminary runs, based on ranges reported in the literature, were conducted to determine the minimum amount of acetic acid required to solubilize chitosan. Acid solutions containing 1 %, 2 %, and 3 % of acetic acid were evaluated, with 3 % being selected as the optimal concentration. Solutions containing 1–2 % acetic acid formed a two-phase system, where chitosan remained agglomerated within the starch phase instead of dissolving completely.

Subsequently, experiments were performed to identify the maximum chitosan concentration capable of forming a homogeneous blend with the starches. After evaluating blends with 0, 15, 30, 45, 60, and 75 % of chitosan (*w*/w), extrudates with shark-skin defects were observed for concentrations above 45 % (Table 1) – demonstrating an upper limiting value. Therefore, samples were produced with proportions of chitosan/starch (native or modified) of 0/100 %, 15/85 %, 30/70 %, and 45/55 % (w/w; reported in the following sections by the chitosan concentration in the solid phase).

Table 1
Qualitative results of the preliminary runs with native starch and chitosan.

Chitosan concentration (%)	Is it possible to produce?	Is the material homogeneous?	Associated problem:
0	✓	✓	N/A
15	✓	✓	N/A
30	✓	✓	N/A
45	✓	✓	N/A
60	✓	✗	Corrugated structure.
75	✗	N/A	Disintegrated material.

N/A indicates not applicable.

It is essential to highlight that a solid:liquid ratio of 57:43 was kept constant for all experiments, with only the starch-to-chitosan proportion being adjusted. While the polymer phase consisted of starch–chitosan blends (0–45 % chitosan, w/w), the liquid phase varied depending on the formulation: for starch-only formulations, it consisted of a mixture of glycerol (62 %) and water (38 %), as proposed by [12]. For blends containing chitosan, the liquid phase was composed of glycerol (62 %) and an aqueous acetic acid solution (38 %), in which acetic acid accounted for 3 % of the aqueous content.

The solid blend was fed into the first zone of the extruder at a flow rate of 0.35 kg/h (dry basis, i.e., considering the moisture of both starch and chitosan, including the changes due to DHT process). The liquid mixture was introduced into the second zone via a peristaltic pump at 0.230 L/h. The samples were extruded with the temperature profile reported by [18] (60, 75, 90, 100, 110, 110, 110, 110, 105, 100) °C, at a screw speed of 150 rpm.

Processing data including torque and pressure were recorded.

2.3. *Materials characterization*

2.3.1. *Composition, thickness, density and surface morphology*

The material thickness was measured using a digital caliper (Tolland, Belgium) at nine randomly selected points of three samples [37]. Specimens measuring 30 mm × 20 mm were conditioned for at least 5 days at 25 °C and 50 % relative humidity. Their length and width were also measured in triplicate using the same caliper, and the volume calculated as the product of the 3 average values. Mass measurements were performed using an electronic balance (New Classic MS, Mettler-Toledo, Columbus, Ohio, USA) with a precision of 0.1 mg. The density was then calculated by dividing the mass by the calculated volume.

Fourier transform infrared (FT-IR) spectrometry (Agilent, 630 FTIR, Malaysia) was performed to assess the presence and interaction of molecular functional groups. The spectra were recorded in the range of 4000–650 cm^{-1}, with a resolution of 4 cm^{-1}, 128 scans per point, and using the attenuated total reflection (ATR) module.

Surface roughness was assessed using a profile-analyzing Laser Microscope (Keyence, VK-H1XME, United States) at 20× magnification. This instrument measures the arithmetical mean height (Sa), which represents the average of the absolute value of height deviations from the mean plane.

2.3.2. *Mechanical properties*

Prior to mechanical testing, the samples were conditioned for at least five days at 25 °C and 50 % relative humidity. Tensile testing (ISO 527-3:2018) was conducted on Type 1B samples, the standard format for the test specimens, using a universal testing machine (Shimadzu, AGS-X 100®, Japan) at a displacement speed of 5 mm/min until fracture.

True strain (ε_{true}) and true stress (σ_{true}) were calculated following Eqs. (1) and (2), using the engineering strain (ε_{eng}) and engineering stress (σ_{eng}) data, assuming incompressibility of the sample, i.e., its volume remained constant during the test. Tensile strength (TS) was determined as the maximum stress the material could withstand before failure, whereas Young's modulus (YM) was calculated from the slope of the initial linear region of the true stress–strain curve. The linear region was defined by selecting the portion of the curve where the coefficient of determination (R^2) of the linear fit was approximately 0.99, with data selected up to a maximum strain of 1 %. For selected samples, both engineering and true stress-strain curves were plotted to illustrate the impact of the calculation method. Each material was evaluated at least nine times to evaluate reproducibility.

$$\varepsilon_{true} = ln\left(1 + \varepsilon_{eng}\right) \tag{1}$$

$$\sigma_{true} = \sigma_{eng}\left(1 + \varepsilon_{eng}\right) \tag{2}$$

2.3.3. *Material interaction with liquid water*

2.3.3.1. Water uptake after immersion. Liquid water absorption was carried out in triplicate according to the method described by [38]. Thin specimens with dimensions of 75 mm × 25 mm were conditioned for four days at 50 °C in an oven (Memmert, Model 100–800, Germany) and then immersed in water at room temperature for up to 72 h. Masses and dimensions of the samples were taken after several immersion periods (t = 0.5, 1, 2, 3, 4, 5, 24, 48, and 72 h) at 20 °C. Sample weights were measured, after removing excess surface water with a dry paper towel, using a balance (New Classic MS, Mettler-Toledo, Columbus, Ohio, USA; precision of 0.1 mg). Due to the small sample thickness, volume swelling measurements were unreliable. Instead, surface area changes were evaluated by measuring both width and length using a digital caliper (Tolland, Belgium). The relative change in weight and surface area were calculated according to Eqs. (3) and (4), respectively.

$$Water\ uptake\ (\%) = \frac{(M_t - M_0)}{M_0}\ (\times)\ 100 \quad (3)$$

$$Area\ change\ (\%) = \frac{(A_t - A_0)}{A_0}\ (\times)\ 100 \quad (4)$$

where M_t and A_t represent the mass and surface area values at time t, and M_0 and A_0 the initial mass and surface area of the samples, respectively, after drying at 50 °C.

2.3.3.2. Surface wettability. The wettability of the sample surface was evaluated at ambient temperature using the sessile drop method with a Digidrop device (GBX Scientific LTD, Dublin, Ireland). Deionized water droplets with a volume of 10 μL were deposited on the sample surface using an automatic micro syringe. Contact angle measurements were automatically determined using the Visiodrop software (Version 1.02.01 GB) at the left and right boundaries, 1 s after deposition. The analysis was conducted in triplicate.

The correlation between the contact angle and the arithmetical mean height was assessed through Pearson correlation using Microsoft Excel®. A power trendline was also added, along with its equation describing the relationship between the two variables.

To specifically analyze interactions between the sample surface and liquid water, 10 μL of deionized water was placed in contact with the sample surface for 5 min and then removed using a dry paper towel. Reflectance images were captured using the Apotome.2 Microscope (ZEISS, AXIO Zoom.V16, France) at 10× magnification.

2.3.4. *Experimental design and statistical evaluation*

This work comprised 12 independent treatments. The sample containing native starch (i.e., no blending, no modification) was considered as the control. All experiments were conducted in triplicates ($n = 3$) for all measurements, except for the mechanical properties, which were tested with a minimum of nine replicates ($n = 9$). The results were statistically analyzed using analysis of variance (ANOVA) followed Tukey's test in Origin® software to compare mean values, with significance set at $p < 0.05$.

3. Results and discussion

3.1. *Visual appearance, thickness, and density characteristics of the samples*

Fig. 2 presents images of all the produced samples, highlighting the effects of starch modification and chitosan incorporation on their appearance. Samples prepared with only starch appeared transparent, whereas the addition of chitosan led to a gradual change in color and opacity - the samples became increasingly brown and opaque as the chitosan content increased. This color change is primarily attributed to the intrinsic color of chitosan, which naturally ranges from off-white to light brown, depending on its degree of deacetylation and molecular weight [39–41].

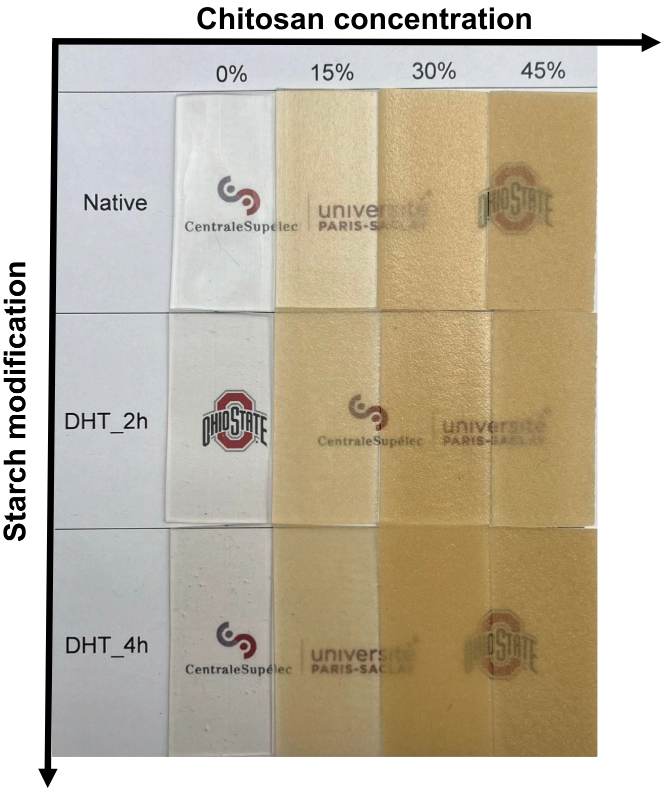

Fig. 2. Images of native and modified starch (DHT process at 130 °C for 2 or 4 h) materials added with different concentrations of chitosan (0–45 %).

Thickness and density of the materials are illustrated in Fig. 3. For samples prepared with pure starch, the density is not affected by the DHT treatment, while the thickness is slightly reduced, likely due to the formation of entanglements and chain rearrangement occurring during the heating [42], promoting less expansion. For materials containing chitosan, both thickness and density decreased as the chitosan content increased. This trend may be attributed to the intrinsically lower bulk density of chitosan compared to wheat starch [43,44], leading to less dense blends.

The simultaneous reduction in density and thickness, if achieved without compromising material properties, offers advantages for packaging applications [45]. A lower density translates to lighter materials, which contributes to reduced material costs and lower environmental impact, minimized post-consumer waste, and a decreased manufacturing and logistics processes carbon footprint. Further characterization of the samples obtained was then carried out.

3.2. *Molecular changes and interactions*

FT-IR analysis was conducted to investigate potential molecular interactions between starch and chitosan. Fig. 4 shows the FT-IR spectra for the raw powders and the samples. All samples exhibited characteristic peaks in the region of 3300–3200 cm^{-1}, corresponding to O—H stretching vibrations [46], which overlap with N—H stretching in the same region [33]; 2900–2850 cm^{-1}, associated with C—H stretching [47]; 1650–1600 cm^{-1}, related to δ (O—H) bending from absorbed water; 1412–1392 cm^{-1}, assigned to C—H bending and wagging; and 1144–989 cm^{-1}, corresponding to C—O stretching vibrations [48]. The spectra of pure chitosan also presented a typical amide I (C=O) stretching at 1651 cm^{-1}, and N—H bending at 1578 cm^{-1} (amide II) [4].

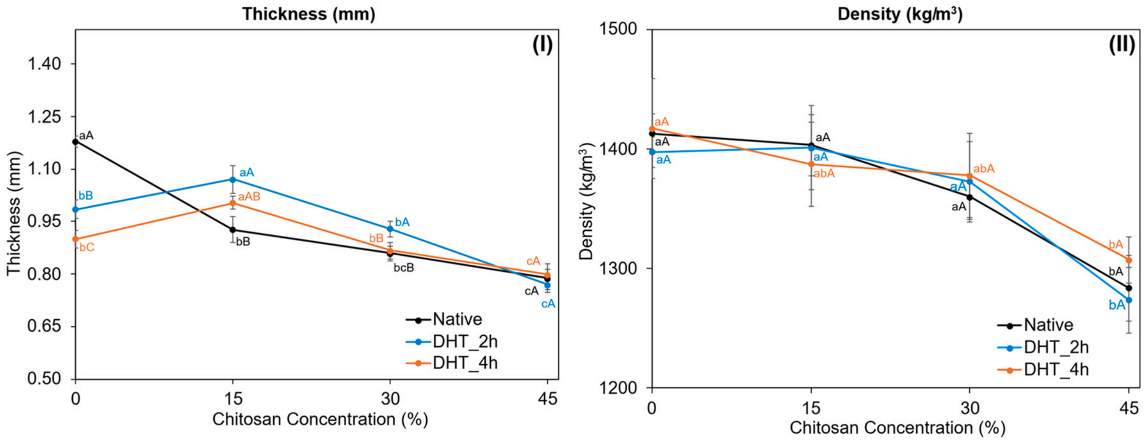

Fig. 3. Thickness (mm) and density (kg/m3) of native and modified starch (DHT process at 130 °C for 2 or 4 h) materials with different concentrations of chitosan. Statistical differences ($p < 0.05$) are marked by different lowercase letters (abc – chitosan concentration effect) and capital letters (ABC – starch modification effect). Error bars indicate the standard deviation.

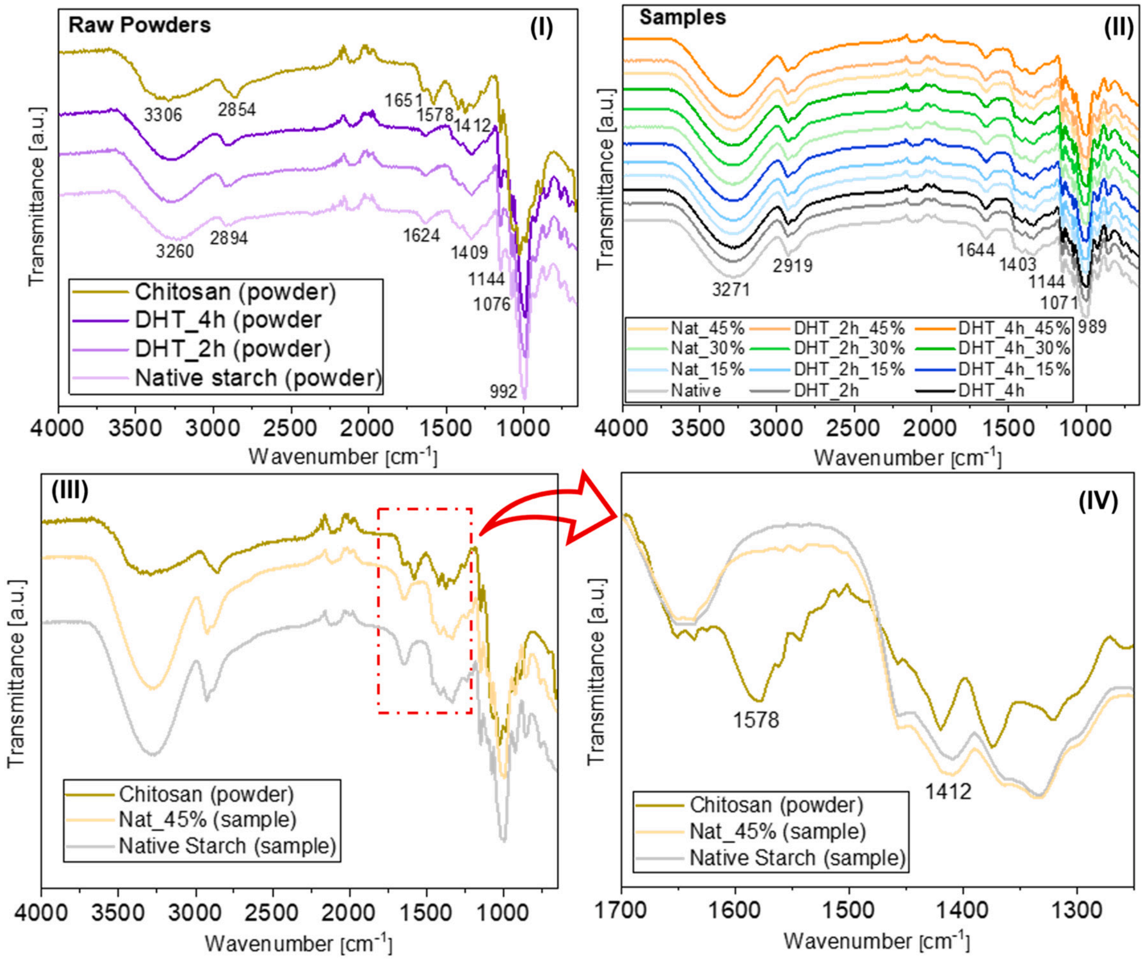

Fig. 4. FT-IR spectra of raw powders (I), samples produced using native and modified starch (DHT process at 130 °C for 2 or 4 h) with different concentrations of chitosan (II), and in the region of 1700–1250 cm^{-1} of chitosan powder, native starch material, and the blend with native starch and 45 % of chitosan (III and IV).

When comparing the samples spectra with those of the native and DHT-modified starches, no significant differences were observed. Similar findings have been reported for cassava [12] and wheat [23] starches, indicating that the changes in starch molecules were not significant enough to be detected by FT-IR analysis.

Fig. 4.III, compares the spectra of chitosan powder, native starch, and the sample containing 45 % chitosan. These samples were selected to assess pattern changes in the samples with the highest chitosan concentration, and to compare it with both native starch and chitosan powder in the region of 1700–1250 cm^{-1}, where typical amide peaks are expected [4,31,33]. It is observed that, in the region of 1578 cm^{-1} (N—H bending), the spectrum for the sample containing chitosan differs from

that of the native starch. [4,33] also noted changes in this region after chitosan addition in starch films produced by casting, which was attributed to the formation of bonds between the hydroxyl groups of starch and the amino groups of chitosan. Moreover, a reaction between glycerol and acetic acid may have displaced bound acetic acid from chitosan, increasing –NH₂ groups available for hydrogen bonding with starch [49].

3.3. *Processing parameters*

Fig. 5 reports the processing parameters (torque and pressure) measured during the extrusion of native and modified starch materials with different chitosan concentrations. Overall, all samples had a smooth extrusion process, with torque ranging from 25 to 35 N-m and die pressure in the range of 3.5 to 7 bars.

At 0 % chitosan, the extruded DHT starch required higher torque and higher die pressure than native starch. It is interesting to notice that DHT modifies starch molecular size distribution and promotes granule expansion, resulting in the formation of entanglements between the polymer molecules [42]. Those structural modifications can potentially increase the viscosity of the melt, resulting in reduced flowability in the extruder. The higher torque is consistent with reports of increased firmness in gels prepared with DHT-modified cassava [22] and wheat [23] starches, which is an indication of the effects of stronger molecular interactions.

Adding chitosan to native starch had no effect on either pressure or torque, regardless of the chitosan concentration. This result is consistent with those obtained by [32], who observed no change in torque following the addition of chitosan during compounding extrusion. However, incorporating chitosan into dry-heated starch reduced both torque and pressure, reducing the difference between native and DHT starch. The new starch molecular and granular structure [23], associated with chitosan, acetic acid and glycerol, probably decrease the extrudate melt viscosity, facilitating flowability. While the exact mechanisms of melting and molecular interaction across the extruder and in the final material still need to be understood, the proposed approach of combining DHT-modified starch and chitosan can also be seen as a possibility of facilitating the extrusion process. Further studies are needed to explore both points.

Among recent studies investigating the effects of chitosan in starch materials produced by extrusion [32,34–36], only [32] presented processing data. Therefore, it is not possible to evaluate the effects of processing parameters on the product properties. This hampers the development of models that are crucial for both industrial applications and optimization of the materials produced – and they are suggested as future studies.

3.4. *Mechanical properties*

Fig. 6 shows tensile strength (TS), elongation at break (EB) and Young modulus (YM) obtained for native and DHT-modified starches blended with chitosan in a range of concentrations from 0 to 45 % (*w/*w). Since three different strategies were applied to enhance the performance of starch-based materials (starch modification, chitosan blending, and the combination of both), the impact of each strategy needs individual analysis.

Modifying starch through DHT increased the material's stiffness (YM) and strength (TS), but reduced maximum elongation (EB), with negligible differences among the two conditions of DHT. Briefly, DHT doubled the YM, and increased by 25 % the TS, while reducing EB only by 25–30 % (Fig. 6). It is worth mentioning that a simple physical, solvent-free and considered sustainable technique of starch modification [19] can significantly increase the mechanical properties of bio-based plastics.

This improvement in mechanical performance can be attributed to the partial depolymerization of wheat starch induced during dry heating, which changes the molecular size distribution and allows better molecular re-association and packing [23]. Furthermore, smaller molecular chains present higher mobility, increasing retrogradation tendency, and, therefore, potentially resulting in a more robust three-dimensional network structure [23,50]. Similarly, DHT was reported to enhance the mechanical properties of films from cassava [12], millet [7] and corn [10] starches.

It is important to note that depending on the starch source and processing conditions, DHT can also promote the oxidation of starch molecules, producing carbonyl and/or carboxyl groups, such as reported in works with cassava [22] and maize/corn [51]. These new functional groups can establish stronger hydrogen bonds with the hydroxyl groups of starch, thereby contributing to the enhancement of films mechanical properties [52]. However, those groups were not observed for wheat starch modifications in the DHT conditions applied here [23]. Therefore, the change of molecular size distribution is the main explanation for the enhancement of mechanical properties.

Regarding the effect of chitosan on materials containing native starch, an overall increase in YM is observed, accompanied by a reduction in TS and EB: adding chitosan makes the materials more rigid,

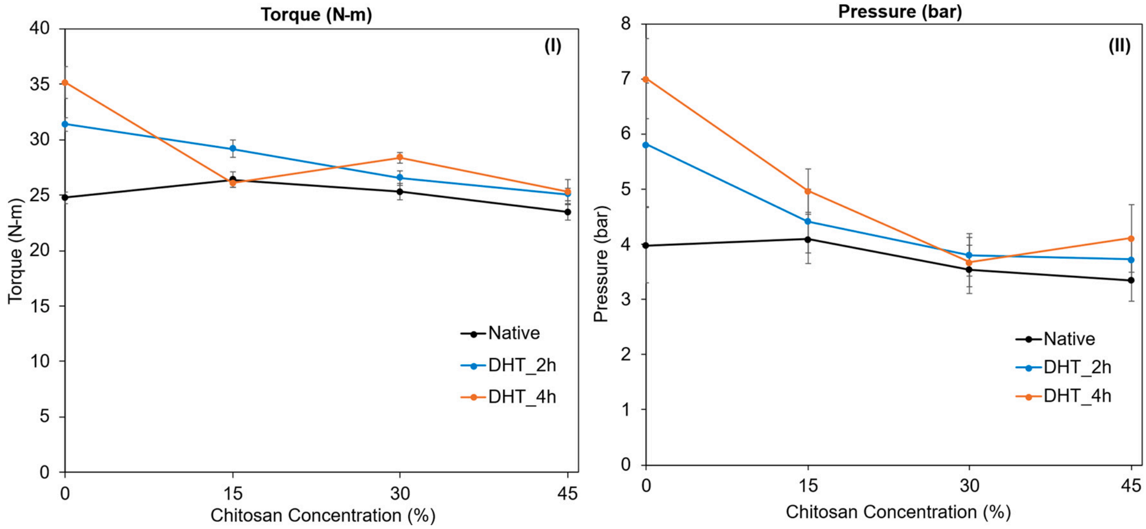

Fig. 5. Processing extrusion parameters – Torque (N-m) (I) and die pressure (bar) (II) - collected during the production of the samples using native and modified starch (DHT processed at 130 °C for 2 or 4 h) and different concentrations of chitosan in the range of 0–45 %. Error bars indicate the standard deviation.

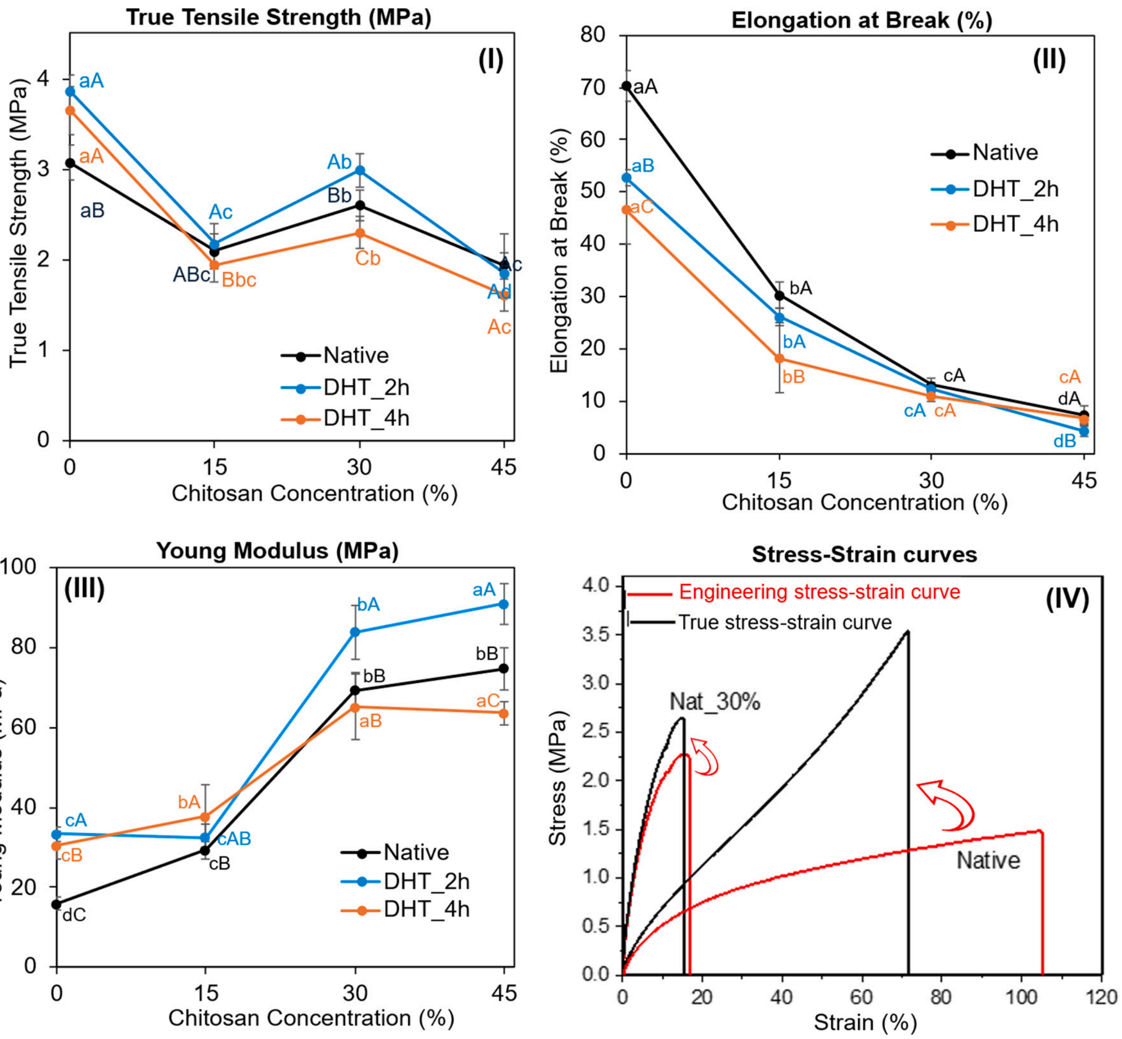

Fig. 6. Tensile Strength (MPa) (I), Elongation at Break (%) (II), Young's Modulus (MPa) (III) for native and modified (DHT process at 130 °C for 2 or 4 h) starch material added with different concentrations of chitosan. In (IV), a comparison between the engineering (in red) and the true (in black) stress-strain curves for native and nat_30% materials. Statistical differences ($p < 0.05$) are marked by different lowercase letters (abc – effect of chitosan concentration) and capital letters (ABC – effect of starch modification). Error bars indicate the standard deviation.

but at the same time weaker and less flexible. Interestingly, this conclusion arises after converting engineering stress-strain data to true-stress strain data, i.e., accounting for changes in the cross-sectional area during deformation, assuming constant volume. While the engineering approach is a conservative method that tends to underestimate strain hardening despite significant plastic deformation, the true stress-strain curve more accurately represents the material response in both the pre and post plastic localization regimes and becomes important when large deformations are considered [53,54]. Fig. 6 IV presents both the engineering (in red) and the true (in black) stress-strain curves of materials produced using native starch and the blend with 30 % of chitosan, which was the native starch blend with the highest TS on the engineering stress-strain analysis. Since the native starch material presented high elongation (greater than 100 %), the conversion increased TS, but also alters the interpretation of chitosan's effect. Chitosan-blended materials had much lower elongation; thus, the conversion did not change their results considerably. It is important to note that most studies rely on stress-strain engineering data, but applying this conversion is essential, especially for flexible packaging materials.

The only study reporting greater TS after chitosan incorporation into starch-based materials via extrusion used very low chitosan concentrations (up to 1.45 % *w*/w) [32]. In contrast, [34] developed films with up to 10 % (*w*/w) chitosan, and [36] with up to 50 % (w/w); extrusion was employed in both studies and lower TS values compared to the control were reported, regardless of the concentration. They attributed this behavior to the presence of smashed chitosan granules not melted [36]. Differently, in our case, materials with up to 45 % (w/w) chitosan appeared homogenous, and just higher concentrations – 60 % and 75 % (see Table 1) – showed signs of phase separation. The reduction in TS may be explained by chitosan disrupting starch-starch interactions, without forming stronger bonds with starch.

Furthermore, when combining dry-heated starch with chitosan, a similar pattern to that of native starch-chitosan blends is observed. Notably, similar FT-IR spectra were observed between blends with native and DHT-modified starch (Fig. 4), suggesting that chitosan interacts with starch in the same way in both cases. Therefore, the addition of chitosan outperformed the effect of starch modification.

3.5. Interaction with liquid water

3.5.1. Water uptake and swelling after water immersion

Fig. 7 shows water uptake (I) and surface swelling (II) over time, as well as the maximum water uptake (III) and maximum surface area change (IV) at 24 h of water immersion. The native starch material exhibited the greatest water interaction, showing approximately a 210 % increase in mass and a 140 % increase in surface area after 24 h of

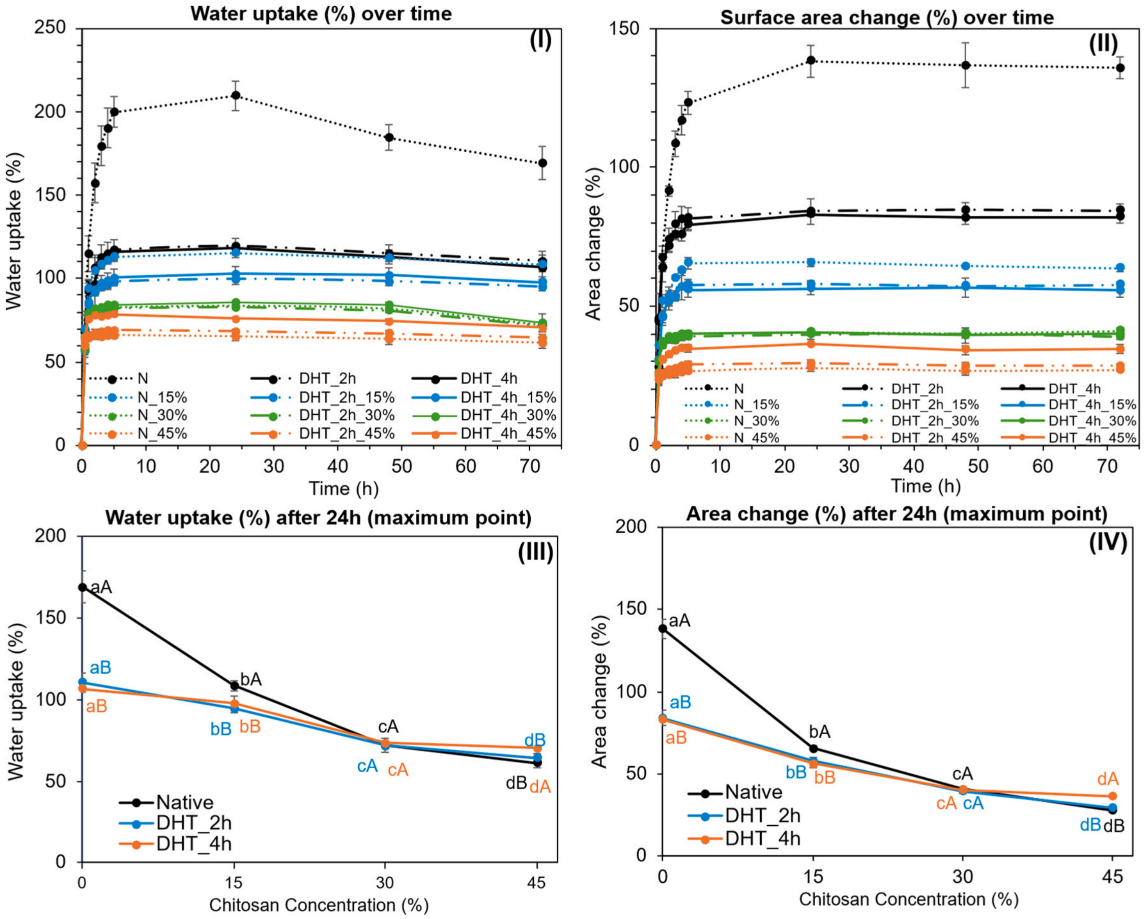

Fig. 7. Time-dependent changes of water uptake (%) (I) and surface area change (%) (II) as well as water uptake and area change after 24 h immersion (maximum point); for native and modified starch (DHT at 130 °C for 2 or 4 h) samples blended with different concentrations of chitosan. For (III) and (IV), statistical differences ($p < 0.05$) are marked by different lowercase letters (abc – effect of chitosan concentration) and capital letters (ABC – effect of starch modification). Error bars indicate the standard deviation.

immersion. An interesting observation in the kinetics curves – Fig. 7.III and .IV – is that after 24 h of water immersion, the mass of the native starch material began to decrease, indicating partial disintegration, whereas the samples containing DHT-modified starch and all the chitosan-blended materials maintained a constant mass, demonstrating improved structural stability in aqueous environments.

Starch is a hydrophilic polysaccharide with several available hydroxyl groups, allowing easy water penetration and consequently weakening inner hydrogen bonds, which can affect the functionality of starch-based materials [55,56]. In this regard, both strategies applied in this work effectively enhance the water stability of starch-based materials.

Compared to native starch, DHT modification reduces the mass change, and the surface area change after water immersion by 43 and 39 %, respectively. These reductions occur regardless of the duration of the DHT treatment, suggesting that 2 h are already sufficient for heat-induced alteration of starch's internal structure, promoting entanglements between polymer molecules [7,42,57]. To the best of our knowledge, there are no other reports in the literature presenting the swelling behavior of DHT-modified starch materials. However, similar reductions in swelling power have been observed in raw starch powder from millet [7], and cassava [58] following DHT treatment.

Incorporating chitosan into native starch materials also enhances the dimensional stability in water, as evidenced by reductions in mass and surface area changes after water immersion. This effect increases with the chitosan content. Although both starch and chitosan are hydrophilic and retain considerable amounts of water, the interaction between water molecules and hydroxyl groups in starch is stronger than with amine groups in chitosan [59]. This explains why swelling is reduced upon chitosan incorporation.

In terms of water uptake, the result achieved by just modifying starch through DHT was comparable to that of blending native starch with 15 % chitosan, both showing around 44 % reduction. Blending native starch with higher chitosan concentrations - 30 % and 45 % - led to an even higher decrease of 60 % and 69 % in water uptake, along with 70 % and 79 % reduction in surface area, respectively. Similar results were reported by [60], that prepared chitosan/starch films by casting in a 1:1 ratio and observed a water uptake ratio of 66.31 %, comparable to the results obtained with 45 % (*w*/w) chitosan.

When chitosan is blended with DHT-modified starch, the effect of chitosan becomes dominant, overriding the influence of DHT alone. The same trend was observed for mechanical properties, suggesting that chitosan interacts similarly with both native and modified starch. This is also confirmed by FT-IR (Fig. 4), where no differences are observed in the spectra of chitosan blends with native and modified starch.

3.5.2. *Surface wettability*

Fig. 8.I shows contact angle for water and materials made with native and modified starch blended with different concentrations of chitosan. As expected, all samples have a contact angle below 90°, indicating a hydrophilic surface.

The sample prepared with native starch exhibits an average contact angle of 47.4°, consistent with the values reported in the literature for native starch films from different sources [12,18,36,61]. However,

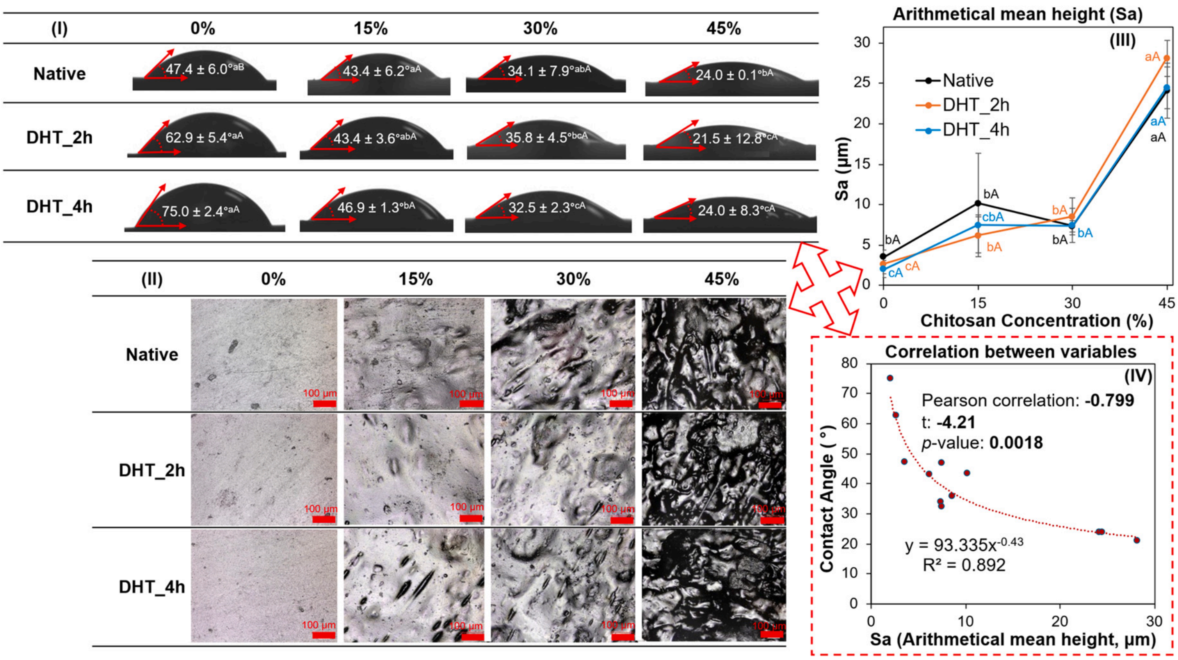

Fig. 8. (I) Contact Angles (°) of native and modified starch (DHT process at 130 °C for 2 or 4 h) samples containing different amounts of chitosan; (II) Surface Images (20×) of all samples, (III) arithmetical mean height of all samples and (IV) statistical correlation between contact angle and arithmetical mean height. For (I) and (III) mean values marked with different superscript lowercase letters indicate statistically significant differences ($p < 0.05$) along the column, while different capital letters indicate statistically significant differences ($p < 0.05$) along the line. In (I) values are represented as mean ± standard deviation, while in (III) error bars indicate the standard deviation.

samples composed solely of DHT treated starch exhibited a higher contact angle, which shifted from 47.4° to 62.9° (for DHT_2h) and 75° (for DHT_4h). This suggests that DHT starch modification alters the wettability of the sample's surface, making it less hydrophilic. A similar effect has been observed for dry-heated-cassava-starch films [12] and for dry-heated-waxy corn starch [62], both produced by casting - in line with our results and hypotheses. This behavior was attributed to the new molecular arrangement resulting from the DHT treatment, which could lead to a decrease in surface hydrophilicity [12].

Adding chitosan reduced the contact angle for all types of starch, with higher chitosan content leading to progressively lower contact angle values. This contrasts with the behavior observed in previous studies where chitosan-starch based films were prepared by casting [47,63] and extrusion in a two-step process [36,61]. Although chitosan was expected to reduce hydrophilicity, it is essential to consider that contact angle measurements can be influenced by several factors, including surface roughness, contamination, deformation and sample geometry [64].

In this work, the surface roughness of the chitosan-containing samples played a critical role in reducing the contact angle. As shown in Fig. 8.II, samples prepared with native and DHT modified starches exhibit relatively smooth and homogeneous surfaces. However, incorporating chitosan increased the surface roughness, which is also confirmed by the higher arithmetical mean height of the surface as the chitosan concentration rises (Fig. 8.III). The correlation between contact angle and arithmetical mean height was evaluated using Pearson's correlation coefficient (Fig. 8 IV), which ranges from −1 to +1. A significant negative correlation of −0.799 was observed, reaffirming the effect of surface roughness on the measured contact angle. As the relation between variables was non-linear, a power-law model was fitted to describe it. On hydrophilic rough surfaces, the liquid can penetrate surface grooves, resulting in contact angles lower than the actual value [64]. Therefore, the measure contact angle may be influenced by the surface geometry rather than the intrinsic hydrophilicity or hydrophobicity of the bulk sample.

Furthermore, to confirm that the decrease in contact angle values was primarily due to the surface geometry rather than a reduction in the material's hydrophilicity, a water droplet test was performed. A 10 μL water droplet was placed on the material's surface, left for 5 min, and then removed using a paper towel to assess any water induced damage. As shown in Fig. 9, materials without chitosan were more affected by water, showing visible signs of weak points and structural deformation upon water exposure. Conversely, samples containing chitosan displayed reduced damage, with higher chitosan content providing greater water resistance. These results corroborate the findings from the water immersion analysis, further supporting the hypothesis that chitosan enhances the water resistance of starch-based materials by limiting water penetration and preserving structural integrity.

3.6. *General discussion on modification strategies*

This section jointly analyses mechanical properties and liquid-water interaction results, comparing them with existing approaches in literature. The goal is to assess the effectiveness of the strategies applied here to improve the performance of starch-based materials. The best results of the present study are highlighted in red in Fig. 10. For mechanical properties, the optimal modification condition was DHT_2h, while the chosen blend with the best results was chitosan/starch 30 % (*w*/w). For liquid water interaction, the optimal blend was chitosan/starch 45 % (w/w), and the best modification condition remained DHT_2h. The best results from the literature are also reported in the same figure, for comparison purposes, despite the differences in composition and process.

A key point to mention is that the properties of starch-based materials are strongly influenced by the starch source. To compare the mechanical properties of modified-starch samples, Fig. 10.I includes results from physical methods, such as DHT, heat moisture treatment (HMT), and annealing, as well as chemical methods, including ozonation and traditional oxidation processes. The results are influenced by several factors, such as processing conditions, starch source, and processing

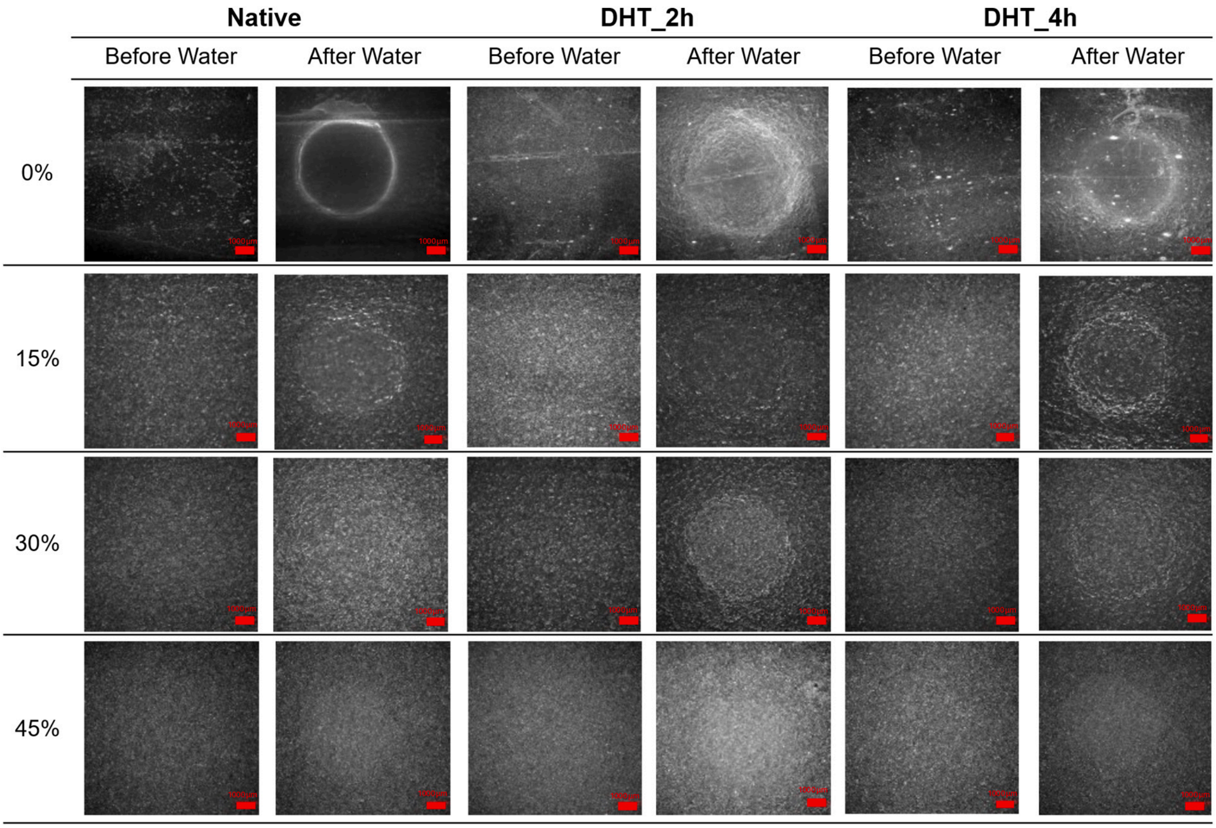

Fig. 9. Surface images (10× magnification) of all samples before and 5 min after the addition of a 10 μL of water droplet on their surface.

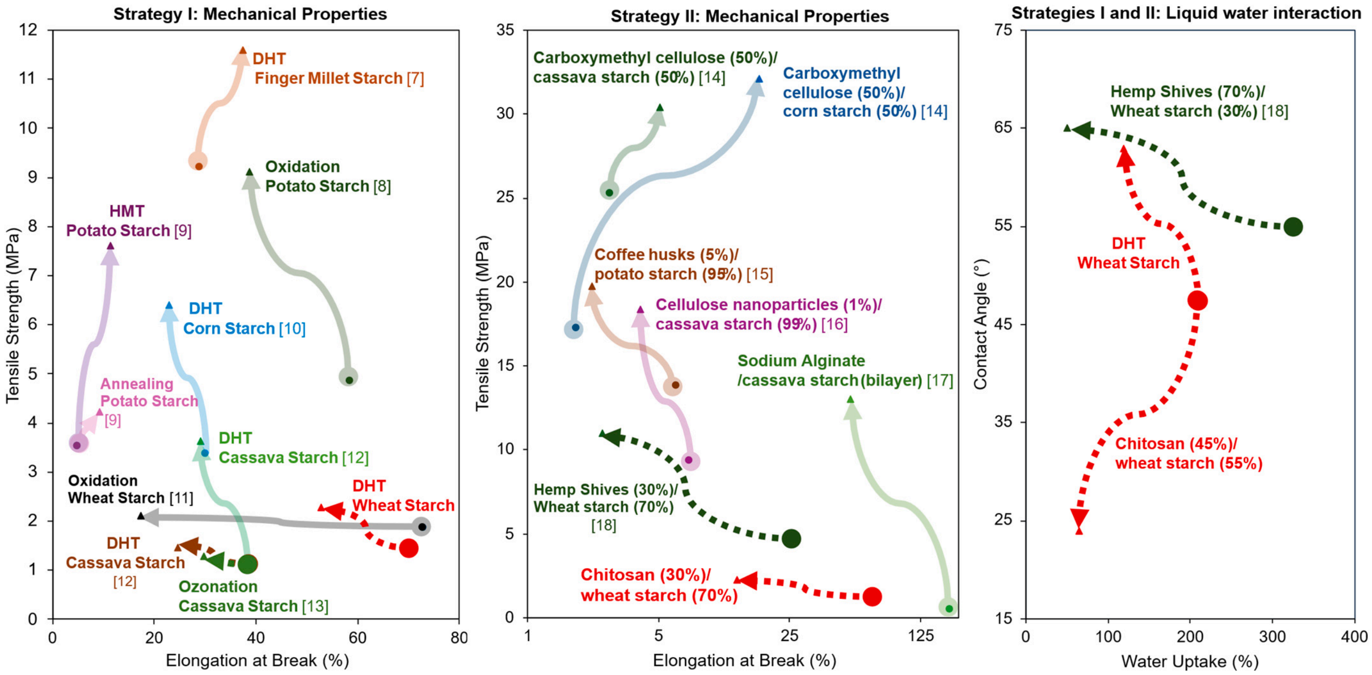

Fig. 10. Comparison of strategies for mechanical properties (I): starch modification techniques and (II) starch blends; and liquid water interaction (III: both strategies). Results from this study are highlighted in red. Continuous lines represent materials produced by casting, and dashed lines represent those produced by extrusion. Arrows indicate changes from control to treated materials highlighting the best results from each study for each property and illustrating the effects of blending and modification techniques.

techniques.

For instance, DHT generally enhances TS of starch-based materials, but this enhancement is generally more pronounced in casting-based materials, when compared to those produced via extrusion. This difference can be attributed to the distinct processing and drying parameters of both techniques. Unlike casting, which occurs under milder conditions (higher water content and lower temperatures), extrusion typically operates under severe processing conditions (lower water content and higher temperatures). Under these extreme processing conditions, biopolymers behave like polymeric melts, and the material transformation does not follow classical mechanisms, since there is a thermal energy input defined by the time-temperature history [65]. During extrusion, starch granules rapidly absorb water and swell, leading to starch gelatinization. However, after extrusion, melted starch molecules could be re-associated into double helices during cooling, leading to starch retrogradation [66]. Therefore, starch undergoes a transition between order and disorder structures [66]. Moreover, during extrusion, materials are quickly dried at high temperature due to the water flashing at the extruder exit. Conversely during casting, materials are dried slowly, which may promote better recrystallization in the matrix, which is further enhanced by DHT treatment [12].

The conditions applied during DHT treatment also impact on the results. For example, [12] observed superior mechanical properties with DHT processed cassava starch at 130 °C for 4 h, when compared to 2 h. Interestingly, in the current study and using the same temperature, the materials treated with DHT for 2 h exhibited higher or equivalent mechanical properties to those treated for 4 h (see Fig. 6). This suggests that for wheat starch, a 4-h treatment is unnecessary, as it demands higher energy without providing additional benefits.

Heat Moisture Treatment (HMT) has also shown promising results, improving both TS and EB by disrupting the starch crystalline structure and strengthening amylose-amylopectin interactions [9,67]. However, to the best of our knowledge, no studies have reported HMT-starch materials produced via extrusion, where these improvements may not be as pronounced as those observed with casting. In contrast, chemical methods have yielded mixed outcomes: oxidation with sodium hypochlorite improved TS in potato starch [8], but left TS unchanged and drastically reduced EB in wheat starch [11]. Ozonated cassava starch-based materials produced by extrusion showed no significant differences to the control [13]. Among all these methods, DHT stands out as one of the most effective and simplest techniques for enhancing starch-based materials.

Another strategy involves promoting blends of starch with other polymers or even using solid particles as fillers (for example, biomass made from agro-industrial by-products). In this case, the final performance largely depends on the compatibility between starch and the selected polymer or particle. As shown in Fig. 10.II, several polymers and by-products have enhanced TS of starch-based materials produced by both casting and extrusion. In comparison with results presented in this study, while the addition of 30 % (*w*/w) chitosan decreased TS by 15 %, other options might be more economically viable for applications such as packaging. For instance, incorporating by-products like coffee husks [15] and hemp shives [18] improved TS by 42 % and 120 %, respectively, while being less expensive and more widely available than chitosan. Other fillers, such as carboxymethyl cellulose [14], cellulose nanoparticles [16], and starch/alginate bi-layer films [17] also have improved the performance of starch-based materials, making them potential candidates for packaging applications. However, chitosan could offer additional benefits such as antimicrobial and antioxidant properties, and greater water resistance [68]. Therefore, to determine the most suitable blend, further considerations are necessary, including the intended final applications, the availability of local by-products, and the scalability of the chosen processing method.

Fig. 10.III presents results for interaction with liquid water. Enhancing the stability of starch-based materials when exposed to liquid water is crucial for packaging applications, as it ensures that the material maintains its structural integrity and performance even when in contact with liquid. However, most studies have primarily focused on water vapor interactions, rather than direct liquid water absorption. Among all the studies analyzed in Fig. 10.I and .II, only [18] assessed both water uptake and contact angle. It is noteworthy in Fig. 10.III that the optimal blend concentrations for water stability differ from those yielding the best mechanical properties, which were generally lower. For example, 30 % (*w*/w) was identified as the optimal concentration for TS in hemp shives blends, whereas 70 % (w/w) resulted in the most water-stable material. This is particularly interesting, as hemp shives are an inexpensive by-product. In our case, 30 % (w/w) chitosan produced the highest TS among the blends evaluated, but 45 % (w/w) led to the lowest water uptake. Hemp shives also increased contact angles, indicating less hydrophilic surfaces, in contrast to our results, where the blending with chitosan promoted surface roughness that influenced the measurement of the contact angles (discussed in Section 3.5.2).

When comparing the two strategies applied in this work, both DHT modification and chitosan blending greatly improved stability in contact with water. Between the evaluated DHT conditions (130 °C for 2 h and 4 h), there was no significant difference in water uptake nor in contact angle, reaffirming that a 2-h treatment is sufficient for wheat starch-based materials. Besides the intended application, the choice between both strategies requires further investigation, considering economic and environmental aspects, such as local availability, energy consumption, and processing costs, as the conversion of chitin to chitosan involves multiple steps [69].

4. Conclusions

In this study, starch and chitosan blends were produced for the first time in a single-step extrusion system, with a focus on evaluating the effects of chitosan addition across a wide range of concentrations. Three strategies for enhancing starch-based materials were evaluated: (I) modifying starch via Dry Heat Treatment (DHT); (II) blending native starch with chitosan at concentrations ranging from 15 to 45 % (*w*/w); and (III) blending DHT-modified starch with chitosan.

DHT treatment alone improved both mechanical properties and water resistance of starch-based materials. No significant differences were observed between the two DHT conditions, suggesting that a 2-h treatment at 130 °C (DHT_2h) is more energy-efficient without compromising performance. Modified materials exhibited a higher contact angle, indicating a less hydrophilic surface.

Blending native starch with chitosan proved to be another effective approach for improving the water stability of starch-based materials but slightly reducing TS. The incorporation of chitosan made the materials more rigid, but weaker, and less flexible. Moreover, water uptake decreased as chitosan increased. The use of chitosan led to materials with rougher surfaces which may have influenced contact angle measurements. However, the reduced water uptake ruled out any potential misconceptions caused by this artifact, confirming that the materials exhibit improved resistance to water exposure.

Interestingly, combining DHT-modified starch with chitosan did not lead to further improvements beyond what was achieved by blending native starch with chitosan. FT-IR analysis confirmed that chitosan interacts in the same manner with native and DHT-modified starch.

These findings demonstrate that DHT modification (Strategy I) is an effective approach for simultaneously enhancing mechanical properties and water stability, while chitosan blending (Strategy II) significantly improves water-stability. To identify the most suitable strategy, economic, industrial and sustainability aspects need to be studied. Overall, the enhanced properties of these materials highlight their strong potential for applications in packaging.

Nomenclature

TS	tensile strength (MPa)
EB	elongation at break (%)
YM	Young modulus (MPa)
DHT_2h	dry-heat treatment at 130 °C for 2 h
DHT_4h	dry-heat treatment at 130 °C for 4 h
HMT	heat moisture treatment
ε_{true}	true strain (%)

σ_{true}	True stress (MPa)
ε_{eng}	engineering strain (%)
σ_{eng}	engineering stress (MPa)
M_t	mass at time t (g)
M_0	initial mass (g)
A_t	surface area at time t (cm^2)
A_0	initial surface area (cm^2)

CRediT authorship contribution statement

Thomás Corrêa Vianna: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Bruna de Oliveira Gomes:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Sylvain Foret:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Patrick Perré:** Writing – review & editing, Validation, Resources, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Osvaldo H. Campanella:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Brahim Mazian:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Pedro E.D. Augusto:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

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.

Acknowledgements

The authors are grateful for T.C. Vianna PhD scholarship in the program "Programme de Financement des Cotutelles Internationales de Doctorat (ADI) 2023" of Université Paris-Saclay. Communauté Urbaine du Grand Reims, Département de la Marne, Région Grand Est and European Union (FEDER Champagne-Ardenne 2014-2020, FEDER Grand Est 2021-2027) are acknowledged for their financial support to the Chair of Biotechnology of CentraleSupélec and the Centre Européen de Biotechnologie et de Bioéconomie (CEBB). Prof. Osvaldo H. Campanella was Visiting Professor through a program of the Direction de la Recherche de CentraleSupélec. Mr. Naji Majoudi and Dr. Aya Zoghlami are acknowledged to help with the contact angle and microscopy evaluations.

Data availability

Data will be made available on request.

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