

RESEARCH ARTICLE

Edible films based on native and phosphated 80:20 waxy:normal corn starch

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Edible films based on 80:20 waxy:normal corn starch were evaluated for their potential use in the food industry. Two types of film were prepared by the casting method: The first from native corn starch and the second from starch modified with glycerol. The influence of this chemical modification on the physicochemical and mechanical properties of both the starch and films was investigated. The phosphorylation of the base material improved the interaction between the plasticizer and the modified starch. This was demonstrated by the lower moisture content and water activity, higher crystallinity, greater elongation under stress and higher thermal resistance observed for the cross-linked films. The results showed that high quality edible films can be prepared from modified 80:20 waxy:normal corn starch, and demonstrated their potential as a coating or packaging material in the food industry.

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1 Introduction

Starch (St) is a widely available, highly biodegradable and economical biopolymer. This has led to its extensive use in the production of edible films and environmentally friendly packaging [1]. The intermolecular forces of this polysaccharide, however, often produce fragile films, and plasticizers are thus added to gain flexibility. Usually, glycerol and water are the conventional plasticizers for this purpose [2]. However, their use increases permeability to water vapor and gases, thus limiting the suitability of the films for food packaging [3]. Two principal strategies have been employed to improve the mechanical properties of biopolymers and decrease their hydrophilicity [4]. The first of these is to add chemical cross-linkers to modify the polymer network [5], and the second by mixing biopolymers with nano-particles [6].

One technique, related to the first strategy mentioned, is to produce starch phosphates by modification. This method can improve the properties of starch films, for example, for their use in the preparation of coatings [7, 8]. Additionally, phosphorylation ensures that the modified materials are edible and, consequently, considered safe for human consumption by the FDA [9].

The aim of this study was to evaluate the effects of phosphorylation on the quality of edible films made from 80:20 waxy:normal corn starch by comparing the physicochemical and mechanical properties of native and phosphated starch, and those of the films prepared from these materials.

2 Materials and Methods

2.1 Materials

The 80:20 waxy:normal corn (*Zea mays*) starch used was provided by Alfonzo Rivas & Cia, Cagua, Aragua State, Venezuela, this starch is obtained by mixing approximately 80 g of waxy corn starch with 20 g of normal corn starch. Food grade glycerol (Aldrich, product code: G7893) was used as a plasticizer.

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2.2 Starch modification

The starch was modified using sodium trimetaphosphate according to the method described by Kerr and Cleveland [10], with some small adjustments [11, 12].

2.3 Film preparation

Biodegradable films containing 2% w/v of either native or modified starch, 1.9% w/v glycerol and 500 mL distilled water were prepared following the methodology described by Gutiérrez *et al.* [11]. The starches were heated at 90°C for 30 min in a water bath, stirring continuously to ensure gelatinization [12]. After the onset of gelatinization, the film-forming solutions were degassed for 30 min under vacuum and then poured into 40 × 30 cm stainless steel trays, keeping a constant level to ensure uniform thickness. They were then dehydrated in a tray dehydrator (Mitchell, USA, Model 645159) for 24 h at 45°C. Once dried, the films from native and phosphated 80:20 waxy:normal corn starch (TPS-NC80:20 and TPS-PC80:20, respectively) were peeled from the trays and conditioned over NaBr ($a_w \sim 0.575$ at 25°C) for 7 days before being tested.

2.4 Characterization

The physicochemical properties of both the starches (native and modified) and the prepared films were evaluated. Knowledge of the intrinsic properties of the starches used for producing biodegradable films is crucial for a better understanding of the behavior of the final materials.

Moisture content, ash, fat, crude protein ($N \times 6.25$) (obtained by the micro-Kjeldahl method), were determined using the gravimetric method [13]. Apparent amylose content was assessed using a colorimetric micro-procedure based on the formation of the amylose/iodine complex [14] and total amylose (TAM) content was determined by the differential scanning calorimetry method described by Pérez *et al.* [15, 16]. Phosphorus content and the degree of substitution (DS) were determined by the colorimetric method [17] using the equations proposed by Deetae *et al.* [18]. The purity of the starches was calculated as the sum of the percentages of water content, ash, crude protein, and fatty materials.

The rheological characteristics of the starches were determined by interpreting the data obtained from a Rapid-Visco-Analyser (Brabender® brand, model Micro Visco-Amylo-Graph, Duisburg, Germany) performed using the Viscograph program (Version 2.4.9). A suspension of 7% starch solids (14% moisture on a dry basis) was prepared. This suspension was heated from 30 to 90°C at a constant rate of 6°C/min and then cooled from 90 to 50°C at the same rate to obtain the following data: Onset of gelatinization (A), maximum viscosity (B), stability “breakdown” (BD), settling “setback” (EB) and consistency (ED).

For the films, the following physicochemical and mechanical properties were also studied: Moisture content [19], water activity (a_w) [20], thermogravimetric analysis (TGA) [20], crystallinity (XRD) [20], optical microscopy [8], color [8, 21–24], and uniaxial tensile behavior [8].

2.5 Statistical analysis

Analysis of variance at a significance level of 5% ($p = 0.05$) was performed using the Statgraphics Program (Statistically Educational Graphics, version 6.0 1992, Statistical Graphics Corporation, Manugistics, Inc., Rockville, USA). When statistical differences were found, the means were compared using Duncan’s multiple range test at a significance level of 5% ($p = 0.05$).

3 Results and discussion

3.1 Physicochemical properties of the starches

The moisture contents of native and modified 80:20 waxy:normal corn starch are given in Table 1. As can be seen, the humidity value of the modified corn starch was lower than that of native starch. Similar results were reported for these types of starches by Pérez *et al.* [25] and Sívoli [26]. The moisture levels observed fall within the reported range for a stable shelf life [27].

Crude protein content was low in both the native and the modified starch and varied between 0.08 and 0.604% (Table 1), showing that the purification process was efficient. The modified starch contained less crude protein than the native starch.

Crude fat content was slightly higher in the native corn starch than the modified starch (Table 1). Although minor amounts of fat could influence the gelatinization of the starches.

Table 1. Chemical characterization on dry basis of native and modified corn starch 80:20 waxy:normal (*Z. mays*)

Parameters	Native corn starch 80:20 waxy:normal (%)	Modified corn starch 80:20 waxy:normal (%)
Moisture	12.3 ± 0.2 ^a	9.5 ± 0.6 ^b
Crude protein	0.604 ± 0.005 ^a	0.08 ± 0.02 ^b
Crude fat	0.4 ± 0.2 ^a	0.3 ± 0.2 ^a
Ash	0.02 ± 0.01 ^a	1.04 ± 0.03 ^b
Purity of starch	98.98 ± 0.07 ^a	98.58 ± 0.08 ^b
Apparent amylose	11 ± 2 ^a	12 ± 3 ^a
TAM	8.6 ± 0.3 ^a	9.1 ± 0.2 ^a
Phosphorus	0.005 ± 0.003 ^a	0.13 ± 0.01 ^b
DS	0.0005 ± 0.0003 ^a	0.013 ± 0.001 ^b

Similar superscript letters in the same row indicate no statistically significant difference ($p \leq 0.05$).

Table 2. Rheological (UB) and initial gelatinization temperature (°C) of a 7% solution of native and modified corn starch 80:20 waxy:normal (*Z. mays*)

	Native corn starch 80:20 waxy:normal (%)	Modified corn starch 80:20 waxy:normal (%)
Initial gelatinization temperature (°C)	71.10 ± 0.02 ^a	72.0 ± 0.1 ^b
Maximum viscosity (B) (UB)	320 ± 53 ^a	512 ± 1 ^b
D	113 ± 64 ^a	439 ± 2 ^b
E	170 ± 66 ^a	536 ± 18 ^b
Breakdown (UB)	207 ± 12 ^a	75 ± 1 ^b
Setback (UB)	–150 ± 13 ^a	25 ± 18 ^b
Consistency (UB)	56 ± 0 ^a	97 ± 18 ^b

The values are the average of two determinations; similar superscript letters in the same row indicate no statistically significant difference ($n = 2$, $p \leq 0.05$).

D = hot paste viscosity at the end of the plateau, at 90°C; E = cold paste viscosity at 50°C, the end of the cooling period; BD = maximum viscosity (B) – the hot paste viscosity at the end of the plateau at 90°C (D); SB = cold paste viscosity at 50°C, the end of the cooling period (E) – maximum viscosity (B); CS = the cold paste viscosity at 50°C (E) – hot paste viscosity at the end of the plateau at 90°C (D).

As expected, the ash, phosphorus content and degree of substitution (DS) increased in the modified starch; all DS values reported were within the range approved by the FDA [9].

The purity of both starches was very high, from 98.5 to 99%, corroborating the efficiency of the purification process.

The apparent and TAM content of the two corn starches was similar and thus not affected by modification. However, the structural properties of 80:20 waxy:normal corn starch were substantially modified due to the increase in the DS. As can be observed from Table 2, modification dramatically altered the gelatinization profile of the corn starch, significantly increasing peak viscosity, setback and consistency. Once again, the modification of these structural properties was due to the increase in the DS. In addition, cross-linking

Table 3. Moisture content, water activity (a_w) and color of different films

Parameters	TPS-NC80:20	TPS-PC80:20
Moisture (%)	44 ± 4 ^a	30 ± 3 ^b
a_w	0.481 ± 0.002 ^a	0.453 ± 0.001 ^b
Crystallinity (%)	15.1 ± 0.9 ^a	16.9 ± 0.8 ^b
L	12.32 ± 0.06 ^a	24.82 ± 0.09 ^b
a	0.0 ± 0.2 ^a	–0.5 ± 0.3 ^a
b	–1.6 ± 0.2 ^a	–1.9 ± 0.2 ^a
Total changing (ΔE)	81.28 ± 0.06 ^a	68.81 ± 0.09 ^b
Whiteness index (WI)	12.31 ± 0.06 ^a	24.79 ± 0.09 ^b
Yellow index (YI)	–11.61 ± 0.01 ^a	–10.03 ± 0.01 ^b

Similar superscript letters in the same row indicate no statistically significant differences ($p \leq 0.05$).

and granular swelling produced a starch that can tolerate elevated temperatures, high shear stress, and acidic conditions. The temperature required for the onset of gelatinization was slightly higher for the modified starch.

3.2 Physicochemical and mechanical properties of the films

Figure 1 shows the images of the film surfaces obtained by optical microscopy. On the TPS-PC80:20 films, small fragments can be observed (Fig. 1b). These fragments were identified as starch grains that had not dissolved completely during the heating process suggesting the need for a higher initial gelatinization temperature (Table 2). This phenomenon was not observed for the TPS-NC80:20 films (Fig. 1a) indicating that native starch grains dissolve at a lower temperature (Table 2). García et al. [28], have shown that the un-dissolved crystalline particles often act as nuclei, inducing the recrystallization of amylose macromolecules during the cooling and storage stages, resulting in a brittle film.

Table 3 shows the moisture content of the films. The humidity of the films made with native corn starch was

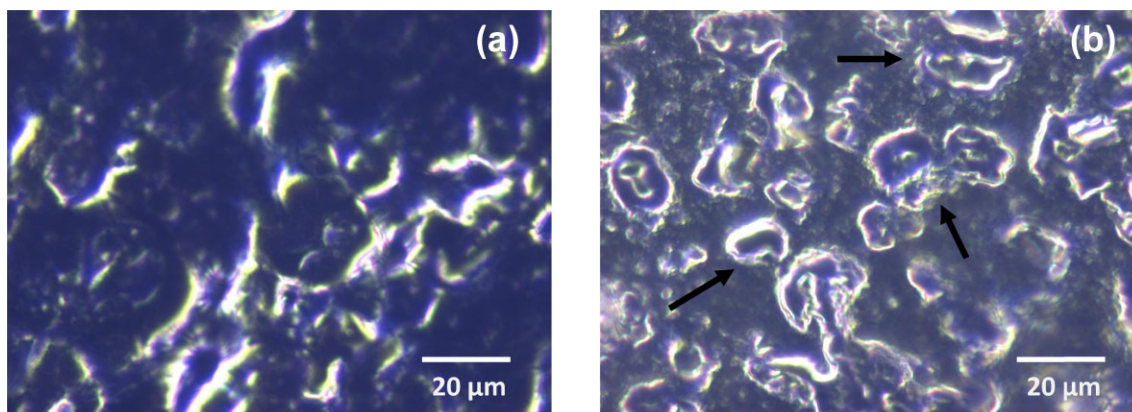


Figure 1. Optical micrographs of the films based on: (a) Native corn 80:20 (TPS-NC80:20) and (b) phosphated corn 80:20 (TPS-PC80:20) at 50× of magnification. Black arrows indicate starch granules with incomplete disruption within edible films.

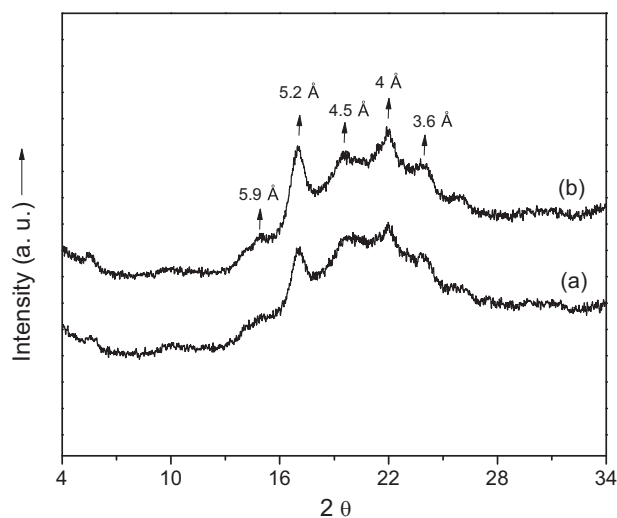


Figure 2. X-ray diffraction pattern of the different films studied: (a) Native corn 80:20 (TPS-NC80:20) and (b) phosphated corn 80:20 (TPS-PC80:20).

significantly higher (20%; $p \leq 0.05$) than those made from modified starch. This suggests that phosphation improved chemical interactions in this thermoplastic material. These results agree with those reported by Cyras *et al.* [29] for films prepared from potato starch modified by acetylation.

Table 3 also shows that water activity (a_w) was higher in the samples formulated with native corn starch; this was expected as native starch showed a higher moisture content. Similar results have been found in the literature for potato starch-based films [30] as well as edible coatings containing native and modified white *Dioscorea trifida* [25].

The X-ray diffraction (XRD) patterns of the films can be observed from Fig. 2. Both materials showed a large amorphous phase and a small crystalline fraction, in agreement with previous studies [28, 31–34]. Generally, starches show A, B and C-type crystal structures. When glycerol is present the double helix configurations are disrupted by the formation of stable single chain V-conformation helices, resulting in the conformation of the amylose-glycerol complex [35–37]. In a previous study, these authors reported that films based on native and modified cush-cush yam starch showed A-type patterns, probably due to a local order generated by associated amylopectin chains, while cassava films showed B-type patterns, due to the presence of amylose which favored interactions with the glycerol [20]. Angellier *et al.* [32] and Mathew and Dufresne [38] reported that waxy maize granules recrystallize in B-type structures when completely dissolved in water after which they develop a smooth A-type structure.

As can be seen from Fig. 2, both films studied presented diffraction peaks in the following d -spacing \cong 3.6, 4.0, 4.5, 5.2, and 5.9 Å. These values represent a combination of known peaks observed for A-type and B-type patterns [20, 28, 31].

The percentage of crystallinity of the films is shown in Table 3. The slight increase in the values observed for the TPS-PC80:20 samples were probably due to the higher phosphorus content of the modified starch (Table 1). According to Osella *et al.* [39], the development of A or B-type XRD patterns on starch films depends partly on their water content. A decrease in moisture is highly correlated with an increase in the crystal phase of a semi-crystalline material [7, 31, 40]. The slight increase observed in the crystallinity of the films made with phosphated 80:20 waxy normal corn starch agrees with the lower humidity values obtained for this material.

Table 3 presents the results of the color parameters of the films studied. TPS-PC80:20 showed higher mean L and $W1$, compared to TPS-NC80:20, indicating that the modified starch film tends to be whiter and less transparent than native starch film. According to the luminosity values obtained, the stronger interactions between the modified starch and the plasticizer (glycerol) results in more opaque films. Fakhouri *et al.* [41] indicate that the opacity of films tends to be produced by hydrogen bonds. Despite the differences in L and $W1$ between the films, it is important to note that both materials showed excellent transparency, and were thus both suitable for consumers. However, the more opaque TPS-PC80:20 films could be more appropriate when protection against incident light is necessary, especially for wrapping products that are sensitive to degradation reactions catalyzed by light [42]. The values of a of the films were both around zero, and no significant differences ($p \leq 0.05$) between them were observed. Negative values of the parameter b indicated that the color of both films tended towards blue, and despite the fact that the difference between them was not significant ($p \leq 0.05$), this effect was more evident for TPS-PC80:20. As there were no significant differences between the films as regards either the values a or b , the changes in color, ΔE , observed must have been due to the values of L . Finally, the results of the yellow index, $Y1$, were consistent with the parameters of b , being negative in all cases, indicating a very low degree of yellowing of both materials, although a more negative $Y1$ value was obtained for the film made from native corn starch.

The thermal stability of the films was evaluated by TGA (Fig. 3). According to the literature, the thermal decomposition of glycerol-starch biofilms occurs in three main stages [28, 43–46]. The first stage corresponds to water loss, the second is due to the decomposition of the glycerol-rich phase, which also contains starch, and the third stage is the result of the oxidation of the partially decomposed starch [28, 43]. As can be seen from Fig. 3, the most important thermal degradation was observed at temperatures between 200 and 330°C, when the decomposition of the glycerol-rich phase occurs [28]. It is well known that the addition of glycerol decreases the intra and intermolecular interaction of starch-starch chains and strengthens hydrogen-bonding

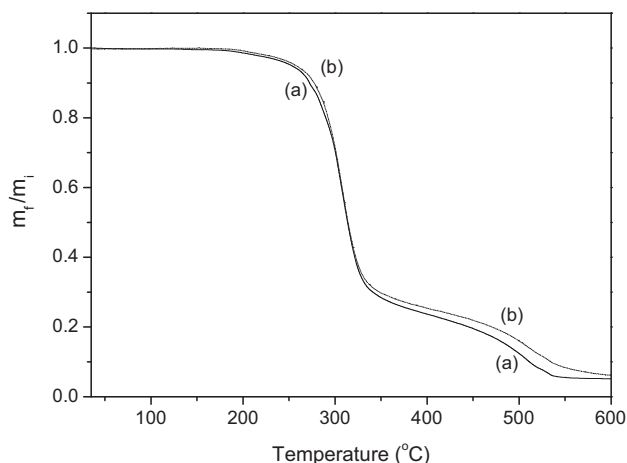


Figure 3. Thermogravimetric analysis (TGA) curves of the different films studied: (a) Native corn 80:20 (TPS-NC80:20) and (b) phosphated corn 80:20 (TPS-PC80:20), in dry basis.

interactions between the hydroxyl groups of the starch chains and the glycerol molecules [29]. From Fig. 3, we can also observe that the onset of the thermal degradation stage corresponding to the decomposition of the glycerol in the modified starch films starts at a slightly higher temperature than that of the native starch films, indicating that the phosphorylation of starch leads to films with a higher thermal resistance.

Typical stress–strain curves were found: A lineal zone following to a no linear, until their break, regardless of the starch used (Fig. 4). However, a little brittle behavior was observed in the films with native 80:20 waxy:normal corn starch, accompanied by the highest Young's modulus and a trend towards an increase in their maximum stress values. Moreover, the modification of both the starches led to the decrease of Young's modulus and maximum stress, and to an

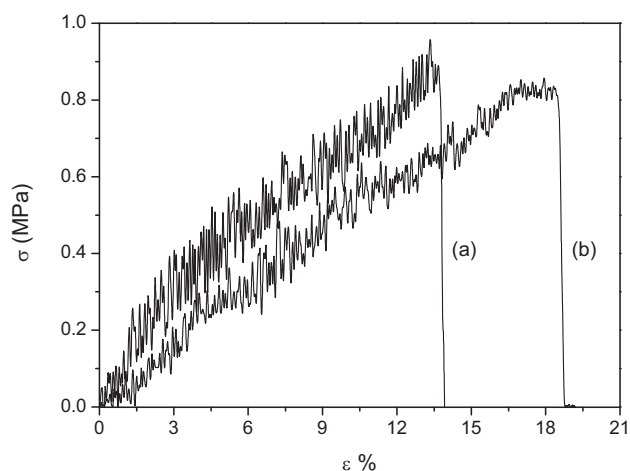


Figure 4. Stress (σ)–strain (ε) curves of the films based on: (a) Native corn 80:20 (TPS-NC80:20) and (b) phosphated corn 80:20 (TPS-PC80:20).

Table 4. Parameters of the uniaxial tensile tests: Young's modulus (E), maximum stress (σ_m), strain at break (ε_b) and tenacity (T)

Materials	E (MPa)	σ_m (MPa)	ε_b (%)	T ($\times 10^5$) (J/m ²)
TPS-NC80:20	9.5 ± 0.9^a	0.9 ± 0.1^a	14 ± 1^a	0.69 ± 0.10^a
TPS-PC80:20	5.6 ± 0.6^b	0.82 ± 0.08^a	18.5 ± 1.5^b	0.86 ± 0.10^a

Similar superscript letters in the same column indicate no statistically significant difference ($p \leq 0.05$).

increase in strain at break, influencing favorably the elasticity (Table 4). Similar behavior was reported by Tapia et al. [47], for films based on *D. trifida* white starches. In our study, the results could be attributed to increased interaction between the phosphorylated starch and the glycerol (Fig. 5). As previously mentioned, once this starch gelatinizes, its interaction with the plasticizer is evident, preventing its re-crystallization within the matrix of the film. Generally, the permanence of crystalline particles act as nucleus for inducing recrystallization of starch macromolecules, leading to more fragile materials [28]. Besides recrystallization, there is other opposite process that acts in the heating of starch, causing softer plasticization [48]. The effect of stiffness retrogradation is a typical phenomenon of plasticization by moisture. Therefore, it may indicate that based 80:20 waxy:normal corn starch films have had moist plasticization. Water acted primarily as a plasticizer, resulting in a less glycerol–starch interaction. These results support the hypothesis that glycerol interacts more strongly with the phosphorylated starch than with the native (Fig. 5). These interactions could be due to the formation of hydrogen type bridge links between the glycerol and the phosphorylated starch groups. This glycerol–phosphorylated starch interaction would decrease intra and intermolecular interactions between starch macromolecules, increasing the movement and rearrangement of their chains, and leading to an increase in the flexibility of TPS-PC80:20. According to Mina et al. [48] these results were due to the role of the glycerol that during the plasticization was more effective for the chemically modified starch than for the native one. What is more, the effect was more notorious in the phosphorylated corn starch films, because the modified starch also has greater degree of substitution (see Table 1), which allows higher starch–plasticizer interactions.

4 Conclusions

The physico-chemical properties of edible films derived from native and phosphorylated 80:20 waxy:normal corn starch were investigated. The purity of the starches was very high; although the modified starch contained more phosphorus, all the starches tested were found to be within the limits permitted by the FDA. Phosphorylation modified the

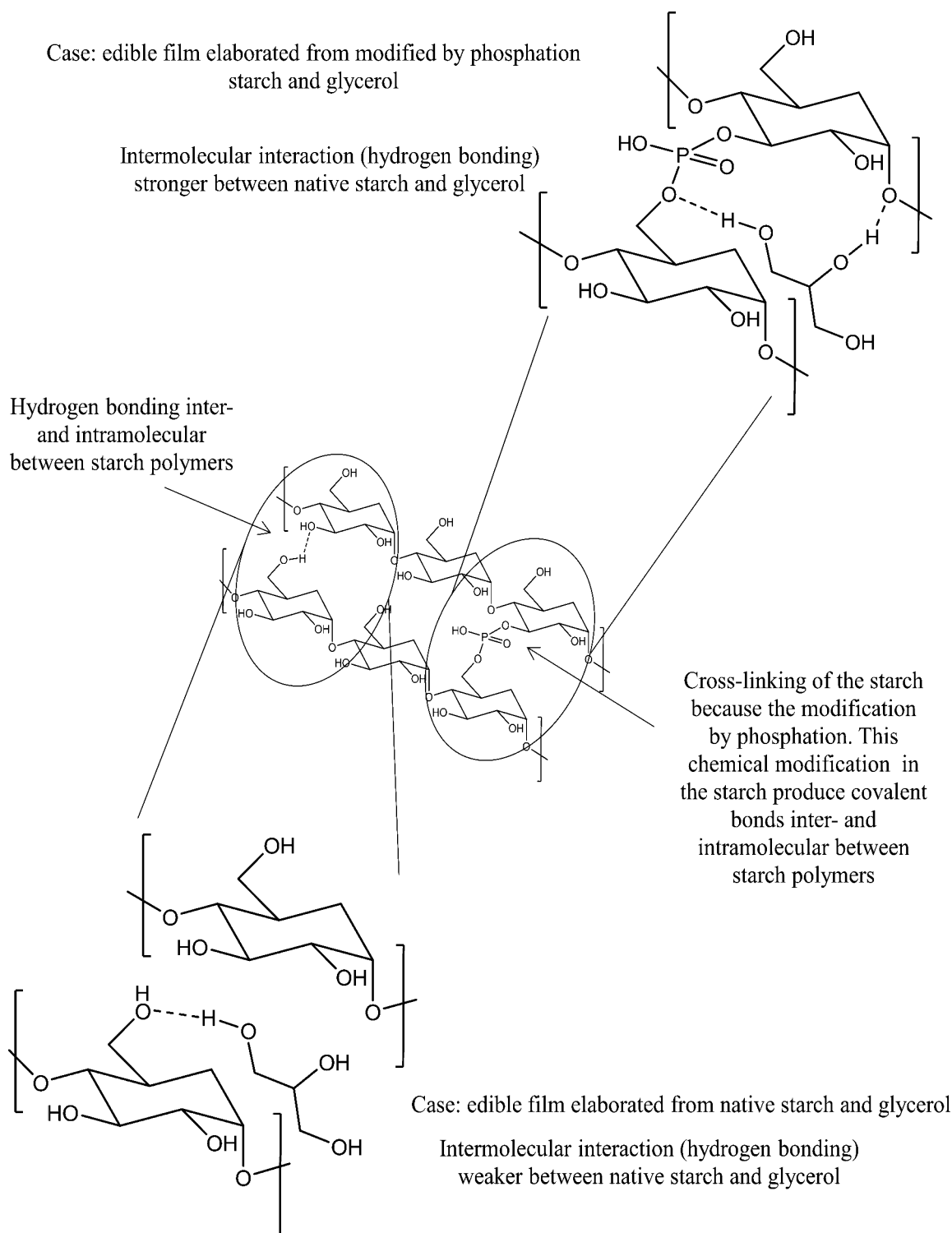


Figure 5. Mechanism proposed to explain the benefits of modified by phosphation in starches used for the preparation of edible films.

gelatinization profile of 80:20 waxy:normal corn starch due to reticulation between the starch polymers, resulting in an increase in the temperature required for the onset of gelatinization, peak viscosity, consistency, and setback. Starch granules were observed in the modified starch films which

could possibly act as nuclei during storage leading to the recrystallization of the amylaceous macromolecules. The films made from both starches had a uniform thickness with no holes and both also showed a high degree of transparency. However, the edible films made from the modified 80:20

waxy:normal starch were more opaque as a result of stronger glycerol–starch interactions. These films could be used in foods susceptible to oxidation in the presence of light. The phosphorylation of the starch produced more crystalline films with a lower moisture content and water activity, probably due to a weakening of the interactions between the starch–starch chains and a strengthening of the hydrogen links between the glycerol and the phosphate groups. This behavior suggests that the glycerol interacts more strongly with modified starch than with native starch. This hypothesis was reaffirmed by the slight increase in the thermal degradation temperature of the modified starch films, giving a material with a higher thermal resistance. A further consequence of the stronger phosphate starch–glycerol (plastification) interaction was a reduction in Young's modulus and maximum stress values as well as an increase in strain at break of the reticulated films making them more flexible. Based on these results, we can conclude that modified 80:20 waxy:normal starch-based films have a great potential as coatings or packaging materials in the food industry.

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