Influence of different commercial modified cassava starches on the physicochemical properties of thermoplastic edible films obtained by flat-die extrusion

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Abbreviations:

NS, native cassava starch; HS, hydrolyzed cassava starch; CMS, carboxymethyl cassava starch; DS, degree of substitution; RH, relative humidity; TGA, thermal gravimetric analysis; RC, relative crystallinity; M, moisture content; S, solubility; WVP, water vapor permeability; DMTA, dynamic mechanical thermal analysis; \(\sigma\), stress; \(\varepsilon\), strain; \(E\), Young’s modulus; \(\sigma_b\), stress at break; \(\varepsilon_b\), strain at break.
Keywords: Crystalline structure; flat-die extrusion; Mechanical behavior; Modified commercial starches; Starch-based films.

Abstract
Starch constitutes a promising resource for biodegradable packaging, but it presents several processing drawbacks when using conventional industrial equipment, such as flat-die extrusion. This work demonstrates that the replacement of native cassava starch (NS) with commercial hydrolyzed (HS) or carboxymethyl (CMS) starches diminishes the amount of energy required to process starch granules into thermoplastic films by extrusion. Homogeneous films with starch-glycerol interactions improvements were obtained when using HS or CMS instead of NS. HS films exhibited the lowest crystallinity and the highest susceptibility to water among the studied systems. Likewise, CMS films maximized $V_h$ crystalline structure fraction and presented higher Young’s modulus and stress at break, and lower WVP values. In order to combine the positive properties and to overcome the limitations of each modified starch, blend films from HS:CMS (50:50 wt.%) were also developed and investigated. Phase separation was observed in this system, and there were no improvements in the overall properties. The results of this investigation show that employing commercial CMS, even in very low carboxymethylation degree represents a successful strategy to improve mechanical and barrier properties of flat-die extruded films, while HS can be used to obtain water-soluble films for specific applications.
1. Introduction

Cassava (*Manihot esculenta*) reached an estimate global production of 278 million tons in 2018, and it is currently growing at over 3% annually [1,2]. Argentina is the fourth producer of cassava in Latin América, behind Brazil, Paraguay and Colombia, reaching an estimate of 192000 tons in 2018 (0.06 % of global production) [1]. Cassava flour is gluten free and can be used in diets for gluten intolerant people since its flour is employed in a wide variety of foods within the diet of many people, such as celiacs [3,4]. Nowadays, cassava starch is commercially available in different forms: native or modified granules [5–9]. Since only 30% is used to supply domestic and export markets, the investigation of cassava starch as raw material of thermoplastic films for biodegradable packaging has been the focus of many investigations for years [10–13].

Modified starches were reported to improve thermoplastic films properties [14–18]. Chemical modifications can impact specific properties on starch materials [19]. Acid hydrolysis and carboxymethylation are some of the most common chemical modifications implemented on starches for different sources [20–22].

The acid hydrolysis process can decreases amylose content of starch. In the hydrolysis process, a decrease in the amylose content of starch occurs [21, 22]. This is due to the result of a fast hydrolysis of the granules amorphous regions, followed by slow hydrolysis of crystalline regions [23,24]. The plasticizing effect of hydrolyzed or carboxymethyl starches improved mechanical resistance and water solubility of thermoplastic starch films. Some authors have investigated the effect of hydrolyzed or carboxymethyl starches on the properties of thermoplastic starch films. Particularly, mechanical resistance and water solubility are generally improved, owing to stronger plasticizer-starch interactions [14,25]. Biduski et al. [28] showed that hydrolyzed sorghum starch films exhibited higher tensile
strength and Young’s modulus but lower strain at break than native starch samples. Likewise, Zhang et al. [27] observed higher tensile strength and water vapor permeability in hydrolyzed pea starch films. Lucchese et al. [29] reported a significantly higher solubility in hydrolyzed pinhão starch films made by casting methodology. On another hand, some investigations on carboxymethyl starch based films showed several advantages over native starch materials, such as lower gelatinization temperature and slower retrogradation, which demonstrates that carboxymethylation improves stability of starch films [14,28].

Most research about thermoplastic films from modified starches are performed on samples produced by casting technique [15,22,27,31–36]. This methodology results in low production yield and, consequently, high cost, making it very difficult to implement in packaging industries. Considering that materials properties depend on the processing method, the evaluation of the characteristics of starch films obtained by scalable techniques, in accordance to new technologies, is a critical and essential point for their possible commercialization. In this sense, one of the most common process currently employed by the plastics industry is extrusion, thus thermoplastic starch films obtained by this technique have been investigated [37–41]. Particularly, it was demonstrated that starch films processed by extrusion presented greater mechanical resistance and lower susceptibility to water than films prepared by casting [42,43]. However, the development of cassava starch film by extrusion technique is still a challenge.

To the best of our knowledge, there are no contributions on the effect of using hydrolyzed and carboxymethyl starch granules on the properties of thermoplastic films obtained by flat-die extrusion and calendering process. Some researchers have used extrusion combined with chemical reagents to modify starch granules but not to perform films.
In this work, the effect of commercial native and modified cassava starches (hydrolyzed and carboxymethyl) on the morphology and physicochemical properties of biodegradable edible starch-based films obtained by flat-die extrusion and calendering process was investigated. Additionally, the combination of hydrolyzed and carboxymethyl starches (50/50 wt.%) on starch blend films was evaluated in order to determine a possible synergistic interaction of both modified starches. The results will help to provide new knowledge in order to design cassava starch-based films with desired physicochemical properties by selecting the appropriate starch chemical modification.

2. Materials and methods

2.1. Materials

Commercial native cassava starch (NS) (18 wt.% amylose and 82 wt.% amylopectin), acid hydrolyzed cassava starch (HS) and sodium carboxymethyl cassava starch (CMS) (DS ~ 0.1), were supplied by Cooperativa Agrícola e Industrial San Alberto Limitada (C.A.I.S.A., Costa Rica, Misiones, Argentina). Morphology, molecular weight, thermal analysis (DTA) curves, Fourier transform infrared (FTIR) spectra and X-ray diffraction patterns of the commercial starches were analyzed before used (Supplementary Data). Sodium bromide (NaBr, Sigma Aldrich), calcium chloride (CaCl₂, Sigma Aldrich), sodium chloride (NaCl, Biopack) and glycerol (Sigma Aldrich) were of analytical grade.

2.2. Starch films preparation

Starch films were prepared using the set-up methodology reported previously [37,41,43,44] with some modifications. Firstly, the selected starch (NS, HS or CMS) (70 wt.%) were
manually mixed with glycerol (15 wt.%) and distilled water (15 wt.%). For HS/CMS blend, 35 wt.% of each starch was used. All systems were stored 24 h in sealed containers and then processed using a co-rotating twin-screw extruder (Nanjing Kerke Extrusion Equipment Co., Ltd., China) with a screw diameter of 16 mm, L/D ratio of 40, 10 individual heating zones and a flat film forming die with a wide of 80 mm connected to a homemade two-roll calendar. The temperature profile and screw speed of the extruder were 90/100/110/120/120/130/130/140/130/120 °C and 120 rpm, respectively (feeding rate: 12 g/min), and calendar was set at 120 °C. The obtained films of (0.20 ± 0.05) mm thickness were stabilized for 4 weeks at 56% RH before testing.

2.3. Characterizations

2.3.1. Morphological characterization
The cryogenic fracture surface of the films was observed using a field emission scanning electron microscope (FE-SEM, Zeiss Supra 40, Germany). Samples were sputter-coated with platinum (15 s, 0.06 mbar of Ar) using a Kurt J. Lesker 108 sputter coater (Kurt J. Lesker Co., PA, USA) before testing.

2.3.2. Thermal analysis (TGA/DTA)
Simultaneous thermogravimetric (TGA) and differential thermal analysis (DTA) tests were carried out in a TGA-DTA analyzer (DTG-60 Shimadzu, Japan). Film pieces of ~10 mg were heated from 30 °C to 450 °C at a rate of 10 °C/min in a dry nitrogen atmosphere and flow rate of 30 mL/min.
2.3.3. Fourier transform infrared spectrum (FTIR)

FTIR-ATR spectra were obtained between 4000 cm$^{-1}$ and 600 cm$^{-1}$ using a FT/IR 4100 spectrophotometer (Jasco Inc., Japan) at 64 scans and a resolution of 2 cm$^{-1}$.

2.3.4. X-ray diffraction (XRD)

X-ray diffraction patterns were obtained using a Siemens D 5000 X-ray diffractometer (Siemens AG, Germany). The radiation was Cu Kα with wavelength of 1.54 Å, and the X-ray generator tension and current were 40 kV and 30 mA, respectively. Patterns were scanned over a diffraction angle (2θ) range of 5–40° at a step size of 0.026° and a count time of 2 s. The relative crystallinity (RC) of the films was estimated as the ratio of the area of the main crystalline peaks to the total area of the X-ray curves using the Origin software (Version 8.0, OriginLab Corporation, Northampton, MA, USA) [45].

2.3.5. Susceptibility to water

Moisture content (M) of the different films was determined using standard methods of analysis according to AOAC Method 934.01 (1995) [46]. Pieces of ~ 0.5 g ($m_i$) were dried in an oven at 95 °C until constant weight ($m_f$). The percentage of moisture content was calculated using equation (1):

$$M = 100 \times \frac{(m_i - m_f)}{m_i} \quad (1)$$

Water solubility of films (S) was determined following Estevez-Areco et al. (2019) [41] Maizura et al. (2007). Disks of 2 cm of diameter of each film sample were dried at 95 °C for 24 h and weighed to obtain the initial dry mass ($m_{di}$). Other disks with the same diameter were placed in sealed containers with 50 mL of distillate water at room temperature (~ 25 °C)
for 24 h, and after that, they were dried at 95 °C for 24 h to obtain the final dry mass (m_{df}).

The percentage of the water solubility of the films was calculated using equation (2):

$$S = 100 \times \frac{(m_{di} - m_{df})}{m_{di}}$$  \hspace{1cm} (2)

Water vapor permeability (WVP) of films was determined at room temperature following ASTM E96-00 standard recommendations with some modifications [47,48]. Samples were placed into circular acrylic cells filled with CaCl$_2$ and collocated inside desiccators at RH of ~70% (saturated NaCl solution). WVP (g Pa$^{-1}$ s$^{-1}$ m$^{-1}$) values were calculated using equation (3):

$$WVP = \frac{G \times e}{\Delta P \times t \times A}$$  \hspace{1cm} (3)

Where $G$ is the weight gain in time (g s$^{-1}$), $e$ the film thickness (m), $\Delta P$ the vapor pressure difference (1681 Pa), $t$ is the time measured in hours and $A$ the cell area (~0.00038 m$^2$).

All tests were performed in triplicate.

2.3.6. Mechanical properties

Uniaxial tensile and dynamic mechanical tests were carried out in a dynamic mechanical thermal analyzer (DMTA IV, Rheometric Scientific, USA), at rectangular tension mode, on pieces of 10 mm x 5 mm x 0.2 mm (effective length, width and thickness). For uniaxial tensile assays, a fixed strain rate of 5 x 10$^{-3}$ s$^{-1}$ and room temperature (~25 °C) were employed. Nominal stress ($\sigma$) – strain ($\varepsilon$) curves were obtained and Young’s modulus (E), stress at break ($\sigma_b$) and strain at break ($\varepsilon_b$) values were determined.

Dynamic mechanical tests were performed from -80 °C to 120 °C, at 1 Hz and heating rate of 2 °C/min. The strain was set at 0.01% to assure that the mechanical response of the samples
was within the linear viscoelastic range [49]. The transition processes were analyzed from the loss tangent dependence on temperature curves. In both tests, ten independent samples for each film were evaluated.

2.3.7. Statistical analysis

Data was analyzed through a two-way ANOVA and Tukey’s Post-Hoc test at a significance level of 5% (p < 0.05). In the case of the starch assays, the independent treatments (factors) were the different types of commercial starches (native, hydrolyzed and carboxylated). Regarding the films, the factors were the films prepared from the different starches (NS, HS, CMS and HS/CMS). Other parameters involved in the film’s development, such as plasticizer concentration, process variables or storage conditions, were not modified throughout the work. The results were informed as average and standard deviation.

3. Results and discussion

3.1. Influence of chemical modification in starch films morphology (SEM)

The advantages of using commercial modified starches against NS in the processing are clear when comparing cryogenic fracture surfaces (Fig. 1). Native starch films presented many broken granules (Fig. 1A), whereas HS and CMS samples exhibited smooth surfaces without starch granules (Fig. 1B and Fig. 1C, respectively), indicating complete starch gelatinization [43,50]. According to Pushpadass et al. [51], the presence of partly melted starch granules in extruded thermoplastic films indicates that starch did not completely gelatinize. The smooth surfaces without holes or broken starch granules observed in HS and CMS films therefore suggests that these chemical modifications contribute to starch gelatinization in the extrusion process, indicating that less
specific mechanical and thermal energy is necessary to gelatinize the modified starches. This result presents great relevance when evaluating costs of thermoplastic starch films process for its use in plastic and/or packaging industries. Nevertheless, broken starch granules in NS films were homogeneously distributed and no defined interface between them and the matrix was observed, indicating good adhesion (Fig. 1a). According to Li et al. [52] and González-Seligra et al. [53], homogeneously dispersed starch granules in a thermoplastic starch matrix could act as self-reinforcement, improving films mechanical resistance and slowing their retrogradation. However, the presence of an interface zone between the granules and the matrix may provoke cracks, leading to decreases in the strain at break of the films.

Neither broken granules nor holes in HS/CMS films (50:50 wt.%) were observed. However, formations of about 10 µm homogeneously distributed within a continuous zone could be detected, suggesting phase separation between both modified starches (Fig. 1D). When two polymers are miscible, a homogeneous blend occurs but when miscibility is partial, rich areas of each polymer appear. If polymers are immiscible, a total phase separation can be observed [54]. Therefore, with regard to HS/CMS films, a partial miscibility between both starches was reflected.

The morphology of the commercial starches granules are shown in S1 (Supplementary Data).

3.2. Influence of chemical modification on the starch films physicochemical properties.

Fig. 2 exhibits the normalized FTIR spectra of the extruded films. All samples show the typical cassava starch-based film bands; one at 3600-3000 cm\(^{-1}\) corresponding to hydrogen bond vibration, a double band at 3000-2900 cm\(^{-1}\) (C-H stretching), at 1150-1100 cm\(^{-1}\) (C-O, C-C and C-O-H stretching), a double band at 1100-990 cm\(^{-1}\) (C-O-H bending), and bands at 930 cm\(^{-1}\), 860 cm\(^{-1}\) and 760 cm\(^{-1}\) (vibrations of glycosidic ring) [55]. An additional band at
~1730 cm\(^{-1}\), related to C=O stretching of esters groups between the acid and glycerol, was observed in CMS and HS/CMS films. The low intensity of this band indicates a very low degree of carboxymethyl substitution [56], in accordance with the information proportioned by the supplier (DS ~ 0.1). CMS films showed higher intensity in C-H stretching band and a narrower and less intense band in OH vibration region. Besides, OH band slightly shifted towards lower wavenumber values (from 3310 cm\(^{-1}\) to 3305 cm\(^{-1}\), Fig 2B), indicating a slight increase in intermolecular hydrogen bonds between starch and glycerol [57]. This behavior suggests a different molecular arrangement in CMS films compared to NS sample.

Commercial hydrolyzed starch films exhibited a slight higher relative difference between intensities of the bands at ~ 995 cm\(^{-1}\) and ~ 1015 cm\(^{-1}\), compared to the other systems (Fig. 2C), indicating that HS films are more amorphous [55,58]. This behavior is associated to the acid hydrolysis process, in which α-D-(1,4) glycoside linkages are the most susceptible bonds to be cleaved. In this sense, the fragmentation occurs mainly into the amorphous regions, instead of the crystalline regions [59], leading to an increase in the number of chain ends and a decrease in starch molecular weight (Table S1, Supplementary Data). On another hand, the band related to OH vibration (3600-3000 cm\(^{-1}\)) in HS samples did not show significant differences in intensity compared to NS films but it was slightly shifted to a lower wavenumber (from 3310 cm\(^{-1}\) to 3298 cm\(^{-1}\)) (Fig. 2B). These results indicated that the hydrolysis did not significant modify the amount of available OH groups but an increase in hydrogen bond interaction between starch molecules was produced. FTIR of commercial starch granules are presented in Figure S2 (Supplementary Data).

Using commercial modified starches led to significant changes in XRD patterns of the films and, therefore, in its relative crystallinity (RC). The typical structure for thermoplastic cassava starch films could be observed in NS samples (Fig. 3a), with peaks at 2\(θ\) = 15°,
23°, 17.3° and 18.3° (d = 5.9 Å, 3.9 Å, 5.1 Å and 4.8 Å, respectively) corresponding to C type and at 20 = 13°, 19.8° and 20.2° (d = 6.8 Å, 4.5 Å and 4.4 Å, respectively) corresponding to Vh type [60,61], which occurs as a consequence of insertion of glycerol molecules within the starch helical structure [62]. NS films showed the highest value for relative crystallinity (RC = 13.5 ± 0.3 %). This fact was a consequence of the presence of starch broken grains, as observed in SEM images (Fig. 2A), that were not found in the films obtained from modified starches [63,64].

The films prepared from CMS showed an increase in the intensity of the peaks corresponding to the Vh type structure (Fig. 3c), possibly due to a better interaction between starch and glycerol, as it was discussed in FTIR section, suggesting that more glycerol molecules are within the helical structure of the starch. Additionally, lower relative crystallinity than NS samples was obtained (RC = 6.9 ± 0.1 %). This could be consequence of the morphology of CMS samples, which did not show broken starch grains, as exhibited in SEM images (Fig. 1C).

HS films presented the lowest crystallinity among the studied systems (RC = 4.2 ± 0.1 %). Besides, diffraction pattern of HS showed some peaks corresponding to Vh crystalline structure (Fig. 3b). The decrease of relative crystallinity in HS films could be related to the presence of more hydrogen bond interaction between starch molecules, in accordance with FTIR analysis (Fig. 2B), and greater glycerol-starch bonding. The more amorphous structure suggests that a less efficient chains packing occurred in HS samples.

The crystalline structure of the blend films prepared with both modified starches (HS/CMS) behave as the sum of the HS and CMS films structures, with a tendency to Vh type structure, and with higher crystallinity than HS but lower than CMS films (RC = 6.3 ± 0.1 %). This fact suggests that a phase separation occurs in HS/CMS films, in agreement with SEM images.
(Fig. 1d). XRD patterns of the commercial starches granules are exhibited in Figure S3 (Supplementary Data).

Relevant differences on thermal (TGA) and differential thermal (DTA) curves behavior between the studied films were observed (Fig. 4). All samples presented three thermal degradation processes (Fig. 4A), typically found in thermoplastic starch materials [65,66]. The first degradation process between 100 °C and 150 °C corresponds to water evaporation, the second, between 150 °C and 280 °C is associated to the glycerol-rich phase degradation, and the last and most important degradation process, around 280 °C and 350 °C, is related to the thermal degradation of starch [67].

CMS and HS/CMS films exhibited slightly lower weight loss than NS samples in the glycerol-rich phase degradation, indicating homogeneous distribution of the plasticizer in the starch matrix and greater interaction between starch and glycerol that hinders the evaporation of the plasticizer. According to FTIR results, strong interactions of starch chains with glycerol and/or the generation of different molecular rearrangement between starch chains occurs in CMS and HS/CMS films, which could make the plasticizer evaporation more difficult.

In HS films, a greater weight loss was observed between 250-300 °C that could be attributable to the early beginning of the starch-rich phase degradation due to the low molecular weight of HS (Table S1, Supplementary Data). Particularly, the more amorphous structure of HS (Figure S3, Supplementary Data) possibly favored the inclusion of the plasticizer molecules in the starch; so higher temperatures to degrade the glycerol were required.

The starch-rich phase degradation temperature ($T_d$) of all films did not present significant differences (Table 1) and occurred in the same range of temperatures reported in the literature.
resulting in thermally stable films up to temperatures below 280 °C.

In DTA curves (Fig. 4B), three endothermic processes were observed for all films. The first, between 50 °C and 150 °C, is associated to a complete gelatinization of starch films [53], while the second and third process, at around 295 °C and 320 °C, correspond to the thermal decomposition of amylose and amylopectin, respectively.

CMS films presented DTA curves with no significant differences than those of NS samples, indicating that the low degree of carboxymethylation did not affect the thermal behavior of the materials.

Hydrolyzed starch film showed a small shoulder at around 258 °C, corresponding to free glycerol evaporation, also observed by TGA. In addition, a decrease of amylose degradation temperature in about 10 °C with respect to NS samples was observed. This behavior is associated to the acid hydrolysis process, in which the α-D-(1,4) glycoside linkages are the most susceptible bonds to be cleaved [59], and it is a consequence of the strong change in the molecular weight of the amylose fraction in HS (Table S1, Supplementary Data).

DTA curves behavior of HS/CMS films did not behave as the sum of those of HS and CMS samples. This is possibly due to the partial miscibility between both modified starches, as previously discussed from their morphology.

Thermal analyses (DTA) of the commercial starches are presented in Figure S3 (Supplementary Data).

The most notable differences in susceptibility to water were observed in the films obtained from commercial hydrolyzed starch (Table 1), which showed increases of more than 70% in the value of water solubility (S) and almost 20% in moisture content (M). Higher solubility values in films made from hydrolyzed pinhão and or potato starch were reported by Luchese.
et al. [29] and Olsson et al. [70], respectively. Generally, water solubility increased with higher acid concentrations and longer hydrolysis times [29,71]. The increase of M in HS system can be associated to the lower molecular weight of this starch. The hydrolysis process broke the chains of the starch, leading to a more amorphous and open molecular arrangement that facilitated the absorption of water [72]. This fact also explains the increase in WVP values of HS samples compared to the rest of the films.

Commercial carboxymethyl starch led to a decrease in the WVP values of CMS films compared to NS (~ 60%) and HS (more than 70%) samples. Regarding the difference with NS system, this could be associated to the presence of carboxyl groups in CMS and to the lower availability of glycerol, as discussed in XRD section. The relevance of these results is related to the fact that a very low degree of carboxymethyl substitution led to cassava starch-based films prepared by extrusion with much better water vapor barrier properties. In addition, it is important to notice that the price of commercial CMS is similar to that of NS (1.00 USD/kg vs. 1.07 USD/kg, according to the supplier). This could generate great interest since it is possible to improve the barrier properties of thermoplastic starch edible films almost without increasing investment costs.

HS/CMS films did not show significant differences on WVP values compared to those of CMS samples. The results showed that the susceptibility to water of HS/CMS films did not satisfy the law of mixtures, indicating phase separation between both chemical modified starches and a non-additive behavior. Considering morphology of HS/CMS samples and the literature, it suggest that these films present a structure of a continuous matrix of CMS with inclusions of HS [73]. In this sense, it is expected that water vapor permeation path occurs through the CMS continuous phase, while the zones formed by HS accelerates diffusion, leading to a WVP value slightly higher than that of CMS.
Tan δ curves of all films exhibited two relaxation processes, typical of partial miscibility of starch-glycerol systems (Fig. 5) [64,68,74]. The first, between -52 °C and -40 °C, corresponds to the α-relaxation of glycerol-rich phase (identified as glass transition temperature, $T_g1$) and the other, from around 40 °C to 80 °C, is associated to the starch-rich phase ($T_g2$)[53,75].

The films obtained from the chemical modified starches showed significant differences on tan δ behavior compared to those from native starch. NS samples exhibited the lowest value of $T_g1$ (at around -52 °C), the highest $T_g2$ (at ~ 77 °C) and a shoulder at about 20 °C that corresponds to the retrogradation of the amorphous amylopectin chains in presence of moisture [75], which is highly dependent on the relative humidity [74]. Moreover, Additionally, the intensity and the temperature at which this peak is observed depends on the starch type [58].

Films obtained from modified starches showed closeness between $T_g1$ and $T_g2$, as a consequence of an improvement in starch-glycerol interactions, as it was previously evidenced on FTIR and TGA assays (Fig. 3B and Fig. 4A, respectively). Particularly, the significant higher value of $T_g1$ in HS films indicates a better miscibility and interaction between the glycerol and the starch, probably due to the lower molecular weight of HS and to the more open structure of the films that enables a better penetration of the plasticizer.

None of the films prepared by the modified starches granules presented the shoulder associated to starch retrogradation, indicating that lower recrystallization occurs in these systems, in agreement with other authors [28,76].

Regarding to HS/CMS films, the relaxation associated to the starch-rich phase resulted wider than in HS and CMS samples. The wider peak is associated to the phase separation in HS/CMS system that led to a combination of the relaxation processes of each starch, which occur at slightly different temperatures.
Modified starch films present several differences in the mechanical parameters determined from the uniaxial tension assays (Table 2), showing increases in the Young’s modulus (E) and the stress at break (σₐ) and decreases in the strain at break (εₐ).

Regarding to HS films, these results could be consequence of the strongest affinity between the glycerol and the hydrolyzed starch, as it was discussed from dynamic mechanical properties and TGA studies. Particularly, decrease of εₐ is related to absence of starch granules, which led to lower free plasticizer per unit area than in NS films.

CMS films showed the highest values of E and σₐ and a decrease in εₐ with respect to NS and HS. This behavior could be explained considering the Vₜ type structure observed in CMS samples (Fig. 1c), which indicates that glycerol molecules have been encapsulated inside the helical structure of the starch, leaving less free plasticizer molecules to act. Thus, increments in the Young’s modulus and stress at break were expectable.

E and σₐ values of HS/CMS films resulted between those of CMS and HS samples, while the strain at break was similar to CMS films, indicating a non-additive behavior. Vu et al. [73] reported a similar behavior on cassava and mung bean starches blend films plasticized with glycerol, proposing that it occurred due to the formation of a network of amylose clusters surrounding granule remnants. As shown in SEM image (Fig. 1D), HS/CMS films presented granule remnants, which could explain the observed results in uniaxial tensile parameters.

Beyond the changes obtained by the use of the commercial modified starches, it was observed that NS films presented higher values of E and σₐ than different extruded thermoplastic films from cassava starch reported in the literature [53,77]. This behavior is probably associated to the presence of homogeneous distributed starch granules with great adhesion to the matrix, as shown in NS films morphology (Fig. 1A), which could be acting as reinforcement [52,78]. Besides, the greater εₐ of these films could be due to a higher
concentration of glycerol in the areas without starch granules grains which, combined with the presence of well-added granules to the matrix, led to increases in the strain at break of NS samples.

4. Conclusions

Commercial modified cassava starches (hydrolyzed and carboxymethyl) led to significant changes in the molecular arrangement of thermoplastic films obtained by flat-die extrusion process, affecting their overall properties. While broken granules grains were observed in native starch samples, films prepared with HS and CMS resulted homogeneous, indicating that less process energy was necessary to gelatinize these starches. Modified starch films presented a more marked Vh type crystalline structure with lower relative crystallinity compared to NS samples and it showed improvements in the starch-glycerol interactions. In particular, CMS films showed a lower amount of free glycerol to act as plasticizer. The use of commercial modified cassava starches strongly modified the mechanical response of the films, leading to increases in both Young’s modulus and the stress at break and decreases in strain at break, for HS and CMS films. Hydrolyzed starch films were the most hydrophilic, showing increases of 50% in WVP and almost 75% in water solubility values, while carboxymethylation led to the films with the highest water resistance, exhibiting decreases in WVP of about 55%. Films obtained from the combination of HS and CMS (50:50 wt.%) presented phase separation, which provoked that their overall properties did not compete with those of the films prepared with only CMS.

In the context of the discussed results, commercial modified starches from cassava could be used for different applications almost without increasing investment costs, reducing the energy required during the starch extrusion process. Carboxymethyl starch could be
employed to produce films with better mechanical and barrier properties than native starch, while hydrolyzed starch could be used in water-soluble packaging for specific products.

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Data Availability Statement

Data available on request from the authors.

Declaration of interest

The authors declare no conflict of interest.

References


Fig. 1. FE-SEM micrograph of cry-fracture surface of (a) NS, (b) HS, (c) CMS and (d) HS/CMS films.
Fig. 2. (A) FTIR spectra of the extruded films, (B) FTIR around the OH vibration region (3600-3000 cm\(^{-1}\)) and (C) FTIR around the 1000 cm\(^{-1}\) region of: (a) NS, (b) HS, (c) CMS and (d) HS/CMS films.
**Fig. 3.** X-ray diffraction patterns of the studied films of: (a) NS, (b) HS, (c) CMS and (d) HS/CMS films.
Fig. 4. (A) Thermal (TGA) and (B) differential thermal (DTA) curves of: (a) NS, (b) HS, (c) CMS and (d) HS/CMS films.

Fig. 5. Tan δ as a function of temperature of (a) NS, (b) HS, (c) CMS and (d) HS/CMS films.
Table 1. Water vapor permeability (WVP), moisture content (M), solubility (S) and starch-rich phase degradation temperature (T_d) of the developed films.

<table>
<thead>
<tr>
<th>Sample</th>
<th>WVP $^{10^-10}$ (g m$^{-1}$ seg$^{-1}$ Pa$^{-1}$)</th>
<th>M (%)</th>
<th>S (%)</th>
<th>T_d (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS</td>
<td>8.3 ± 0.8$^a$</td>
<td>16 ± 1$^a$</td>
<td>19 ± 2$^a$</td>
<td>315 ± 4$^a$</td>
</tr>
<tr>
<td>HS</td>
<td>12 ± 1$^b$</td>
<td>19 ± 1$^b$</td>
<td>33 ± 2$^b$</td>
<td>318 ± 4$^a$</td>
</tr>
<tr>
<td>CMS</td>
<td>3.5 ± 0.5$^c$</td>
<td>15 ± 1$^a$</td>
<td>17 ± 1$^a$</td>
<td>315 ± 4$^a$</td>
</tr>
<tr>
<td>HS/CMS</td>
<td>4.5 ± 0.5$^c$</td>
<td>15 ± 1$^a$</td>
<td>19 ± 2$^a$</td>
<td>318 ± 4$^a$</td>
</tr>
</tbody>
</table>

Different superscript letters in the same column mean significant differences ($p<0.05$).

$^1$Assays were performed in triplicate

Table 2. Young’s modulus (E), elongation at break ($\varepsilon_b$) and stress at break ($\sigma_b$) of the developed films.

<table>
<thead>
<tr>
<th>Sample</th>
<th>E (GPa)$^1$</th>
<th>$\varepsilon_b$ (%)$^1$</th>
<th>$\sigma_b$ (MPa)$^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS</td>
<td>0.30 ± 0.03$^a$</td>
<td>24 ± 1$^a$</td>
<td>7.6 ± 0.8$^a$</td>
</tr>
<tr>
<td>HS</td>
<td>0.45 ± 0.06$^b$</td>
<td>20 ± 2$^b$</td>
<td>10 ± 1$^b$</td>
</tr>
<tr>
<td>CMS</td>
<td>0.83 ± 0.05$^c$</td>
<td>6 ± 2$^c$</td>
<td>19 ± 2$^c$</td>
</tr>
<tr>
<td>HS/CMS</td>
<td>0.63 ± 0.05$^d$</td>
<td>7 ± 2$^c$</td>
<td>13 ± 1$^d$</td>
</tr>
</tbody>
</table>

Different superscript letters in the same column mean significant differences ($p<0.05$).

$^1$The reported results are the average of at least 10 independent experiments.
This work demonstrates that replacing cassava starch (NS) with commercial hydrolyzed (HS) or carboxymethyl (CMS) starches reduces the energy required to process starch granules into thermoplastic films by extrusion. Also, employing commercial CMS represents a successful strategy to improve mechanical and barrier properties of extruded films, while HS can be used to obtain water-soluble films for specific applications.