



Effects of exposure to pulsed light on molecular aspects of edible films made from cassava and taro starch



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ABSTRACT

Pulsed light (PL) has been proposed as a method for modifying the physico-chemical properties of edible films by photo-polymerization. Films made from cassava or taro starch plasticized with glycerol were developed and divided into two groups. Films in group 1 were exposed to the maximum PL intensity permitted by the U.S. Food and Drug Administration (FDA) (12 J/cm^2), while those in group 2 acted as the controls. The thickness, opacity, density, water solubility, water activity, stability in acidic or alkaline solutions, thermogravimetric (TGA) curves, differential scanning calorimetry (DSC), infrared spectra (ATR-FTIR), nuclear magnetic resonance ($^1\text{H NMR}$) and morphology of the films were then evaluated. Edible films prepared from taro starch (lower amylose content) exposed to PL were physically modified through photo-polymerization (cross-linking), resulting in a slight improvement in starch-glycerol interactions. Conversely, starch cross-linking was not evident in the films made from the cassava starch (higher amylose content), and a photo-degradation effect (deterioration) was confirmed. *Industrial relevance:* This study confirmed that pulsed light (PL) caused the deterioration of edible films made from a starch with a high amylose content. In contrast, PL treatment improved the physico-chemical properties of films made from a starch with a low amylose content. PL can thus be proposed as a quick physical method for the modification of edible films on an industrial scale.

1. Introduction

The materials currently most widely used in the food packaging industry are petroleum-derived polymers. Nonetheless, multiple problems associated with their use such as: maintaining a dependence on oil prices, a lack of renewability and the creation of potential pollutants have been identified (Gutiérrez, Tapia, Pérez, & Famá, 2015a). The use of biodegradable polymers with special emphasis on starches has been proposed as an alternative. Starches are cheap, plentiful, renewable and nontoxic (Talja, Helén, Roos, & Jouppila, 2007). Some of the characteristics of starch-based films, such as water sensitivity, mechanical strength and barrier properties are, however, not as good as those of synthetic polymer films (Dhakal & Zhang, 2012; Gutiérrez, Tapia, Pérez, & Famá, 2015a,b). In order to improve these several alternatives have been proposed, among which are procedures that chemically and/or physically modify the starch (García-Tejeda et al., 2013). Recently, Gutiérrez and González (2016) suggested that starch-based films could be modified by exposing them to pulsed light (PL). PL is a non-thermal emerging technology based on UV radiation (Izquier & Gómez-López, 2011). According to Gutiérrez and González (2016), the high intensity

of PL and the broad spectrum over which it operates suggest that it could be an efficient way to initiate the photo-polymerization process in starch-based films. Photo-polymerization can generate one or more of the following chemical processes in biopolymer-based films: depolymerization, cross-linking, and the formation of double bonds in polymer chain and other low-molecular compounds (Cui, Hanus, & Kessler, 2013).

In this regard, Gutiérrez and González (2016) observed a deteriorative effect of PL on cassava (*Manihot esculenta* C.) and taro (*Colocasia esculenta* L. Schott) starch-based films. It was unclear, however, why the films prepared from taro starch exposed to PL did not suffer as much deteriorative damage as the cassava starch-based films under the same conditions.

The effects of PL on the physico-chemical properties of edible films made from cassava and taro starch were studied using several assays, in order to establish whether PL treatment causes cross-linking in starch-based films. Two starches with different amylose contents were selected, so that the effects of PL on films with different amylose/amylopectin ratios could be compared.

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2. Experimental

2.1. Materials

Starches were isolated from taro and cassava tubers using the method described by Pérez, Bahnassay, and Breene (1993) obtaining a yield of approximately 30% in both cases (Gutiérrez, Pérez, Guzmán, Tapia, & Famá, 2014). Total amylose content was determined by the differential scanning calorimetry (DSC) method described by Pérez et al. (2013), which is based on energy changes during the formation of the amylose/lyso-phospholipid complex. An approximate total amylose content of 15.1% for the taro starch and 19.9% for cassava starch was obtained. Glycerol from Prolabo, Sweden, was used as a plasticizer for film formation.

2.2. PL equipment

PL processing was carried out with a XeMaticA-1XL system (SteriBeam GmbH, Kehl, Germany). This consists of a metal housing surrounding a treatment chamber made from polished stainless steel with inner dimensions 35 cm wide, 24 cm deep and 30 cm high. The system is equipped with a 19 cm, cylindrical, xenon-gas filled, ozone-free (TiO doped quartz) flash lamp, located at the top of the chamber, that emits a spectrum ranging from UV to infrared (Fig. 1).

2.3. Film formation

Films containing 4.5% w/v of starch and 1.5% w/v of glycerol in 500 mL distilled water were prepared following the methodology described by Gutiérrez and González (2016). One group of films was exposed to PL while the other was used as a control. The experimental group was treated with PL using the equipment described above, at the maximum intensity (12 J/cm²) permitted by the U.S. Food and Drug Administration (FDA) (FDA, 2013). The resulting thermoplastic starch films: cassava treated with pulsed light (TPS-CPL), untreated cassava control (TPS-C), taro treated with pulsed light (TPS-TPL), and untreated taro control (TPS-T) were obtained. Films were conditioned with a saturated NaBr solution ($a_w \sim 0.575$ at 25 °C) for seven days prior to each test. During this period containers were protected from light in a dark room in order to avoid photodegradation. Film samples used for determining water activity (a_w) were not conditioned.

2.4. Film characterization

2.4.1. Determination of film thickness (e)

A digital micrometer (Micromaster ®) with an accuracy of 0.001 mm was used to determine the thickness (e) of the films. Measurements



Fig. 1. PL equipment.

were taken at 18 random positions.

2.4.2. Opacity

The opacity was determined according to the method proposed by Sukhija, Singh, and Riar (2016). The ultraviolet (UV) and visible light barrier properties of dried films were measured at selected wavelengths between 400 and 800 nm using a UV-Vis spectrophotometer (u-2001, Japan). Film opacity was measured at 600 nm and calculated using the following equation given by Han and Floros (1997):

$$\text{Opacity} = A_{600}/e \quad (1)$$

where: A_{600} = the absorbance at 600 nm and “ e ” = film thickness (mm).

2.4.3. Density (ρ)

Film density (ρ) was determined by cutting samples of each film type into 12 mm diameter (ϕ) discs. The thickness (e) of the discs was then determined by taking 18 random measurements of each one. After this, the discs were dried at 105 °C for 24 h and weighed, and the density was then calculated as the ratio between the weight and volume (thickness \times area) of each disc, using Eq. ((2)).

$$\rho = \frac{W_i - W_f}{\left(\frac{\phi}{2}\right)^2 \times \pi \times e} \quad (2)$$

where W_i is the initial dry weight and W_f the final dry weight.

The density experiments were performed in triplicate, and data was reported as mean values.

2.4.4. Water solubility (WS)

Water solubility was defined as the percentage of film dry matter solubilized after 24 h immersion in distilled water at 25 °C (Gontard, Guilbert, & Cuq, 1992). The water solubility (WS) of the films was determined according to Romero-Bastida et al. (2005) using the modifications proposed by Hu, Chen, and Gao (2009). For this, ~ 0.5 g of each film sample was weighed, placed in a well containing 50 mL distilled water, and left for 24 h at 25 °C. The water was then drained and the samples dried in an oven at 105 °C for 24 h. The dried samples were then weighed and the percentage of water solubility was obtained using the following equation:

$$WS = \frac{(W_i - W_f)}{W_i} \times 100 \quad (3)$$

where W_i is the initial dry weight and W_f the final dry weight.

2.4.5. Water activity (a_w)

A psychrometric a_w meter Aqualab Cx-2 (Decagon Devices, Pullman, USA) previously calibrated with water at 25 °C was used to determine the water activity of the films. The mean value of three measurements was reported.

2.4.6. Stability in acidic or alkaline solutions

The stability of the films in acidic and alkaline solutions was evaluated. Film pieces (25 \times 25 mm) were immersed in containers with 20 mL of standard HCl (pH 1) or NaOH (pH 13) solutions. The containers were then sealed and maintained at 25 °C for 24 days. Changes in the appearance of samples were recorded with an 8.1 mega-pixel Cyber-shot Sony camera, model DSC-H3 (Japan). At least six samples of each film system were tested in order to ensure good reproducibility.

2.4.7. Thermogravimetric analysis (TGA)

The thermal behavior of the films was analyzed using a thermogravimetric analyzer (Shimadzu DTG-60, Japan). Film samples of approximately 10 mg were gradually heated from room temperature to 500 °C at 10 °C/min, under nitrogen flow (30 mL/min). Analyses

were performed in triplicate.

2.4.8. Differential scanning calorimetry (DSC)

Film samples were evaluated using a Mettler Toledo DSC 823, (Schwerzenbach, Switzerland). Samples of 6–7 mg were weighed, placed individually in aluminum pans and hermetically sealed; an empty pan was used as a reference. Pans were heated from -70 to 120 °C at a heating rate of 10 °C/min. The glass transition temperature (T_g) was measured as the temperature in the middle of the relaxation range and the enthalpy (ΔH) as the difference in heat flow. All measurements were taken in duplicate.

2.4.9. Attenuated total reflectance Fourier transform infrared spectroscopy (ATR/FTIR)

Fourier transform infrared spectroscopy in total attenuated reflection mode (ATR-FTIR) was used to study the interactions between the components of the films. Measurements were carried out at 25 °C with the aid of a Nicolet 8700 (Thermo Scientific Instrument Co., Madison, Wisconsin, USA). Spectra were recorded between 700 and 4000 cm^{-1} from an average of 40 scans at 4 cm^{-1} resolution. Each sample was scanned three times observing good reproducibility.

2.4.10. Nuclear magnetic resonance (^1H NMR)

The nuclear magnetic resonance of the films (^1H NMR) was determined using a Bruker Avance 300 MHz NMR spectrometer (Rheinstetten, Germany). The films examined were dissolved in $\text{DMSO-}d_6$ at 25 °C for 24 h. All analyses were performed in triplicate.

2.4.11. Light microscopy

Small pieces (2 $\text{cm} \times 1$ cm) of each film were mounted onto glass slides and examined using an optical microscope (Olympus BX60M, Japan) at $50\times$. At least three microphotographs of each system were then taken using a video camera imaging system (Olympus IMAGE RS).

2.5. Statistical analysis

Experimental data were analyzed to determine whether differences in the properties measured were statistically significant. Results were expressed as means \pm SD. Statistical comparisons were done by analysis of variance followed by Tukey's multiple range tests using the "Statistical Analysis Systems" software (version 9.2, SAS, Statistical Analysis Systems, North Carolina, USA). Differences were considered to be significant when $p < 0.05$.

3. Results and discussion

3.1. Thickness

Table 1 shows the thicknesses of the different systems studied. The films derived from cassava starch (TPS-CPL and TPS-C) were thicker than the taro starch-based films (TPS-TPL and TPS-T). According to Gutiérrez, Morales, Tapia, Pérez, & Famá (2015), a greater interaction between the starch and the plasticizer could lead to thicker films. This suggests that the films prepared from cassava starch interact more

strongly with the glycerol than the taro starch films. This fits well with the results reported by Gutiérrez, Tapia et al. (2015a), since films with a higher amylose content are generally thicker.

In addition, the TPS-TPL films were thicker than their respective controls (TPS-T), whereas the opposite was the case for the cassava starch films, i.e. the PL treated cassava starch films (TPS-CPL) were significantly thinner ($p \leq 0.05$) than the controls (TPS-C). This suggests that starch-glycerol interactions decreased in the TPS-CPL films (compared to their controls, TPS-C), possibly due to a decrease in their molecular weight and the occurrence of starch retrogradation processes as reported recently by Gutiérrez and González (2016) for these types of systems. In contrast, the increase in the thickness of the taro starch films shows that stronger hydrogen-bonding interactions between the starch and the glycerol were generated after exposure to PL. Similar results were reported for films made from cross-linked "waxy" cush-cush yam starches with sodium trimetaphosphate (Gutiérrez, Morales, Pérez, Tapia, & Famá, 2015). This demonstrates a cross-linking effect in the taro starch films (with a lower amylose content) after being exposed to PL (TPS-TPL), and explains the slight, albeit not significant ($p \geq 0.05$), increase in the molecular weight of the TPS-TPL films reported by Gutiérrez and González (2016). A photo-degradation effect (cross-linking) due to PL may thus occur in the taro starch-based films (lower amylose content), whereas a deteriorative effect caused by PL was confirmed for the cassava starch-based films (higher amylose content).

According to Gutiérrez and González (2016), an additional phenomenon, known as starch retrogradation, takes place independently of the amylose content when the films were exposed to PL. This phenomenon was more significant, however, in the cassava starch-based films (TPS-CPL): the percentage of crystallinity of the TPS-CPL film ($21.0 \pm 0.2\%$) was higher than that of the TPS-TPL film (11.0 ± 0.1), indicating that the TPS-CPL film has a greater tendency towards retrogradation compared to the TPS-TPL film (Gutiérrez & González, 2016).

3.2. Opacity

The opacity values of the different systems studied (Table 1) indicate that opacity can vary with the amylose content of starches, also see Fakhouri et al. (2007). This is because molecules in solution, due to their orientation in parallel lines, get close enough to each other to form hydrogen bonds between the hydroxyl groups of adjacent chains. As a result, the affinity of the polymers for water is reduced, favoring the formation of opaque films. This fits with the results obtained, since the cassava starch-based films (TPS-CPL and TPS-C) were more opaque than the films prepared from taro starch (TPS-TPL and TPS-T).

On the other hand, the films exposed to PL were more opaque than the control films for both the starches studied. This increase in the opacity of the PL-treated systems possibly occurred as a result of the molecular reorganization of carbohydrate polymers as suggested by Karbowski, Debeaufort, Champion, and Voilley (2006). However, the opacity of the taro starch films exposed to PL (TPS-TPL) increased by 1.38 times compared to the control films (TPS-T), whereas the opacity of the TPS-CPL films increased by only 1.07 times compared to their

Table 1
Thickness (e), opacity, density (ρ), water solubility (WS) and water activity (a_w) of the different films.

Parameter	TPS-C	TPS-CPL	TPS-T	TPS-TPL
e (mm)	0.18 ± 0.01^c	0.15 ± 0.01^d	0.09 ± 0.01^a	0.12 ± 0.01^b
Opacity	0.28 ± 0.01^c	0.30 ± 0.01^d	0.18 ± 0.01^a	0.25 ± 0.01^b
ρ (g/cm^3)	1.11 ± 0.02^d	1.05 ± 0.03^c	0.94 ± 0.03^a	0.99 ± 0.01^b
WS (%)	38 ± 1^a	41 ± 1^b	52 ± 1^d	49 ± 1^c
a_w	0.453 ± 0.002^a	0.455 ± 0.001^a	0.488 ± 0.001^c	0.484 ± 0.001^b

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

Thermoplastic starch films: Cassava control (TPS-C), cassava treated with pulsed light (TPS-CPL), taro control (TPS-T) and taro treated with pulsed light (TPS-TPL).

respective controls (TPS-C). This greater relative increase in the opacity of the taro starch films exposed to PL could be due to cross-linking reactions, since, as is well known, these reactions promote the alignment of the polymeric chains converting them into physical obstacles to the passage of light, thus increasing film opacity (Gutiérrez & González, 2016).

3.3. Density

The density values of the films are shown in Table 1, and were statistically different ($p \leq 0.05$) between the systems studied. Density can be affected by different factors, such as the type of polymeric structure, which results from the composition, molecular weight and interactions between the components present in the films (Pelissari, Andrade-Mahecha, & do Amaral Sobral, P.J., & Menegalli, F.C., 2013). The densest films were those made with the starch with the higher amylose content (cassava starch). Following Pelissari et al. (2013) more compact structures lead to denser films. According to Gutiérrez and González (2016) the films based on cassava starch (TPS-CPL and TPS-C) were more compact than the taro starch-based films (TPS-TPL and TPS-T). The density values obtained in this study thus correspond to the microstructures previously reported for these materials. The densities of the films developed were, however, lower than those reported by Müller, Yamashita, and Laurindo (2008) for cassava starch films plasticized with glycerol ($\sim 1.98 \text{ g/cm}^3$), but similar to those obtained by Pelissari et al. (2013) for banana flour and starch films.

On the other hand, the density values of the PL treated cassava starch-based films (TPS-CPL) were lower than their respective controls (TPS-C). This is probably associated with a decrease in their molecular weight, as reported by Gutiérrez and González (2016) for the same systems under study. In contrast, the TPS-TPL films were significantly denser ($p \leq 0.05$) than their respective controls (TPS-T). This was possibly related to a weak cross-linking reaction in the taro starch films (lower amylose content), associated with the slight (albeit statistically not significant; $p \geq 0.05$) increase in their molecular weight as reported by Gutiérrez and González (2016). Gómez-Guillén, Ihl, Bifani, Silva, and Montero (2007) also reported an increase in the density of starch-based films, which they attributed to a decrease in the availability of hydrogen groups due to cross-linking between the tuna-fish gelatin and the antioxidant extracts from murta leaves.

3.4. Water solubility

The solubility of edible films provides an indication of their integrity in an aqueous medium, such that higher solubility values indicate a lower resistance to water (Romero-Bastida et al., 2005).

The water solubility values of the different systems studied at 25 °C (Table 1) show that the taro starch films (TPS-TPL and TPS-T) were significantly more water soluble ($p \leq 0.05$) than the cassava starch films (TPS-CPL and TPS-C). According to Almasi, Ghanbarzadeh, and Entezami (2010), a strong hydrogen bonding interaction between the hydroxyl groups of the starch chains and the glycerol molecules decreases sensitivity to water. The taro starch, with a lower amylose content, interacts more weakly with the glycerol. The uninterrupted glycerol dissolves in the water, thereby decreasing the water resistance and increasing the water solubility of the starch.

The TPS-TPL films were significantly less soluble ($p \leq 0.05$) than their respective controls (TPS-T), possibly due to the cross-linking of the taro starch, which has a lower amylose content. Similar results were reported by Gutiérrez, Morales, Pérez et al. (2015) for films derived from native starches and starches modified by cross-linking with sodium trimetaphosphate. These results also suggest that the photo-induced polymerization (cross-linking) by PL is apparently preferably exerted on starches with a lower amylose content.

In contrast, the water solubility of the TPS-CPL films was significantly higher ($p \leq 0.05$) than that of their respective controls (TPS-C).

This was possibly due to the photo-degradation effect, which could generate the fragmentation of the starch chains, thus reducing film molecular weight and increasing solubility.

3.5. Water activity (a_w)

The water activity of the films is reported in Table 1. Higher a_w values were obtained for the samples made from taro starch. According to Gutiérrez, Morales, Pérez et al. (2015), a_w is directly proportional to moisture content in edible films. The greater a_w values observed for the TPS-TPL and TPS-T films thus resulted from weaker interactions between the glycerol and the taro starch (lower amylose content). Exposure to PL in the taro starch-based films resulted in a significant decrease in a_w . This could be related to the cross-linking of the taro starch, since stronger starch-glycerol interactions would compromise the Lewis sites (polar sites) thus preventing water adsorption from the environment. Similar results have been found in the literature for native and cross-linked white *Dioscorea trifida* edible coatings (Pérez, Segovia, Tapia, & Schroeder, 2012). The opposite occurred in the cassava starch films, where PL treatment slightly increased a_w , although this was not significant ($p \geq 0.05$). Finally, all the a_w values obtained indicate that it is unlikely that the edible films studied would suffer significant micro-biological growth.

3.6. Film stability in acidic or alkaline solutions

The aim of this assay was to evaluate whether the films developed were stable against changes in pH. This due to the fact that edible films may interact with the food products they come into contact with and it is thus important to determine whether their stability is affected by acidic or alkaline conditions.

All the films placed in the acidic solution remained visually unchanged for at least 24 days, demonstrating their stability in this medium. This means that they could be used for packaging slightly acidic foods such as meats and citrus fruits. Similar results were obtained by Hu et al. (2009), Gutiérrez, Morales, Pérez et al. (2015) and Gutiérrez, Suniaga, Monsalve, and García (2016) for thermoplastic polymer films derived from potato, cassava and cush-cush yam starches, as well as for films based on native and modified plantain flours.

In contrast, immersion for one day in the alkaline solution generated a swelling reaction in the films (Fig. 2), although the TPS-TPL and TPS-T films were more stable in this medium than the TPS-CPL and TPS-C films (Fig. 2 c and d). This may be because taro starch is less susceptible to swelling than cassava starch, due to its lower amylose content.

It is important to note that after 24 days all the studied systems showed lower stability in the alkaline medium compared to other TPS films reported in the literature (Gutiérrez, Morales, Pérez et al., 2015; Gutiérrez, Morales, Tapia et al., 2015). These matrices could thus be proposed as vehicles for transporting active substances for their release in the small and large intestines after the swelling process. As is well known, the intestinal environment has a pH of about 5 to 8 (Evans et al., 1988). The pH of the stomach, however, lies between 1 and 3 (Evans et al., 1988). This means that these starch-based materials could tolerate the pH of the stomach without causing any swelling, and could thus deliver active substances directly to the intestines. This has been already accomplished for some active compounds and probiotic bacteria (Piermaria, Diosma, Aquino, Garrote, & Abraham, 2015).

In a previous study, Gutiérrez, Morales, Pérez et al. (2015) reported that the disintegration and dissolution of films in an alkaline medium could be due to the reaction between sodium hydroxide and the hydroxyl groups of starch molecules. This destroys and reduces intra and intermolecular interactions (hydrogen-bonding interactions) between starch macromolecules, thus facilitating the swelling of the starch (Hu et al., 2009).

The TPS-TPL films showed a lower degree of swelling (and were

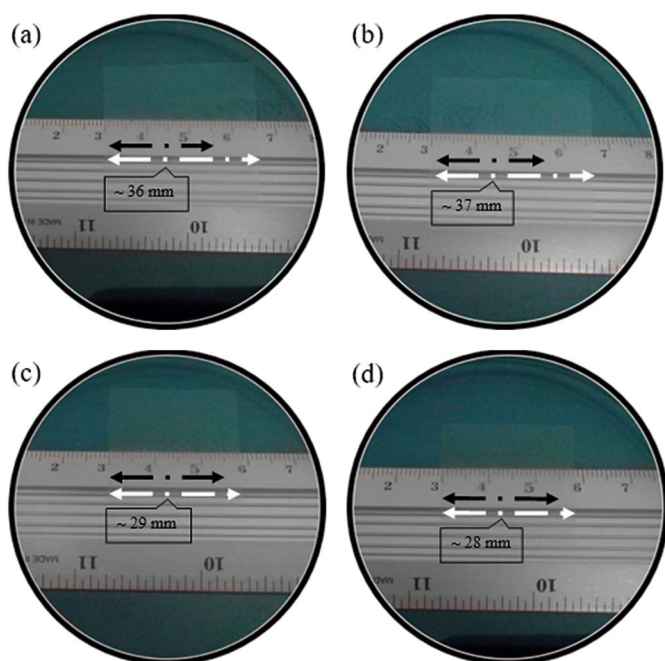


Fig. 2. Digital photographs of the thermoplastic starch films immersed in alkaline medium after 1 days: (a) cassava control (TPS-C), (b) cassava treated with pulsed light (TPS-CPL), (c) taro control (TPS-T) and (d) taro treated with pulsed light (TPS-TPL). Black line highlights initial length of the films. White line highlights final length of the films after 1 days immersed in alkaline medium.

thus more stable) than their controls (TPS-T). This increase in the stability of the taro starch polymeric system could be because the cross-linking of the starch chains occurs on the starch hydroxyl groups leading to their loss, resulting in films that are less reactive in an alkaline medium (NaOH). Treatment with PL thus improved the physicochemical properties of the taro starch films, possibly due to the photo-polymerization effect (cross-linking) which reduces swelling in these systems. Similar results were reported by Gutiérrez, Morales, Pérez et al. (2015) for films derived from native starches and starches modified by cross-linking with sodium trimetaphosphate.

In contrast, the TPS-CPL films were less stable than their respective controls. This was probably due to a photo-degradation effect (depolymerisation of starch), which exposes the starch chains, making them more likely to react with hydroxyl ions in the medium and therefore become more swollen.

3.7. Thermogravimetric analysis (TGA)

Thermogravimetric analysis (TGA) was performed in order to analyze the thermal stability of the four film systems (Fig. 3A). According to Pelissari et al. (2013) and Gutiérrez et al. (2016), the thermal decomposition of glycerol-starch films occurs in three main stages: the first stage corresponds to water evaporation which occurs at about 100 °C; the second stage is associated with the decomposition of the glycerol-rich phase which also contains starch, and occurs between ~180–260 °C; and lastly, the third stage occurs from 330 °C, due to the oxidation of the partially decomposed starch (Gutiérrez et al., 2016; Sanyang, Sapuan, Jawaed, Ishak, & Sahari, 2015).

As can be seen from Fig. 3A, the first stage of thermal degradation was not observed, since the weight loss of the films was recalculated on dry basis to avoid distortions resulting from their different moisture values, reported by our research group in Gutiérrez and González (2016).

In the second stage, a double weight loss was observed for the taro starch films (TPS-TPL and TPS-T) (Fig. 3A; and also evident from the DTGA curves, Fig. 3B). This could be related to phase separation

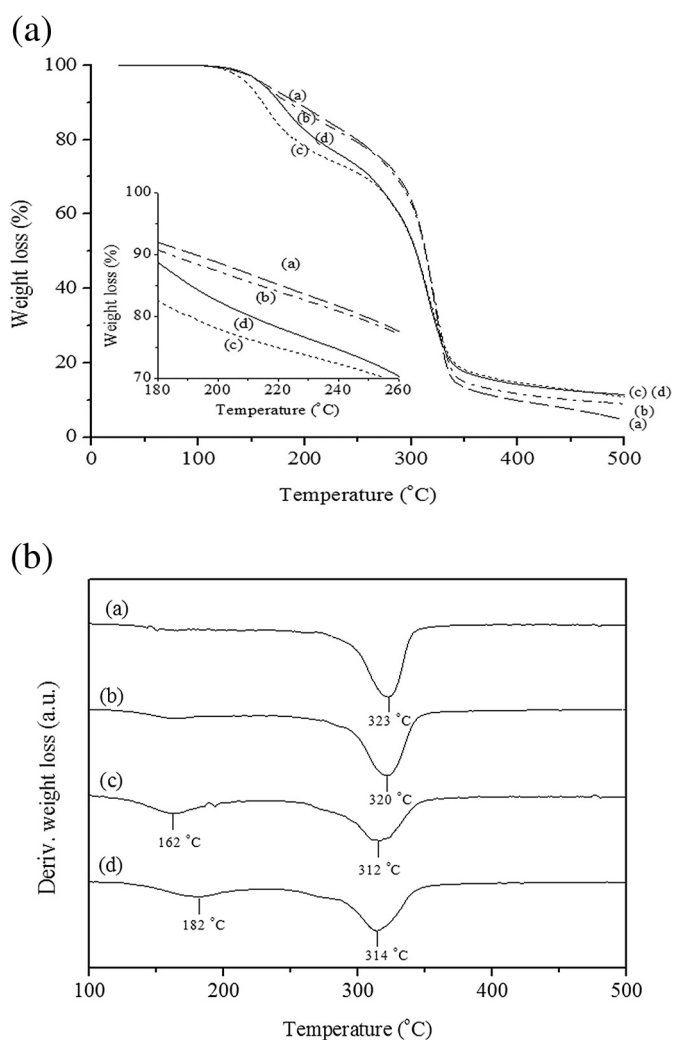


Fig. 3. (A) TGA and (B) DTGA curves of the different films studied: (a) cassava control (TPS-C), (b) cassava treated with pulsed light (TPS-CPL), (c) taro control (TPS-T) and (d) taro treated with pulsed light (TPS-TPL), in dry basis.

brought about by the low compatibility between the taro starch and the glycerol (Gutiérrez et al., 2016; Sanyang et al., 2015). These glycerol-free TPS-TPL and TPS-T films can then absorb moisture from the environment, thus explaining the slight increase in their moisture values compared to the cassava starch-based films (TPS-CPL and TPS-C) (also see results from Gutiérrez and González (2016)).

Exposure to PL lowered the degradation temperature of the cassava starch films from 323 °C to 320 °C (Fig. 3B). In other words, the TPS-CPL films showed lower thermal resistance than their respective controls (TPS-C). PL treatment thus weakened the intermolecular interactions (hydrogen bonding interactions) between the starch chains and hydroxyl groups of glycerol in the cassava starch-based films (Cyras, Tolosa Zenklusen, & Vazquez, 2006; Pelissari et al., 2013). As before, this may be because photo-degradation causes the fragmentation of the starch chains, thus reducing the molecular weight and thermal resistance of these materials.

This behavior contrasted with that of the taro starch-based films where the degradation temperature increased from 312 °C to 314 °C after exposure to PL (Fig. 3B). As is well known, cross-linking occurs preferentially in amylopectin chains which are more prevalent in taro starch (lower amylose content) (Jane, Xu, Radosavljevic, & Seib, 1992). Similar results have been reported by Gutiérrez, Morales, Pérez et al. (2015) for films prepared from “waxy” cush-cush yam starch cross-linked with sodium trimetaphosphate. In addition, the increase in the

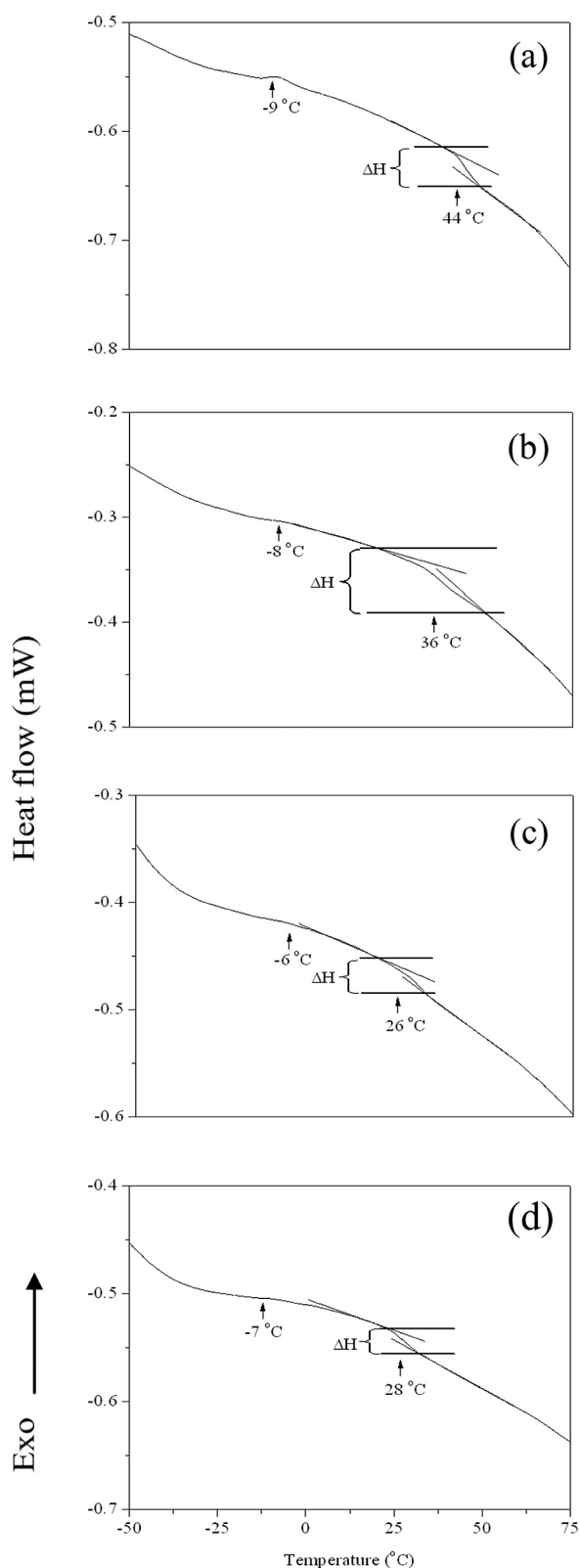


Fig. 4. Heating thermograms of the films based on: (a) cassava control (TPS-C), (b) cassava treated with pulsed light (TPS-CPL), (c) taro control (TPS-T) and (d) taro treated with pulsed light (TPS-TPL).

interactions between the taro starch and the glycerol, after PL treatment, would explain the slight decrease in the water solubility of these films (TPS-TPL) compared to the controls (TPS-T).

Finally in the third stage, stable thermal degradation curves were

observed to 500 °C (Patel et al., 2010; Ruiz, 2006). In addition, a further phase at around 475 °C was detected in the cassava starch-based films (TPS-C and TPS-CPL) associated with the thermal decomposition of starch Ruiz (2006).

3.8. Differential scanning calorimetry (DSC)

The DSC thermograms of the studied films (Fig. 4) reveal two drops in heat flow for both the taro and the cassava starch samples. According to Mali, Grossmann, Garcia, Martino, and Zaritzky (2002); Pelissari et al. (2013) and Gutiérrez et al. (2016), the first drop, located between -12 °C and -6 °C, could be attributed to the water melting point; and the second, between 26 and 44 °C, to the glass transition temperatures of the films.

The melting point of water in the developed films was affected by both a_w and moisture content. Gutiérrez and González (2016) indicate that taro starch-based films have a higher moisture content than cassava starch films, which could be related to the higher melting temperatures measured for these systems (Fig. 4 c and d).

A decrease in the Tg has been associated with more mobile starch chains (Mali, Grossmann, Garcia, Martino, & Zaritzky, 2006). Lower enthalpy (ΔH) values associated with glass transition have been correlated with the weakening of inter and intramolecular interactions between starch-starch chains (Mali et al., 2002; Pelissari et al., 2013). According to Bertuzzi, Vidaurre, Armada, and Gottifredi (2007), this leads to a decrease in the growth rate of the crystalline phase in these semi-crystalline biomaterials, as the alignment and recrystallization of starch molecules is impeded.

The DSC curves of the thermoplastic cassava starch films treated with PL (TPS-CPL, Fig. 4b) showed higher ΔH compared to their controls (TPS-C, Fig. 4a). According to that established by Mali et al. (2002) and Pelissari et al. (2013), this demonstrates that PL favors intermolecular interactions between the starch chains, i.e. PL induces starch retrogradation, thus reducing the stability of these films, which could also be correlated with their stability in an alkaline medium.

In contrast, the ΔH of the TPS-TPL films was lower than that of the controls (TPS-T) (Fig. 4), demonstrating that treatment with PL improved their stability. This fits well with the results regarding the stability of the TPS-TPL films in an alkaline medium.

The decrease in the Tg after exposure to PL in the cassava starch films (TPS-CPL, Fig. 4b) can be associated with the fragmentation of the starch chains caused by photo-degradation enabling the polymeric chains to become more mobile.

Finally, an increase in Tg was observed for the PL treated taro starch-based films (TPS-TPL, Fig. 4d) compared to their controls (TPS-T, Fig. 4c). The cross-linking of the starch chains limits the movement of polymeric chains, thus, the increase in Tg could be related to cross-linking caused by PL in these film systems. Similar results have been reported by Reddy and Yang (2010) for films derived from native starches and starches modified by cross-linking with citric acid.

3.9. Attenuated total reflectance Fourier transform infrared spectroscopy (ATR/FTIR)

Fig. 5A shows the FTIR spectra of the different films studied over the entire absorption range, as well as the functional groups associated with each band. No differences were observed between spectra before and after treatment with PL for the cassava starch films, or at least, no obvious changes in the matrix were detected. Similar results were reported by Umami-Shafiqah, Fazilah, Karim, Kaur, and Yusup (2012) for films made from sago starch exposed to UV radiation for 2 h. Nonetheless, the band located at 1720 cm^{-1} for the PL treated taro starch films (TPS-TPL) could be assigned to C=O bonds from ester groups formed between starch chains, confirming the cross-linking effect proposed for these systems. Similar results were reported by Wang, Ren, Li, Sun, and Liu (2014) for films prepared from cassava

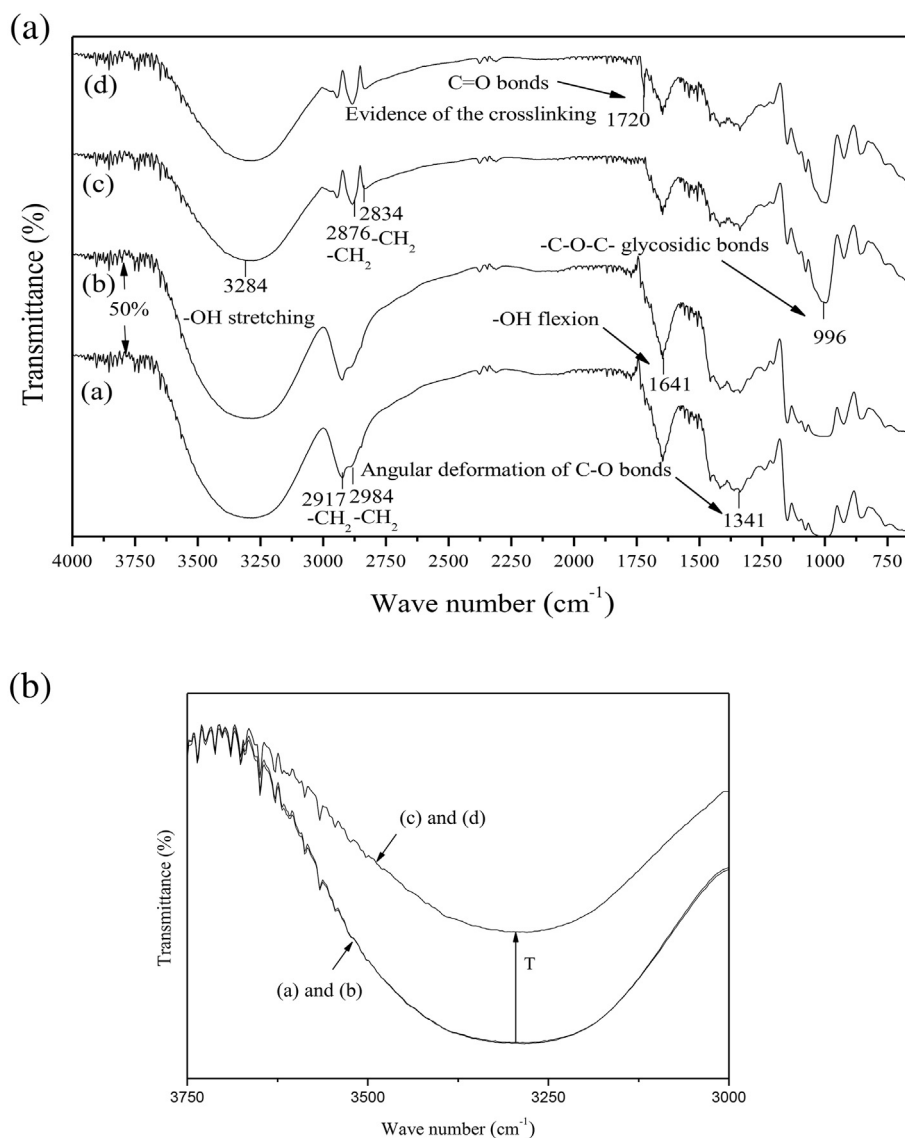


Fig. 5. Panel A- FTIR spectra of the different films studied in all the absorption range: (a) cassava control (TPS-C), (b) cassava treated with pulsed light (TPS-CPL), (c) taro control (TPS-T) and (d) taro treated with pulsed light (TPS-TPL). Panel B-FTIR spectra in the range of absorption corresponding to C–O group (OH stretching) of the different films studied: (a) cassava control (TPS-C), (b) cassava treated with pulsed light (TPS-CPL), (c) taro control (TPS-T) and (d) taro treated with pulsed light (TPS-TPL).

starch cross-linked with citric acid.

The cassava starch-based films showed a single peak at $\sim 2917\text{ cm}^{-1}$ not present in the films developed from taro starch. This was possibly due to the higher amylose content of the cassava starch, which could have suppressed the signal of the starch CH_2 groups. Fig. 5B shows the differences in the band shape and intensity of the films. Specifically, the taro starch films showed higher percent transmittances in the region between 3750 and 3000 cm^{-1} than the cassava starch films. This could be related to an increase in the degrees of freedom of the OH groups, which itself is attributable to lower Tg values. Similar results were reported by Gutiérrez et al. (2016) for films made from native and modified plantain flour. This means that an increase in the transmittance could be associated with a decrease in the Tg.

Additionally, it should be remembered that the taro starch films (TPS-TPL and TPS-T) were thinner than the cassava starch films (TPS-CPL and TPS-C). According to the Beer-Lambert law, the distance light moves through a substance is directly proportional to the absorbance. The shorter distance the light had to travel through the thinner taro starch films (TPS-T and TPS-TPL) decreased the amount absorbed, thus increasing their transmittance values with respect to the cassava starch-

based films (TPS-C and TPS-CPL) in the above mentioned region (3750 to 3000 cm^{-1}).

3.10. Nuclear magnetic resonance (^1H NMR)

Previous results have suggested that PL induces the cross-linking of taro starch (higher amylopectin content). In order to confirm this we decided to use the ^1H NMR technique. According to Kapelko-Żeberska, Buksa, Szumny, Zięba, and Gryszkin (2016), the signal of the $-\text{CH}_2$ group associated with the methylene protons generated by the cross-linking reactions of the starch chains is found at around 2.5 ppm. Based on this and taking into account the ^1H NMR spectra of the developed films (Fig. 6), we can see that exposure to PL produced a slight cross-linking reaction in the taro starch films (Fig. 6b). There was no evidence of this behavior, however, in the cassava starch films (Fig. 6a). This suggests that PL generated cross-linking reactions are more likely to occur in starch-based films with a low amylose content.

In addition, Mizoguchi and Ueda (2008) indicated that the cross-linking of polymers leads to an increase in their average molecular weight. This would explain the slight increase in the molecular weight of the TPS-TPL films reported by Gutiérrez and González (2016), even

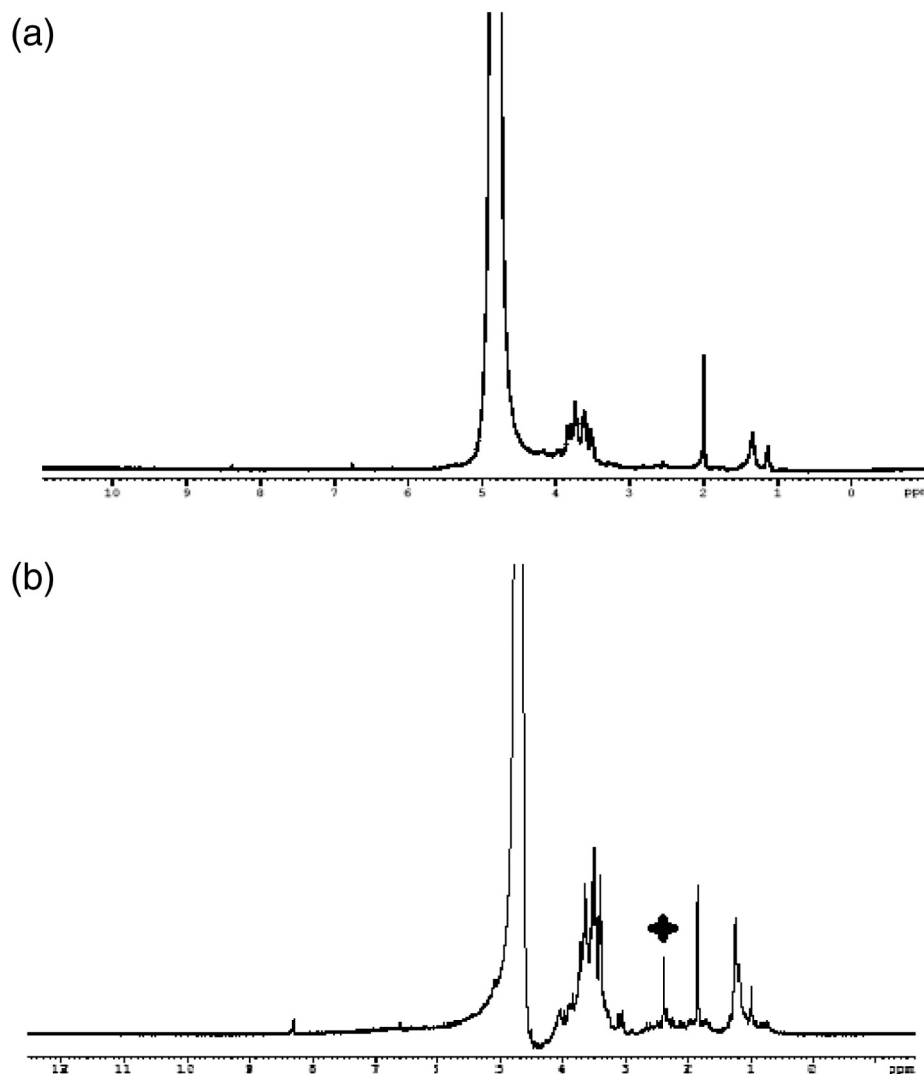


Fig. 6. ^1H NMR spectra of the films based on: (a) cassava treated with pulsed light (TPS-CPL) and (b) taro treated with pulsed light (TPS-TPL).

though this was not statistically significant ($p \geq 0.05$).

3.11. Light microscopy observations

Fig. 7 shows the images of the films as observed by optical microscopy. The TPS-CPL and TPS-C films (Fig. 7 a and b) contained small fragments identified as granular particles, products of the retrogradation of the cassava starch (higher amylose content). Gutiérrez and González (2016) found that the cassava starch films they studied (TPS-CPL and TPS-C) were more crystalline, $21.0 \pm 0.2\%$ and $19.0 \pm 0.2\%$, respectively, than those derived from taro starch (TPS-TPL and TPS-T; 11.0 ± 0.1 and 10.0 ± 0.1 , respectively). Similar results were reported by García, Famá, Dufresne, Aranguren, and Goyanes (2009) who indicated that the recrystallization of starch macromolecules produces crystalline particles that may act as nuclei. These granular particles could disperse light resulting in the decrease in transmittance observed in the FTIR spectra of the cassava starch films (Fig. 5B). The particles could also explain the higher T_g values for these systems. The lower amylose content of the taro starch-based films (Fig. 7 c and d) limited starch recrystallization, thus producing less grainy films with higher percent transmittance.

The TPS-CPL films had a higher particle density than the TPS-C films (Fig. 7 a and b). This may have been a product of the PL induced starch retrogradation. As has been reported in the literature, UV radiation generates different changes in polymeric materials, such as retrograda-

tion and the fragmentation of biopolymeric chains.

In contrast, fewer granules were observed on the taro starch films after exposure to PL (TPS-TPL) compared to untreated films (TPS-T). This is consistent with the results outlined earlier, since their physical modification by cross-linking, resulting from PL induced photo-polymerization, would inhibit the retrogradation process. According to Gutiérrez and González (2016), the increase in the crystallinity of films made from cassava starch after exposure to PL was primarily due to starch retrogradation, whereas the higher crystallinity values of PL treated taro starch films were mainly due to cross-linking. It is worth remembering that the cross-linking of starch chains promotes molecular alignment, thus increasing crystallinity.

4. Conclusions

In this study, cassava and taro starch films plasticized with glycerol were developed in order to determine whether or not cross-linking occurred. A recent study conducted by our research group found that in cassava starch-based films (higher amylose content) exposure to PL generated a deteriorative effect, due primarily to starch retrogradation and starch depolymerization. This led us to question if the lower amylose content of taro starch could produce a cross-linking effect after PL treatment. This new study showed that PL did have a slight photo-polymerization (cross-linking) effect on the taro starch-based films, thus improving the starch-glycerol interaction. Specifically, PL gener-

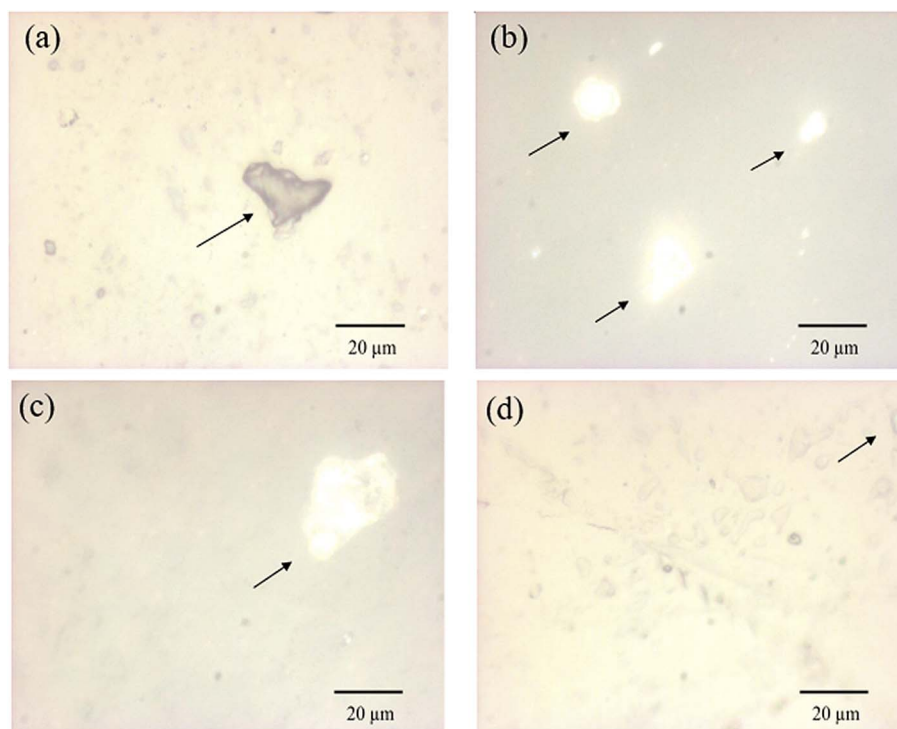


Fig. 7. Optical micrographs of the films based on: (a) cassava control (TPS-C), (b) cassava treated with pulsed light (TPS-CPL), (c) taro control (TPS-T) and (d) taro treated with pulsed light (TPS-TPL). At 50 × of magnification. Black arrows indicate starch particles in retrogradation process within of the edible films.

ated two contrasting phenomena in the taro starch films: on the one hand, it produced starch retrogradation, but on the other created crosslinks between the starch chains. This contrasts with its effects on the cassava starch-based films (higher amylose content), which suffered photo-degradation (depolymerisation and starch retrogradation).

Conflicts of interest

The authors declare no conflict of interest.

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