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Effect of beet flour on films made from biological macromolecules: Native and modified plantain flour

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ABSTRACT

Biological macromolecules such as starches of different amylaceous sources have been used in the formulation of edible films. However, there are few studies aimed at evaluating edible and intelligent films with response to pH changes from natural pigments, this despite the importance of these materials. In this context, films from native and modified plantain flour, plasticized with glycerol, with or without the addition of beet flour were developed. The chemical and structural composition of the flours, and its incidence on thickness, water solubility, contact angle, and mechanical and microstructural properties were evaluated, thus as its response to pH changes of the developed films. The observations showed that the incorporation of beet flour allowed to obtain intelligent films front to pH changes alkaline. Likewise, the betalains that were found in beet flour interacted more efficiently with the phosphated plantain flour, limiting well its immediate response to pH changes. In the same way, proteins and sugars of beet flour allowed to obtain more flexible films, due to the hydrogen bond interactions between these constituents and the plantain flours. This latter could justify the decrease of contact angle, and the increase on thickness and solubility of these films.

1. Introduction

Edible and biodegradable films elaborated from biological macromolecules have been studied over the last two decades, because these thermoplastic materials are considered environmentally friendly due they degrade more easily, compared with the common synthetic plastics [1–6]. However, the properties of these films depend strongly on the chemical composition of the biomatrix, as it has been shown that small variations in the content of protein, fat and fiber, can markedly alter the properties of these materials [7]. Likewise, the properties of these biopolymers can be modified by the addition of plasticizers, antimicrobials, pigments, among others; this with the purpose of improves their properties [8–15]. Another of the alternatives that have been employed with the objective of improve the properties of the edible films is the chemical and/or physical modification of the starch [16–20].

Starch is an excellent raw material for the production of biodegradable packaging, due to it being a botanical source renewable, economical and highly available [21]. The functionality of starch is provided by amylose and amylopectin [22]. Pelissari et al. [23] reported that the amylose content in plantain starch and flour (*Musa paradisiaca*) was 23.1% and 35%, respectively. Such materials have been used in some studies as matrix in the preparation of films based on native and modified polysaccharides [24–26]. Similarly, recent researchers have reported excellent properties of edible films made from starch and flour plantain [16,27–29]. However, plantain flour has not been evaluated as potential biomatrix of edible and intelligent films. The implement of the intelligent films in the food industry can alert to consumers on the quality and safety of the products. The incorporation of the pigments as the betalains found in the beet [30], can act as front indicator to pH changes. The observation of these changes would be associated with fraudulent modifications of the foods, breach of cold chain, and would be of great use to simply tell to the consumer the freshness degree of the food product [31,32]. Likewise, edible and intelligent

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films could indicate to the consumer that the food is ending its useful life, allowing decrease the great amount of food that is discarded by consumers [33–37]. FAO [38] has estimated that about a third of the food produced annually for human consumption are wasted, this would represent 1.3 billion tons of food lost, and that are valued by over USD 750 billion, which could feed the more than 842 million of people that still suffer from chronic hunger globally [39].

Additionally, to technological level, the employment of beet flour in these films could improve the properties of the polymer matrix due to its fiber content and simple sugars [40–45]. The fiber could act as reinforcement of the matrix and the simple sugars as plasticizer [46,47].

The aim of this study was to evaluate the chemical composition of the flours employed and its incidence on the response to pH changes and physicochemical properties of the edible and intelligent films developed.

2. Experimental

2.1. Materials

The plantain flour (*Musa* ssp., group AAB, sub-group clone Harton) was obtained by the method described by Pacheco [48]. The plantain used had a degree of maturation of 1, according with the scale of Loesecke [49] that was acquired at a local market in Caracas, Venezuela. Also, the beet flour was obtained following the methodology of Pacheco [48], but with some modifications: the beet was not immersed in a solution of citric acid (1%) before of dehydrate the tuber slices. Glycerol from Prolabo, Sweden, was employed as plasticizer in the formation of the films.

2.2. Preparation of the modified flour

The modified plantain flour was obtained following the methodology described by Kerr and Cleveland [50], modified by Lim and Seib [51]. The maximum concentration of sodium trimetaphosphate allowed by the FDA [52] was used, i.e. 3% w/w of the modifying agent with respect to the weight of the flour.

2.3. Characterization of the flours

The chemical composition of the flours evaluated was determined. Moisture content, ash and fat were determined using the gravimetric method [53]. Total protein was determined in based on the biuret reaction [54]. Determination of crude fiber was performed by the method proposed by Van Soest et al. [55]. Apparent amylose content was assessed using a colorimetric microprocedure based on the formation of the amylose/iodine complex [56]. Total sugars were determined by the methodology proposed by Lane and Eynon [57]. Phosphorus content and degree of substitution (DS) were determined by the colorimetric method ICONTEC [58], using the equations proposed by Deetae et al. [59]. Total carbohydrates were determined by subtraction of the percentages of water content, ash, crude protein and fatty materials, to one hundred percent of sample. Additionally, color parameters of all the flours were obtained by a Colorimeter Macbeth model Coloreye 2445 using CIE LAB scale, water activity (a_w) of the flours were determined using a psychrometric a_w meter Aqualab Cx-2 (Decagon Devices, Pullman, USA) previously calibrated with water at 25 °C and morphology of the granules were also determined. This latter determination was performed by optical microscopy with and without polarized light [60,61].

2.4. Film formation

Biodegradable films containing 2% w/v of either native or modified flour, 1.9% w/v glycerol and 500 mL distilled water were prepared following the methodology described by Gutiérrez et al. [17–20]. In the case of the films with incorporation of beet flour, this was added 10 min before the end of the process of obtaining the film forming solution (*FFS*), this in order to preserve the pigments of the beet, and give the opportunity of a homogeneous inclusion in the FFS. The incorporated quantity of beet flour was of 4% with respect to plantain flours used. The gel obtained was poured into stainless steel trays 40 cm × 30 cm, and dried in a Mitchell dehydrator (Model 645 159) for 24 h at 45 °C. The resulting thermoplastic starch films: native plantain flour (*TPS-NPF*), native plantain flour with incorporation of beet flour (*TPS-NPFB*), phosphated plantain flour (*TPS-PPF*) and phosphated plantain flour with incorporation of beet flour (*TPS-PPFB*) were then carefully removed from the casting molds. Before characterization, the films were conditioned at ~57% relative humidity (*RH*) for a week at 25 °C.

2.5. Characterization of the films

2.5.1. Determination of film thickness

The thickness (e) of the films was determined at 18 random positions using a digital micrometer (Micromaster®, Mitutoyo, USA) with an accuracy of 0.001 mm. The results were used for the determination of the tensile properties.

2.5.2. Water solubility

Water solubility was measured according to Romero-Bastidas et al. [24] using the modification proposed by Hu et al. [62]. For this, were weighed ~0.5 g of the films and were carried to an oven at 100 °C for 24 h. This is to obtain the initial dry matter. Next, the samples were immersed in 50 mL distilled water during 24 h at 25 °C. After, the water content was removed to dry the samples in an oven (100 °C for 24 h) to determine the final weight of the dry matter. Solubility is reported as the difference between the initial and final dry matter with respect to initial dry matter.

2.5.3. Scanning electron microscopy (SEM)

The films were cryofractured by immersion in liquid nitrogen, mounted on bronze stubs and sputter coated with a thin layer of gold for 35 s. The fracture surface of each material was analyzed using a Philips XL series 30 (Amsterdam, The Netherlands) scanning electron microscopy.

2.5.4. Contact angle

Contact angles were determined using a USB Digital Microscope (model DIGMIC200X, Perkin, China) equipped with Image Analysis Software 220X 2.0MP video, with 0.01° precision. A drop of distilled water (2 μL) was placed on the surface of each material. The contact angles (θ) were calculated by analyzing the images to determine the angle formed by the intersection of the liquid-solid interface (drop of water-surface of the film) and the liquid-vapor interface (tangent to the boundary of the drop) [63].

Contact angles were determined at a temperature of 25 °C and measured on the side of the surface exposed to the drying air during the film preparation. Measurements were taken just at the moment when the drop of water came into contact with the film surface. This was done in order to avoid false results caused by phenomena such as dehydration, swelling and dissolution [64]. A total of 12 contact angles were measured *per* film discarding two maximum and minimum values in each case. Thus, the mean and standard deviation of 8 measurements was reported.

Table 1
Chemical composition on dry basis of the flours used.

Parameter	Native plantain flour (%)	Phosphated plantain flour (%)	Beet flour (%)
Moisture	9.3 ± 0.3	8.6 ± 0.6^{a}	8 ± 1^{a}
Total protein	2.62 ± 0.05^{b}	1.00 ± 0.03^{a}	10.3 ± 0.9^{c}
Crude fat	0.37 ± 0.09^{b}	0.081 ± 0.009^{a}	0.66 ± 0.03^{c}
Ash	2.41 ± 0.03^{b}	0.81 ± 0.04^{a}	8.2 ± 0.2^{c}
Crude fiber	0.297 ± 0.002^{a}	0.36 ± 0.06^{b}	5.1 ± 0.4^{c}
Total carbohydrates	85.3 ± 0.3^{b}	89.5 ± 0.6^{c}	68.0 ± 0.1^{a}
Apparent amylose	24 ± 1^{c}	10 ± 1^{b}	0 ± 0^{a}
Total sugars	<1.3	<1.3	15.7 ± 0.4^{a}
Phosphorus	0.019 ± 0.001^{a}	0.022 ± 0.001^{b}	0.070 ± 0.007^{c}
DS	0.0019 ± 0.0001^{a}	0.0022 ± 0.0001^{b}	0.0073 ± 0.0007^{c}

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

2.5.5. Uniaxial tensile strength

The mechanical properties were measured using a universal test machine (Instron Ltd., High Wycombe, UK) and following the norm ISO 527-2 [65]. Equilibrated samples (15 per formulation) were mounted in the film-extension grips of the testing machine and stretched at a rate of 0.02 in/sec until breaking. The relative humidity of the environment was maintained at nearly 57% during the tests, which were performed at 25 °C.

The force-distance curve obtained in the tests was transformed into stress-strain curves, which allowed to obtain the following parameters: Young's modulus (E), maximum stress (σ_m), strain at break (ε_b) and toughness (T).

2.5.6. Response to pH changes

In order to observe the response of the films front to pH changes, samples of each system developed were placed in solutions of pH equal to 1, 7 and 13, elaborated from NaOH and HCl. The response of the materials was evaluated from the images photographed with a Cyber-shot Sony camera, model DSC-H3 (Tokyo, Japan) with 8.1 mega pixels [66].

2.6. Statistical analysis

The analysis of data was performed through the analysis of variance (ANOVA) using the Statgraphics Plus 5.1. software (ManugisticsCorp., Rockville, MD). Fisher's least significant difference (LSD) procedure was used at the 95% confidence level.

3. Results and discussion

3.1. Characterization of the flours used for the formation of the films

Table 1 shows the moisture content of the flours employed. As can be observed, the values obtained for plantain flours and beet flour were within the range reported for a lifespan stable for both flours [67]. However, no statistically significant differences ($p \geq 0.05$) were observed in moisture content between the plantain flours. Similar results were reported by Gutiérrez et al. [61], Pérez et al. [68] and Sívoli et al. [69], for phosphated starches of different sources amylaceous.

The crude protein content was relatively low in the native and modified plantain flours, between 1.00% and 2.62% (Table 1). The reduction of the 50% of crude protein content was recorded for the modified plantain flour, possibly this has been due to the leaching of the proteins during the modification of the flour [61]. So the same, the beet flour had a protein content (10.3%), an order of magnitude greater than plantain flours [70].

The crude fat content in the modified plantain flour was significantly ($p \leq 0.05$) lower compared to native plantain flour (Table 1). These small variations in the content of fatty material could influence in the plasticity of the edible films [61].

The fiber content in modified plantain flour was increased around of 20%, possibly as result of the leaching of proteins and fatty material during the modification. Likewise, higher values of crude fiber were found in beet flour (5.1%). This result was expected, considering the studies found in the literature [41,42,45].

As expected, phosphorus content and degree of substitution (DS) in modified flour was increased; all values of DS reported were within the range approved by the FDA [52].

The beet flour revealed a total sugars content of about 15.7%, this high total sugars content was verified with results previously reported [40,43,44]. In the case of the native and phosphated plantain flours, total sugars resulted less than 1.3%, this being consistent with the values obtained by Pérez-Sira [71] in native plantain flour.

The apparent amylose content of the modified plantain flour decreased about of the 50% as result of the cross-linking of the amylose. This behavior would correspond with the results obtained of DS: a greater value of DS showed a lower apparent amylose content. In the case of beet flour, it can be presumed that no contain starch, since the apparent amylose content was of 0% [72].

Table 2 shows the values of water activity (a_w) of the flours used. A direct relationship between moisture content and a_w was found, confirming that the flours used are not susceptible to the growth of molds and yeasts.

Color parameters were evaluated, being determined the lower values of L for beet flour. Therefore, it can say that is a dark flour compared to plantain flour (Table 2). The greatest value of the

Table 2
Water activity (a_w) and color parameters of the flours used.

Parameter	Native plantain flour	Phosphated plantain flour	Beet flour
a_w	0.430 ± 0.002^{b}	0.318 ± 0.001^{a}	0.500 ± 0.001^{c}
L	88.77 ± 0.01^{c}	84.93 ± 0.02^{b}	33.45 ± 0.01^{a}
a	1.51 ± 0.01^{a}	1.81 ± 0.01^{b}	33.98 ± 0.02^{c}
b	17.11 ± 0.01^{c}	7.07 ± 0.02^{b}	3.13 ± 0.04^{a}
Color differences (ΔE)	16.41 ± 0.01^{b}	10.54 ± 0.01^{a}	69.45 ± 0.02^{c}
Whiteness Index (WI)	79.48 ± 0.01^{b}	83.26 ± 0.01^{c}	25.21 ± 0.02^{a}
Yellow Index (YI)	33.10 ± 0.02^{b}	16.00 ± 0.04^{a}	82.1 ± 0.1^{c}

The values are the average of three determinations, similar letters in the same row indicates non-significant differences ($n = 3$, $p \leq 0.05$).

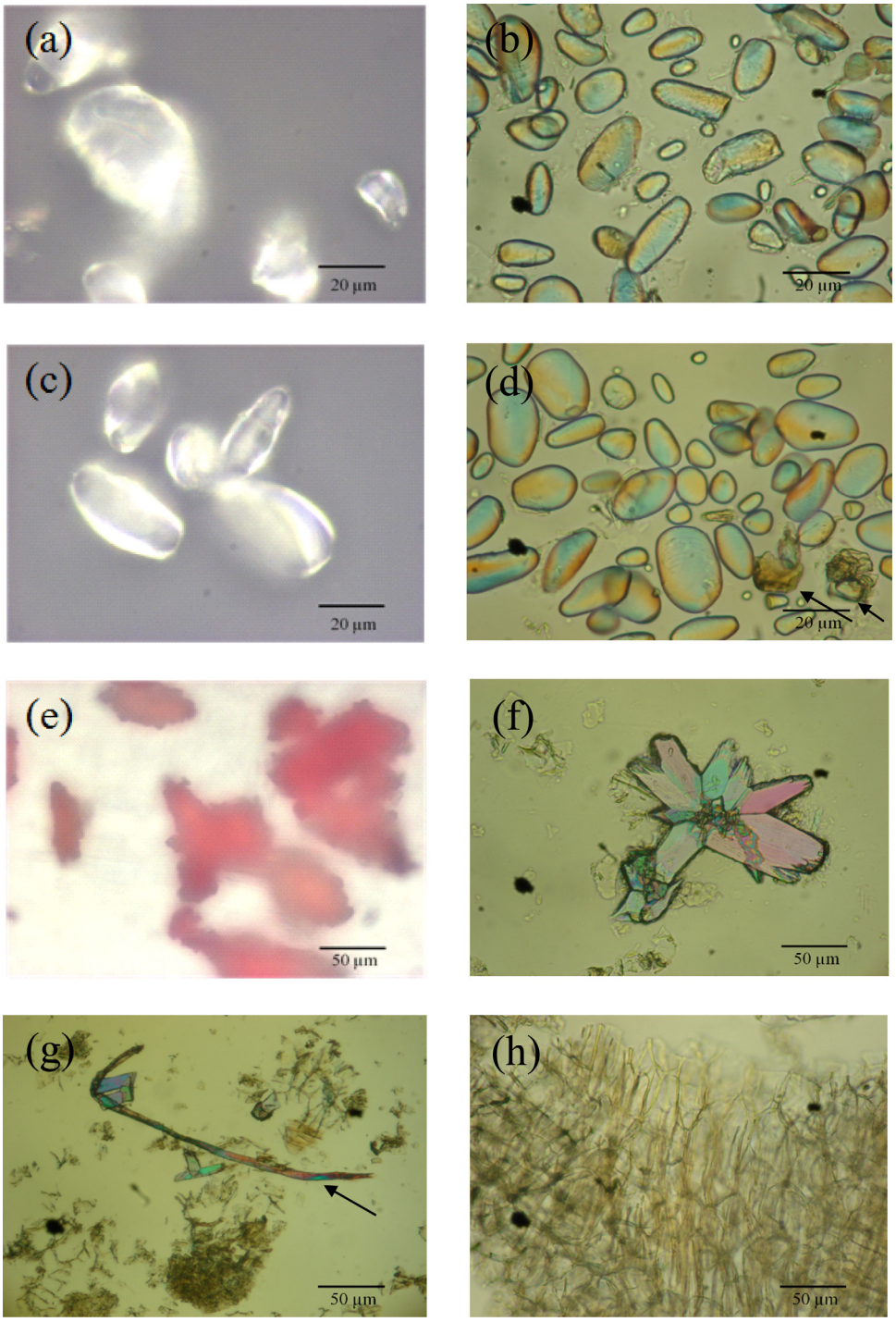

Fig. 1. Optical micrographs of the granules of: (a) native plantain flour and (c) phosphated plantain flour, at 50× of magnification; (e) Optical micrographs of beet flour at 20× of magnification. Optical micrographs with polarized light of: (b) native plantain flour, (d) phosphated plantain flour, (f) betalain crystal obtained of beet flour, (g) lignin fragments belonging to the wall of the sclerenchyma of the beet and (h) cellulose associated to the wall of the cell parenchyma of the beet, at 20× of magnification. In (d) black arrow indicate possible particles of fibrous material.

parameter *a* was obtained for beet flour, indicating a natural tendency toward the red color, own of the coloring of the pigments of this tuber. So the same, the highest value of the parameter *b* was determined for native plantain flour, this would indicate a tendency toward yellow color, that would be associated with the carotenoids of the plantain, and that are lost by leaching during modification by phosphation.

The minor color difference (ΔE) was determined in modified plantain flour. Again, this result may be related with the loss of pigment during the modification, which increased the whiteness index (*WI*) and decreased the yellow index (*YI*).

In Fig. 1a–d, it can be seen the images of starch granules contained in native and modified plantain flours observed by optical microscopy with and without polarized light. Both flours presented starch granules of elongated and longitudinal oval shape with irregular sizes and within the range reported in the literature: 10–50 μm [71,73]. However, the starch granules contained in modified plantain flour (Fig. 1c and d) have a slight increase in grain

Table 3
Thickness (e), solubility and contact angle of the different films.

Parameter	TPS-NPF	TPS-NPFB	TPS-PPF	TPS-PPFB
e (mm)	0.20 ± 0.03^{a}	0.22 ± 0.02^{a}	0.24 ± 0.08^{a}	$0.28 \pm 0.01^{b,a}$
Solubility (%)	52.5 ± 0.6^{a}	53.5 ± 0.1^{b}	$53 \pm 2^{a,b}$	$54 \pm 5^{a,b}$
Contact angle (°)	42 ± 2^{b}	42 ± 3^{b}	39 ± 2^{b}	27 ± 2^{a}

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

size. Similar results have been reported by Gutiérrez et al. [61] in phosphated starches of cassava and dark-cush-cush yam. Additionally, in Fig. 1d was observed that the crystallinity of the starch is maintained, since the cross of Malta was not altered by the modification performed, i.e. that the modification did not cause drastic changes on the crystalline structure of the starch. In said figure, also was possible observe fibers particles contained in modified plantain flour, which could be related with the increase of crude fiber content in this flour, since the leaching of proteins, fats and pigments during the modification of the plantain flour, allowing the relative increase in crude fiber content. Fig. 1e–h shows the images of light microscopy of beet flour. In Fig. 1f was observed a betalain crystal that has crystallized and grown up, after of the loss structural of the parenchyma of the cells of the beet (Fig. 1h). It was also possible to detect lignin fragments belonging to the wall of the sclerenchyma of the beet (Fig. 1g) and cellulose associated to the wall of the cell parenchyma of the beet (Fig. 1h).

3.2. Characterization of the films

3.2.1. Thickness

Table 3 shows the thicknesses of the different systems studied. It can be seen that the films made from phosphated plantain flour (TPS-PPF) and phosphated plantain flour with incorporation of beet flour (TPS-PPFB), resulted be thicker than native plantain flour-based films (TPS-NPF and TPS-NPFB). Pérez et al. [68] reported a significant increase in the thickness of cross-linked starch-based films derived from *Dioscorea trífida*. So the same, Gutiérrez et al. [18,20] have reported that the increasing of the thickness in the edible films is due to the higher interaction starch-plasticizer during gelatinization.

On the other hand, a trend in the increase of the thickness was observed when beet flour was incorporated in the matrix (TPS-NPFB and TPS-PPFB). This increase could be ascribed to two phenomena: (1) to the particles of cellulose and lignin (fiber) that are contained in beet flour, and that are occluded in the polymer matrix [26,74], and (2) to the proteins and sugars associated to beet flour that are compatible with the plantain flours, i.e. it would be established hydrogen bonding interactions between the plantain flours and the sugars, and between the plantain flours and proteins contained in beet flour. This last would justify the more pronounced increase in the thickness of films made from phosphated plantain flour with incorporation of beet flour (TPS-PPFB), respect of the elaborated from native plantain flour with incorporation of beet flour (TPS-NPFB). Therefore, is could establish that the sugars and proteins contained in beet flour have better hydrogen bond interaction with phosphate groups incorporated in modified plantain flour, in contrast with native plantain flour.

3.2.2. Water solubility

Table 3 shows the water solubility values of the different systems studied at 25 °C. Both native plantain flour-based films (TPS-NPF and TPS-NPFB) showed a slight tendency to decrease its water solubility compared to the samples elaborate from phosphated plantain flour (TPS-TPP and TPS-PPFB); however, these differences were not significant ($p \geq 0.05$). This tendency possibly is due to phosphates groups incorporated in the modified plantain flour that increased the polar character of the films. In a previous study, Gutiérrez et al. [20] found that films made from cross-linked starch were more hydrophilic than native starch-based films. Additionally, these results agree with the trend reported by Pérez et al. [68] for native and phosphated *D. trifida* white starch based edible films. Other similar results were provided by Garcia-Tejeda et al. [16], where it can see that the films elaborated from oxidized banana starch increased its water solubility compared to native banana starch-based films. So the same, Pitak and Rakshit [28] reported similar solubility values for films made with mixtures of plantain flour and chitosan.

In the same way, it can be seen that the incorporation of beet flour to evaluated films produced an increase in water solubility, being significant after of the modification of the flour. This would be related with the sugars contained in beet flour, which confer to the films a more polar character.

According to Romero-Bastidas et al. [24], the solubility of the edible films provides an indication of integrity of the material in an aqueous medium, so that higher values of solubility indicate a lower water resistance. However, edible films with high values of water solubility, have been proposed for its application as vehicle of bioactive substances, since easily dissolve in the mouth [75]. Whereby, it can establish that all films evaluated, in this work represent an excellent biomatrix that can serve as vehicle of food additive.

3.2.3. Scanning electron microscopy (SEM)

Fig. 2 shows the SEM images of the cryo-fracture surfaces of the different films. It can be seen that the films produced are non-porous. All systems have compact structures, although this resulted to be more marked in the phosphated plantain flour-based films (Fig. 2c and d), where the white strokes or lines that divide the dark areas seem to form dark regions closer [76]. Similar structures have been reported in cassava starch-protein films by Saavedra and Algecira [77], and in studies of cassava-glycerol films by Gutiérrez et al. [18] and García et al. [78]. This pattern is probably produced by the amylose present in the systems evaluated [79–81]. Likewise, Fig. 2a and b shows a higher density of white lines, which may be areas where occurs the build-up of the stress [76].

It may also be indicated that all the films studied evidenced insoluble particles, which may be associated with fibrous material, since these particles is located within the films. However, a higher content of fiber particles were observed in phosphated plantain flour-based films (Fig. 2c), compared to films made from native plantain flour (Fig. 2a). These results would be adjusted with the chemical composition of the flours used.

3.2.4. Contact angle

Table 3 shows the results of contact angle of the films studied. Native plantain flour-based films (TPS-NPF and TPS-NPFB) presented the higher values of this parameter although not significantly ($p \geq 0.05$). In this regard, Ojagh et al. [82] indicate that it is well known that the contact angle with the water is greater with the increase of the surface hydrophobicity. So the same, Karbowiak et al. [63] have indicated that the increase of the contact angle with water in biopolymer materials could be due to a strong hydrogen bond inter-molecular by below of the surface of the film, i.e. the more polar sites (Lewis sites) would be affected, thus generating a reduction in the surface polarity of biopolymer-based films. Taking this into account, is possible assert that the surface of native plantain flour-based films (TPS-NPF and TPS-NPFB) is less moisturized because it does not have enough energy to break the cohesive force of water [83].

Considering the results shown in Fig. 3, TPS-NPF and TPS-NPFB present a surface more hydrophobic than phosphated plantain

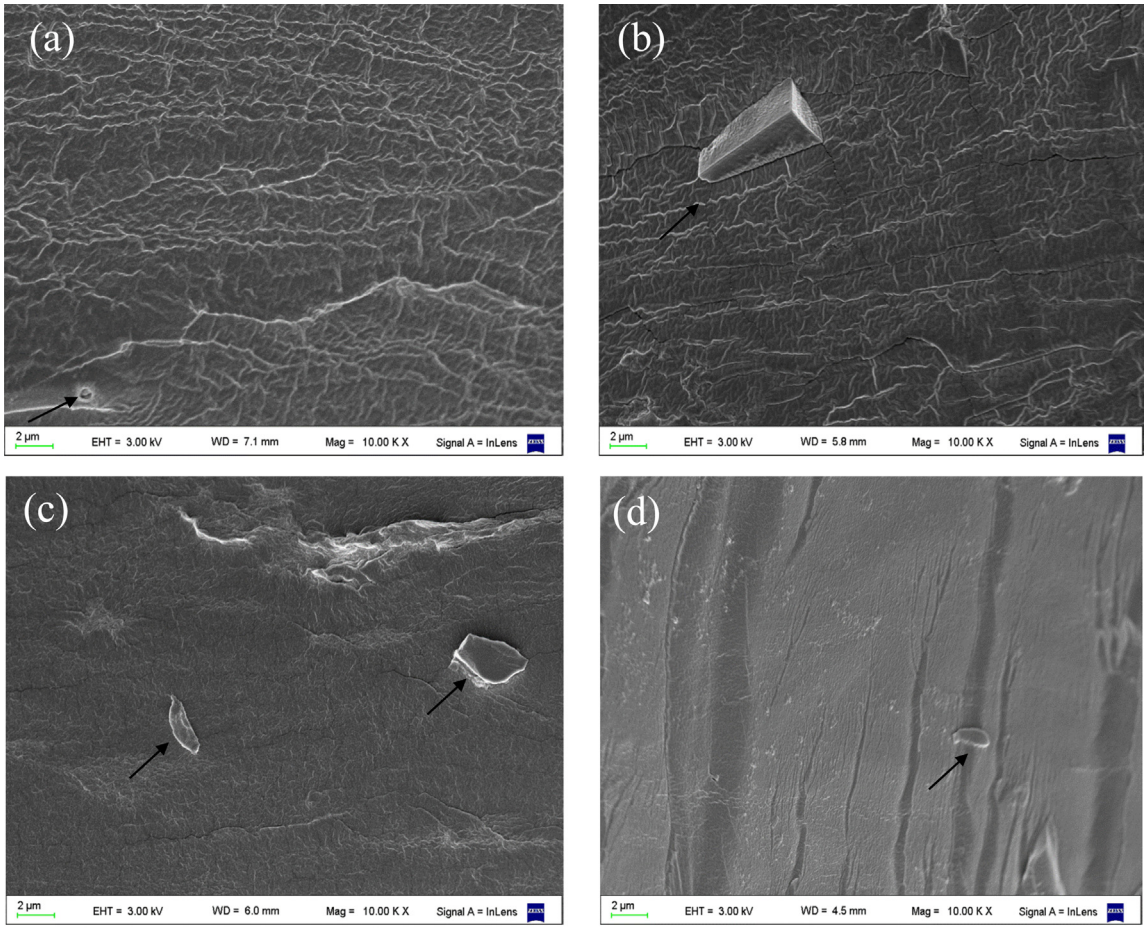

Fig. 2. SEM micrographs of the cryogenic fracture surface of the films based on: (a) native plantain flour (TPS-NPF), (b) native plantain flour with incorporation of beet flour (TPS-NPFB), (c) phosphated plantain flour (TPS-PPF) and (d) phosphated plantain flour with incorporation of beet flour (TPS-PPFB). At 10k× of magnification. Black arrow indicates possible particles of fibrous material.

flour-based films (TPS-TPP and TPS-PPFB). This would correspond with the observed in the SEM images (Fig. 2a and b), since a hydrophobic surface requires of Lewis sites for to be wetted [83]; and in the case of the films made from native plantain flour (higher amylose content) presented a closed structure, thus forming a physical impediment to be wetted.

Interestingly, such a water structure requires that the hydrogen-bond network of water directly adjacent to a non-polar surface be interrupted, yielding "dangling hydrogen bonds". These dangling hydrogen bonds have been theoretically predicted [84] and resolved by spectroscopically from hydrogen bonds in bulk water [85–87]. Whereby, the increase in the contact angle between the

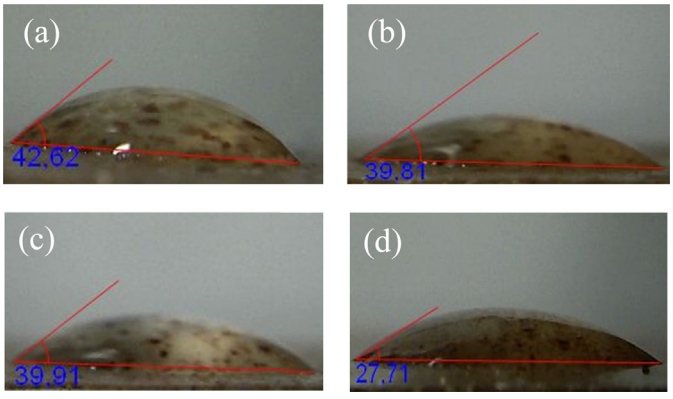

Fig. 3. Contact angle of the films: (a) native plantain flour (TPS-NPF), (b) native plantain flour with incorporation of beet flour (TPS-NPFB), (c) phosphated plantain flour (TPS-PPF) and (d) phosphated plantain flour with incorporation of beet flour (TPS-PPFB).

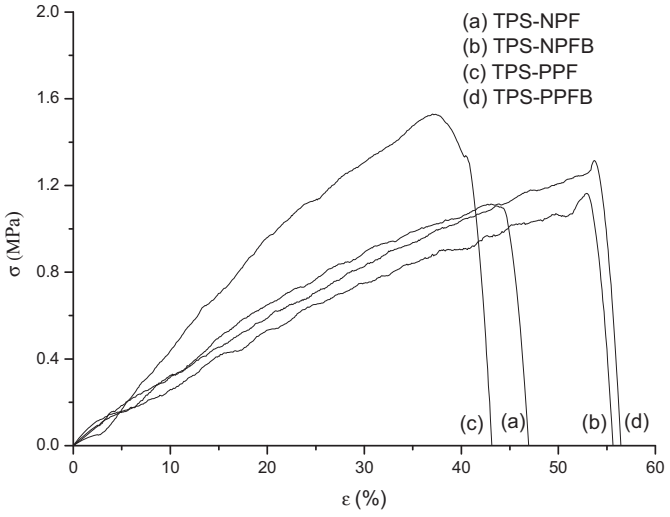

Fig. 4. Stress (σ)–strain (ε) curves of the films based on: (a) native plantain flour (TPS-NPF), (b) native plantain flour with incorporation of beet flour (TPS-NPFB), (c) phosphated plantain flour (TPS-PPF) and (d) phosphated plantain flour with incorporation of beet flour (TPS-PPFB).

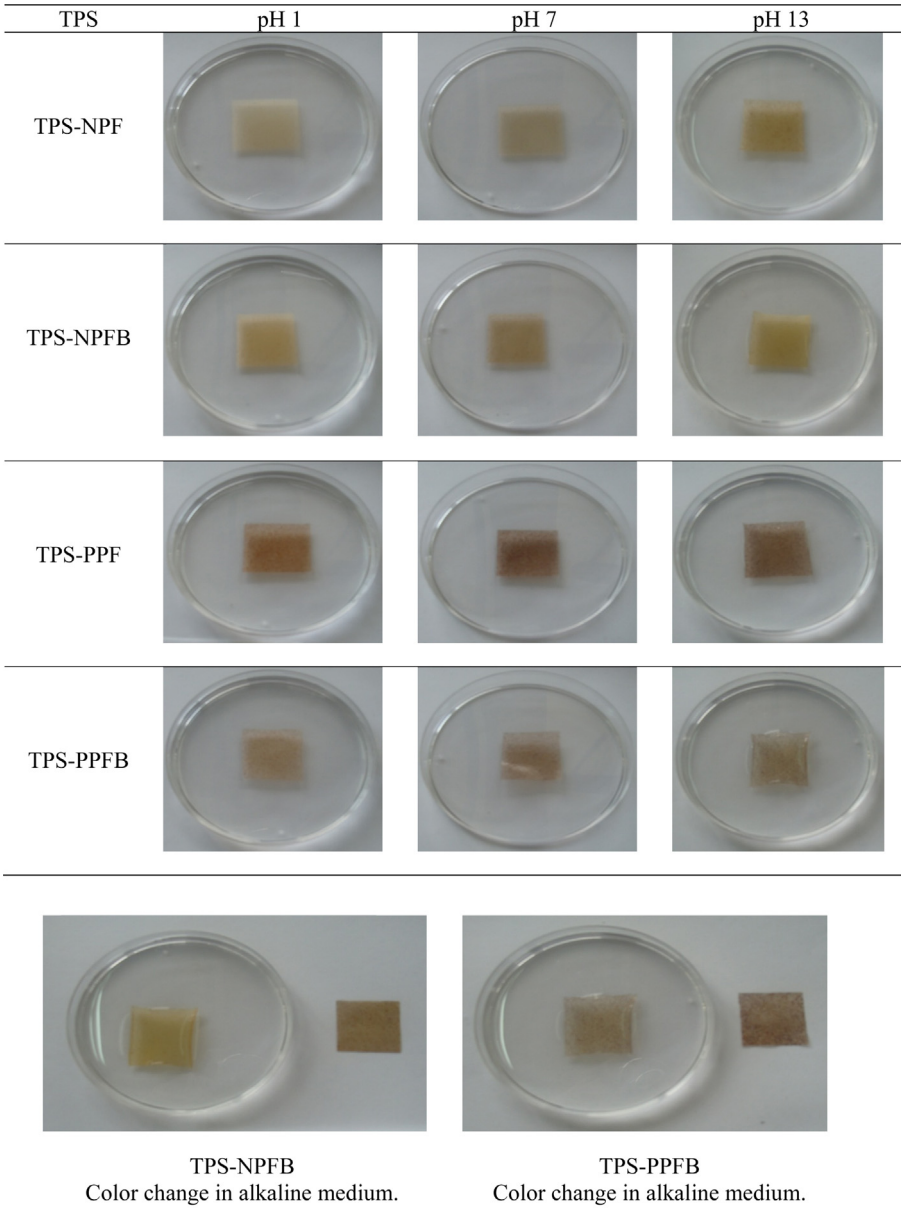

Fig. 5. Response of the films evaluated at different conditions of pH.

surface and water would be the manifestation of the dangling hydrogen bonds in native plantain flour-based films.

Conversely, phosphated plantain flour-based films presented lower contact angle. This behavior was possibly due to the addition of phosphates groups that strengthened the polar character of the surface, thus increasing the density of Lewis sites in the surface. These sites located in the surface of phosphated plantain flour-based films would approach to the hydrogen bond network in water, thus competing with the cohesive forces, leading to the collapse of water structure on the hydrophilic surface, thus decreasing the contact angle.

Additionally, the incorporation of beet flour in the systems evaluated (TPS-NPFB and TPS-PPFB) tended to decrease the contact angle as a consequence of the polar character of the sugars contained in beet flour.

3.2.5. Uniaxial tensile strength

The stress-strain curves of each developed films are shown in Fig. 4. A small elastic linear zone was observed followed by a non-linear zone until breaking point, regardless of the flour used. In case of the films with the incorporation of beet flour (TPS-NPFB and TPS-PPFB), it was observed a significant increase in the strain at break, demonstrating a plasticizer effect by part of this flour (Table 4). This would confirm that proteins and sugars contained in beet flour would act as plasticizers, possibly due to the strong hydrogen bond interactions between the starch contained in the plantain flours, and the sugars and proteins. Therefore, beet flour may be recommended in films that require a more flexibility.

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

Material	E (MPa)	σ_m (MPa)	ε_b (%)	T ($\times 10^5$) (J/m^3)
TPS-NPF	2.7 ± 0.7^a	1.0 ± 0.1^a	49 ± 2^a	3.0 ± 0.1^a
TPS-NPFB	3.3 ± 0.1^a	1.14 ± 0.03^a	53 ± 1^b	3.6 ± 0.1^b
TPS-PPF	5.8 ± 0.2^b	1.4 ± 0.1^b	44 ± 4^a	3.8 ± 0.1^b
TPS-PPFB	3.4 ± 0.1^a	1.3 ± 0.1^b	55 ± 6^b	4.3 ± 0.1^c

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

Similar results have been reported by Talja et al. [88], in films made from potato starch plasticized with various polyols. So the same, all the films evaluated lengthened almost the double than plantain flour-based films (*M. paradisiaca*) reported by Pelissari et al. [26], this probably due to the variety of the amylaceous source and the manufacturing process of the films.

Moreover, considering that the modification by phosphation reduced the content of lipids and proteins in modified plantain flour, this possibly caused the slight decrease in the elongation of the films made from phosphated plantain flour (TPS-PPF), this with respect to the native (TPS-NPF). Although no statistically significant differences ($p \geq 0.05$) were observed between TPS-NPF and TPS-PPF. Pelissari et al. [26] reported a similar phenomenon in films made from starch and flour of plantain (*M. paradisiaca*).

Likewise, higher elasticity values have been associated with more compact structures [18,77]. This is consistent with the SEM images obtained for the films made from modified plantain flour with the incorporation of beet flour (Fig. 2d).

In the case of native plantain flour-based films, the addition of beet flour did not lead to significant changes in Young's modulus and maximum stress; while that beet flour in phosphated plantain flour-based films caused a decrease of Young's modulus of about 40%, this being consistent with the increase in the elongation of this material [89], and with its plasticizer character (Table 4).

Likewise, since that the modification of the plantain flour increased fiber content in this biomatrix, this caused a positive effect in the significant increase ($p \leq 0.05$) of Young's modulus and maximum stress of phosphated plantain flour-based film (TPS-PPF), this compared to native plantain flour-based film (TPS-NPF). This because the fibrous material creates a reinforcement within the polymeric structure [38].

Overall, the results of the mechanical tests showed that the Young's modulus of the samples ranges from 2 to 5 MPa. Whereby, the films obtained are less rigid compared to the films made Gutiérrez et al. [18] from cassava and cush-cush yam starches; this even using the same methodology. Similarly, more rigid films based on plantain flour (*M. paradisiaca*) of another variety were reported by Pelissari et al. [26]. For this reason, the films obtained are some recommended for food packaging that requires a higher elastic modulus.

Moreover, the incorporation of beet flour increased the toughness in the films evaluated with respect to its analogous without incorporation of this flour. This would be very useful, since these films would absorb more energy without being transmitted to a food, this could minimize the damage caused by blows in the foods during transport and storage.

3.2.6. Response to pH changes

Fig. 5 shows the images of the response of the films evaluated at different pH. It was observed that films that contained beet flour (TPS-NPFB and TPS-PPFB) color change in alkaline medium, this due to the pigment found in beet flour (betalains). However, the response was more rapid when was employed as biomatrix the native plantain flour. This would confirm that the interaction between the native plantain flour and the beet flour is weaker that the produced between the phosphated plantain flour and the beet flour. Likewise, these intelligent films could be applied in products of fishery, since the histamine production as result of microbial growth would act of alkaline medium, causing a color change in the films with incorporation of beet flour, which could indicate the loss of the quality in this food type.

4. Conclusions

The modification of the plantain flour reduced the lipid, protein and amylose content, and increased the degree of substitution by phosphation and the fiber content. The granule size of the phosphated plantain flour increased slightly as result of the modification. However, the granular structure of the phosphated plantain flour no evidenced structural damage. It was also evidenced that the use of beet flour improved the hydrogen bonding interactions as result of plasticizer effect of the sugars and proteins contents in beet flour, which led to the decreased of contact angle, and to the increase of the solubility and the thickness of the films. Similarly, a more open and compact structure has been linked with the plasticizing effect of the sugars contained in beet flour, since is well known that plasticizer effect of the sugars allow to obtain similar structures. Finally, the response to pH changes in the intelligent films made from phosphated plantain flour with incorporation of the beet flour was slower, which confirmed a greater interaction between the betalains and modified plantain flour. Therefore, this limited its response to pH changes in alkaline medium, but caused a positive effect on the flexibility of these films.

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