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Biofilms Production from Avocado Waste[*]

Producción de biopelículas a partir de residuos de aguacate

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Abstract

Objective: To obtain biofilms from starch and cellulose present in the avocado (*Persea americana*) peel and seed. *Materials and methods*: The starch characterization included humidity, gelatinization temperature, paste clarity, absorption index, solubility index, swelling power, amylose, amylopectin, amount, and starch yield. Five mixtures were made with 3 g of starch, 5 mL of 30 % NaOH (w/v), 3 g of cellulose, and different proportions for glycerin: 2 g; 2.5 g; 3 g; 3.5 g; 4 g, and PVA: 2 g, 3 g, 4 g, 5 g, and 6 g. Films were formed on acrylic plates, using the casting method. The bioplastic was characterized in terms of moisture, solubility in water, density, thickness, biodegradability, stress, deformation, and modulus of elasticity. *Results and discusión*: The addition of cellulose to the mixture does not contribute to film formation, unlike PVA which did. The film had the best physical appearance with a mixture of 2 g of glycerin and 6 g of PVA. The bioplastic characterization was 23.43 % humidity, 39.39 % for water solubility, 1.52 g/cm^3 density, 0.58 mm thickness, 21.03 % weight loss for the biodegradability test, 1.53 MPa for tension, 21.25 % deformation, and 10,04 MPa for the modulus of elasticity. *Conclusions*: The bioplastic obtained did not show the resistance of traditional plastic. However, the results obtained serve as a starting point for the realization of other formulations, aimed at producing a bioplastic capable of competing with its synthetic relatives.

Keywords: Bioplastic, biodegradable, biopolymers, food waste.

Resumen

Objetivo: obtener películas plásticas a partir de almidón y celulosa presentes en la cáscara y semilla de aguacate *(Persea americana)*. *Materiales y métodos*: la caracterización del almidón incluyó humedad, temperatura de gelatinización, claridad de la pasta, índice de absorción, índice de solubilidad, poder de hinchamiento, amilosa, amilopectina, cantidad y rendimiento de almidón. Para la realización del bioplástico se hicieron cinco mezclas con 3 g de almidón, 5 mL de NaOH al 30 % (m/v), 3 g de celulosa y diferentes proporciones para glicerina: 2 g; 2,5 g; 3 g, 3,5 g; 4 g y PVA: 2 g, 3 g, 4 g, 5 g y 6 g. Las películas se formaron sobre placas acrílicas, utilizando el método de *casting*. El bioplástico se caracterizó en términos de humedad, solubilidad en agua, densidad, espesor, biodegradabilidad, tensión, deformación y módulo de elasticidad. *Resultados y discusión*: los resultados obtenidos mostraron que la adición de celulosa a la mezcla no contribuyó con la formación de película, a diferencia del PVA que sí lo hizo. La película con mejor aspecto físico se obtuvo con la mezcla de 2 g de glicerina y 6 g de PVA. El bioplástico caracterizado mostró 23,43 % de humedad; 39,39 % de solubilidad en agua, 1,52 g/cm^3 de densidad; 58 mm de espesor; 21,03 % de pérdida de peso para la prueba de biodegradabilidad; 1,53 MPa para tensión; 21,25 % deformación y 10,04 MPa para el módulo de elasticidad. *Conclusiones*: el bioplástico obtenido no mostró la resistencia del plástico tradicional. Sin embargo, los resultados obtenidos sirven como punto de partida para la realización de otras formulaciones, encaminadas a producir un bioplástico capaz de competir con sus parientes sintéticos.

Palabras clave: bioplástico, biopolímeros, biodegradable, residuos alimentarios.

Introduction

Nowadays the plastic is present in the most of human's activities. The production of plastics registered an exponential increase over the years, going from 1,50 million of metrics tons (Tm) in 1950 to around 350 million Tm in the 2017 [1]. The conventional plastics are made of fossil resources and are characterized for slow degradation rate and may take decades and even centuries [2]. The accumulation of this material after its use an inadequate final disposal cause irreversible environmental damages.

Most of these wastes can travel long distances to reach the sea. In 2010, between 4,8 to 12,7 million Tm of plastic waste entered into the ocean from 192 coastal countries [3]. The macro plastics and micro plastics represent a risk to the marine ecosystem that may cause damages for ingestion or mutilations due to entanglements.

Recent research indicates that the hydrophobic plastic contaminants can be absorbed by marine fauna and then, pass to the humans by the food chain [4]. Additionally, the burning of plastic waste in the open air releases toxic gases such as dioxins, furans, mercury and polychlorinated biphenyls increasing the atmospheric pollution, also, contributes to the climatic change and represent threat to both animal and human health [5].

An alternative to this situation is the use of biodegradable plastics. This material are capable of decomposing by the natural action of microorganisms and producing as a result CO2, methane, water and biomass [6]. Bioplastics are developed from various sources, including agricultural and agroindustrial wastes, that are environmentally sustainable and economically viable for the production of bioplastics [7]. Agroindustrial solid wastes are mainly composed by horticultural seeds, peels, roots, stems, among others. The avocado (Persea Americana) fruit processing generate seeds and peels [8], characterized by to have starch and cellulose respectively, being a potential second generation raw material that can be used in the development of new materials. Some studies have evaluated cellulose of sugarcane and chitosan, with avocado seed starch and glycerol as polymers blends [9]–[11]. However, they are preliminary investigations that are still under development.

The avocado is a fruit with a soft and yellowish pulp, which has a seed in the central part and between the peel and the seed, the edible pulp [12]. It is consumed fresh in salads or as guacamole, but at an industrial level it also has a varied possibility of uses: base for spreads, cosmetics and oil, the latter having great future potential [13]. At the Latin American level, countries such as Mexico, Chile, Colombia and Ecuador, are dedicated to the manufacture and export of avocado oil [14].

In 2018, 18232 Tm of avocado were produced in Ecuador, of which 18191 Tm were produced in the mountains and 41 Tm in the coastal region [15]. The avocado is consumed fresh and on a smaller scale, it is processed to obtain oils and spreads for snacks. Among the bibliography consulted, there is no record of agroindustrial waste in the country, and although its management is not optimized with respect to the environmental consequences it generates [16], the avocado industrialization produces a large amount of waste since only its seed can represent up to 26 % of the fruit's weight [17].

In this sense, it was proposed to obtain a bioplastic from the avocado seed and peel, to evaluate the respective physical and mechanical properties.

Materials and Methods

The avocado peel and seeds used in the investigation were obtained from the local market in the province of Manabí, Ecuador. In the experimentation were used analytical grade reagents and distilled water to make the solutions.

Extraction of Compounds from the Waste

In the experimentation a total of 450 seeds and 20 peels avocados were used. For the starch extraction, avocado seeds were washed with distilled water to remove dirt and dried in the open air. They were then finely cut and placed in a 0,2 % (w/v) solution of sodium metabisulfite (Na2S2O5) for 24 h. For the reduction of size an Oster blender was used. The suspension obtained was decanted for 24 h to settle the starch granules and the supernatant was removed. The starch was washed with distilled water to make a suspension. Subsequently, the starch was dried at 70 °C for 6 h, in an oven (Thermolyne FD1535M), weighed and screened in a 125 µm mesh [18].

Avocado peels were initially washed with distilled water to remove dirt and then subjected to acid hydrolysis. 1 g of sample free of extractable was taken and 15 mL of 72 % H2SO4 was added for 2 h with stirring. The solution was diluted to 4 % H2SO4 and boiled for 4 h. The sample was decanted and filtered, then the solid obtained was dried in the oven for 24 h at 105 ± 3 °C. The fibers obtained were used as fillers in the mixtures to make the plastic films, adding 3 g of these in each case.

Biodegradable Film Obtaining

Preliminary experiments were carried out in order to identify the mixing proportions with film formation. The first part of the experimentation was carried out with mixtures of starch (3 to 5 g), glycerin (1 to 5,5 g), cellulose (3 g) and CH3OH 25 % (2 to 4 mL). For the second test, starch (3 to 4 g), glycerin (5 to 6 g), cellulose (3 g) and 30 % NaOH (5 to 6 mL) were mixed, according to [9]. With the preliminary formulation, it was not possible to form the film, which is why it was decided to add polyvinyl alcohol (PVA) into the mixture, taking into account the results obtained by Pilla in a similar investigation [19]. The polyvinyl alcohol solution was prepared by weighing 50 g of PVA and mixed with 1 L of distilled water. It was heated at 90 °C with constant stirring for 2 hours. After this time, the solution was cooled to 20 °C and transferred to a closed container. For each sample, 3 g of starch and 5 mL of 30 % NaOH (w/v) were used, varying the amounts of PVA and glycerin (table 1).

Table 1. Formulations for the biodegradable film

Glycerin (g)	PVA (g)
2.0	6
2.5	5
3.0	4
3.5	3
4.0	2

Source: Own elaboration

The procedure consisted of weighing the amount of the starch from the avocado residues using an analytical balance PW254 ADAM and mixing it with distilled water, glycerin and PVA, under constant stirring. The solution was then placed in a thermostatic bath at 80 °C and the NaOH solution was added, to form a paste. The obtained mixtures were placed in Petri dishes and drying in a Thermolyne FD1535M oven at 65 °C for 18 h, after which they were demolded.

Analytical Characteristics

Starch was characterized by the contents of moisture [20], gelatinization temperature for the Grace method [21], water absorption rate, water solubility, swelling power [22], starch [23], amylose, amylopectin [24] and pasta clarity [25]. In addition, the starch yield was determined through the relationship between the starch masses obtained and the avocado seeds used according to equation (1).

$$\%Starchyield = \frac{starchmass}{seedsmass} \times 100 \tag{1}$$

To the avocado peel was determined the cellulose content by the Kurschner & Hoffer method and the amount of lignin by the Klason method [26]. The biodegradable plastic film was characterized determining moisture, water absorption, density, thickness, biodegradability, surface analysis and the mechanicals properties: tension, deformation, modulus of elasticity.

The moisture was determined by gravimetry according to method AOC 934,06 [27], for which 0,50 g of film was weighed and dried at 100 °C for 4 h until constant weight. For water absorption, 0,50 m ± 0,001 m specimens were made and dried at 50 °C for 24 h. They were cooled to room temperature and weighed. They were then introduced into 400 mL of distilled water for 24 h. After this time they dried and weighed [28]. The percentage of water absorption was calculated with the equation (2).

$$Waterabsorption = \frac{Initialweight - Finalweight}{Initialweight} \times 100\% \tag{2}$$

Density was determined by the ratio between weight and volume measured at 23 ± 1 °C, in samples 0,02 m long, 0,02 m wide and 0,003 m thick [29]. The thickness of the films was measured with an IINECO digital caliper [30]. Biodegradability of the material was determined by gravimetry considering weight loss over time. Samples of 0,05 m x 0,05 m were taken and placed in the soil at a depth of 0,10 m under anaerobic conditions, for 45 days [31].

The surface analysis structural of the bioplastic was performed using an atomic force microscope (AFM) Park System NX10 Cantilivier NCHR. For this, the sample was placed in the sample holder and the topography of the material was scanned as a non-contact with the tip of the cantiliver. The mechanical properties of the bioplastic (tension, deformation, modulus of elasticity) were determined using the Zwick/Roell Z010 universal texturometer test machine [32]. Samples of 0,10 m long and 0,025 m wide films were cut and placed between the clamps of the equipment. They were then stretched at 0,05 m until they broke.

Results

All variables were determined in triplicate. Table 2 shows the results obtained in the characterization of starch.

Table 2. Starch characterization

Variable	Unit	Value ± σ
Moisture	%	11 ± 1
Gelatinization temperature	°C	66.67 ± 1.53
Water absorption rate	g gel/g sample	5.45 ± 0.32
Water solubility	%	21.21 ± 0.05
Swelling power	g H_2O/g starch	0.17 ± 0.01
Starch	%	55.68 ± 3.62
Amylose	%	22.34 ± 0.04
Amylopectin	%	77.66 ± 0.04
Pasta clarity	----	43 ± 1.73
Starch yield	%	5.01 + 0.05

Source: Own elaboration

The moisture content of the extracted starch was 11 %, close to the reported value for native starch (11,33 %) and modified starch (11,67 %) of avocado seed [33]. Similar results are reported for quinoa starch [34], yams [35], cassava [36] and corn [37]. The literature reports a maximum humidity level of 14 %, to prevent the growth of microorganisms that contribute to its degradation [38].

The gelatinization temperature was 66,67 ºC, being a value close to 65,7 °C [39] and 67,6 [40], which were reported for avocado seed starch. Close values were reported for corn starch 72 °C [41] and lower than banana with 78 °C [42] and yam that was between 80 and 84 °C [43].

The gelatinization temperature is a property that depends on factors, such as size of the granules, the amylose/amylopectin ratio [44]. Furthermore, the higher the moisture content, the gelatinization temperature increases [43]. This property indicates the temperature at which the native starch can be gelatinized to transform it into a thermoplastic material.

The swelling power is a property of the starch amylopectin content and is related to the water absorption capacity [45]. Water solubility index indicates the amount of amylose that is released from inside the starch granule when it begins to lose its structure by effect of water absorption [35]. Water absorption, water solubility and swelling power were 5,45 g/g, 21,21 % and 0,17 g/g, respectively. These results were inferior to those obtained for the isolated starch of avocado seeds where the water absorption index was 22-24 g/g, the water solubility 19-20 % and the swelling power was 28-30 g/g [39]. Lower values of native starch represent limitations in its use because it affects the gelatinization process of starch and properties such as texture, stability, retrogradation [46].

The starch was 55,68 %, lower than 91,48 % [33] and 73,62 % [47], reported for avocado seed native starch. The variations may be due to the purity obtained for the starch during its extraction [11]. The amylose was 22,34 % and amylopectin was 77,66 %, similar values were obtained for starch isolated from avocado seeds [39], corn starch [41] and lower for the modified starch avocado seeds [33]. Amylopectin contents between 79 and 83 % favor the formation of more stable gels, improving the strength and elasticity of the formed plastic materials [48].

The clarity of the paste was much greater than the values reported for the native (1,17 %) and modified (0,56 %) starch of avocado seeds [33]. This variable affects the appearance of the product and the difference may be due to the method used for starch extraction. The starch yield was 5,01 % lower than 8,05 % obtained for native avocado starch [33], potato 16,5 % [49], quinoa 18-30 % [34], mango cotyledon 42-50 % [50]. It is possible to achieve better yields in starch extraction by increasing the precipitation time of the process [47].

Cellulose and lignin content in the avocado peel were 33,33 % and 29,01 %. For the peels of three different varieties of avocado, cellulose was between 8 and 50 % [51]. Cellulose and lignin content in avocado peels and seeds were 27,58 % and 4,37 %, respectively [52]. Cellulose is a filler material [53], used to increase resistance in bioplastics. Its effect on the mechanical properties of bioplastic obtained from avocado seed starch has been studied with satisfactory results [54]. However, in the study carried out, cellulose was not observed to improve the characteristics of the films formed. On the contrary, when adding the cellulose fibers, the film became lumpy and made the drying operation difficult.

Although with the preliminary experiments it was possible to form the film, only the one formed a film without breaking easily could be characterized, whose composition was 3 g of starch, 5 ml of 30 % NaOH solution (w/v), 2 g of glycerin and 6 g from PVA. The rest of the formulations had some of these problems: they formed sticky pastes that made their handling impossible, they broke after drying or when they were removed from the Petri dish in which they were formed, making their characterization impossible. On the other hand, cellulose did not contribute to film formation.

The preparation of each of the bioplastic formulations was carried out in triplicate. The results corresponding to the characterization are shown in table 3.

Table 3. Bioplastic characterization

Variable	Unit	Value ± σ
Moisture	%	23.43 ± 1.25
Water solubility	%	39.39 ± 8.75
Density	g/cm^3	1.52 ± 0.06
Thickness	mm	0.58 ± 0.04
Biodegradability	%	21.03 ± 7.28
Tension	MPa	1.53 ± 0.06
Deformation	%	21.25 ± 1.95
Modulus of elasticity	MPa	10.04 ± 1.43

Source: Own elaboration

The moisture content is high when compared to other bioplastics reported between 9 and 18 % [55]–[57]. In a polymeric formulation the humidity should preferably be in a range of 5 to 8 % (w/w), since greater contents do not produce a continuous or uniform film [58]. The high moisture content causes negative effects on the properties of the bioplastic, making it a low resistance material [59].

Water solubility was close to 40 %. Potato bioplastics showed a percentage of water solubility of 17 to 24 % [60]. The bioplastics made of corn, rice and mixtures thereof, presented a water absorption rate between 10 and 29 %. Starch-based bioplastics have the disadvantage of their low moisture barrier. For this, it is recommended to add a hydrophobic fluid to the thermoplastic mixture [61].

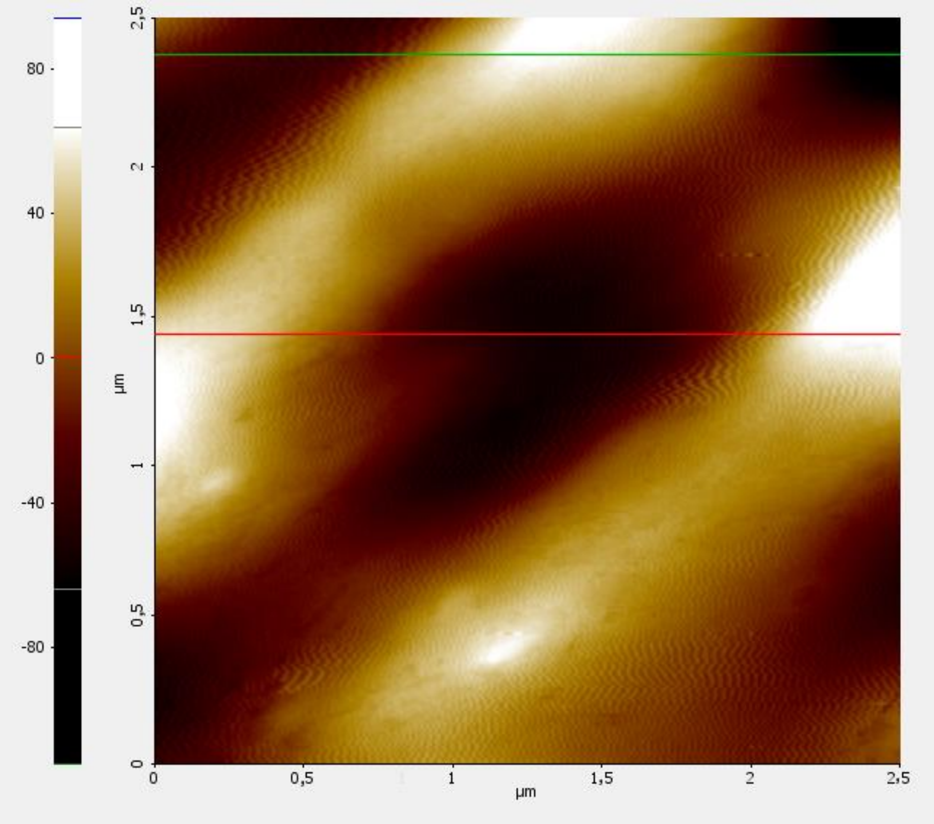
However, density values of 0,19 to 0,68 g/cm^3 have been reported in foamed materials and biodegradable trays made from cassava starch and cellulose fibers. A product with a lower density would have a lower weight, thus favoring its marketing conditions [29], [62].
The thickness of the film depends on the relationship between amylose / amylopectin [60]. Its value was 0,58 mm, similar to that found for corn flour based bioplastics (0,25 mm - 0,45 mm) for use in the footwear industry [63], animal bone meal bioplastics for plastic tableware [64], glycerol polymer for additive manufacturing based on the extrusion of the material [65]. The thickness of the bioplastic influences the tension of the material and varies according to its application.

The biodegradability evaluated for 45 days under anaerobic conditions was verified through weight loss, which was 21,03 %. This result resembles the evaluation carried out to evaluate the biodegradability of starch-based bioplastic by immersion in sterile water and the soil burial test for 10 days, where a weight loss of 29,89 % was recorded [66]. The resulting biodegradability was low compared to the biodegradability test carried out for 24 weeks for mixtures of biomass with polylactic acid, where weight losses of 64.3 % and 76.3 % were reported [67].

The mechanical characteristics of bioplastic: tension, deformation and modulus of elasticity, were 1.53 MPa, 21.25 % and 10.04 MPa, respectively. Similar values were obtained for a cassava starch bioplastic, with 1,5 MPa for maximum tensile strength, 42.5 MPa for elastic modulus and 26 % deformation [68]. The mechanical properties of bioplastics have certain disadvantages compared to conventional plastics, but they can be improved. In the development of a cassava flour bioplastic, 3.2 MPa and 748.7 MPa were obtained for tension and modulus of elasticity, with the flour gelatinization [69] and with addition of graphene oxide [70]. It has been proven that the use of TiO2 improves the mechanical strength and elasticity of thermoplastic materials [71].

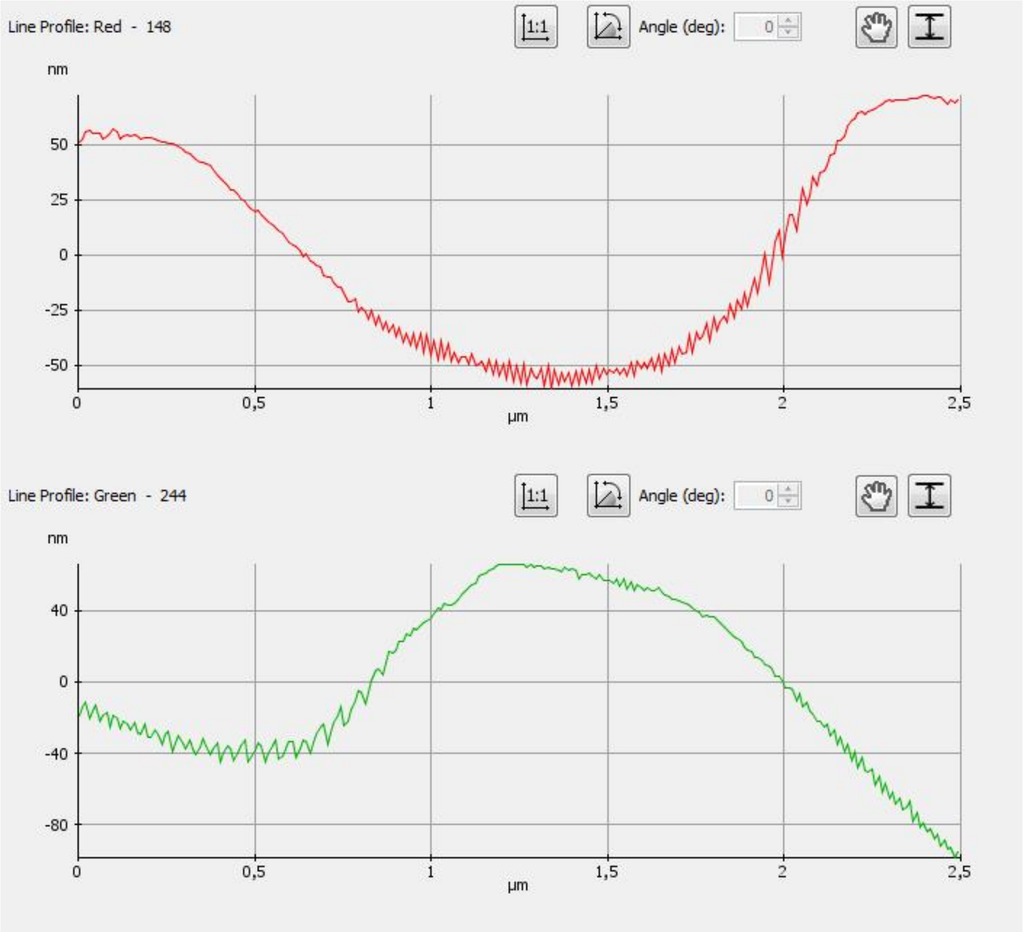
The surface and roughness of the film obtained by AFM are shown in figures 1 and 2, respectively. The AFM indicates the areas of roughness of the material, as well as irregular structures. This may be related to the quantity and quality of starch used [72], plasticizer used, the distribution of the material in the film [73]. It has been determined that bioplastics with irregular structure negatively influence the stress of the material [74].

Figure 1. Surface of the bioplastic obtain for AFM



Source: Own elaboration

Figure 2. Roughness of the film obtained by AFM



Source: Own elaboration

Conclusions

Avocado residues (seeds and peels) have easily removable starch and cellulose for use in the production of bioplastics. The characterization of starch showed that it is a starch with high solubility, low water absorption and low swelling power, which is reflected in the percentage of amylose and also influences the formation of biodegradable films. The best results in terms of film formation and durability were obtained with 3 g of starch, 2 g of glycerin, 6 g of PVA and 5 mL of 30 % NaOH. Despite the results recorded in the research, there are still characteristics of the biofilm such as water absorption, tension and biodegradability that must be improved either by improving the quality of the starch used or by adding additives, in order to compete with synthetic relatives.

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