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Obtaining cassava starch biofilms with hydroxyapatite using egg shells

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ABSTRACT: Biofilms consist of biodegradable compounds which can be replaced by materials that are not degradable, in order to reduce the impacts that they can generate for the environment. The main objective of this paper was to synthesize cassava starch membranes using hydroxyapatite, in which it represents a ceramic biomaterial. The biofilms were obtained using 2%w cassava starch, 30%w glycerol and hydroxyapatite varying in five different concentrations (0.0%; 1.0%; 2.5%; 3.5%; 5.0%). The preparation of the solution consisted of the mixture of cassava starch, glycerol, hydroxyapatite and distilled water, which were subjected to magnetic stirring with heating until reaching the gelation phase. The solution was put to rest, then submitted to an ultrasonic bath and later deposited in acrylic plates for drying in oven at 50ºC for 5 hours. The hydroxyapatite reduced the rate of water vapor permeability by 18%, decreased water solubility by 20% and increased opacity by 8%. The addition of the ceramic compound changed the physicochemical properties of the film, making it promising for using as biodegradables bags, for example.
Keywords: Biodegradable films, ceramic compound, biomaterial.

Obtenção de biofilmes de fécula de mandioca com hidroxiapatita utilizando cascas de ovos

RESUMO: Biofilmes consistem em compostos biodegradáveis os quais podem ser substituídos por matérias que não são degradáveis, com o intuito de reduzir os impactos que estes podem gerar para o meio ambiente. O principal objetivo deste trabalho foi sintetizar membranas de fécula de mandioca utilizando hidroxiapatita, em que esta representa um biomaterial cerâmico. A obtenção dos biofilmes foi realizada utilizando 2% de fécula de mandioca, 30% de glicerol e a hidroxiapatita variando em cinco concentrações distintas (0.0%; 1.0%; 2.5%; 3.5%; 5.0%). O preparo da solução consistiu na mistura da fécula, do glicerol, da hidroxiapatita e água destilada, os quais foram submetidos à agitação magnética com aquecimento até atingir a fase de geleificação. Em seguida a solução foi posta em repouso, depois submetida ao banho ultrassônico e posteriormente, depositada em placas de acrílico para sua secagem em uma estufa a 50ºC durante 5 horas. O aumento da quantidade da hidroxiapatita reduziu a taxa de permeabilidade ao vapor de água em aproximadamente 18%, diminuiu a solubilidade em água em 20% e aumentou a opacidade em 8%. A adição do composto cerâmico alterou as propriedades físico-químicas do filme, tornando-o promissor para uso em sacolas biodegradáveis, por exemplo.
Palavras-chave: Filmes biodegradáveis, composto cerâmico, biomaterial.

INTRODUCTION

The search for materials from renewable sources has been studied in order to replace petroleum-derived materials, such as petroleum plastics, in which they directly attack the environment of living beings. Based on this foundation, the investigation and use of biodegradable polymers has been gaining ground due to its high capacity to contribute to degradation in order to cause lesser consequences to the environment, being a fundamentally important means to reverse the worrying situation of the decomposition of petroleum plastics (Leites Luchese et al., 2015).

Biodegradable polymers, also known as biopolymers, have as one of their main characteristics, biodegradability, unlike some polymers from petroleum (Figueiredo Brito et al, 2011).

Part of the materials from renewable sources, the cassava starch can act directly as an agent to assist in the replacement of common plastics, that is, they are not degradable (Salazar-Sánchez et al., 2019). Cassava starch is found in large quantities, obtained in a renewable and low cost way (Oludayo Oluwasina et al., 2019). In addition, this biopolymer offers properties that favor the formation of films, such as those mentioned above, as well as the presence of viscosity and amylopectin (Mansur Tavares et al., 2019).

Cassava starch has hydrophilicity as one of its characteristics. Thus, it is necessary to use a material that is of fundamental importance to reduce its affinity with water. Ceramic biomaterial in which it presents peculiarities that are analogous to bones and teeth, hydroxyapatite has as one of its functionalities the potentiation of certain characteristics of certain compounds due to belonging to the phosphate group, more specifically due to the presence of calcium (Menezes Sousa et al., 2017). It has characteristics such as bioactivity and biocompatibility, making it a fundamentally important compound that can act in several applications (Niranjan Ramesh et al., 2020). It presents $Ca_{10}(PO_4)6(OH)_2$ as a molecular formula and one of its properties is osteoconductivity. Among its mechanical properties is found in the ceramic

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compound a high fragility and low mechanical resistance. These characteristics can be changed when there is a combination of hydroxyapatite with some material that favors the change without its degradation, such as the formation of the HAp-zirconia composite (Ferreti Bonan et al., 2014).

Hydroxyapatite can be obtained through aqueous methods (VALENTE, 1999) such as wet precipitation, known as an acid-base reaction (Menezes Sousa et al., 2017) and hydrothermal synthesis, thus obtaining biomaterial nanoparticles (Jayabal Prakash et al., 2020). Synthesization by means of the acid-base reaction consists of mixing the orthophosphoric acid with a calcium hydroxide solution, called the neutralization reaction (Sousa Farias et al., 2019). This method has a high amount of purity, making it quite advantageous (VALENTE, 1999).

The synthesis of hydroxyapatite can be performed through the use of egg shells. The importance of reusing them is in the justification of reusing elements for which they generate a significant amount of waste every year. In addition, the uses coming from egg shells have a greater aspect of crystallinity, a peculiarity by which it makes hydroxyapatite an important biomaterial (Sousa Farias et al., 2019).

In order to develop a biofilm that would contribute in a way to replace petroleum plastics, it is evident that the ceramic biomaterial has high potential when combined with certain compounds, a study was prepared for the production of a biodegradable film made of starch of cassava, using hydroxyapatite in its composition, obtained through the powder of the shell of the white chicken egg.

MATERIALS AND METHODS

Obtaining Hydroxyapatite

Hydroxyapatite was obtained from white chicken egg shells using phosphoric acid P.A., supplied by VETEC Química fina LTDA, using the wet precipitation method. Obtaining calcium oxide and hydroxyapatite synthesis happened according to the methodology of Menezes Sousa et al. (2017) with some modifications.

To prepare the hydroxyapatite-forming solution, it was necessary to collect white chicken egg shells. hese were washed with running water and distilled water and dried in oven - model TE-394/2 Tecnal brand - with air circulation at 100ºC for one hour to remove impurities. Then, a mortar and pistil was used to macerate the shells manually, so that they formed a powder. In Figure 1, egg shells are observed after washing, crushing using the mortar and pistil and storage in a plastic container.

Figure 1: Powder obtained by chicken egg shells.

The stored powder was sent to a Leucodema sieve shaker with meshes of 1000mm, 0.500mm, 0.250mm, 0.125mm and 0.053mm for approximately eight minutes and according to its granulometry, it was stored. For the preparation of the calcium oxide solution, the size of 0.053mm meshes and below this value (bottom of the sieve container) were used. The powder was exposed to a muffle at 800ºC for 3 hours, with a heating rate of 10ºC per minute, consisting of the thermal cycle to result, as a product, calcium oxide.

The calcium hydroxide solution was prepared by mixing 150 mL of distilled water in a beaker and 5.6 g of calcium oxide obtained through egg shells. The beaker was taken to a magnetic stirrer with heating - brand Lucadema - with a temperature of 80ºC for the addition of phosphoric acid. This was added by means of a burette with a speed of ± 1mL per minute. In Figure 2, the mixture of phosphoric acid and calcium hydroxide solution is identified by means of a burette.

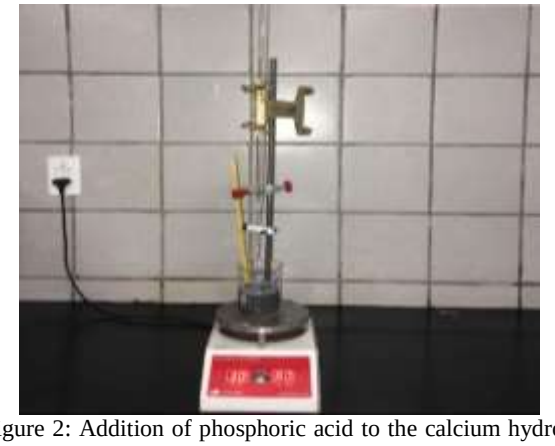

Figure 2: Addition of phosphoric acid to the calcium hydroxide solution.

When the reagent was added to the beaker, it was stirred for 55 minutes until it reached a temperature of 100ºC so that the liquid could evaporate, forming a viscous paste as a product. The resulting material went back to the air circulation oven and remained for 24 hours at 110ºC. The final material was removed from becker with the aid of a spatula, fragmented in a mortar and pistil, sieved, stored in porcelain crucibles

and submitted to a muffle with the purpose of making a new calcination for 2 hours at 900ºC.

To confirm the presence of hydroxyapatite, the powder obtained, after calcination, was submitted to the X-ray diffraction characterization (XRD) technique. This was performed on an X-ray tube device, with a reading on a drive shaft of Thetha-2Thetha, with a voltage of 40kV, an electric current of 30mA, with a scanning range of 5,000 - 90,000, a scanning speed 0.6000 (deg / min), sampling step of 0.0200 (deg) and a preset hour of 2.00 (sec).

Figure 3 identifies the presence of a flow chart that allows a better understanding of the hydroxyapatite synthesis process.

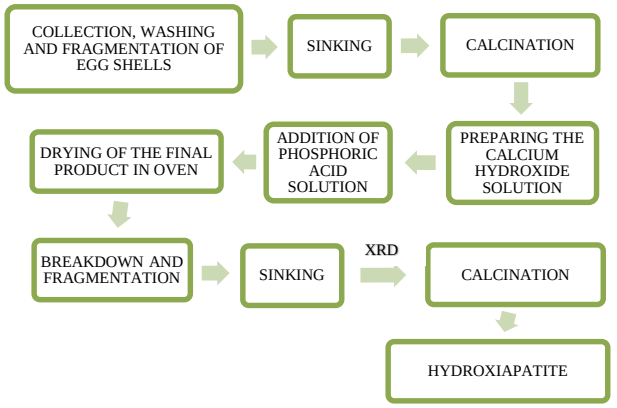

Figure 3. Flowchart showing the obtaining and characterization of hydroxyapatite.

Obtaining cassava starch biofilms with Hydroxyapatite

The filmogenic solutions forming the cassava starch film were adapted from Oliveira et al. (2018). The 2% (w/w) aqueous starch dispersions were heated to 70 ± 5ºC and kept at this temperature for 30 minutes under constant agitation to complete the gelatinization process. The films were prepared in five different formulations (0.0%; 1.0%; 2.5%; 3.5%; 5.0%) of hydroxyapatite, called formulations A, B, C, D and E, respectively, in 100 mL of distilled water. Glycerol was used as a plasticizer (30% w/w), in relation to the dry mass of the biopolymer. The solution was subjected to an ultrasonic bath, in a Q3350 model, for 5 minutes to remove bubbles that the solution could contain and, finally, left to rest for 30 minutes. Then, 60g of solution were deposited on acrylic plates 15 cm wide, 15 cm long and 2 cm deep and left to dry by evaporating the solvent for 5 hours using an air circulation oven, according to casting method. All films were prepared in triplicate.

To evaluate the characteristics, the biofilms of cassava starch with hydroxyapatite were subjected to the techniques of characterization of thickness, rate of permeability to water vapor, opacity and water solubility.

Figure 4 identifies the presence of a flow chart that allows a better understanding of the process of obtaining starch biofilms using hydroxyapatite.

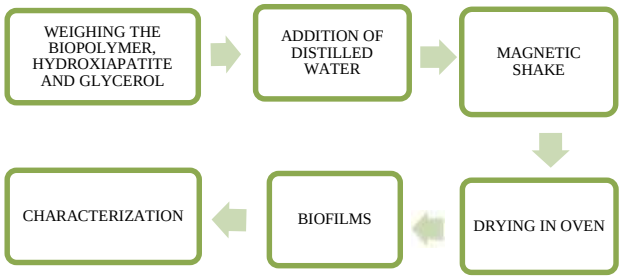

Figure 4. Flowchart showing the production of cassava starch biofilms with hydroxyapatite.

Characterizations of starch biofilms with hydroxyapatite

The thickness of the biofilms was carried out with the aid of a digital micrometer from Mitutoyo. The measurements were made at five different points in each sample.

The water vapor permeability rate was obtained in cuttings of 2 cm in diameter of each biofilm formulation and deposited sequentially on top of the WVPR measuring cells. These contained approximately 6mL of distilled water and were sealed so that the only passage of water vapor was through the films. The cell-film sets were weighed and deposited in a desiccator at 29ºC internal temperature and approximately 10% relative humidity. The weight of the cells was measured every hour for a period of 8 hours. The WVPR of the films was calculated in g./h.m^2 as follows in Equation 1:

$$WVPR = w / (A * t) \quad (1)$$

where: w: Weight of water that permeated through the film (g); A: Exposed permeation area (m²); and t: permeation time interval (h).

To determine the opacity of the films, a portable colorimeter (Konica Minolta Sensing Inc. Japan, model Color Reader CR - 10) was used. The parameter selected for study was the 'L', which consists of the luminosity on the white and black backgrounds, taken as standard. Thus, the calculation for obtaining opacity was done using Equation 2:

$$Op = (opp / opb) * 100 \quad (2)$$

where: Op: Opacity of biofilms (%); Opp: Opacity on the black background; and Opb: Opacity on the white background.

The solubility of the films was determined according to a methodology adapted from Oliveira et al. (2018). Samples of 3 cm squares were dried at 105ºC for 40 minutes and weighed (± 0.001g), considering them as the initial mass of the film. Then, the films were placed in conical flasks that contained approximately 50mL of distilled water and were stirred for 24 hours on a shaking table - Tecnal brand. The films were again dried at 105ºC for 40 minutes

and weighed again until constant weight, considered as final mass. The tests were performed in triplicate for each group of synthesized films. Finally, solubility can be calculated from Equation 3:

$$S = ((mi - mf) / mi) * 100) \quad (3)$$

where: S: Solubility of films (%); mi: initial mass of the film (g); and mf: final mass of the film (g).

A completely randomized design was used, with five treatments and three repetitions, and the means were compared using the Tukey test, with a 5% probability of error, for the smallest minimum difference in multiple comparisons.

RESULTS AND DISCUSSION

Figure 5 shows the X-ray diffractogram of the hydroxyapatite. Obtaining this by the wet precipitation method using white chicken egg shells was confirmed by the diffractogram. The peaks by which they compose the Figure indicate the crystalline phase of the ceramic compound, revealing the presence of hydroxyapatite in the powder from the shells of white chicken eggs. It is noted pure hydroxyapatite phase containing a hexagonal geometry in its structure, with the ceramic compound obtained by the hydrothermal method (Jayabal Prakash et al., 2020). It is also verified in Sousa Farias et al. (2019) that the results depend on the means by which hydroxyapatite is obtained, being more efficient when it comes from egg shells compared to commercially derived calcium hydroxide. It is justified that obtaining the hydroxyapatite by the egg shells, the crystalline phase is more elevated, making it more effective. Likewise, in Menezes Sousa et al. (2017) they managed to obtain the crystalline phase in hydroxyapatite by the method of wet precipitation using chicken egg shells, confirming the presence of the compound through the characterization by X-ray diffractograms. Similarly, in Munarin et al. (2015), the DRX presented the pure phase of the ceramic compound, being represented by the characteristic peaks of the analyzed material.

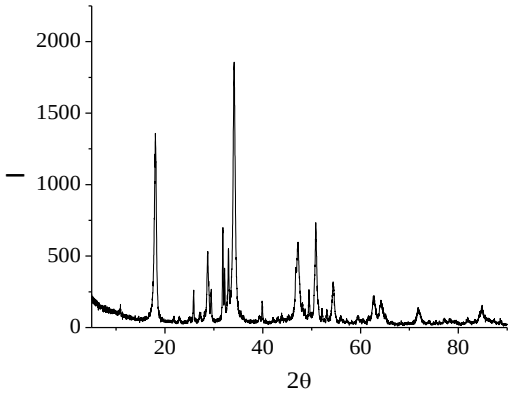

Figure 5. X-ray diffractogram of hydroxyapatite obtained from white chicken egg shells.

In order to obtain the calcium hydroxide solution, mesh sizes of 0.053 mm and below 0.053 mm were selected. The choice of grain sizes was due to the fact that they have a smaller diameter when compared to the others. This causes the contact of the particles in the solution to increase, accelerating the reaction speed, facilitating the occurrence of the chemical reaction. The selected granulometries were mixed and then the amount needed to prepare the calcium hydroxide solution was weighed. Table 1 shows the storage of granulometry according to their diameters.

Table 1. Granulometry for the preparation of the calcium hydroxide solution.

Granulometry (mm)	Mass (g)
0.500	26.5020[b]
0.250	35.1170[a]
0.125	21.7261[c]
0.053	15.6875[d]
<0.053	11.5703[e]

Data are reported with an overall average of 22.1206. Different lowercase letters indicate mean values with a significant difference ($p \leq 0.05$).

Table 2 identifies the thickness results for each biofilm. The values obtained varied between 0.060mm and 0.078mm, showing that the hydroxyapatite caused a slight increase in thickness, however, it was not significant. This suggests that this was caused by the ceramic compound as its concentration changed in biofilms. Formulation C did not follow the standard presented. This may have occurred because the film did not result in a uniform matrix or the ceramic compound did not dissolve in a way that would allow a homogeneous biofilm, causing some agglomeration and, consequently, divergence. Oliveira et al. (2018) showed the concentration of beeswax increased the surface area of biofilms of cassava starch.

Table 2. Thickness of films A, B, C, D and E.

Formulations	THICKNESS (mm)
A	0.060[a]±0.002
B	0.061[a]±0.005
C	0.081[a]±0.017
D	0.076[a]±0.002
E	0.078[a]±0.030

Data are reported with a general mean and standard error of 0.0714 ± 0.008. Same lowercase letters indicate that the means do not differ according to the tukey test ($p \leq 0.05$).

Table 3 shows the results of the water vapor permeability rate. It is noted that the addition of hydroxyapatite in the formulations causes a reduction in the permeability rate, leading to the conclusion that the hydroxyapatite acted in a way to block the passage of water. Sales Monteiro et al. (2018), obtained lower WVP using modified clay, which suggests that the increase in compounds may favor the formation of biocomposites based on cassava starch with improved properties. Likewise, in Sales Monteiro et al. (2017), there was an improvement in the water vapor barrier

using natural clay, which may show that hydroxyapatite, as well as the compound used in such research, was of fundamental importance to contribute to the reduction of the water vapor permeability rate.

Table 3. Water vapor permeability rate of films A, B, C, D and E.

Formulations	WVPR (g/h.m^2)
A	52.26 ^{b} ±2.75
B	48.98 ^{ab} ±5.62
C	48.06 ^{ab} ±2.37
D	46.76 ^{ab} ±4.76
E	40.34 ^{a} ±2.02

Data are reported with a general mean and standard error of 47.2822 ± 2.05. Different lowercase letters indicate mean values with a significant difference ($p \leq 0.05$).

Table 4 shows the opacity of biofilms. Note that as the hydroxyapatite concentration is increased, the opacity increases accordingly. If formulation A is compared, which contains only the biopolymer and formulation D, which in addition to the biopolymer contains a certain concentration of hydroxyapatite, it is possible to observe that in this biofilm it is more opaque, that is, it has less transparency than that biofilm that it contained only the cassava starch in its constitution. Formulation E, even containing a higher concentration of ceramic compound compared to other formulations, did not comply with the standard presented. This can be explained by the fact that the elaborated biofilm did not have a homogeneous matrix. Studies by Sales Monteiro et al. (2017) showed an increase in opacity with the addition of natural clay when compared to the biofilm that had only cassava starch in its composition. Similarly in Oludayo Oluwasina et al. (2019) showed an increase in biofilm opacity when adding oxidized starch. The increase can also be explained due to the formation of cross-linked starch molecules, in which they make the biofilm more opaque and, consequently, reduce its light permeation due to the formation of very strong intermolecular bonds.

Table 4. Opacity of films A, B, C, D, and E.

Formulations	OPACITY (%)
A	47.81^{d}±0.26
B	49.03^{c}±0.23
C	50.54^{b}±0.14
D	51.74^{a}±0.41
E	50.39^{b}±0.16

Data are reported with a general mean and standard error of 49.90 ± 0.15. Different lowercase letters indicate mean values with a significant difference ($p \leq 0.05$).

Table 5 shows the water solubility. The results showed that the increased concentration of the hydroxyapatite ceramic compound produced an effect on biofilms, making them less soluble in water, leading to the conclusion that the hydroxyapatite acted in a way to reduce the solubility of the films. Sales Monteiro et al. (2017) noted that were able to decrease solubility in water using natural or modified clay.

Table 5. Solubility of films A, B, C, D, and E.

Formulations	SOLUBILITY (%)
A	73.38^{a}
B	68.56^{a}
C	64.60^{a}
D	51.77^{ab}
E	29.98^{b}

Data are reported with a general mean and standard error of 57.66 ± 6.56. Different lowercase letters indicate mean values with a significant difference ($p \leq 0.05$).

CONCLUSIONS

It was concluded that it is possible to obtain the hydroxyapatite ceramic compound using white chicken egg shells, making the method efficient and promising since it destines the egg shells, reusing them. The use of the compound for the production of cassava starch biofilms resulted in membranes with a greater thickness, water vapor permeability and reduced solubility and an increase in opacity of the biofilms.

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