

## USE OF UNSUPERVISED PATTERN RECOGNITION TO EVALUATE THE CORRELATION OF BIODEGRADABLE FILM PROPERTIES<sup>1</sup>

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### ABSTRACT

Biodegradable films were produced using different amounts of corn starch, glycerin and propolis-green extract in a complete factorial design with three central points. The results of the physical, mechanical and optical properties of the materials obtained were evaluated through the use of Principal Component Analysis (PCA), which aimed to verify the similarity between the materials produced, as well as to stimulate the relationship between the determined characteristics. According to the results of the score chart and the analysis of CP1, which retained 55.56% of the information, it was found that the materials that were synthesized with lower amounts of corn starch and glycerin (films 1 and 2) were the ones that most distanced themselves from others. It was also possible to indicate that the propolis-green extract did not influence the differentiation of the samples, which may be associated with the fact that this additive was inserted in a smaller amount. Using the weight chart, it was possible to identify an increase in thickness with increasing opacity. The simultaneous analysis of the score and weight graphs showed that the weight is higher for samples that contain a greater amount of corn starch. In view of the results obtained, it can be inferred that the synthesis proposed in the present study was adequate and promising in the production of low-cost, easily accessible materials, with great potential to be applied as environmentally friendly packaging.

**Keywords:** Packaging. Sustainable development. PCA.

## USO DO RECONHECIMENTO DE PADRÕES NÃO-SUPERVISIONADO PARA AVALIAR A CORRELAÇÃO DAS PROPRIEDADES DE BIOFILMES

### RESUMO

Foram produzidos filmes biodegradáveis empregando quantidades distintas de amido de milho, glicerina e extrato de própolis-verde em um planejamento fatorial completo com três pontos centrais. Os resultados das propriedades físicas, mecânicas e ótica dos materiais obtidos foram avaliados através do emprego da Análise de Componentes Principais (PCA), a qual tinha como objetivo verificar a similaridade entre os materiais produzidos, bem como estimular a relação entre as características determinadas. Conforme resultados do gráfico de

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scores e pela análise da CP1, a qual reteve 55,56% das informações, verificou-se que os materiais que foram sintetizados com menores quantidades de amido de milho e glicerina (filmes 1 e 2) foram os que mais se distanciaram dos demais. Também foi possível indicar que o extrato de própolis-verde não influenciou na diferenciação das amostras, o que pode estar associado ao fato desse aditivo ter sido inserido em menor quantidade. Pelo gráfico de pesos foi possível identificar um aumento da espessura com o aumento da opacidade. A análise simultânea dos gráficos de scores e pesos evidenciou-se que a gramatura é maior para amostras que contêm maior quantidade de amido de milho. Diante dos resultados obtidos, pode-se inferir que a síntese proposta no presente estudo foi adequada e promissora na produção de materiais de baixo custo, fácil acesso, com um grande potencial para serem aplicados como embalagens ambientalmente favoráveis.

**Palavras-chave:** Embalagens. Desenvolvimento sustentável. PCA.

## 1 INTRODUCTION

When the production of packaging for the food sector is reported today, there is a great concern with projections aimed at extending the shelf life of products, maintaining their nutritional, sensory and microbiological safety quality (MATTA *et al.*, 2019; SILVA *et al.*, 2020).

However, it is worth mentioning that the accelerated production is accompanied by the generation of waste, which, being of petrochemical origin, degrades slowly. In this aspect, there is a growing threat to the environment, which makes it unsuitable for its use when thinking about sustainable development (AZIZ *et al.*, 2018; VESPUCCI, 2021).

To minimize this problem, there is a growing search for the development of new environmentally friendly materials. In this context, biodegradable films, formed by the insertion of a forming agent, solvent and plasticizer, can be considered an effective alternative to avoid the problems resulting from packaging disposal (ARAÚJO *et al.*, 2012; AZIZ *et al.*, 2018).

Among a great possibility of forming agents that can be used, corn starch stands out in this work as a sustainable and abundant material in nature. In addition, it is low cost, easy to dissolve in water, and has an adequate thermoplastic behavior (DAZA *et al.*, 2018; SIRVIÖ *et al.*, 2018).

Therefore, it should be noted that the composition of starch-based films requires the use of plasticizing agents that have an affinity for this type of forming agent, with emphasis on glycerin, which interacts with the carbohydrate through strong intermolecular forces such as hydrogen bonds. When added to the reaction medium, the plasticizer tends to disrupt native starch and reduce polymer-polymer interactions, increasing the mobility of polymer chains

OLIVEIRA *et al.*, 2011; NASCIMENTO *et al.*, 2012; BRODNJAK, 2017; COSTA, 2018; SIRVIÖ *et al.*, 2018; NANDI; GUHA, 2018).

The films obtained are highlighted for having multiple benefits, which include wide occurrence, low cost, in addition to being odorless and colorless, biocompatible and environmentally sustainable (CHUNG *et al.*, 2010; FAKHOURI *et al.*, 2012; SOUZA *et al.*, 2012; MEDINA-JARAMILLO *et al.*, 2017).

As reported by Vespucci (2021), these types of biodegradable films provide the option of being ingested, knowing that they are synthesized from non-toxic and safe materials to be used in food.

Recently, films are being prepared to reach the term “active packaging”, which may contain antioxidants to delay the harmful effects related to the loss of food quality (MATTA *et al.*, 2019). The incorporation of substances obtained from the Brazilian fauna and flora are known, highlighting the use of green propolis, which consists of a composition of resinous substances that bees of the species *Apis mellifera* collect from various plants, and use it to protect their young, and the food stored in its hive (ARAÚJO *et al.*, 2012; LAPA *et al.*, 2020). As the mixture can be found in the city of Bambuí - MG, its use as an additive in synthesized materials stands out.

It should be noted that, once synthesized, biodegradable films must undergo a thorough characterization, since the determination of their physical, mechanical and optical properties is crucial to validate the applications and possible choice of materials obtained for a particular purpose (AVELINO, 2019).

However, it is worth mentioning that when analyzing a significant amount of characteristics of materials synthesized with different amounts of precursors, a wide range of data is generated, which makes it necessary to use advanced approximations for the analysis of the data, according to reports described in the literature (LAPA *et al.*, 2020). It is then highlighted the use of chemometric techniques, such as the use of unsupervised Pattern Recognition composed of Principal Component Analysis (PCA).

In view of what has been discussed, the objective of this work was to employ a multivariate analysis technique that could be used to evaluate the interrelationships between the physical, mechanical and optical properties of biofilms, as well as to identify the similarity of materials synthesized with different amounts of the precursors.

## 2 MATERIAL AND METHODS

The materials used in the synthesis were purchased from local pharmacies and supermarkets in order to make the synthesis low-cost and easy to access. For this purpose, corn starch (Brand: MAIZENA), plasticizing agent double-distilled glycerin (Brand: FARMAX) and propolis-green extract (Brand: Natucentro) were used as forming agent.

The synthesis process consisted of preparing a mixture composed of corn starch and glycerin. Then, the colloidal suspension was heated to a temperature of 65 °C, and the system was kept under constant agitation. Then, the green propolis extract was added in the desired amount and the resulting solution was kept under heating and manual stirring for 10 minutes. After the completion of this procedure, the film-forming solutions were placed in a flat container, being dried at room temperature for three days for complete evaporation of water and formation of biofilms (VEIGA-SANTOS *et al.*, 2007; SOUZA *et al.*, 2011).

To evaluate the effect of the parameters: corn starch mass ( $X_1$ )- variable amount between 3.0 g e 5.0 g, glycerin ( $X_2$ ) and- variable amount between 0.4 g e 1.0 g, propolis-green extract ( $X_3$ )- variable amount between 0.1 g e 0.2 g, in the preparation of biodegradable films, the experiments were carried out using the Full Factorial Design with Central Point. For the three variables studied, this type of design involved eight factorial points and three central points, accounting for the synthesis of 11 biofilms.

The thickness was obtained with a micrometer (Carbographite  $\pm$  0.01 mm) at five points of each 4 cm<sup>2</sup> specimen and the results were expressed in millimeters (mm). Density was determined through the ratio between the mass and the thickness measured at five random points on the 4 cm<sup>2</sup> specimen, whose results were expressed in g cm<sup>-3</sup>. The grammage was determined by the ratio between the mass and area of biofilms with 2 cm<sup>2</sup>. The results were expressed in g m<sup>-2</sup> (SARANTÓPOULOS *et al.*, 2002). The swelling index (SI) was performed with 2 cm<sup>2</sup> specimens. The initial dry mass was obtained after weighing the materials. After the predetermined intervals (1 minute, 30 minutes and 60 minutes), the specimens were removed and the excess water was absorbed on filter paper for 1 minute. The hydrated films had their mass measured again. The swelling index ( $I_i$ ) was calculated according to equation 1, and the results were expressed as a percentage (%).

$$SI (\%) = \left( \frac{w_f - w_i}{w_i} \right) \cdot 100 \quad (1)$$

$w_f$  is final weight and  $w_i$ , the inicial weight.

The transparency of the films was determined in a UV/Visible spectrophotometer (METASH-Model V-5000). For this, the specimens were cut into rectangles and adhered to

the inner wall of the quartz cuvette. Under these conditions, transmittance at 600 nm was measured. The transparency of biofilms was calculated by equation 2:

$$\text{Transparency} = \frac{-\log T}{x} \quad (2)$$

Where T is the transmission (%) at 600 nm and X is the film thickness in mm. Results were presented as the ratio between absorbance at 600 nm and film thickness in millimeters ( $\text{Abs}_{600\text{nm}} \text{ mm}^{-1}$ ) (DOU *et al.*, 2018).

To carry out the tensile tests, the films were cut into 10 cm x 1 cm strips and evaluated in triplicate. The equipment used to perform the analysis was the Stable Micro Systems Texture Analyzer (model TA-XT2, England), with a 1 kN load cell. Each strip was fixed in the equipment with 30 mm clamps and a displacement speed of  $0.8 \text{ mm s}^{-1}$ . The modulus of elasticity (ME, MPa) was found by tracing a tangent to the tensile stress x strain curve in the linear region (elastic region), calculating the ratio between stress and corresponding strain (ASTM, 2001).

The unsupervised pattern recognition analysis was applied to the data in order to verify the correlation between the analyzed properties, as well as to evaluate the similarity between the biodegradable films that were synthesized under different conditions. For this purpose, Principal Component Analysis (PCA) was applied. The samples went through the auto-scaling pre-processing, using Euclidean distance, in which the data were connected by the nearest neighbor. Chemoface software version 1.5 was used for the chemometric analysis (NUNES *et al.*, 2012).

### 3 RESULTS AND DISCUSSION

There was a range of variation for all the properties analyzed. The differences in the values obtained are justified by the different amounts of the precursors (corn starch, glycerin and propolis-green extract) that were used during the synthesis, as observed in Table 1.

Table 1- Range of variation of properties analyzed in biodegradable films

Type	Property	Minimum value	Maximum value
Physical	Thickness (mm)	0.04	0.10
	Density ( $\text{g cm}^{-3}$ )	0.968	1.382
	Grammage ( $\text{g m}^{-2}$ )	40	135

	Swelling index (%)	50.09	173.11
Mechanical	Young's module (MPa)	4.43	96.73
	Breaking stress (MPa)	8.81	151.74
	Elongation (%)	0.72	10.92
Optical	Transparency (Abs600 mm <sup>-1</sup> )	5.46	15.80

Source: Authors (2022).

It should be noted that in all characterization analyses, it was the average of the results that were consistent with the data set was established (SALES et al., 2021). As the samples from the central point were synthesized under the same conditions, the average of similar results obtained in tests 9, 10 and 11 was used.

As the results obtained were different in the analyzed samples, it became necessary to use Principal Component Analysis (PCA) in order to analyze the data in a detailed and comparative way, and this chemometric tool was also used in order to verify which properties that allowed the approximation or distancing of the different films produced. The results obtained from the applied PCA are described in Figure 1.

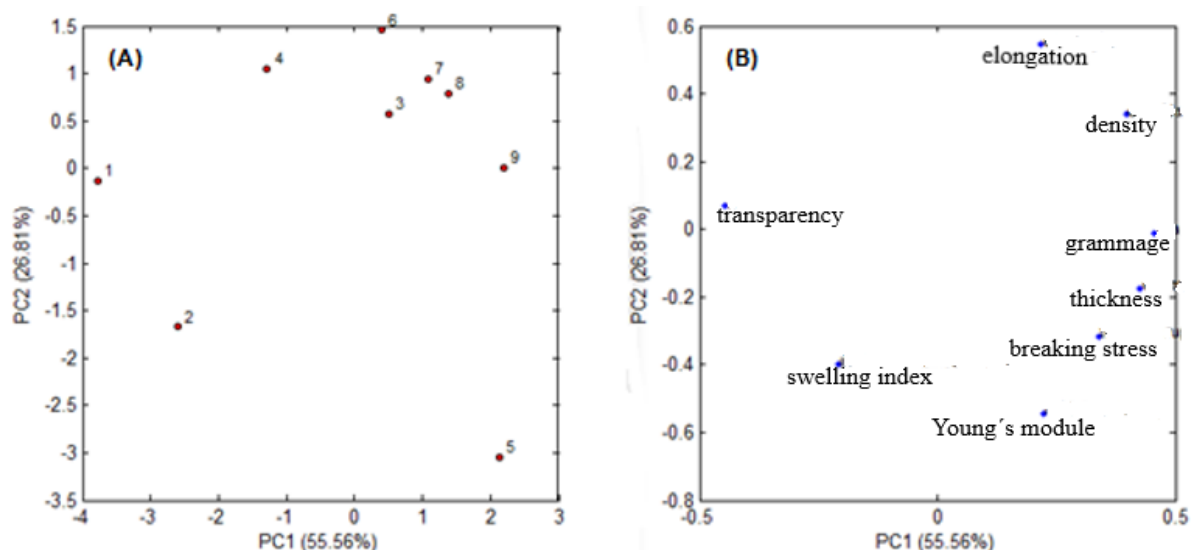


Figure 1- Score (A) and weight (B) graph of properties analyzed in biofilms through PCA analysis  
Source: Authors (2022).

The score chart showed that the first two principal components (PC1 and PC2) together explained 82.37% of the data variability, with 55.56% being explained by the first principal component (PC1) and 26.81% by the second (PC2). Based on the analysis of principal component 1, which retains a greater amount of information, the samples synthesized in conditions 1 and 2 were the ones with the lowest similarity when compared to the other synthesis conditions.

In this case, it can be reported that the conditions in which the minimum amounts of corn starch and glycerin are used cause the results to be more distinct in relation to the other samples for the properties that were analyzed, being observed that the amount of extract of green propolis did not differentiate the two samples, as can also be analyzed in the comparison of samples 7 and 8, which were synthesized in the highest amounts of forming agent and plasticizer. The results obtained may be associated with the fact that the additive is added in small amounts when compared to other precursors.

When analyzing the weight chart, it is worth mentioning that the film weight, defined as the ratio between the mass and area of a given material, is directly related to the mechanical strength of the films, with higher weights offering greater mechanical strength.

The simultaneous analysis of the score and weight graphs shows that the grammage is higher for samples that contain a greater amount of corn starch. Almeida et al. (2013) synthesized films formed by a blend of bacterial cellulose and potato starch and indicated that blends with higher amounts of cellulose have a higher grammage value.

The same tendency is shown for the analysis of stress at rupture, since according to CP1, these two properties are located in the same quadrant in the weight graph. In this case, it is possible to infer that the films that presented the highest values for the tensile strength were those that presented the highest value for the weight. The results can also be attributed to the fact that polysaccharides have high tensile strength and low elongation (FAKHOURI *et al.*, 2012). The data obtained indicate a consonance with results available in the literature (ALMEIDA *et al.*, 2013).

Another evident observation of the analyzed properties indicates that an increase in the thickness of the biofilms causes an increase in their opacity in the film. In fact, it is observed in the weight chart that the most transparent materials are the ones with the smallest thickness. The increase in opacity in biofilms as the amount of corn starch is increased is related to the fact that it is dependent on the amylose content of the starches. With larger amounts of starch in suspension, there is a higher amount of amylose molecules in solution, which, because they are linear, tend to orient themselves in parallel, approaching enough to form interactions such

as hydrogen bonds between hydroxyl chains of adjacent. As a result, the polymer's affinity for water is reduced, favoring the formation of opaque pastes and resistant films (FAKHOURI *et al.*, 2007).

It is also worth mentioning the importance in determining transparency, since although more transparent films allow the visualization of the quality of the coated food, the entry of light can lead to oxidation and degradation reactions (FERNANDES *et al.*, 2015; CARISSIMI, 2017).

According to the analysis of Fig. 1, and as predicted, it is verified by the analysis of the weights graph that although principal component 2 represents 26.81% of the information in the data set, elongation and rupture stress are opposite properties, which is coherent in that while the first property analyzes the elasticity of the materials produced, the second is correlated with the rigidity and resistance of the biofilms (McHUGH; KROCHTA, 1994).

A simultaneous analysis of the score and weight graph allows the synthesis of materials to be correlated with the analyzed properties. It is evident that the most transparent film (biofilm 1) is the one with the lowest amount of the three precursors. This biofilm is also evidenced by having lower results for weight, tensile strength and elongation. As corn starch is the forming agent, it can be inferred that such results are associated with lower amylose and amylopectin content and the fact that polysaccharides exhibit high strength and low elongation values (FAKHOURI *et al.*, 2012).

On the other hand, it appears that biofilm 2 has the highest swelling index, justified by having the lowest amounts of corn starch and glycerin. In this case, there are fewer interactions of the hydrogen bond type between the components, increasing molecular mobility and interaction with water.

Biofilms 7 and 8 are the ones with the highest results for density, justified by the synthesis conducted with the highest amounts of corn starch, a carbohydrate with a high molecular mass.

It is worth mentioning that the propolis-green extract was added to provide antioxidant properties to the produced films, since according to another work developed by the research group, the films that were most suitable could be used in the packaging of bananas (SALES *et al.*, 2021). It was found that the fruits that were packaged showed lower mass losses than the sample used as control (without packaging). The fact observed that the additive is a resinous mixture consisting of components that can act as antioxidants and antimicrobials (BANKOVA, 2000; LAPA *et al.*, 2019).



It is noteworthy that the FTIR analysis carried out in a previous work by the research group (LAPA et al., 2020). The analysis showed characteristic bands of the additive used, demonstrating that the appropriate synthesis temperature for the gelatinization of corn starch and subsequent biofilm formation was not sufficient to evaporate the inserted extract. This additive is adequate when considering the application of biofilms as packaging that prevent fruit oxidation, in line with work developed by the team involved (SALES et al., 2021).

#### 4 CONCLUSION

It was observed that the use of Pattern Recognition unsupervised by Principal Component Analysis (PCA) was essential and of fundamental importance to verify the similarities and differences between the biodegradable films that were synthesized, as well as allowing to correlate the analyzed properties, being that the results obtained were consistent with those available in the literature. It can also be inferred that the synthesis proposed in the present study was adequate and promising to the production of low-cost and easily accessible materials, with great potential to be applied in packaging.

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