Revista Colombiana de Investigaciones Agroindustriales
Volumen 9 (2). Julio – Diciembre. [p. 119-129]
https://doi.org/10.23850/24220582.5211



# Evaluation of mechanical properties of matrices derived from fish scale collagen

## Evaluación de propiedades mecánicas de matrices derivadas a partir de colágeno de escamas de pescado

*Juan Manuel Sánchez-Soto*[1]

*Ruth López-Alcántara* [2]

*Andrea del Pilar Sánchez-González*[3]

*Eyleen Jeniffer Torres-Mendoza*[4]

[1]Research group SENAGROTIC, Tecnoparque Nodo la Granja, Servicio Nacional de Aprendizaje - SENA, Colombia. Doctorado en Proyectos, Universidad Internacional Iberoamericana, México juan.sanchez@doctorado.unini.edu.mx

[2]Universidad Autónoma de Campeche, México. Doctorado en Proyectos, Universidad Internacional Iberoamericana, México rutlopez@uacam.mx

[3]Research group SENAGROTIC, Tecnoparque Nodo la Granja, Servicio Nacional de Aprendizaje - SENA, Colombia. Doctorado en Ingeniería con énfasis en Ingeniería de Alimentos, Universidad del Valle, Colombia apsanchezg@sena.edu.co

[4]Eyleen Jeniffer Torres-Mendoza. Research group SENAGROTIC, Tecnoparque Nodo la Granja, Servicio Nacional de Aprendizaje - SENA, Colombia etorresm@sena.edu.co

Recibido: 17/10/2022 Aceptado:20/10/2022

**Abstract** The development of biodegradable materials arises as an alternative to reduce the pollution caused by plastic waste to the environment, with this premise this study was proposed to develop plastic biopolymers from bioactive compounds with different matrices modified cassava starch (5 – 12 %), fish scale collagen (10 – 40 %), lemon essential oil (0,5 - 1,5 %) and gelatinization temperature (70 – 80 °C); A Box Behnken response surface experimental design was used; with the determination of their mechanical properties (maximum stress, Young's modulus, shear strength, stress at break and percentage elongation at break). According to the results found, it was determined that the modified cassava starch had the greatest influence on the mechanical properties, taking into account its importance to create more resistant materials, but it evidences plasticizing difficulties, where the fish scale collagen has a significant influence. In addition, it is evidenced that lemon essential oil had a great influence on Young's modulus (46,28 ± 2,31 MPa) and the percentage of elongation (69,69 ± 2,16 %); while the gelatinization temperature of 80 °C is not recommended for this type of starch-protein matrices due to damage of the structure; determining a better mechanical resistance and a great increase of Young's modulus. In conclusion, the characteristics and performance of the film based on cassava starch, collagen flakes and lemon essential oil have a positive impact on the maximum level of mechanical efficiency of the biodegradable films, achieving a better performance in their mechanical properties.

**Keywords:** bioplastics, package, starch, collagen, mechanical properties, cassava, essential oil, fish scale.

**Resumen** La elaboración de materiales biodegradables, surge como alternativa para disminuir la contaminación causada por los desechos plásticos al medio ambiente, con esta premisa, se propuso este estudio comprendido en el desarrollo de biopolímeros plásticos, a partir de compuestos bioactivos con diferentes matrices de almidón modificado de yuca (5 – 12 %), colágeno de escamas de pescado (10 – 40 %), aceite esencial de limón (0,5 - 1,5 %) y temperatura de gelatinización (70 – 80 °C). Se utilizó un diseño experimental de superficie de respuesta Box Behnken; con la determinación de sus propiedades mecánicas (esfuerzo máximo, módulo de Young, fuerza de corte, esfuerzo a la ruptura y porcentaje de elongación de ruptura). Según los resultados encontrados, se determinó que el almidón modificado de yuca fue el de mayor influencia en las propiedades mecánicas, teniendo en cuenta su importancia para crear materiales más resistentes, pero evidencia dificultades plastificantes, donde el colágeno de escamas de pescado incide significativamente. Además, se evidencia que el aceite esencial de limón presentó gran influencia en el módulo de Young (46,28 ± 2,31 MPa), y en el porcentaje de elongación (69,69 ± 2,16 %); mientras que la temperatura de gelatinización de 80 °C no es recomendada para este tipo de matrices almidón - proteína por daños de la estructura; determinando una mejor resistencia mecánica y un gran aumento del módulo de Young. En conclusión, las características y el rendimiento de la película a base de almidón de yuca, colágeno de escamas, y aceite esencial de limón; tienen un impacto al considerar el nivel máximo de eficacia mecánica de películas biodegradables y alcanzar un mejor comportamiento en sus propiedades mecánicas.

**Palabras clave:** bioplástico, envase, almidón, colágeno, propiedades mecánicas, yuca, aceite esencial, escamas de pescado.

## Introduction

In the industry, packaging production uses indistinctly different materials with properties of conservation, that enhance distribution and marketing of various products. Packaging plays a key role in extending the shelf life of food and serves as protection for non-food products. In addition, the environmental problems caused by plastic polymers and the concern of consumers for food safety and health is well-known, which has generated an increase in the search for new biodegradable and edible materials based on biopolymers, such as polysaccharides (starch, agar, cellulose, chitosan, among others), proteins (collagen/gelatin, soy, myofibrillar protein, whey, wheat, amorphous zein, etc.) and lipids (wax, fatty acids) (Hou *et al.*, 2019).

According to Assis (2019) the main functions of packaging are: to protect the product against mechanical, physical, chemical, and microbiological damage. Its application in food, especially in highly perishable products, is based on some properties, such as cost, availability, functional attributes, mechanical properties, optical properties, barrier against gas flow, structural resistance to water and microorganisms among others (Cardozo & Puerto, 2019).

In the case of collagen, as indicated by Perez- Puyana *et al.* (2020), the addition of chemical agents induces changes in both the mechanical properties and the architecture of the matrices, helping to overcome the loss of mechanical strength and structural stability, the author has proposed the formation of hybrid matrices, i.e., the mixture of collagen with other natural polymers that makes possible to obtain films with different mechanical and architectural characteristics.

When starch is used, it must have a gelatinization process, which is defined as the loss of semi-crystallinity of starch granules in the presence of heat and high amounts of water, with little or no occurrence of depolymerization. Gelatinization occurs over a narrow range of temperatures that varies depending on the starch source. Cassava starch gelatinizes in water at temperatures between 60 °C and 67 °C, which consists of a swelling of the starch molecules due to water penetrating their molecular structure (León *et al.*, 2020).

However, for film production, starch alone does not produce mechanical and barrier properties suitable for packaging (dos Santos *et al.*, 2018). Therefore, components such as distilled water, which is commonly used as a plasticizer to achieve starch destructuration in mixtures to obtain thermoplastic starches, must be considered in order to achieve better mechanical and barrier properties, besides being the most abundant and economical plasticizer. Another important component is glycerol, which has often been used for starch films due to its inherent compatibility with amylose chains, decreasing the intermolecular forces between starch molecules and promoting better mechanical properties. In turn, glycerol-plasticized starch films are more flexible and feasible for various uses (Nordin *et al.*, 2020).

At low starch concentrations, glycerol can act as an anti-plasticizer, decreasing the elongation at break, as well as the permeability to moisture and oxygen, due to the formation of cross-links between hydrogen bonds, small plasticizer molecules and starch side chains (Assis, 2019). However, glycerol produces hygroscopic films with high water vapor permeability, and these properties can be improved by adding hydrophobic components such as lipids to the films (Marín, 2019).

Essential oils are used as natural ingredients to improve film characteristics such as mechanical and barrier properties. In addition, they offer other beneficial properties in film development such as antioxidant and/or antimicrobial activity. According to the scientific literature studied, oregano essential oil is well-known for its antimicrobial, antioxidant and anti-inflammatory properties, thus being a natural alternative for food preservation (Cabezas, 2020). According to dos Santos *et al.*

(2018), the essential oil contributes to increase the elongation and reduce the tensile strength of films.

On the other hand, Susilawati *et al.* (2019), determined that thickness is one of the most important properties that determine the quality characteristics of bioplastics. The thickness should be adjusted to the product to be packaged. Thick packages increase the tensile strength value, but reduce the elongation value (Rusli *et al.*, 2017). The process temperature is another important variable to take into account because biopolymers are thermosensitive to high temperatures (Murillo, 2020), consequently, mechanical and barrier properties may limit the characteristics required for a package such as: protecting, preserving, distributing and storing. The research group of Wang *et al.* (2016), studied the mechanical and water solubility properties of collagen-starch films demonstrating that the mechanical properties of the hybrid film are improved as a function of increasing concentration (in the range of 10 % to 50 %) and the type of starch used. Likewise, this group evidenced an improvement in the thermostability of the collagen-starch material (Denaturation temperature [Td] of 95 °C) as the proportion of starch in the formulation increased compared to the collagen film (Td of 92 °C).

On the other hand, Ulyarti *et al.* (2020) investigated the effect of adding different concentrations of gelatin (0 %, 1 %, 1,5 %, 1,5 %, 2 %, 2,5 % and 3 %) to 1 % cassava starch to produce edible bioplastic films, concluding that the best formulation was to use 3 % gelatin or to work with 15 % modified cassava starch. In other words, increasing the concentration of gelatin in starch or using modified cassava starch increases the mechanical properties of the film. Alzáte-Pérez *et al.* (2018) reported on the development of a partially hydrolyzed collagen-based bioplastic from bocachico (*Prochilodum magdalenae*) scales, reinforced with rice husk at 3 %, 5 % and 10 %. Based on this material the bioplastic was elaborated, which was characterized by its resistance and deformation, therefore it could be used as a traditional plastic. Considering the above, the objective of this work was to evaluate the mechanical properties of bioplastics made with fish collagen, cassava starch, lemon essential oil and different gelatinization temperatures.

## Methodology

In the literature there are few studies on obtaining and characterizing the properties of starch-collagen based films and even less information on cassava-fish protein modified starch-based bioplastics. (Mroczkowska *et al.*, 2021), (Alias & Ishak, 2020), (Oluwasina & Awonyemi, 2021).

### *Materials*

The physicochemical characterization of the modified cassava starch OXIGEL®3 had an amylose content of 22,49 % and amylopectin of 77,51 %, moisture of 13,51 %, protein of 0,55 %, carbohydrate of 85,36 %, fat of 0,39 % and ash of 0,16 %. In general, it can be said that comparing a modified cassava starch and a native cassava starch, the former exhibits better functional properties in terms of texture, mechanical properties, solubility and consistency for gel formation (higher presence of amylopectin and protein).

The characterization of the fish scale collagen obtained a moisture content of 8,63 %, fat of 0,05 %, dietary fiber of 0,16 %, protein of 86,33 % and ash of 2,68 %, and the characterization of the lemon essential oil showed a specific weight of 0,85 at 25 ºC, a refractive index of 1,47 and a density of 0,85 g/ml method AOAC (2003). The lemon essential oil for the study produces a crosslinking effect that leads to a structure that is more solid and resistant to the bioprocesses it undergoes, decreasing the free volume and molecular mobility of the polymer in this case the modified cassava starch.

### *Experimental design*

A large number of variables affect the production process and the resulting physical and mechanical properties of natural polymer-based films. However, the factors with the higher impact in these properties will be evaluated using statistical methods. In other words, to optimize the process parameters, the Box-Behnken surface method is used over the following four factors (modified cassava starch, fish scale collagen, lemon essential oil and gelatinization temperature) while the independent or response variables (caliper, maximum stress, Young's modulus, breaking strength, stress at break, percent elongation at break) were employed. For the Box-Behnken design, the design points are placed at combinations of the high and low levels of the factors and their midpoints as shown in Table 1.

**Table 1**

*Matrix of factors and variables using a Box Behnken response surface design to define plastic films*

| Factors | Levels | | |
|---|---|---|---|
| | -1 | 0 | 1 |
| A = Modified cassava starch (%) | 5 | 8,5 | 12 |
| B = Fish scale collagen (%) | 10 | 25 | 40 |
| C = Lemon essential oil (%) | 0,5 | 1 | 1,5 |
| D = Gelatinization temperature (°C) | 70 | 75 | 80 |

*Source: Authors.*

### *Film production by the Casting Method*

The polymeric films were prepared by the 'casting' method, first the ingredients were mixed: Fish scale collagen, modified starch, lemon essential oil, distilled water, chitosan and glycerin; this mixture was subjected to heating in a continuous stirring plate at 4000 rpm to ensure the homogenization of the materials until reaching the different gelatinization temperatures ranging between 70 – 80 °C. After the solution of the film components was formed, it was deposited in aluminium trays and then evaporated at 70 °C in a forced convection oven for 12 hours, finally obtaining the biodegradable films.

### *Mechanical properties*

Mechanical properties (maximum stress, Young's modulus, rupture strength, stress at break and percent elongation at break) were measured according to ASTM standard method D882 (American Society for Testing and Materials [ASTM], 1980). Films with dimensions of 5 * 1 cm were used and loaded into a texturometer (LS1, Lloyd Ltd., Largo, FL, USA) with a force capacity 1000 N. The specimen speed and initial distance were set at 5 mm/min and 4 cm, respectively. They were analyzed using Nexygen Plus software. (Lloyd ltd., Version 3.0). Results are reported as the average of 10 measurements.

## Results and discussion

In this section are summarized the results found after determination and optimization of the base matrix of the biopolymer to be developed by combining concentrations of modified cassava starch, fish scale collagen, lemon essential oil and gelatinization temperatures, mentioned above and using the Box Behnken 4-factor response surface method for a total of 27 treatments.

### *Caliber (mm)*

With respect to this variable, starch was the only factor that had a statistically significant effect ($p < 0,05$) on the size of the processed films. Figure 1 shows the contour plots of starch versus the other factors and its behaviour against the film size, where it can be observed that the higher the starch content, the larger the film size. Starch contents between 5 – 9 % allow us to obtain films with calibers between 0,4 – 0,6 mm, whilst films with starch higher than 9 % one could obtain calibers between 0,6 - > 0,8 mm.

As indicated by Guarás (2018), the packages that have starch as material coming from cereals present random type-A schemes, while the diffractograms of starches coming from tubers, rhizomes and corn have high amylose content with type-B patterns influencing the crystal structures of both types A and B to obtain double, parallel, six-row helices, with a crystallographic repeat distance of 1,05 nm, giving rise to larger gauges, as corresponds in this study. According to Adamiak and Sionkowska (2020) the caliber increases with the addition of chitosan reinforcing the collagen structure and increases the pore size. It should be noted that the swelling properties of chitosan/collagen composites depend on the amount of crosslinking agent and the weight ratio of the components.

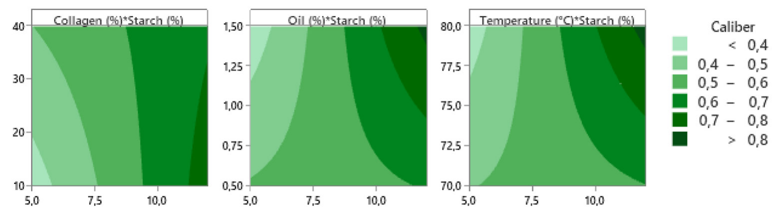


**Figure 1**

*Contour plot for caliber in relation to starch vs. collagen, oil and gelatinization temperature*
*Source: Authors.*

According to the results obtained by Abarca and Hidalgo (2021) the best derived matrix obtained with the combination of 11,5 % starch, 6 % collagen, 70 % water, 5 % vinegar, 6,50 % glycerin, 0,40 % citric acid, 0,6 % talc yielded a caliber of 0,231 mm compared to the present study which was 0,6 - > 0,8 mm, leading to an increase in resistance to mass transfer and vapor pressure; this effect on the structure of the film may be caused by the swelling of water in the polymer.

## *Maximum stress (MPa)*

Maximum stress can be defined as the stress that a material can withstand before breaking by stretching from both ends under specified conditions of temperature, humidity and speed. The films produced obtained maximum stress values ranging from 0,19 – 1,98 Mpa.

The combination of A: 12 %, B: 10 %, C: 1 % and D: 75 °C obtained the highest values of 1,89 ± 0,07 Mpa. Just like the study given by the maximum stresses performed by Gastelum *et al.* (2018) where it indicates that the material significantly increases its mechanical properties compared to the other treatments when one of its components, in this case the starch in the polymeric matrix helps to decrease the percentage of deformation, preventing the composite material from reaching a rupture.

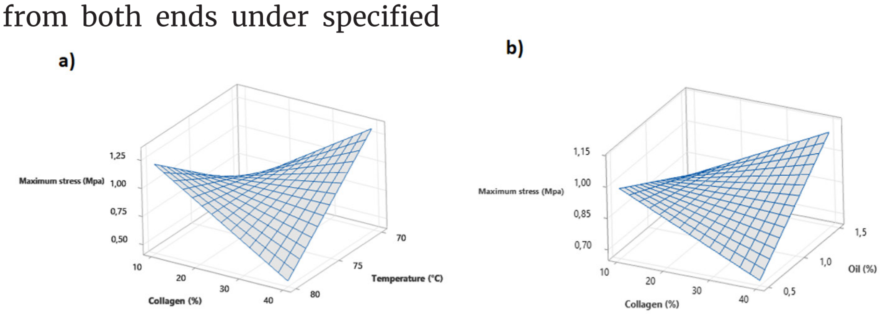


**Figure 2**

*Response surface for maximum stress in relation to the most influential interactions*
*Note. a) collagen*temperature, b) collagen*oil. Source: Authors.*

Figure 2 presents the response surface graphs for these interactions, with respect to B*D, an inversely proportional relationship is observed, as the temperature increases but the amount of collagen decreases the maximum stress increases or as the temperature decreases but the amount of collagen increases the maximum stress also increases. The highest points for this interaction are encountered in: 0,94 ± 0,049 Mpa for 80 °C; 10 % collagen and 70 °C; 40 %. On the other hand, Figure 3, presents the interaction between B*C, a proportional relationship is observed that as the collagen and oil content increases, a higher value of maximum stress is obtained (1,12 ± 0,09 Mpa), however, a greater influence is observed with the oil factor. With respect to the other interactions, it is observed that the maximum stress value is only influenced by the % starch, regardless of whether the % collagen or gelatinization temperature increases or decreases.

The increase in the mechanical properties in this material for elaborated packaging containing fish scale collagen can be influenced by the molecular weight of the protein. Palma-Rodríguez *et al.* (2017) report on the dependence of this type of protein in relation to the plasticizing power they have, when the elements with low molecular weight materials produce greater plasticizing power in the films than those with high molecular weights which would seriously indicate that the limit of the thermal properties of proteins is being reached; concordant with the results of 10% and 40% of collagen for maximum effort. In addition, the same study relates the combination of the concentration of poly-vinyl alcohol (PVOH) with starch, affected with a reduction in the melting temperature data, a variable directly related to the maximum stress behaviour of the element with which it is combined.

A similar case study is presented by Holguin-Cardona (2019) where he applies to 8 prototypes of bioplastics mechanical tests that present a maximum stress of 3,25 MPa, compared to the ASTM D-638 standard of 3,46 MPa (minimum value 3 MPa), recommending the use of thickening agents such as polysaccharides that will increase volume performance and lower production costs. This last point is relevant for any process of elaboration of biopackaging and for the case of the present research study, this value is below the standard of conventional plastics, but for the study the value of 1,89 ± 0,07 MPa for biodegradable derived matrices is important to take into account since it was observed that the incorporation of modified cassava starch reinforced the matrix increasing the maximum stress, behaviour that relates Bejarano-Martínez (2018), where he obtains a value by increasing the starch content increasing in his proposal 12,5 % presenting a result of 1,35 MPa of higher maximum stress.

### *Young's modulus (MPa)*

Young's modulus or modulus of elasticity (ratio of stress to strain over the linear part of the stress-strain curve) is a measure of film stiffness (Granda *et al.*, 2014), i.e. the higher the modulus, the higher the stiffness of the films. In this study the obtained values varied between 1,94 – 49,2 MPa, the combination of A: 12 %, B: 25 %, C: 1 % and D: 70 °C obtained the highest values with 46,28 ± 2,31 MPa. This could be explained by the fact that as the amount of starch increases, the stiffness of the material increases, and therefore the Young's modulus values.

With respect to oil, according to Slavutsky and Bertuzzi (2015) the incorporation of some lipid component produces some changes in the film such as an increase in Young's modulus and tensile strength and a decrease in elongation, which may be correlated with the water content. According to Malihi *et al.* (2022) hydrophobic compounds, such as essential oils, can improve the moisture barrier properties of the film, improving its strength, conclusion is similar for this study as increasing the oil content increased Young's modulus values. However, the effect of the addition of essential oils on the mechanical properties of films is a relatively complex process and contradictory results are found in literature.

With respect to the interactions, those with statistically significant influence were: B*D, A*B and B*C. Figure 3 shows an inversely proportional relationship between the two factors (BD), where the highest Young's modulus values occur at points B: 10 % and D: 80 °C or B: 40 % and D: 70 °C. According to Alias and Ishak (2020), the temperature and time of the protein during molding affects the tensile strength and Young's modulus of the films, behaviour that is evidenced in this study. On the other hand, A*B interactions are more influenced by the % starch, regardless of whether it increases or decreases the % collagen, and B*C by oil, or of whether it increases or decreases the % collagen.

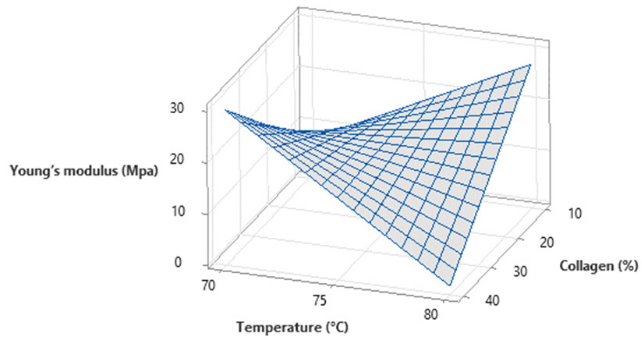


**Figure 3**

*Response surface for Young's modulus with BD interaction*
*Source: Authors.*

## *Load at break (N)*

The results obtained for the load at break, defined as the maximum load (N) necessary to stretch the composite material to its breaking point, in the films produced, obtained values ranging from 0,62 N – 5,97 N. The combination A: 12 %, B: 40 %, C: 1% and D: 75 °C obtained the highest values of 5,19 ± 0,42 N. Regarding the interactions obtained, the only one that had a statistically significant influence was that of A*B. Figure 4 shows an inversely proportional relationship both in the response surface and in the contour plot; as starch increases but collagen content decreases, the breaking strength value increases; however, it is more influenced by the % starch than by collagen.

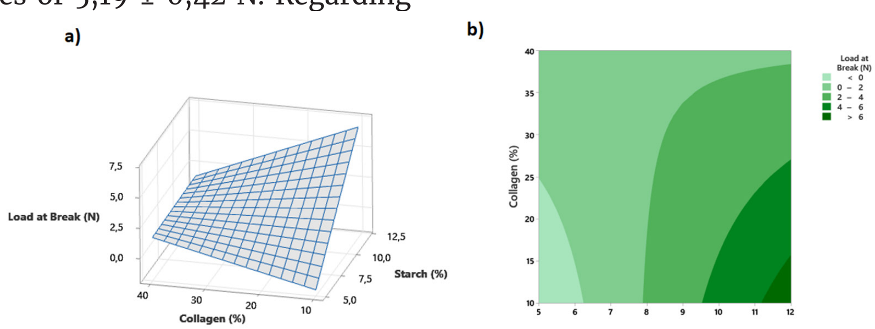


**Figure 4**

*Response surface and contour plot for Load at break with starch*collagen interaction*
*Source: Authors.*

In comparison with the films composed of starch and collagen at 10 % in the study by Wang *et al.* (2016), the films that have a higher percentage of collagen show a greater presence of starch granules in the material obtained, indicating a more compact distribution, showing a greater fragility to breakage when subjected to heating, for the case of this study the modified cassava starch has 22,49 ± 0,19 of amylose which allowed its shape to be maintained in the films made.

According to Guarás (2018) when starch uses high temperatures for gelatinization it is recommended not to use extrusion processes, since this unitary operation aims to decrease the content of plasticizers with boiling point above so that it does not denature. In addition, it should be taken into account that viscosity increases when there is high humidity, directly affecting the load at break with a higher degree of gelatinization of the material, without forgetting that this variable is the main one for the polymer degradation processes.

Studies carried out by Gutierrez (2020) shows the negative statistical effect of collagen on the force necessary to cut the films, considering that the collagen concentration measure increases, it is necessary to apply less cutting force, according to the results found, it is shown that the films present a force to rupture of 25,25 ± 1,17 N, these results are much higher than those found with collagen from fish scales and modified cassava starch with maximum values of 5,97 N, being closer to those of tilapia skin with force values of 8,5 ± 1,6 N, referenced by the same author in his study.

## *Breaking stress (MPa)*

It is important to bear in mind that the maximum stress is different from the breaking stress, the latter refers to the minimum force per unit section capable of producing a rupture. The films produced obtained values that varied between 0,15 - 1,19 MPa, the combination of A: 12 %, B: 10 %, C:1 % and D: 75 °C obtained the highest values with 1,11 ± 0,23 MPa. According to the Pareto diagram (Figure 1d), starch was the only factor that showed a positive and direct effect on the variable ($p > 0,05$), behaviour similar to that of maximum stress.

With respect to the interactions, those with statistically significant influence were: B*C, A*B, B*D and A*C. The breaking stress value of the A*B and A*C interactions is only influenced by the % starch, regardless of whether the amount of collagen or oil increases or decreases. On the other hand, Figure 5 presents the response surface plots of the interactions with significant influence (B*C and B*D), both showing an inversely proportional relationship between the two factors. The behaviour of the B*C interaction is comparable to the study reported by Jiang *et al.* (2022), who found the inversely proportional relationship of bovine collagen with oregano essential oil, causing changes in the mechanical properties, possibly since the protein is affected by the phenolic compounds of the essential oil.

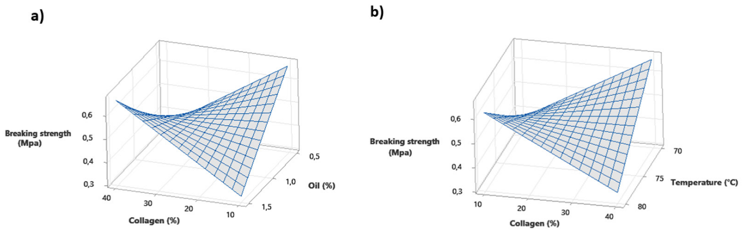


**Figure 5**

*Response surface for the breaking strength in relation to the interactions*

*Note. a) B*C and b) B*D. Source: Authors.*

Regarding the B*D interaction, Chisenga *et al.* (2019) in their study concludes that at higher gelatinization temperatures a structural loss occurs within the starch granule collapsing its structure, therefore, in this case decreases the values of breaking stress if combined with higher concentration of collagen creating more elastic films but weak in its configuration.

### *Total elongation percentage (%)*

The elongation percentage represents the longitudinal increase relative to the initial length of the sample after withstanding a tensile force, being a measure of the extensibility of the film until it breaks (Alias & Ishak, 2020). In this study the films produced obtained values ranging from 7,81 % - 72,54 %. The combination of A: 8,5 %, B: 25 %, C: 0,5 % and D: 70 °C obtained the highest values with 69,69 ± 2,16. Regarding collagen, the addition of this factor can provide a flexible nature of the film produced, however, high values make it more rigid and the elongation percentage value decreases, similar behaviour to the study of Susilawati *et al.* (2019) who also used fish gelatin.

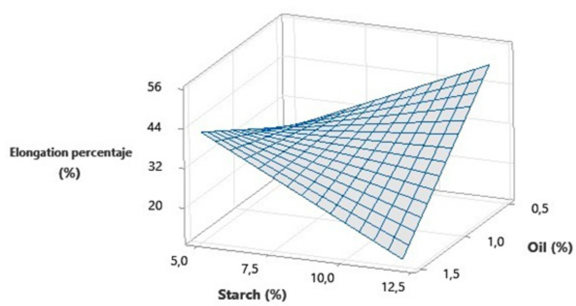


**Figure 6**

*Response surface for the total elongation percentage with AC interaction*
*Source: Authors.*

With respect to the interactions, those with statistically significant influence were: AC and BC. Figure 6 shows that as the concentration of oil and starch decreases, the elongation percentage increases. That is to say that by incorporating oil to the formulation of the films these become less plasticized, these results are comparable to those of Slavutsky and Bertuzzi (2015) who used sunflower oil and as its concentration increased, Young's modulus increased but the elongation percentage decreased. On the other hand, the BC interaction the elongation value is only influenced by the % of oil, regardless of whether it increases or decreases the % of collagen.

## Conclusions

This study shows that starch has a positive and direct effect on the variables of caliber, maximum stress, Young's modulus, load at break and stress at break, the higher the starch content the value of these variables increases, however, the percentage of deformation of the film decreases, which leads to form a more resistant but less flexible material. On the other hand, collagen and oil increase the total elongation percentage, considering that these factors influence the plasticizing power of the films, the incorporation of starch in the film combined with collagen serves as a potential solution for biodegradable packaging addressing the challenges faced by the environment.

The incorporation of lemon essential oil does influence mechanical changes of the biodegradable films, increasing the oil content, increasing the Young's modulus value, but decreasing the elongation percentage, the EOs have great potential in multifunctional and controlled release applications such as bioactive food packaging. It was also concluded that the optimum gelatinization temperature for these factors should be within a range of 70 – 75 °C, since the temperature of 80 °C generated a structural loss within the starch granule thus collapsing the film structure and its mechanical characteristics.

## Acknowledgment

The authors would like to thank the Servicio Nacional de Aprendizaje - SENA, Regional Tolima del Centro Agropecuario la Granja, for the support provided as well as the permission to conduct the experiments in the laboratory facilities at Tecnoparque nodo la Granja, and finally to the Universidad del Tolima for the development of the study.

## References

Abarca, P. F. J., & Hidalgo, L. D. M. (2021). *Obtención de película biodegradable a partir de colágeno (Gallus gallus domesticus) y almidón (Musa balbisiana) para el recubrimiento de frutas.* (Undergraduate thesis, Universidad de Guayaquil). Institutional repository. http://repositorio.ug.edu.ec/handle/redug/53887

Adamiak, K., & Sionkowska, A. (2020). Current methods of collagen cross-linking: Review. *International Journal of Biological Macromolecules*, 161, 550–560. https://doi.org/10.1016/j.ijbiomac.2020.06.075

Alias, S. A., & Ishak, K. M. K. (2020). Preparation and characterization of protein bioplastics from fish waste using different plasticizers. *Materials Science Forum*, 982 MSF, 67–72. https://doi.org/10.4028/www.scientific.net/MSF.982.67

Alzáte-Pérez, S. I., Quintanilla, J. P., Paredes, C. R., & Velasco, E. (2018, september, 13-14). Bioplásticos de colageno parcialmente hidrolizado obtenido de las escamas del bocachico (*Prochilodum magdalenae*), reforzados con cáscarilla de arroz. In *Memorias del IV Simposio de Materiales Poliméricos, Biopolímeros. Informador Técnico*, 82 (2), 103–106. Cali, Colombia. https://doi.org/10.23850/22565035.1983

American Society for Testing and Materials [ASTM]. (1980). D882-80, Standard Test Method for Tensile Properties of Thin Plastic Sheeting. In *Manual book of ASTM standards*. ASTM. https://www.astm.org/

AOAC. (2003). *Official Methods of Analysis*. Association of Analytical Washington, DC, USA. https://www.aoac.org/

Assis, A. M. (2019). *Conservação de melão "cantaloupe" minimamente processado com diferentes recobrimentos.* [Master thesis, Universidade Federal De Campina Grande]. Institutional repository. http://dspace.sti.ufcg.edu.br:8080/jspui/bitstream/riufcg/3533/3/ANAMARINAASSISALVES–DISSERTAÇAOPPGSAACADÊMICO2019.pdf

Bejarano-Martínez, N. L. (2018). *Rendimiento del proceso de extracción de almidón a partir de frutos de plátano (Musa paradisiaca).* [Undergraduate thesis, Universidad Nacional de San Agustín de Arequipa]. Institutional repository. http://repositorio.unsa.edu.pe/handle/UNSA/7578

Cabezas, J. A. (2020). *Recubrimientos biodegradables obtenidos a partir de cáscara y semilla de sandía (Citrullus lanatus).* [Degree thesis, Universidad Agraria del Ecuador]. Institutional repository. https://cia.uagraria.edu.ec/Archivos/CABEZASRODRIGUEZ JOSE ALBERTO.pdf

Cardozo, J. R., & Puerto, R. L. (2019). *Agregado de valor a frutos silvestres de agraz a través de la aplicación de recubrimientos comestibles a base de almidones de papas andinas.* [Degree thesis, Universidad Pedagógica y Tecnológica de Colombia]. Institutional repository. https://repositorio.uptc.edu.co/bitstream/001/2815/1/TGT_1419.pdf

Chisenga, S. M., Workneh, T. S., Bultosa, G., & Alimi, B. A. (2019). Progress in research and applications of cassava flour and starch: a review. *Journal of Food Science and Technology*, 56 (6), 2799–2813. https://doi.org/10.1007/s13197-019-03814-6

dos Santos, K. C., Almeida, N. L., Haas, T. M. C., Brandelli, A., Rodrigues, E., Hickmann, S. F., & Cladera, F. O. (2018). Characterization of active biodegradable films based on cassava starch and natural compounds. *Food Packaging and Shelf Life*, 16, 138–147. https://doi.org/10.1016/j.fpsl.2018.03.006

Gastelum, A. N., Hernández, M. S., Gonzalez, B., Vega, Y., & Muñoz, I. M. (2018). Análisis comparativo de las propiedades mecánicas de un material compuesto reforzado con fibras de carbono y las de su matriz polimérica de resina epóxica. *Matéria (Rio de Janeiro)*, 23 (2). https://doi.org/10.1590/s1517-707620180002.0428

Granda, D., Medina, Y., Culebras, M., & Gómez, C. (2014). Desarrollo Y Caracterización De Una Película Activa Biodegradable Con Antioxidantes (Alfa-Tocoferol) a Partir De Las Proteínas Del Lactosuero Tt - Development and Characterization of an Active Biodegradable Film With Antioxidants (Alpha-Tocopherol) From. *Vitae*, 21 (1), 11–19. http://www.scielo.org.co/pdf/vitae/v21n1/v21n1a2.pdf

Guarás, M. P. (2018). *Desarrollo de Nanocompuestos Basados en Almidón Termoplástico a Escala Planta Piloto.* [Doctoral thesis, Universidad Nacional de Mar del Plata]. Repositorio Institucional CONICET Digital. Argentina. https://ri.conicet.gov.ar/handle/11336/91843

Gutierrez, R. S. (2020). *Películas biodegradables a partir de colágeno de piel de pez diablo (Pterygoplichthys pardalis).* [Master thesis, Instituto Tecnológico de Tuxtla Gutiérrez]. Institutional repository. http://repositorio.digital.tuxtla.tecnm.mx/xmlui/handle/123456789/2034

Holguin-Cardona, J. S. (2019). *Obtención de un Bioplástico a partir de Almidón de Papa.* [Undergraduate thesis, Fundación Universidad de América]. Institutional repository. https://repository.uamerica.

edu.co/bitstream/20.500.11839/7388/1/6132181-2019-1-IQ.pdf

Hou, C., Gao, L., Wang, Z., Rao, W., Du, M., & Zhang, D. (2019). Mechanical properties, thermal stability, and solubility of sheep bone collagen–chitosan films. *Journal of Food Process Engineering*, 43 (1), 1–8. https://doi.org/10.1111/jfpe.13086

Jiang, J., Watowita, P. S. M. S. L., Chen, R., Shi, Y., Geng, J.-T., Takahashi, K., Li, L., & Osako, K. (2022). Multilayer gelatin/myofibrillar films containing clove essential oil: Properties, protein-phenolic interactions, and migration of active compounds. *Food Packaging and Shelf Life*, 32, 100842. https://doi.org/10.1016/j.fpsl.2022.100842

León, G., León, D., Monroy, M., Espriella, S., & Herrera, A. (2020). Modificación química de almidones mediante reacciones de esterificación y su potencial uso en la industria cosmética. *Archivos Venezolanos de Farmacología y Terapeútica*, 39 (5), 620–626. https://doi.org/10.5281/zenodo.4263410

Malihi, N., Danafar, F., & Moosavi-nasab, M. (2022). The effect of Oliveria decumbens Vent. essential oils and lysozyme on physicochemical and functional properties of fish gelatin film. *Journal of Food Measurement and Characterization*, 16 (3), 2356–2364. https://doi.org/10.1007/s11694-022-01344-y

Marín, D. (2019). *Nanoliposomas a partir de productos naturales infrautilizados y residuos agroalimentarios como ingrediente funcional en alimentos.* [Doctoral thesis, Universidad Complutense de Madrid]. Institutional repository. https://eprints.ucm.es/id/eprint/57956/1/T41485.pdf

Mroczkowska, M., Culliton, D., Germaine, K., & Neves, A. (2021). Comparison of mechanical and physicochemical characteristics of potato starch and gelatine blend bioplastics made with gelatines from different sources. *Clean Technologies*, 3 (2), 424–436. https://doi.org/10.3390/cleantechnol3020024

Murillo, R. G. (2020). *Aplicación de la tecnología de extrusión en productos con alto contenido en proteína* [Degree thesis, Universidad Politécnica de Valencia]. Institutional repository. https://riunet.upv.es:443/handle/10251/150018

Nordin, N., Othman, S. H., Rashid, S. A., & Basha, R. K. (2020). Effects of glycerol and thymol on physical, mechanical, and thermal properties of corn starch films. *Food Hydrocolloids*, 106, 105884. https://doi.org/10.1016/j.foodhyd.2020.105884

Oluwasina, O. O., & Awonyemi, I. O. (2021). Citrus peel extract starch-based bioplastic: effect of extract concentration on packed fish and bioplastic properties. *Journal of Polymers and the Environment*, 29 (6), 1706–1716. https://doi.org/10.1007/s10924-020-01990-7

Palma-Rodríguez, H. R., Salgado-Delgado, R., Páramo-Calderón, D., Vargas-Torres, A., & Meza-Nieto, M. (2017). Caracterización parcial de películas biodegradables elaboradas con almidón de plátano y proteínas séricas de la leche. *Acta Universitaria*, 27(1), 26–33. https://doi.org/10.15174/au.2017.1215

Perez-Puyana, V., Alonso-González, M., Rubio-Valle, J. F., Guerrero, A., & Romero, A. (2020). Use of genipin to crosslink collagen and chitosan-based biomaterials. En 24th *International Congress on Project Management and Engineering July*, 656–665. http://dspace.aeipro.com/xmlui/bitstream/handle/123456789/2459/AT03-017_20.pdf?sequence=1&isAllowed=y

Rusli, A., Metusalach, M., & Tahir, M. M. (2017). Characterization of Carrageenan Edible films Plasticized with Glycerol. *Journal Pengolahan Hasil Perikanan Indonesia*, 20 (2), 219. https://doi.org/10.17844/jphpi.v20i2.17499

Slavutsky, A. M., & Bertuzzi, M. A. (2015). Formulation and characterization of nanolaminated starch based film. *LWT - Food Science and Technology*, 61 (2), 407–413. https://doi.org/10.1016/j.lwt.2014.12.034

Susilawati, Rostini, I., Intan, R. P., & Rochima, E. (2019). Characterization of Bioplastic Packaging from Tapioca Flour Modified with the Addition of Chitosan and Fish Bone Gelatin. *World Scientific News*, 135, 85–98. www.worldscientificnews.com

Ulyarti, U., Nazarudin, N., Surhaini, Lisani, Ramadon, R., & Lumbanraja, P. (2020). Cassava Starch Edible Film with Addition of Gelatin or Modified Cassava Starch. *IOP Conference Series: Earth and Environmental Science*, 515 (1), 6–11. https://doi.org/10.1088/1755-1315/515/1/012030

Wang, K., Wang, W., Ye, R., Liu, A., Xiao, J., Liu, Y., & Zhao, Y. (2016). Mechanical properties and solubility in water of corn starch-collagen composite films: Effect of starch type and concentrations. *Food Chemistry*, 216, 209–216. https://doi.org/10.1016/j.foodchem.2016.08.048