Starch/chitosan/glycerol films produced from low-value biomass: effect of starch source and weight ratio on film properties

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Abstract. Films based on chitosan from giant squid (Dosidicus gigas) residual endoskeleton, starch from different low-value agroindustrial byproducts and glycerol were successfully obtained. Four starch sources and three different weight ratios (1/1, 2/1 and 5/1 of starch/chitosan) were used. Applying a statistical design of completely randomized blocks, the effect of starch source and weight ratio on the film properties were evaluated. The starch source did not affect any of the tested properties. However, film properties such as thickness, water solubility, water vapor permeability, elongation, and tensile strength revealed significant differences (p<0.01) related to the starch weight ratio in the films. A higher starch weight ratio led to lower water solubility; nevertheless, the higher starch weight ratio led to films with higher thickness, water vapor permeability and tensile strength.

1. Introduction

In modern society, synthetic plastics have become a serious environmental concern. Due to their low biodegradability, these materials can remain in the environment for a long time and cause problems such as saturation of landfills and greenhouse gas production [1]. In most cases, these materials can even reach terrestrial and aquatic ecosystems, affecting living organisms [2].

Finding new renewable packaging materials is still an important challenge [3-6]. Biocompatible materials such as chitosan, starch, gelatin, and proteins have been used to obtain new generations of biodegradable packages [7-9]. Starch and chitosan are two of the most commonly used biopolymers to produce biodegradable packages with specific advantages and disadvantages. Starch has such properties as being homogeneous, odorless, and colorless and having very low oxygen permeability [10]. However, it is highly hydrophobic, highly water soluble, and brittle [11] and shows poor mechanical and barrier...
properties [12]. On the other hand, chitosan is able to form films with a lack of water resistance and poor mechanical properties [13].

Chitosan is usually prepared from low-value abundant wastes; however, the most popular production method (chemical) still negatively affects the environment [14]. In contrast, starch raw materials and production process are low cost and cause less pollution compared to chitosan production.

Adding a plasticizer into films prepared with biopolymers improves film properties [15]. Versino, Lopez, Garcia and Zaritzky [16] observed that plasticized starch films showed lower water vapor permeability compared to unplasticized ones. Additionally, the presence of the plasticizer induces structural modifications of the starch network, leading to a less ordered but compact structure [17]. It is very important to maintain moisture content in some edible products and maintain their quality. In addition, Zhong and Xia [18] concluded that the presence of glycerin increases the flexibility of the final films because of the reduction of the intramolecular bonds between polymeric chains.

According to Versino, Lopez, Garcia and Zaritzky [16], the starch source is critical for film optical properties, which strongly influence consumer acceptability of the packaging material. Additionally, it has been well known that the origin of starch (variety and part of the plant) influences starch composition. Different authors [19-21] have considered that the amount of amylose in starch has an influence on the properties of chitosan/starch films. Properties such as water vapor permeability and tensile strength are inversely proportional to amylose content, while elongation at break is directly proportional to the amylose content in starch.

From the above studies, it appears that combining both chitosan and starch may be useful for the production of packaging films with improved properties. Many authors have studied the combination of both materials with or without the addition of plasticizer agents [11, 20, 22-25]. However, the combination of biopolymers made of low-cost and abundant sources, such as residual biomasses, is still an attractive topic to investigate. Moreover, reports on the influence of characteristics such as effective diffusivities on the overall properties of food packaging materials have been limited or omitted in the literature so far.

Within agroindustrial production, there is a portion of raw material with low quality that is not considered for elaborating the final product. These surplus materials are usually sold in the local markets as animal feed for very low prices. However, some of those materials are rich in starch, giving low-cost materials an aggregate value. Some of those raw materials are broken rice grain (“ñelen”) produced in the process of rice peeling, hard yellow corn surplus, banana surplus from exportation activity (variety Cavendish) (BS) and low-quality cassava. In the case of chitosan production, there are unconventional residual biomasses with a high content of chitin that could be used as raw material. Giant squid (Dosidicus gigas) is a widely distributed species in the South Pacific Ocean (coast of Peru and Chile). The residual material of giant squid processing is used to produce animal feed or is disposed of in illegal dumps, causing environmental problems.

In this framework, the present study aimed to produce starch/chitosan/glycerol films using chitosan derived from residual biomass of giant squid (Dosidicus gigas) endoskeleton and four sources of starch from low-value agroindustrial products in different weight ratios and to evaluate the effect of the source and weight ratio of starch on film properties.

2. Materials and Methods

2.1. Chitosan and starch samples

Chitosan (molecular weight of 1000 kDa, deacetylation degree of 85.5%) produced via a chemical method from giant squid (Dosidicus gigas) endoskeleton [26] was used for the experiments. Various starch samples were obtained using low-value raw materials, such as broken rice grains (RS) (“ñelen”), hard yellow corn surplus (CS), banana surplus from exportation activity (variety Cavendish) (BS) and low-quality cassava (CaS). Starch isolation was carried out by mixing dried and ground raw materials with a solution of sodium metabisulfite (5%). The mixture was filtered and then decanted at 5 °C for 24 h. Afterwards, the obtained starch was washed repeatedly with distilled water and ethanol.
Finally, the starch samples were dried at 55 °C for 48 h and were subsequently crushed and sieved. The yield (wt.%) was calculated by dividing the weight of starch by the total weight of used raw materials multiplied by 100.

The starch samples were characterized to determine moisture, ash, lipid and amylose contents (wt.%) using classical methods [27-29].

2.2 Film preparation
Chitosan/glycerol/starch films were obtained by a casting method. A solution of chitosan 1% (w/v) in 1% (v/v) acetic acid was prepared. Aqueous solutions of different starch weight ratios (1/1, 2/1 and 5/1 starch/chitosan) were produced by agitation at 30 °C for 30 min and then at 90 °C for 60 min to ensure that the total gelatinization of starches occurred. The solutions were filtered through a cellulose filter (pore size 0.10 mm) to remove insoluble particles. The chitosan solution and the solutions with different starch weight ratios were mixed to prepare the samples, as summarized in table 1. The mixture was stirred for 30 min, and a glycerol solution (1.5% w/w) was added in a volumetric ratio corresponding to 2:1 with respect to the starch solution. The mixture was stirred for an additional 30 min and then placed into an ultrasonic bath (frequency 40 kHz) for 20 min. Finally, the mixture was dispersed over acrylic molds. The samples were dried at 60 °C for 23 h and subsequently, the produced films were stored between plasticized papers inside plastic bags.

Table 1. Composition of the prepared films (taken as base 100 ml of solution before drying).

| Samples   | Chitosan (g) | Glycerol (g) | Starch Source | Starch (g) | Starch/Chitosan Ratio |
|-----------|--------------|--------------|---------------|------------|-----------------------|
| Ch-G-RS1  | 0.4          | 0.3          | Rice          | 0.4        | 1/1                   |
| Ch-G-RS2  | 0.4          | 0.3          | Rice          | 0.8        | 2/1                   |
| Ch-G-RS5  | 0.4          | 0.3          | Rice          | 2.0        | 5/1                   |
| Ch-G-CS1  | 0.4          | 0.3          | Hard yellow corn | 0.4     | 1/1                   |
| Ch-G-CS2  | 0.4          | 0.3          | Hard yellow corn | 0.8     | 2/1                   |
| Ch-G-CS5  | 0.4          | 0.3          | Hard yellow corn | 2.0     | 5/1                   |
| Ch-G-BS1  | 0.4          | 0.3          | Banana        | 0.4        | 1/1                   |
| Ch-G-BS2  | 0.4          | 0.3          | Banana        | 0.8        | 2/1                   |
| Ch-G-BS5  | 0.4          | 0.3          | Banana        | 2.0        | 5/1                   |
| Ch-G-CaS1 | 0.4          | 0.3          | Cassava       | 0.4        | 1/1                   |
| Ch-G-CaS2 | 0.4          | 0.3          | Cassava       | 0.8        | 2/1                   |
| Ch-G-CaS5 | 0.4          | 0.3          | Cassava       | 2.0        | 5/1                   |

2.3 Film characterization
To take SEM images, a triangular section (0.5 cm each side) of the film samples was cut, placed on a carbon support and covered with a gold coating. The conditioned sample was placed into the vacuum chamber of an SEM (Vega3 Tescan, Czech Republic). SEM images were taken at two magnifications to obtain view fields of 5 and 20 μm and with a 15 kV acceleration voltage for only the samples with a starch/chitosan ratio of 2/1.

The thickness of the films was measured using a digital micrometer (Mitutoyo IP 65 Coolant Proof, Japan) with 0.001 mm accuracy. Ten different points were taken across the films to measure the thickness. The average value was calculated and reported.

For water solubility, 2 cm × 3 cm sections of the film samples were cut and weighed using an analytical balance (Sartorius Quintix, USA). Every section was placed into a 100 mL flask, and 80 mL of distilled water was added. The samples were stirred for 1 h at room temperature, taking care that the
film fraction was still submerged in the water. The remaining pieces of the samples after soaking were recovered and dried at 60 °C until a constant weight was reached [30]. Water solubility was calculated according to the following equation:

$$\text{Solubility (\%) = \frac{[\text{Initial dry weight} - \text{Final dry weight}]}{\text{Initial dry weight}}} \times 100$$

(1)

To determine the luminosity factor of the films (%), a colorimeter (Konica Minolta CR400, Japan) was used with white color as the reference.

The water vapor permeability of the films was determined by a desiccant method according to standard test method ASTM E96M/E96-05. A section of the films (3.5×3.5 cm) was placed into probe tubes (130 × 15 mm) with 2 g of silica gel. Every tube was covered with a double layer composed of aluminum paper and paraffin and was closed hermetically. The closed tubes were weighed and then placed into a desiccator with 65% relative humidity at room temperature (30 °C). At the beginning, the sample was weighed every hour for the first two days; then, it was weighed every 2 h for one week; finally, it was weighed every day until the final sample weight exceeded 4% of the initial weight. Weight vs. time was graphed, and the slope was calculated. A linear dependence is observed, and the slope represents the amount of water vapor transmitted per unit time. To determine the water vapor permeability, equation 2 was used:

$$WVP = \frac{\text{WVTR} \times e}{p_w \times (HR_1 - HR_2)}$$

(2)

where $WVP$ is the water vapor permeability (g·Pa⁻¹·m⁻¹·s⁻¹), $\text{WVTR}$ is the ratio of the slope of the curve to the exposed area of the films (g·m⁻²·s⁻¹), $e$ is the thickness (m), $p_w$ is the water vapor pressure at the temperature of the experiments (Pa), $HR_2$ is the relative humidity inside the chamber and $HR_1$ is the relative humidity inside the tube.

The tensile strength (MPa) and elongation at break (%) were calculated using a standard test method according to ASTM D882-01 (ASTM, 2001b). The measurements were performed with a universal texturometer (Brookfield CT3, Canada) with an activation charge of 0.05 N. Samples of 10 × 2.5 cm (length x width) were cut off and loaded in the fixing accessory of the texturometer. The crosshead speed was set at 0.5 mm·s⁻¹ until break.

Tensile strength was calculated by dividing the maximum force on the film during fracture by the cross-sectional area (width × thickness). Elongation at break was calculated by dividing the maximum extension of the film by the length of the samples.

Binary countercurrent gas diffusion measurements were performed in a Graham’s diffusion cell [31]. Samples with a circular shape (20 mm diameter) were prepared from the parent film sheet using a die-cutting tool. All gas transport measurements were performed very carefully owing to the soft and easily deformable structure of the films, following the procedure described by Soukup, Hejtmánek, Petráš and Šolcová [32]. Two gas pairs involving Ar/He and He/H₂ (99.99% grade, Linde Gas, Czech Republic) were used for the diffusion tests. All diffusion tests in the Graham’s cell were performed under room temperature and pressure.

**Figure 1.** Graham’s diffusion cell setup. (1) -- diffusion cell, (2) -- tested sample, (3) -- 4-way valve, (4) -- digital bubble flowmeter. A -- argon or helium, B -- helium or hydrogen.
2.4 Statistical design
A randomized complete block design was used to statistically evaluate the influence of the starch source and/or the starch/chitosan weight ratio (blocks) on the qualitative parameters of the produced films. In the case of a significant difference ($p<0.01$), Tukey’s test [33] was applied to establish the difference between levels of treatment or blocks.

3. Results and discussion

3.1 Starch samples
The calculated yields of different starch isolates ranged from 4.06 to 23.47% (table 2). The botanical source of the raw material affects the starch yield; thus, banana, which is a fruit, presents the lowest yield level compared with cereals (rice and corn) and tuberous root (cassava). In the case of bananas, the yield of starch is directly related to the stage of ripening; thus, ripe bananas give significantly less starch yield than unripe bananas. This phenomenon is caused by the decomposition of starch to simple carbohydrates during the ripening process. In this study, physiologically mature bananas (still green and unripe) were used.

Table 2. Properties of starches prepared for this study.

| Sample                  | Yield (wt.% ) | Moisture (wt.% ) | Ash content (wt.% ) | Lipids (wt.% ) | Amylose (wt.% ) |
|-------------------------|---------------|------------------|---------------------|----------------|-----------------|
| Rice starch (RS)        | 23.47 ± 0.01  | 5.81 ± 0.45      | 5.96 ± 0.37         | 0.31 ± 0.01    | 19.85 ± 0.07    |
| Hard yellow corn starch (CS) | 8.83 ± 0.02  | 5.21 ± 0.05      | 5.69 ± 0.28         | 6.40 ± 0.49    | 20.40 ± 0.05    |
| Banana starch (BS)      | 4.06 ± 0.02   | 4.74 ± 0.26      | 4.95 ± 0.22         | 0.20 ± 0.11    | 28.96 ± 1.32    |
| Cassava starch (CaS)    | 17.58 ± 0.02  | 3.37 ± 0.11      | 3.44 ± 0.12         | 0.11 ± 0.01    | 23.17 ± 0.49    |

The moisture, ash and lipid contents were between 3.37 – 5.81, 3.44 – 5.96 and 0.11 – 6.40 wt.%, respectively (table 2). Because the amylose content in the starch is a key parameter that is correlated with different properties of starch [21], this parameter was thoroughly studied. The concentration of amylose in the starch samples was significantly variable. In the literature, different levels of amylose can be found that are not necessarily similar to the levels obtained in this study. In the case of vegetal biomass, factors such as variety, agronomic conditions, climatic conditions [34], soil type, geographical location and extraction method might influence the amylose content in starches. Ashwar, Shah, Gani, Shah, Gani, Wani, Wani and Masoodi [21] observed that the amylose content in rice starch was 24.55%, while López and García [35] obtained 23.9% amylose with regular corn starch. Zhang, Whistler, BeMiller and Hamaker [36] collected data from other authors related to the amylose content in various cultivars of bananas, which varied between 9.1 and 12%. However, other authors observed 19.5% for the Cavendish variety of banana and 40.7% for the Valery variety of banana [37]. López and García [35], Pelissari, Yamashita, Garcia, Martino, Zaritzky and Grossmann [38], and Zaritzky and Grossmann [18] obtained an amylose content between 15.5 and 16.8% in cassava starch.

3.2 Film characterization
The size, shape and distribution of starch granules in the films with a starch/chitosan ratio of 2/1 are depicted in figure 2. All samples revealed two phases: the first is a compact structure that consists of chitosan/glycerin and a soluble fraction of starch. The second is composed of granules of starch distributed in the first phase. The granules of rice starch revealed irregular shapes and elliptical forms.
and were distributed throughout the entire surface of the film. The granules of corn starch were round, more regular and homogeneously distributed in the film. The granules of cassava starch were oval and round with edges and were distributed rather anisotropically. López and García [35] observed that materials based on corn starch present more homogeneity and compactness than films produced from tuberous starches such as cassava starch, in agreement with this study. Additionally, the granules of banana starch are faintly recognizable with irregular round shapes and irregular distributions.

The size of starch granules decreased in the order of Ch-G-RS>Ch-G-RS>Ch-G-CaS>Ch-G-BS. Additionally, it can be seen from the SEM images that all starch granules are higher than the films except for banana starch. The thickness of the films varied significantly \((p<0.01)\) as a function of starch weight ratio (figure 3a and table 3). The higher the amount of starch in the films was, the higher the amount of solids (the higher the amount of dry matter) in the film; thus, the amount of water was lower. Therefore, the higher the ratio of starch/chitosan was, the higher the thickness of the films. Santacruz, Rivadeneira and Castro [20] observed that an increase in starch weight ratio led to an increase in film thickness due to the larger starch granule size at high swelling power. From a statistical point of view, thickness did not vary significantly \((p>0.01)\) with respect to starch source.

Water solubility and water vapor permeability depended significantly \((p<0.01)\) on the starch weight ratio in the film (table 3, figures 3b and 3c) but not on the starch source. The higher starch weight ratio increases the water solubility and WVP of the films. The higher starch weight ratio also increases the amount of free hydroxyl groups in the films that are able to form hydrogen bonds with water molecules and thus increased solubility. During the preparation of the films at the aforementioned experimental conditions, gelatinization as well as retrogradation take place. The amino groups of the chitosan were protonated to \(\text{NH}_3^+\) in the acid solution, whereas the ordered crystalline structures of the starch molecules were destroyed by gelatinization during solubilization, exposing the OH groups to readily form hydrogen bonds with \(\text{NH}_3^+\) of chitosan [22]. Related to WVP, Santacruz, Rivadeneira and Castro [20] mentioned that the explanation for the observed tendency is that the high starch weight ratio may promote retrogradation and that this phenomenon leads to a higher WVP. Statistically, there was no significant difference in WVP between the samples prepared with starch/chitosan ratios of 1/1 and 2/1. However, the sample containing a 5/1 ratio of starch/chitosan had significantly higher WVP than the other samples. According to Liu, Qin, He and Song [10], when the starch weight ratio is increased, numerous microcracks are formed due to the incompatibility between different polymers, which favors water penetration.

According to the statistical analysis (table 3), the luminosity factor does not depend on either the weight ratio or the type of starch \((p<0.01)\). However, films made of corn starch are slightly yellow, possibly due to the oxidation of the high amount of lipids that content this type of starch.

Tensile strength (TS) and elongation at break (E) varied significantly \((p<0.01)\) as a function of the starch weight ratio in the films (table 3). However, both parameters are independent of the starch source. TS increases with the increasing starch weight ratio (figure 4b) due to the formation of the intermolecular hydrogen bonds between \(\text{NH}_3^+\) cations of the chitosan backbone and \(\text{OH}^-\) anions of the starch [11]. Statistically the films containing 1/1 and 2/1 ratios of starch/chitosan were similar, while the films prepared with the ratios of 5/1 presented lower elongation at break. It appears that films containing chitosan/starch/glycerol revealed a limit starch weight ratio that changes the flexibility of the films. This finding is fully in agreement with Xu, Kim, Hanna and Nag [11], who observed that increasing the starch weight ratio (brittle agent) in the film reduces its flexibility.
Figure 2. SEM images of the produced films: Ch-G-RS2 (a-e), Ch-G-CS2 (b-f), Ch-G-BS2 (c-g), and Ch-G-CaS2 (d-h).
Table 3. Statistical analysis of the results according to the randomized complete block design and Tukey’s test.

| Starch weight ratio | Parameter                               | Thickness (mm) | Water solubility (%) | Water vapor permeability ($\times 10^{12}$) (gPa$^{-1}$ m$^{-1}$ s$^{-1}$) | Elongation (%) | Tensile strength (MPa) | Luminosity factor (%) | Net diffusion molar flux density (Ar/He) ($\mu$mol cm$^{-2}$s$^{-1}$) | Net diffusion molar flux density (He/H$_2$) ($\mu$mol cm$^{-2}$s$^{-1}$) |
|---------------------|-----------------------------------------|----------------|----------------------|------------------------------------------------------------------|----------------|------------------------|-----------------------|-----------------------------------------------------------------|---------------------------------------------------------------------|
| Ch-G-S1             | Ch-G-S2                                 | 0.070$^c$      | 46.27$^c$            | 1.09$^b$                                                          | 10.99$^a$      | 3.75$^a$               | 92.27$^a$             | 0.09$^a$                                                        | 0.07$^a$                                                            |
| Ch-G-S5             | Ch-G-S2                                 | 0.088$^b$      | 61.49$^b$            | 1.38$^b$                                                          | 11.90$^a$      | 9.23$^b$               | 90.83$^a$             | 0.09$^a$                                                        | 0.06$^a$                                                            |
| Ch-G-S5             | Ch-G-S2                                 | 0.134$^a$      | 75.52$^a$            | 2.38$^a$                                                          | 2.95$^b$       | 21.79$^a$              | 87.09$^a$             | 0.09$^a$                                                        | 0.07$^a$                                                            |

**Statistical analysis with respect to starch source**

| Parameter                      | Films with different starch source | Ch-G-RS | Ch-G-CS | Ch-G-BS | Ch-G-CaS |
|--------------------------------|-----------------------------------|---------|---------|---------|----------|
| Thickness (mm)                 |                                   | 0.106$^{**}$ | 0.092$^a$ | 0.085$^a$ | 0.106$^a$ |
| Water solubility (%)           |                                   | 57.67$^a$   | 64.13$^a$ | 62.95$^a$ | 59.61$^a$ |
| Water vapor permeability ($\times 10^{12}$) (gPa$^{-1}$ m$^{-1}$ s$^{-1}$) |                                   | 1.56$^a$    | 1.65$^a$  | 1.71$^a$  | 1.56$^a$  |
| Elongation (%)                 |                                   | 7.59$^a$    | 7.68$^a$  | 9.13$^a$  | 10.05$^a$ |
| Tensile strength (MPa)         |                                   | 8.98$^a$    | 9.32$^a$  | 12.85$^a$ | 15.22$^a$ |
| Luminosity factor (%)          |                                   | 91.24$^a$   | 89.33$^a$ | 85.80$^a$ | 93.89$^a$ |
| Net diffusion molar flux density (Ar/He) ($\mu$mol cm$^{-2}$s$^{-1}$) |                                   | 0.11$^a$    | 0.11$^a$  | 0.06$^a$  | 0.07$^a$  |
| Net diffusion molar flux density (He/H$_2$) ($\mu$mol cm$^{-2}$s$^{-1}$) |                                   | 0.06$^a$    | 0.09$^a$  | 0.05$^a$  | 0.05$^a$  |

a-c levels of Tukey’s test

*Values are the averages of the different starch sources.

**Values are the averages of the three starch/chitosan ratios.
Figure 3. Thickness (a), water solubility (b), water vapor permeability (c) and luminosity (d) of the films.

Figure 4. Mechanical properties of the films: (a) elongation at break and (b) tensile strength.
Gas diffusion measurements were conducted with inert gas pairs (Ar/He and He/H₂) based on the fact that these gases are neither able to react with the components of the films nor able to be adsorbed on the surface. Every experimental point was replicated three times, and the corresponding mean values for both the volumetric diffusion fluxes and the net diffusion molar flux densities are summarized in table 4. Statistically, neither the weight ratio nor the type of starch affected the net diffusion molar flux density of the produced films.

| Sample       | Gas pair | t (°C) | P (Torr) | d (cm) | Vd (cm³/min) | Nd (µmol cm⁻²s⁻¹) |
|--------------|----------|--------|---------|--------|--------------|-------------------|
| Ch-G-RS1     | Ar/He    | 25.0   | 739.6   | 2.0    | 0.42         | 0.09*             |
| Ch-G-RS1     | He/H₂    | 25.0   | 739.6   | 2.0    | 0.32         | 0.07              |
| Ch-G-RS2     | Ar/He    | 25.2   | 734.0   | 2.0    | 0.60         | 0.13              |
| Ch-G-RS5     | He/H₂    | 25.2   | 734.0   | 2.0    | 0.33         | 0.07              |
| Ch-G-CS1     | Ar/He    | 23.8   | 737.9   | 2.0    | 0.58         | 0.12              |
| Ch-G-CS1     | He/H₂    | 23.6   | 737.9   | 2.0    | 0.40         | 0.08              |
| Ch-G-CS2     | Ar/He    | 23.8   | 740.0   | 2.0    | 0.49         | 0.10              |
| Ch-G-CS2     | He/H₂    | 23.8   | 740.0   | 2.0    | 0.46         | 0.10              |
| Ch-G-RS5     | Ar/He    | 25.6   | 733.9   | 2.0    | 0.55         | 0.11              |
| Ch-G-RS5     | He/H₂    | 25.2   | 733.9   | 2.0    | 0.47         | 0.10              |
| Ch-G-BS1     | Ar/He    | 24.6   | 739.7   | 2.0    | 0.35         | 0.07              |
| Ch-G-BS1     | He/H₂    | 24.6   | 739.7   | 2.0    | 0.29         | 0.06              |
| Ch-G-BS2     | Ar/He    | 24.4   | 739.7   | 2.0    | 0.18         | 0.04              |
| Ch-G-BS2     | He/H₂    | 24.4   | 739.7   | 2.0    | 0.16         | 0.03              |
| Ch-G-BS5     | Ar/He    | 24.4   | 739.7   | 2.0    | 0.36         | 0.08              |
| Ch-G-BS5     | He/H₂    | 24.6   | 739.7   | 2.0    | 0.29         | 0.06              |
| Ch-G-CaS1    | Ar/He    | 24.6   | 740.0   | 2.0    | 0.31         | 0.07              |
| Ch-G-CaS1    | He/H₂    | 24.6   | 740.0   | 2.0    | 0.26         | 0.05              |
| Ch-G-CaS2    | Ar/He    | 25.2   | 739.6   | 2.0    | 0.32         | 0.07              |
| Ch-G-CaS2    | He/H₂    | 24.8   | 739.6   | 2.0    | 0.24         | 0.05              |
| Ch-G-CaS5    | Ar/He    | 24.2   | 739.3   | 2.0    | 0.31         | 0.07              |
| Ch-G-CaS5    | He/H₂    | 24.0   | 739.3   | 2.0    | 0.24         | 0.05              |

*Values are the average of three measurements.

List of symbols:
- t: temperature during measurement
- p: atmospheric pressure during measurement
- d: diameter of the sample fixed into an impermeable disc of the diffusion cell
- Vd: volumetric diffusion flux directly measured by a digital bubble flowmeter
- Nd: net diffusion molar flux density calculated from Vd

4. Conclusions
Films based on chitosan from giant squid (Dosidicus gigas) residual endoskeleton mixed with glycerin and four sources of low-value raw materials were successfully obtained.

In terms of the effect of starch weight ratio on chitosan/glycerin/starch film properties, it was observed that a higher starch weight ratio led to the production of starch/chitosan/glycerol films with lower water solubility and elongation and higher thickness, water vapor permeability and tensile strength.

The type of starch did not affect any of the evaluated properties of the chitosan/glycerin/starch films.
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