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**Chemistry**

# Influence of reduced graphene oxide on the properties of corn starch films containing antimicrobials

## A influência da adição de óxido de grafeno reduzido nas propriedades de filmes de amido de milho contendo antimicrobiano

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## ABSTRACT

Natural polymers, such as corn starch, have been studied as replacements for petrochemical polymers; however, their mechanical, structural, and barrier properties must be improved. This study aimed to evaluate the incorporation of reduced graphene oxide (rGO) into starch films (SFs) prepared using ultrasound to maintain the antimicrobial activity of the bacteriocin nisin. Dynamic mechanical analysis (DMA) demonstrated a reduction in the modulus of elasticity up to a maximum of 0.422 MPa and a tensile strength of no more than 0.619 MPa in films containing graphene and nisin or "Gan." Compared with traditional polymers, there was an increase in the elongation at break of the two formulations, reaching 28.87% of that of commercial PVC. The barrier capacity, measured by the water vapor permeability (PVA), showed no significant difference between the films. Scanning electron microscopy (SEM) with energy-dispersive spectroscopy (EDS) showed that the surfaces of the materials were predominantly smooth, but with some particulate concentrations, especially in those containing bacteriocin. The application of the films to mozzarella cheese demonstrated the antibacterial capacity of the material, significantly inhibiting the bacterium *Listeria monocytogenes* after seven days of storage.

**Keywords**: Biopolymer; Bacteriocin nisin; Active packaging

## RESUMO

Polímeros naturais, como o amido de milho, têm sido estudados como substitutos de polímeros petroquímicos; no entanto, suas propriedades mecânicas, estruturais e de barreira precisam ser aprimoradas. Este estudo teve como objetivo avaliar a incorporação de óxido de grafeno reduzido (rGO) em filmes de amido (FS) preparados por ultrassom para manter a atividade antimicrobiana da bacteriocina

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nisina. A análise dinâmico-mecânica (DMA) demonstrou redução no módulo de elasticidade até um máximo de 0,422 MPa e resistência à tração de no máximo 0,619 MPa em filmes contendo grafeno e nisina ou "Gan". Em comparação com polímeros tradicionais, houve aumento no alongamento na ruptura das duas formulações, atingindo 28,87% daquele do PVC comercial. A capacidade de barreira, medida pela permeabilidade ao vapor d'água (PVA), não apresentou diferença significativa entre os filmes. A microscopia eletrônica de varredura (MEV) com espectroscopia de energia dispersiva (EDS) mostrou que as superfícies dos materiais eram predominantemente lisas, mas com algumas concentrações de partículas, especialmente naqueles que continham bacteriocina. A aplicação dos filmes em queijo mussarela demonstrou a capacidade antibacteriana do material, inibindo significativamente a bactéria *Listeria monocytogenes* após sete dias de armazenamento.

**Palavras-chave**: Biopolímero; Bacteriocina nisina; Embalagem ativa

## 1 INTRODUCTION

Indiscriminate resource extraction significantly impacts the environment through unregulated polymeric waste production and improper disposal (Silva et al., 2022).

Biodegradable polymer production from renewable resources, such as sugarcane, cellulose, chitin, and cornstarch, is a viable mean of mitigating natural resource depletion and degradation. (Barcellos et al., 2022; B.M. Carvalho, 2023). These materials decompose via microbial activity and can be derived from animals, microorganisms, and plants, as well as from renewable and sustainable sources. (Justino et al., 2022).

Starch, a high-molecular-weight glucose polymer linked by glycosidic bonds, has great potential as a biopolymer because of its high degradability, low cost, ease of processing, and industrial availability. (Oliveira et al., 2022; Ayyubi et al., 2022; Friedrichsen et al., 2022). Cornstarch forms a film through gelatinization, which occurs in water at elevated temperatures, and retrogradation, where water is expelled and viscosity increases, creating a film. This film can be processed by casting, extrusion, or molding, which affects its thickness, mechanical behavior, and crystallinity (V. de M. Queiroz, 2022). However, starch films require improved oxygen and water barrier properties, and carbon-based nanomaterials such as graphene can provide a solution (Tsou et al., 2022).

Graphene, a two-dimensional carbon structure with a hexagonal "honeycomb-like" network, exhibits high mechanical strength of up to 1100 MPa, making it useful as a polymer reinforcement. Owing to its low production yield, graphene is used in derivatives such as graphene oxide (GO), reduced graphene oxide (rGO), and functionalized reduced graphene oxide (fr GO). These derivatives possess properties that are similar to those of graphene. (Iqbal et al., 2020, Mota et al., 2021). Graphene production methods include the mechanical or chemical exfoliation of graphite, chemical vapor deposition, or chemical synthesis from benzene (Salvatierra et al., 2015; B. M. Carvalho, 2023).

Chemical exfoliation is the predominant method for producing graphene oxide by reacting graphite with strong oxidizers, such as sulfuric acid and potassium permanganate. (Komorizono, 2021). The reduction of GO to rGO involves the removal of oxidized groups and can be achieved chemically using reducing agents to produce rGO or thermally by applying heat to generate rGO thermally. (B. M. Carvalho, 2023). The binding of rGO to polysaccharides, such as starch, enhances its mechanical properties and offers an environmental advantage over petrochemical-derived polymeric matrices. Owing to their antioxidant and antimicrobial properties, as well as their increased resistance to conventional substances, natural materials have prominent applications in food preservation (Maraschin et al., 2019). Unlike chemical preservatives commonly used for food preservation, which pose health and environmental risks, natural antimicrobial agents, including bacteriocins such as nisin, inhibit pathogenic bacteria such as *Listeria monocytogenes* by disrupting the plasma membrane and are safe for human consumption. (Gomes, 2023; Quichaba, 2021). This study evaluated the structural, mechanical, and water vapor barrier properties of starch nanocomposite films containing the antimicrobial agent nisin, reinforced with rGO, and their potential use as active packaging for mozzarella cheese.

Biocomposites with graphene derivatives have demonstrated antibacterial properties in vitro against a broad spectrum of pathogenic microorganisms. Pathogenic microorganisms and modification of the surface of graphene derivatives with essential oils or other metallic compounds such as Ag, ZnO, or $TiO_2$ have already been adopted as a strategy to increase the antimicrobial potential inherent in these nanostructures (Barra et al., 2020). Studies with reduced rGO in films with furcellaran and Ag (Jamróz et al., 2020), as well as with iodine on a starch base (Narayanan et al., 2021) have been described as having an antibacterial effect in vitro. In this innovative study, nisin was added together with rGO for the same purpose, but the antimicrobial activity was determined directly in food.

Although reduced graphene oxide (rGO) and other 2D graphene-based nanomaterials exhibit exceptional properties, such as high surface area, chemical stability, and antimicrobial potential, concerns about their toxicity remain critical, particularly for applications involving direct or indirect contact with food. Recent studies have shown that the cytotoxic effects of rGO-based materials depend on several factors including surface functionalization, concentration, and environmental conditions. For instance, Cebadero-Domínguez et al. (2023) demonstrated that digested rGO exhibited no significant cytotoxicity in intestinal and hepatic cell models at concentrations up to 200 μg/mL, suggesting a mitigating effect of gastrointestinal digestion. In contrast, Oxidative stress and reduced cell viability with functionalized rGO derivatives were observed at higher concentrations, reinforcing the role of surface chemistry in modulating toxic responses. Similarly, Guo et al. (2021) noted that less oxidized graphene structures, such as rGO, tend to show lower toxicity than GO due to their reduced reactivity. Complementing this, da Rosa et al. (2021) highlighted that 2D graphene materials can induce oxidative stress, membrane disruption, and developmental toxicity in model organisms such as *Danio rerio* (zebrafish), *Caenorhabditis elegans*, and *Drosophila melanogaster*. These organisms have been proven valuable for evaluating toxicological endpoints, including locomotor behavior, reproduction, and oxidative biomarkers. Therefore, incorporating rGO into biocompatible systems, such as in combination with natural antimicrobials like nisin, is a promising strategy to balance functionality with safety in food-related applications.

The application of packaging in real food systems differs from many studies that have only evaluated antimicrobial activity in in vitro assays. Cheese is prone to initiate degradation through its surface, resulting in active films that can protect foods, such as mozzarella cheese, against post-processing contamination by *L. monocytogenes* bacteria through contact with primary packaging impregnated with nisin between slices. When evaluating the Gan 5 film in particular, the incorporation of graphene oxide at 5 h of sonication emphasized the best interaction with the other components (also demonstrated in the other analyses carried out), also allowed for better diffusion of nisin and a significant antimicrobial effect inhibition of the *L. monocytogenes* count in food.

Thus, this study explored an innovative approach for developing sustainable and functional food packaging. This is accomplished by the utilization of starch films reinforced with reduced graphene oxide (rGO) and the incorporation of the antimicrobial agent nisin. This study addresses the need for eco-friendly alternatives to petrochemical-based polymers while simultaneously addressing food safety concerns through the integration of antimicrobial properties. This study demonstrates the potential of these biocomposite films to inhibit *L. monocytogenes* in packaged mozzarella cheese and highlights their practical application in the food industry.

## 2 MATERIALS AND METHODS

The reagents used were commercial corn starch (Maizena®) purchased from local stores, glycerol (Sigma-Aldrich®), nisin (Nisin supplied by Tovani Benzaquen®), and graphite (<20 µm; Sigma-Aldrich®). Commercial polymers, polypropylene (PP, Betanin®), high-density polyethylene (HDPE, Valgroup®), and polyvinyl chloride (PVC, RoyalPack®), were comparatively analyzed in the mechanical testing of the films produced in this study.

## 2.1 Preparation of rGO

The rGO was produced using the graphite chemical oxidation method described by Matos (2015). First, graphite oxide (Gr-O) was obtained by the oxidation of graphite, in which 60 mL of $H_2SO_4$ was added to a round-bottom flask containing 1.0 g of graphite (Sigma-Aldrich). This mixture was stirred for 15 min, after which $KMnO_4$ (3.5 g) was added, and stirring continued for another 2 h. Next, 200 mL distilled water was added. Subsequently, approximately 3 mL of $H_2O_2$ (30%) was added, and the resulting solid (Gr-O) was filtered and washed sequentially with distilled water, HCl, acetone, ethanol, and finally with additional distilled water. In the second step, GO was obtained by exfoliating Gr-O in an ultrasonic bath at 40 kHz for 30 min. For two hours, GO was reduced to rGO via a reflux process with sodium borohydride.

## 2.2 Preparation of Nisin Solution

Nisin solution was prepared in two stages according to the method described by Meira et al. (2015) First, it was dissolved at a concentration of 2.5 mg/mL in a solution of sodium phosphate monobasic monohydrate (1.38 g/mol) and centrifuged (5000 rpm for 10 min) using only the supernatant. For incorporation into the films, the nisin solution was diluted to a 1.0 mg/mL concentration.

## 2.3 Corn Starch Film Preparation

The films were prepared following the method developed by Meira et al. (2017) with some modifications. Stirring was performed using an ultrasound probe (Sonics Vibra-Cell, model CV18, amplitude 85%) and rGO was added to some of the formulations. Films solely containing corn starch and the plasticizer glycerol were denoted “C.” Upon incorporation of nisin as a preservative, the films were identified as “N,” and as “G” when only the rGO reinforcement was added. Formulations containing all the components were called “Gan 0.25,” “Gan 2,” and “Gan 5,” where the numbers refer to differences in rGO solution preparation times, as presented in Table 1. In formulations containing rGO, films were prepared by dispersion in distilled water using sonication, according to Tsou et al. (2022). Separately, the corn starch was added to the distilled water, and the solution was heated to gelatinize the starch using a plate heater (Logen Scientific® model Magnetic Stirrer) and ultrasound stirring, while the solution temperature was monitored using a thermocouple (Gehaka® model CG1800) for 10 min at 75 °C (±2 °C). Plasticizer, rGO solution, and nisin were added after cooling to 50 °C and stirred for 10 min. Table 1 lists the compositions used and the duration of the rGO dispersion. A volume of 40 mL of each film solution was then placed on acrylic plates measuring 11.5 X 11.5 $cm^2$ and left in an air circulation oven (Cienlab brand) for 18 h at 36 °C. After this period, the finished films were removed from the plates and stored in a desiccator until characterization.

Table 1 – Starch biopolymer formulations and rGO solubilization times

| Sigla | Tempo * (H) | Amido de Milho(g) | Água destilada (mL) | rGO | (g) (mL) | (mL) | (mL) |
|---|---|---|---|---|---|---|---|
| C | - | 4 | 100 | | - | 1,5 | - |
| N | - | 3,9 | 90 | | - - | 1,5 | 10 |
| G | - | 3,9 | 90 | 0,08 | 10 | 1,5 | - |
| GAN 0,25 | 5 | 3,9 | 80 | 0,08 | 10 | 1,5 | 10 |
| GAN 2 | 0,25 | 3,9 | 80 | 0,08 | 10 | 1,5 | 10 |
| GAN 5 | 2 | 3,9 | 80 | 0,08 | 10 | 1,5 | 10 |

* Time during which graphene was stirred before incorporation into the film solution.
Source: Organized by the authors

## 2.4 Determining thickness

Thickness analysis was carried out in laboratory 1104 at the Federal University of Pampa (UNIPAMPA) Bagé Campus using a Fowler® model Pro-Max digital caliper to measure the perpendicular distance between the surfaces at three different points on the sample.

## 2.5 Mechanical Properties

Tensile tests were conducted on a TA Instruments® Q800 Dynamic Mechanical Analyzer using a tensile support for films and fibers at 25 °C, with a force application rate of 3 N/min and a maximum force of 18 N. The samples were 30 mm in height and 7 mm in width and thickness, as shown in Figure 1. Six tests were performed for each material according to the ASTM D5026-15 standard.

## 2.6 Permeation Water Vapor (PWV) Analysis

The analysis was performed according to the ASTM E96/E96M standard in a desiccator containing 43% sulfuric acid solution and anhydrous calcium chloride as an absorbent material.

## 2.7 SEM and EDS Characterization

Observational analysis of the samples was conducted using a JSM-6610LV scanning electron microscope with exposure to a 10 kV accelerating beam. The samples observed by SEM were previously tested by DMA, coated with gold via sputtering, and packed in an aluminum sample holder. In addition to the concomitant EDS analysis, two sample locations were observed: the top and film fractures.

## 2.8 Antimicrobial properties

The antimicrobial effects of the films were evaluated using commercial mozzarella cheese following a procedure similar to that proposed by Meira et al. (2016) Approximately 10 g of cheese slices were inoculated with a culture of *L. monocytogenes* derived from ATCC® 7644 to obtain an initial bacterial count of approximately 6 log CFU/g. The films were then placed in contact with the surfaces of inoculated cheese samples and stored at 4 ± 2 °C. Uninoculated samples were used as controls. Viable *L. monocytogenes* cells were counted after 0, 1, and 7 d. For analysis, the samples were placed in sterile plastic bags into which 90 mL of sterile 1 g/L peptone water solution was poured and homogenized. Aliquots of the dilutions were inoculated onto Modified Listeria Oxford Agar (HiMedia®, India). All experimental treatments were tested in duplicate, with three independent preparations, and the averages were calculated at each time point. The results are expressed as log CFU/g of cheese.

### 2.9 Statistical analysis

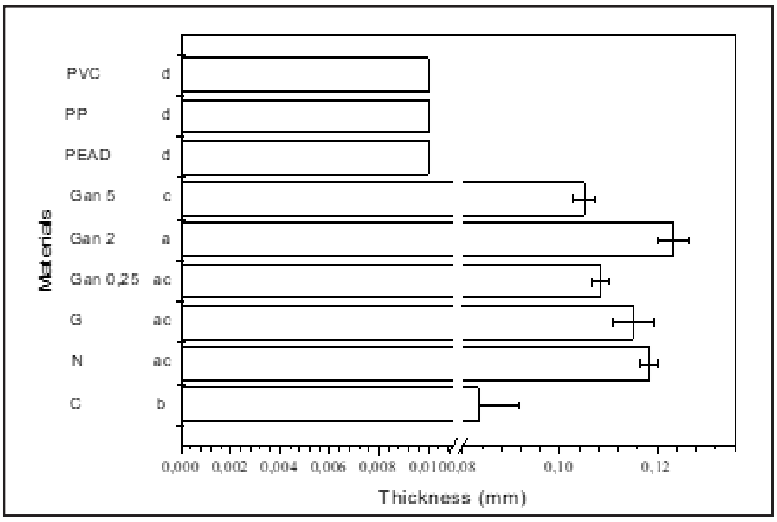
The results were subjected to analysis of variance (ANOVA), and the means were compared using Tukey's range test at the 5% level using the Sisvar program. OriginLab software was used to create the graphs shown in Figures 1, 2, 3, and 4.

## 3 RESULTS AND DISCUSSIONS

### 3.1 Determining thickness

The films studied in this work had thickness measurements between 0.08 mm and 0.12 mm, reaching an overall average of 0.10 mm. Figure 1 shows the variation in film thickness represented by the statistical error, while the commercial materials analyzed had an exact thickness of 0.01 mm in all measurements. In comparison to this study, Queiroz et al. (2022) reported averages between 0.03 mm and 0.08 mm for cassava starch films with added jamon extract and produced films with even lower thicknesses, between 0.01 mm and 0.15 mm, using potato peel with bacterial cellulose and curcumin.

Figure 1 – Graph of the average thickness of the films produced compared to traditional commercial polymers



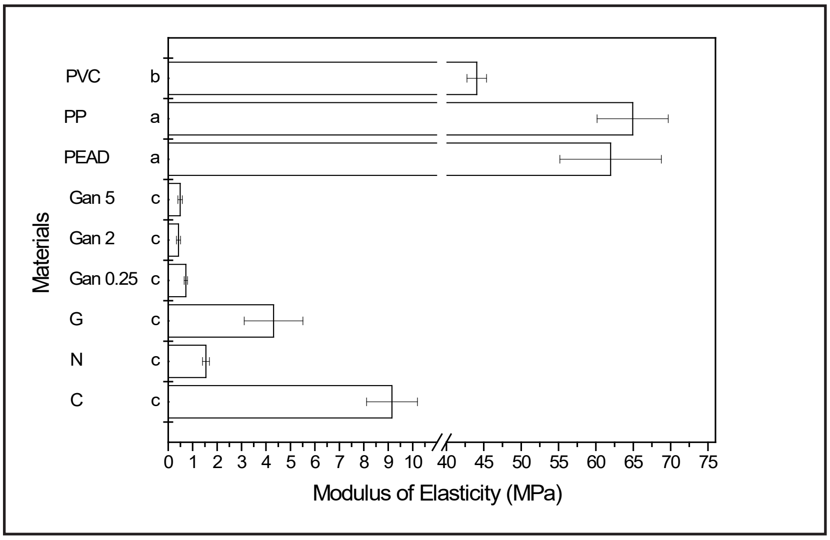

Obs: Identical lowercase letters adjacent to material identifications indicate that the means of these materials do not differ significantly. The letter 'd' signifies that the error presented is not statistically significant.
Source: Authors

### 3.2 Mechanical Properties

#### 3.2.1 Modulus of elasticity

The rigidity of the material, or its ability to deform under a load, was evaluated. (Nolasco, 2023). In this study, as shown in Figure 2, the highest average values were obtained in the tests of films "C" (9.15 ± 1.034 MPa) and "G" (4.30 ± 1.20 MPa). Other films produced had average values below 0.42 MPa (Figure 2) but did not differ significantly from the "C" and "G" films. The modulus of elasticity of film "C" reached 20.78% of that of PVC. Among the reinforced films, the corresponding value is 9.72%. Finally, in films containing the antimicrobial agent, the best result was achieved with film "N," at 3.50% of the modulus of elasticity of commercial PVC. However, the modulus of elasticity of the films produced differed significantly from the values obtained for the commercial materials, which were 61.97 MPa, 64.92 MPa, and 44.06 MPa for PVC, PP, and HDPE polymers, respectively, as shown in Figure 2. These values are higher than those reported by Menegotto et al. (2019), ranging from 0.81–0.07 MPa in chitosan-based films with added lactic acid, but not higher than the 33.61 MPa reported by Othman et al. (2019) for tapioca starch films reinforced with microcrystalline cellulose.

Figure 2 – Graph of the modulus of elasticity of the films produced compared with commercial materials

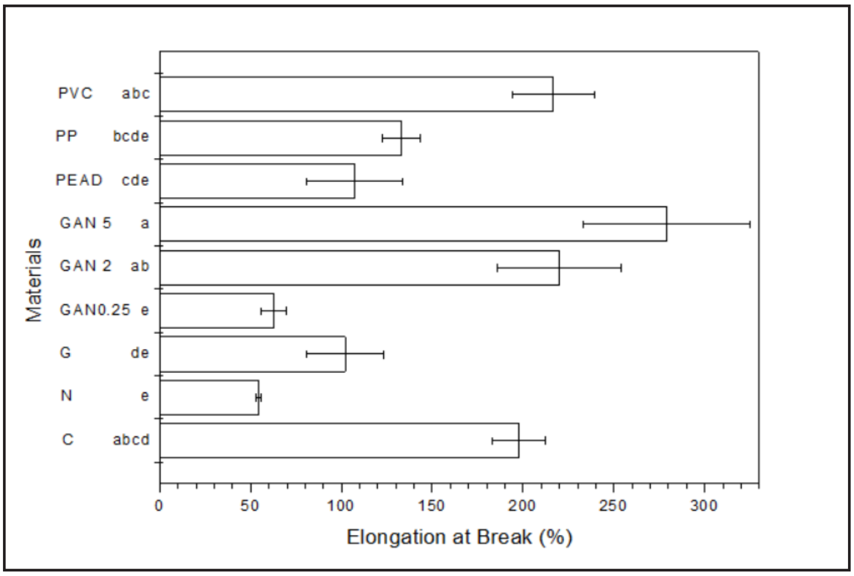



*Equal lowercase letters next to the identification of the materials mean that the averages of these materials are not significantly different.
Source: Organized by the authors

### 3.2.2 Elongation at Break

This property is related to molecular stretching. In this study, the averages ranged from 54.39 ± 3.53% for the “N” film to 279.10 ± 111.88% for the “Gan 5” film, as shown in Figure 3. The commercial materials analyzed exhibited values of 107.18%, 133.00%, and 216.55%, respectively. With respect to this property, the best performance was observed in AF, which was composed of the antimicrobial and reinforcement, with increases of 1.55% for “Gan 2” and 28.87% for “Gan 5” compared to PVC film, a material widely used for food packaging. Moghadam et al., (2020) observed values between 81.18% and 172.96% in films based on mung bean protein enriched with pomegranate peel, whereas E. L. Queiroz et al. (2021) observed a variation between 1.44% and 76.33% in cassava starch films with added jamelon extract. Mollik et al. (2021) achieved values between 67% and 80% in a potato starch nanocomposite reinforced with rGO.

Figure 3 – Elongation at break of biopolymers and commercial materials analyzed

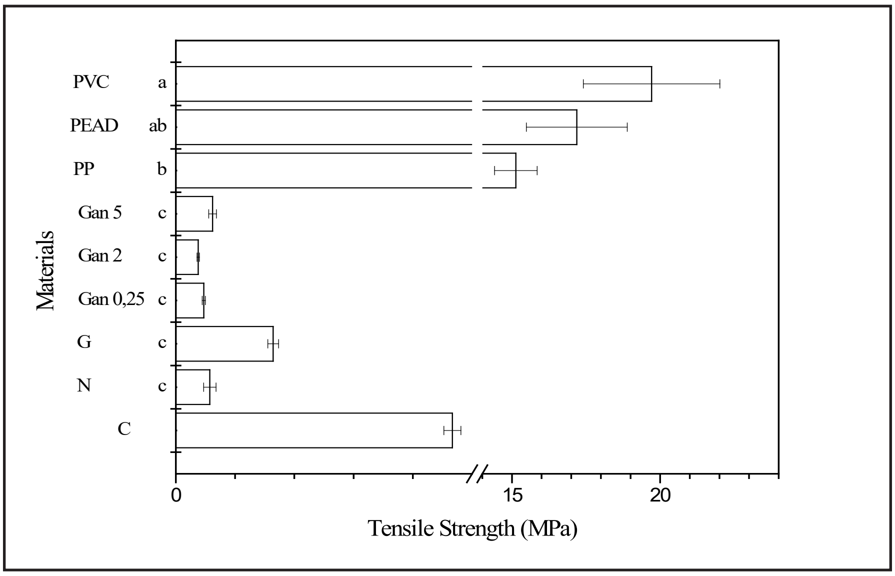



*Identical lowercase letters adjacent to the material identifications indicate that the means of these materials do not differ significantly.
Source: Authors

### 3.2.3 Tensile at break

This property expresses the amount of load that a material can withstand immediately before breaking. In this study, the values shown in Figure 4 ranged from 4.66 ± 0.14 MPa for "C", 0.37 ± 0.02 MPa for "Gan 2", and 0.62 ± 0.06 MPa for "Gan 5" in the reinforced films. Film "G" obtained the highest average of 1.64 ± 0.09 MPa, while the commercial materials showed values between 19.71 ± 2.30 MPa and 15.13 ± 0.72 MPa. González et al. (2023) obtained a value of 2.2 MPa in plasticized starch film incorporated with rGO and added with sage extract. Yusoff et al. (2021) obtained values ranging from 7.0 to 9.6 MPa when polylactic acid was incorporated into tapioca starch. Ayyubi et al. (2022) reported higher values ranging from 9.63 MPa to 31.93 MPa in biodegradable plastics based on chitosan/manioc starch/PVA and crude glycerol.

Figure 4 – Tensile strength of films made from commercial materials



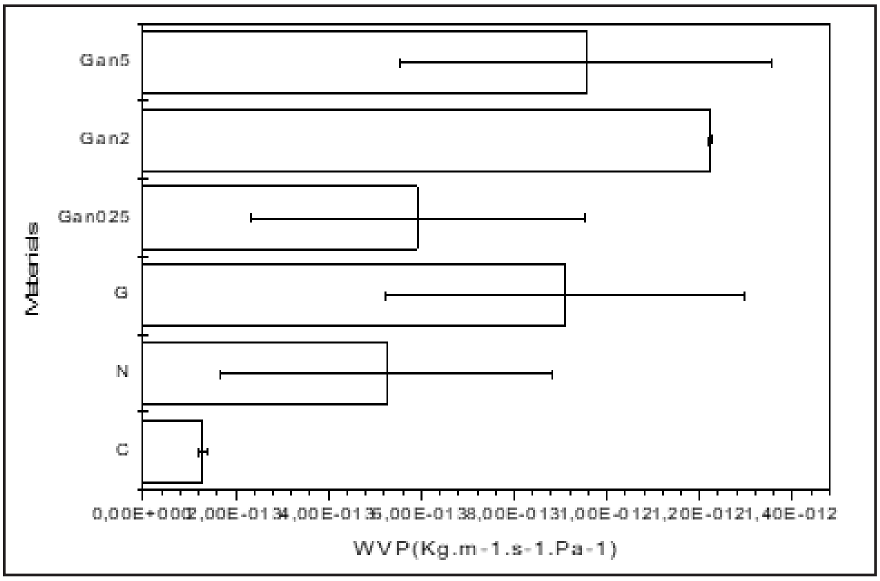

*Identical lowercase letters adjacent to the material identifications indicate that the means of these materials do not differ significantly.
Source: Authors

### 3.3 Water vapor permeability

Water vapor permeability (WVP) analysis determines the potential of a package to inhibit the passage of water in gaseous form (Shaikh et al., 2019). In this case, the analysis revealed no statistically significant differences between the results, as shown in Figure 5. However, the films containing rGO exhibited a higher permeability to water vapor.

Gómez-Aldapa et al. (2020) reported PVA values of 2.25 and 6.25 x $10^{-10}$ in their investigation of the effects of PVA on biodegradable potato starch films, using Sorbitol as a plasticizer.

Figure 5 – Water vapor permeability of starch films




*Analyses were performed in triplicates. Values are presented as mean ± standard deviation. The mean values were not significantly different (P <0.05).
Source: Authors

The WVP values were 5.2 ± 0.6 × $10^{-12}$ g·$m^{-1}$·$s^{-1}$·$Pa^{-1}$ in corn starch/PVA/chitosan films (Caicedo et al., 2022). Similarly, Fonseca-García et al. (2021), observed a reduction in the WVP rate on the order of $10^{-14}$ as the concentration of plutonic F127 in films based on corn starch, chitosan, and poloxamers increased.

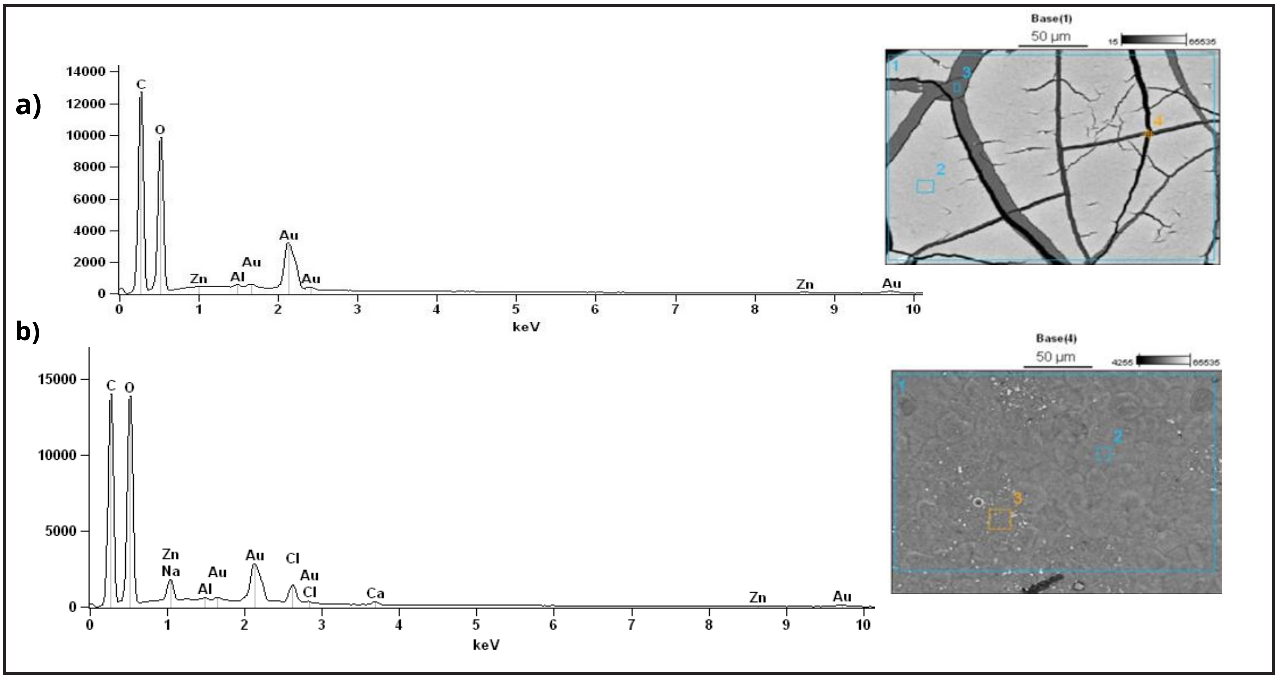
### 3.4 SEM and EDS Characterization

SEM revealed the structure of small cracks and agglomerates that could potentially decrease the fracture strain and failure strength, as discussed in Section 3.2. These microcracks may compromise the integrity of the material, increase its brittleness, and weaken the bonding between the doped reduced graphene oxide (rGO) and surrounding matrix. Furthermore, larger cracks serve as pathways for oxygen infiltration, intensify the damage, and possibly result in material breakdown (Awaja et al., 2016).

In the micrographs of the surface of the SF in Film "C" (Figure 6 (a)), microcracks appeared along with cubic structures, which may be sodium (Na) and potassium (K) salts detected by EDS, as shown in Figure 5. These salts, along with chlorine (Cl) and calcium (Ca), may originate from the commercial materials used in the formulation. In the “N” film (Figure 6 (b)), a greater amount of particulate material was observed, with EDS detecting salts of Chlorine (Cl) and Calcium (Ca), which were present only in SF containing nisin.

Agglomerates suggest that nisin may not be homogeneously distributed, and intergranular segregation may also be observed on the surface of the material. As shown in Figure 5 (b), clusters indicate an uneven distribution of nisin, along with the formation of potential intergranular segregation across the surface.

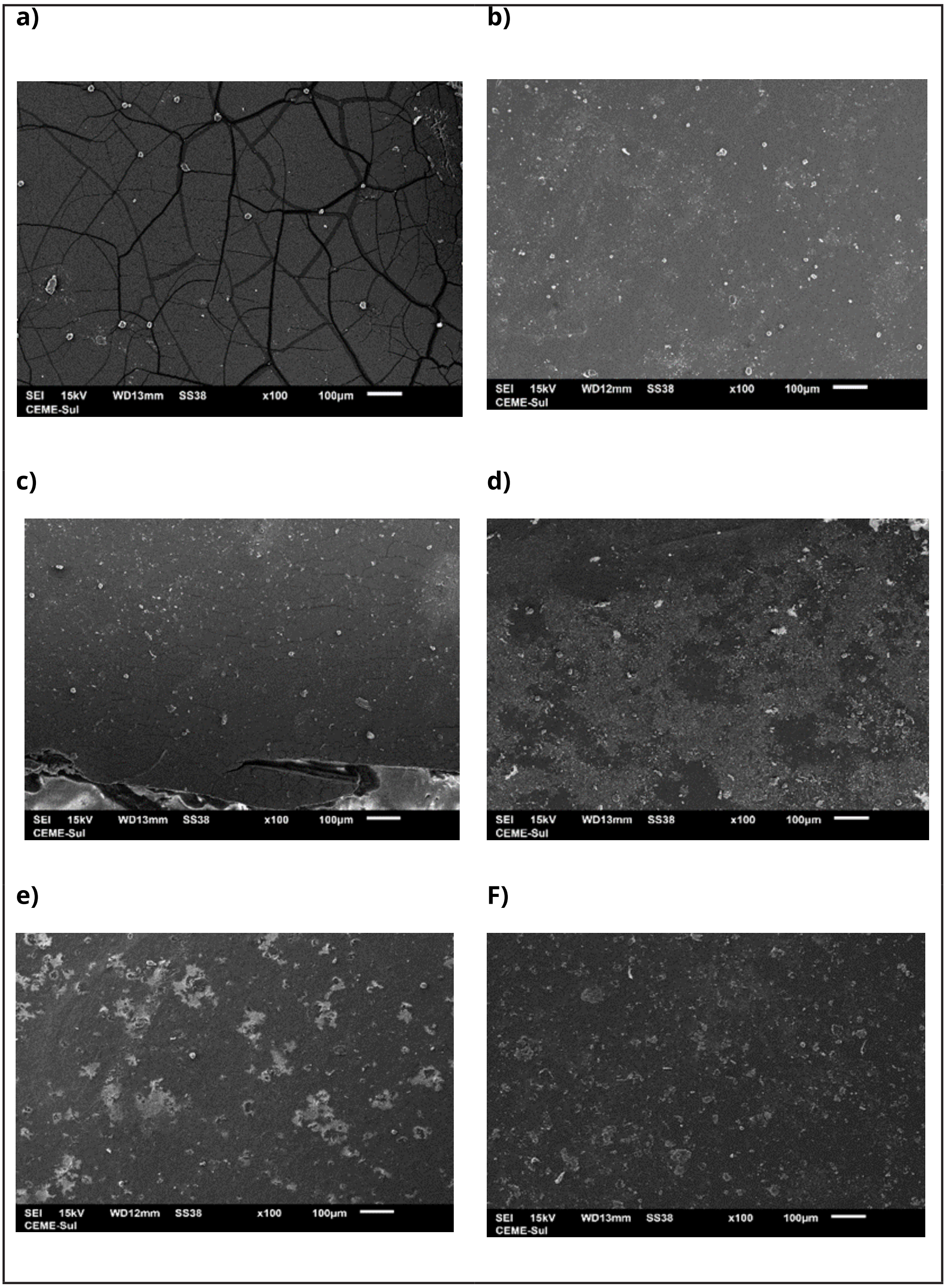
Figure 6 – EDS spectra. (a) Film “C,” (b) Film “N.”




Source: Authors’ private collection (February 2024)

The “G” film shows particulate material distributed over its surface, similar to the films containing nisin, but without the intergranular segregations.

Figure 7 – SEM top micrographs of SF: (a) film “C,” (b) film “N,” (c) film “G,” (d) film “Gan 0.25”, (e) film “Gan 2,” (f) film “Gan 5”



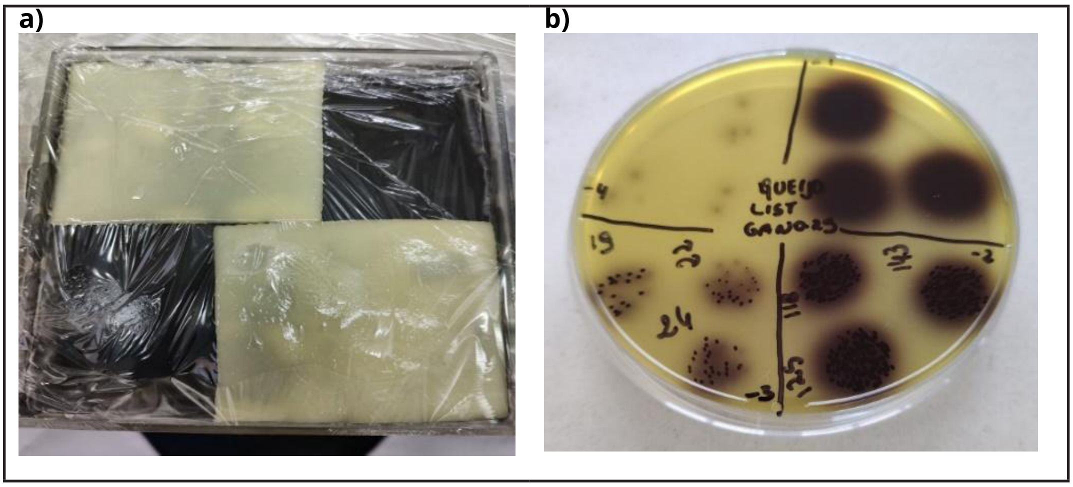

Source: Authors’ private collection (February 2024)

### 3.5 Antimicrobial properties

The antimicrobial activity of films containing nisin was verified by contact with portions of commercial mozzarella cheese previously contaminated with *Listeria monocytogenes* and packaged with SFs, as shown in Figure 8 (a). *L. monocytogenes* is a widely distributed psychrotrophic pathogen that can survive and proliferate in cheese for long periods in the environment, food, and processing plants (SalmierI et al., 2014).

Figure 8 (b) shows the bacterial colony counts on Oxford agar. The initial count was 6.20 ± 0.08 log CFU/g (data not shown).

Figure 8 – (a) Previously contaminated cheese in contact with SF. (b) Oxford nutrient plates with colonies of *L. monocytogenes*




Source: Authors' private collection (February 2024)

The control "C" films did not inhibit *L. monocytogenes*, demonstrating an almost constant level of cell viability over the days (almost 6 log CFU/g, Table 6). The "Gan 0.25" film showed behavior similar to that of the control, with no significant change in the count between days one and seven. In contrast, the other films, including "N," "G," and "Gan 5," significantly reduced the number of viable bacteria after seven days, reaching approximately one logarithmic cycle of the initial count (6.20 logs UFC/g), with the lowest counts observed for the "N" films (Table 6).

Similarly, sodium caseinate films containing nisin (1000 IU/cm$^2$) reduced *Listeria innocua* by 1.1 log CFU/g after one week of storage at 4 °C when applied to semi-soft cheeses (Cao-Hoang et al., 2010).

Table 2 – Colony counts of *L. monocytogenes* (log CFU/g) in mozzarella cheese packaged with the prepared films

| **Films** | **N** | **Day 1** | **Day 7** |
|---|---|---|---|
| C | 3 | $5,95^{Aa} \pm 0,12$ | $5,98^{Aa} \pm 0,4$ |
| N | 3 | $5,62^{Ba} \pm 0,02$ | $5,16^{Cb} \pm 0,15$ |
| G | 3 | $6,02^{Aa} \pm 0,11$ | $5,52^{Bb} \pm 0,03$ |
| Gan 0,25 | 3 | $5,80^{ABa} \pm 0,15$ | $5,63^{Ba} \pm 0,02$ |
| Gan 5 | 3 | $5,54^{Ba} \pm 0,01$ | $5,19^{Cb} \pm 0,01$ |

*Different capital letters in the columns indicate statistical difference by Tukey's test ($p<0.05$); a, b: Different lowercase letters in the rows indicate statistical difference, while equal letters do not differ significantly from each other.
Source: Authors' organization

When comparing treatments on day one, only the "N" and "Gan 5" films differed from the control. However, after seven days of storage, all films significantly reduced the bacterial count compared with Film "C," demonstrating the potential of these films for use in active food packaging (Table 2). The antimicrobial and antifungal properties of graphene-based nanostructures are attributed to their ability to induce cell membrane disruption and oxidative stress, compromising bacterial proliferation and sporulation. Biocomposites with graphene derivatives have shown antibacterial properties in vitro against a broad spectrum of pathogenic microorganisms. Modifying the surface of graphene derivatives with essential oils or other metallic compounds such as Ag, ZnO, or $TiO_2$ has already been adopted as a strategy to increase the antimicrobial potential inherent in these nanostructures (Barra et al., 2020). The application of packaging in real food systems differs from many studies that have evaluated antimicrobial activity only under in vitro conditions. Films with graphene oxide and furcellaran did not exhibit an antimicrobial effect against pathogenic bacteria in vitro, necessitating the incorporation of Ag nanoparticles (NPs) into the polymer matrix to reveal the activity (Jamróz et al., 2020; Narayanan et al., 2021). rGO was also used to prepare iodine-soluble starch nanocomposites that exhibited antibacterial activity against *E. coli and S. aureus.* In this study, nisin was added together with rGO for the same purpose, and its antimicrobial activity was determined in food. Cheeses are prone to surface spoilage, and this study produced active films that can protect foods such as mozzarella cheese from post-process contamination with *L. monocytogenes* through contact as primary packaging impregnated with nisin between slices. When evaluating the “Gan 5” film in particular, the incorporation of rGO with five hours of sonication enhanced the interaction with the other components (as demonstrated in previous analyses), also allowing for better diffusion of nisin and a significant antimicrobial effect in inhibiting the *Listeria monocytogenes* count in food.

## 4 CONCLUSIONS

Cornstarch films containing rGO and nisin produced by casting had an average thickness of 0.108 mm, low modulus of elasticity, and low tensile strength. However, there was a significant improvement in the elongation at break, reaching 279.1% in the “Gan 5” film, surpassing that of the commercial PVC. The formulations did not alter the water vapor barrier capacity of the materials. Structural evaluation revealed materials with predominantly smooth surfaces and some concentrations of particulates, demonstrating the importance of longer ultrasound time when preparing rGO. This facilitated a better interaction between nisin and rGO, highlighting the significance of the antimicrobial activity of the bacteriocin against *Listeria monocytogenes* inoculated into packaged cheeses. Therefore, cornstarch films containing nisin and rGO, particularly “Gan 5,” represent a new strategy for active primary packaging in direct contact with food. The results demonstrate the potential of these bio-based antimicrobial films as an environmentally friendly alternative to traditional food packaging materials, with promising applications in the food industry.

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