Modification of chicha gum: antibacterial activity, *ex vivo* mucoadhesion, antioxidant activity and cellular viability

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Abstract

The aim of the present work was to modify the exuded gum of *Sterculia striata* tree by an amination reaction. The viscosity and zero potential of the chicha gum varied as a function of pH. The modification was confirmed by X-ray diffraction (XRD), infrared spectroscopy (FTIR), size exclusion chromatography (SEC), zeta potential, thermogravimetric analysis (TG), and differential scanning calorimetry (DSC). Furthermore, the chemical modification changed the molar mass and surface charge of the chicha gum. In addition, the gums were used in tests for *ex vivo* mucoadhesion strength, antibacterial activity against the standard strain of *Staphylococcus aureus* (ATCC 25923), inhibitory activity of α-glucosidase, antioxidant capacity, and viability of Caco-2 cells. Through these tests, it was found that amination caused an increase in the mucoadhesive and inhibitory activity of chicha gum against the bacterium *Staphylococcus aureus*. In addition, the gums (pure and modified) showed antioxidant capacity and an inhibitory effect against the α-glucosidase enzyme and did not show cytotoxic potential.

Keywords: Chicha gum; Mucoadhesion; Antimicrobial activity; Antioxidant activity; Cell viability.

Highlights

- The exuded gum from the *Sterculia striata* tree was chemically modified by an amination reaction.
- Chemical modification of the gum was confirmed by XRD, FTIR, SEC, zeta potential and thermal analysis (TG/DTG/DSC).
- Chicha gum aminated showed an increase in mucoadhesive and inhibitory activity against *Staphylococcus aureus* bacteria.
- The chicha gum and modified gum showed no cytotoxic potential.

1. Introduction

Natural gums are polysaccharides exhaled from trees that represent an abundant raw material for the most diverse industrial applications, including the food industry and pharmaceutical and cosmetic fields. This wide industrial application of natural gums is because they are chemically inert, biodegradable, non-toxic, cost saving as raw materials and easily available in nature [1,2].

In addition, natural gums have varied compositions and rheological properties that allow their easy use in various biomedical applications, including tissue engineering, biosensors, drug delivery systems, antibacterial activity and cytotoxic properties. In these applications, natural gums are inexpensive and have few side effects [3,4]. In addition, natural gums are preferred over synthetic materials due to their non-toxicity, low cost, abundance, and they are derived from renewable sources [5].

Among the most varied gums found in nature, chicha gum has attracted much attention from researchers for its application in several purposes. Chicha gum is exuded by *Sterculia striata*, an abundant plant in northeastern Brazil. This gum belongs to the family *Sterculiaceae* and has a high molecular weight ($4.2 \times 10^6 - 3.6 \times 10^7$ g mol^{-1}). Moreover, chicha gum is a heteropolymer formed of partially acetylated chains and composed of uronic acid (42.2–49.2%), rhamnose (23.8–28.8%), galactose (19.3–23.4%), xylose (5.6–7.7%), and acetyl groups (9.6–10.7%) [1,3,4,6]. This polysaccharide has polyanionic character due to the presence of the carboxylic groups present in the glucuronic acids [3,4]. This makes chicha gum sensitive to the pH variation of the medium [7].

Further to the carboxylic groups, chicha gum has several hydroxyl groups in its polymer chain that provide the possibility of inserting new functional groups in its structure through various chemical modifications [1,6]. The modified polymers have improved properties relative to crude material in terms of solubility, mucoadhesion, swelling, coagulation and flocculation, i.e., the modification allows obtaining a customized product according to the appropriate characteristics for the desired application [8]. In this respect, the chemical modification of gums has increased their reactivity potential seeking the development of new delivery systems, among which nanoparticles, thus improving their self-assembly capacity [9] and performance in pharmaceutical applications such as swelling, mucoadhesion and drug release [10,11].

Among the various reactions of chemical modification of the gum surface, the incorporation of alkaline sites from nitrogen compounds (amination) can be highlighted. Many natural polymers have been modified with the incorporation of nitrogen groups and have shown improvements in their properties. For example, fenugreek gum modified with amino groups obtained a greater adhesion force than films prepared with gum without modification [11]. The

xyloglucan of tamarind with amino groups obtained better thermal properties in relation to the polymer without modification. In addition, amino gum showed better antimicrobial activity than chitosan [12].

In this context, studies on the properties of pure and modified chicha gum with amino groups are necessary, considering that there are still few reports in the literature about the characteristics of pure chicha gum and no reports on the properties of this modified gum with amino groups.

Thus, the present study aims to chemically modify chicha gum with amino groups through the reaction of this gum with ethylenediamine. The gums (pure and modified) were characterized by elementary analysis, X-ray diffraction (XRD), infrared spectroscopy (FTIR), size exclusion chromatography (SEC), zeta potential, thermogravimetric analysis (TG), first derivative (DTG) and differential scanning calorimetry (DSC). In addition, the gums were assessed for *ex vivo* mucoadhesion strength, antibacterial activity against *Staphylococcus aureus*, inhibitory activity of α-glucosidase, antioxidant capacity, and human cell viability.

2. Materials and methods

2.1 Materials

Sterculia striata gum (Chicha gum) was obtained from exudates collected manually from native *Sterculia striata* trees located in the city of Teresina, Piauí, Brazil, registered at Herbarium Graziela Barroso under the number TEPB: 30418, sodium chloride (Salado *et al*), sodium hydroxide NaOH), and ethanol (C_2H_6O) were purchased from Dinâmica Química Contemporânea Ltda. Acetone (C_3H_6O), sodium borohydride ($NaBH_4$), acetic anhydride ($C_4H_6O_3$), 1-methylimidazole ($C_4H_6N_2$), dichloromethane (CH_2Cl_2), trifluoroacetic acid ($C_2HF_3O_2$), sulfuric acid (H_2SO_4), and quercetin (95% purity) were purchased from Sigma-Aldrich. Nutrient agar (NA, Himedia, India), brain heart infusion broth (BHI, Himedia, India), Müller Hinton agar (Sigma–Aldrich), and MTT (3-[4,5-dimethylthiazol-zyl]-2,5-diphenyletrazolium) (Sigma-Aldrich), were used for the biological assays, as was analytical grade dimethyl sulfoxide (DMSO) (Sigma-Aldrich). The acid yellow 73 dye (AY73) was supplied by Danny Color Dyes and was used without prior purification. (Brazilian SISGEN Registration nº ABD61DA)

2.2 Chicha gum (CG) isolation

Chicha gum (CG) was isolated in salt form using a method described by Brito et al., 2005 [13] and Brito et al., 2004 [14]. *Sterculia striata* exudate (1.0 g) was dissolved in distilled water (100.0 mL) under stirring (2000 rpm/12 h on a mechanical stirrer - Tecnal, model TE-139) at 25°C for 12 h. NaCl (1.0 g) was added and then filtered (porcelain filter with the aid of a vacuum

pump) to solution, and its pH was adjusted to 7.0 with 0.1 mol L^{-1} NaOH solution. The polysaccharide was precipitated with 95% ethanol, washed with acetone, dried at 40°C in a hot air oven for 24 h, and macerated to obtain it in powder form (Fig. 1).

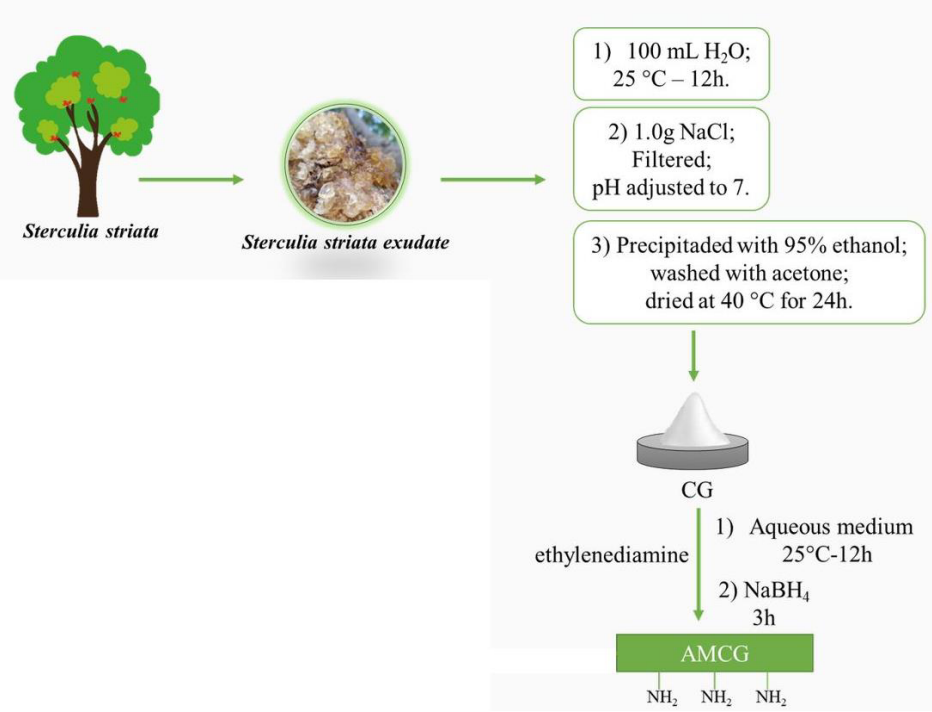


Fig. 1. Isolation scheme and chemical modification of chicha gum (CG).

2.3 Analysis of the composition of monosaccharides

The neutral sugars were determined by gas chromatography as alditol acetates. Hydrolysis was performed with 2 M trifluoroacetic acid (TFA) at 120°C for 1 h. The monosaccharides were reduced with $NaBH_4$ (15% in 3 M NH_3) for 1 h at 30°C and subsequently acetylated with acetic anhydride (3 mL) in the presence of 1-methylimidazole (450 µL) for 30 min at 30°C. Alditol acetate derivatives were separated with dichloromethane and analyzed by gas chromatography with a flame ionization detector (GC-FID) equipped with a 30 m DB-225 column (J & W Scientific, Folsom, CA, USA) with film thicknesses of 0.25 mm and 0.15 µm, respectively. The temperature program of the oven used was as follows: initial temperature 200°C, increase in temperature at a rate of 40°C/min up to 220°C, remaining for 7 min, followed by a rate of 20°C/min to 230°C and maintaining this temperature for 1 min. The injector and detector temperatures were 220 and 230°C, respectively. The flow rate of the carrier gas (H_2) was adjusted to 1.7 mL/min [15].

Uronic acids were determined colorimetrically according to the method of Nunes et al., 2012 [15]. The samples were prepared by pre-hydrolysis in 0.2 mL of 72% H_2SO_4 for 3 h at room

temperature, followed by 1 h of hydrolysis in 1 H_2SO_4 at 100°C. A calibration curve was made with D-galacturonic acid.

2.4 Preparation of CG solutions at different pH values

CG solutions were prepared at a concentration varying from 0.01 % to 5.00 % (w/v) at pH 4.5. To assess the effect of pH of diluted solutions on their sol gel properties, the pH was adjusted to values from 1 to 10 using HCl or NaOH (0.1 and 1.0 mol L^{-1}).

2.4.1 Viscosity

A Thermo Scientific HAAKE MARS III rheometer with a cone geometry (35 mm, 1°). was used to measure the viscosity to assess the effect of pH of diluted solutions on sol-gel properties of CG. Apparent viscosity curves were fitted to Cross model [16].

2.5 Synthesis of aminated chicha gum (AMCG)

The CG was modified by means of an amination reaction using the method previously described in the literature [17]. The synthesis of AMCG was carried out by the addition of ethylenediamine as an aminating agent that replaces the OH group of CG with $-NHCH_2CH_2NH_2$. Then the $-NHCH_2CH_2NH_2$ group was reduced to the $-NH_2$ group by the addition of sodium borohydride ($NaBH_4$) [17]. CG (1.0 g) was dissolved in 250 mL of distilled water under stirring at a temperature of 25°C for 12 h. Subsequently, 25 mL of ethylenediamine was added to the homogeneous solution of CG and allowed to react under continuous stirring for 12 h. Then, 50 mL of sodium borohydride (5%) was added and stirred vigorously for 3 h. After completion of the reaction, the modified polysaccharide was precipitated with ethanol, washed with acetone, dried at 40°C in a hot air oven for 24 h and macerated to obtain it in powder form. The chicha gum amination reaction scheme is shown in Fig. 6 (results section).

2.6 Physicochemical characterization

The elementary analysis of carbon, hydrogen, and nitrogen were evaluated in a PerkinElmer model PE 2400 elemental analyzer. The zeta potential measurements of the gums were performed on Zetasizer Nano ZS equipment (Malvern Instruments). The molar masses of the gums were obtained by size exclusion chromatography (SEC) in a Shimadzu LC-10AD chromatograph with an RID-6A refractive index detector, 7.8x300 mm linear Ultrahydrogel column, with 0.1 mol L-1 NaN3 as the mobile phase. The X-ray diffraction (XRD) patterns of the samples were obtained using an X-ray diffractometer (Shimadzu XR-D600 A) with CuKα as the radiation source and a wavelength of 154 p.m. The infrared spectra (FTIR) were obtained using a Varian 660-IR spectrophotometer by the tablet method in KBr 1% (m/m) of sample in 32 scans

in the region of 400 to 4000 cm-1 with a resolution of 4 cm-1. Thermogravimetric analysis was performed using a DSC-TGA thermogravimetric analyzer (SDT Q600 V20.9) in an atmosphere of inert air (synthetic air). For scanning, the heating rate was between 0°C and 800°C with an interval of 10°C/min.

2.7 *Ex vivo* mucoadhesion strength

The *ex vivo* mucoadhesion strength was assessed on a TA-XT plus texture analyzer (TA Instruments®, UK) using porcine intestinal mucosa as a mucoadhesion model because it resembles the human intestinal mucosa [18]. During the test, CG and AMCG gums were fixed with double-sided tape to a 10 mm cylindrical probe, while sections of mucosa of the porcine small intestine were fixed on a specific lower support for mucoadhesion testing and moistened with pH 7.4 buffer solution preheated to 37°C. The probe was moved perpendicularly toward the mucosa with a constant velocity of 0.1 mm/sec, and contact with the mucosa was maintained for 60 seconds with a compression force of 0.5 N. After the contact time, the probe was removed at a speed of 0.1 mm/sec. The analysis was performed in triplicate [19].

2.8 Antibacterial activity against the standard strain of *Staphylococcus aureus* (ATCC 25923)

2.8.1 Inoculum Preparation

Cultures were obtained by transferring a range of bacterial growth on nutrient agar to a falcon tube containing 3.0 mL of 3% brain heart infusion (BHI) medium, followed by incubation at 37 °C for 24 h. From this culture in BHI, a standard bacterial suspension was prepared to a density equivalent to 0.5 on the Mac Farland scale, approximately 1.5 x 10^8 CFU/mL (colony forming units - CFU) [1,6].

2.8.2 Direct Contact Test

To carry out these tests, 2000 µg of the material to be tested and 2000 µL of bacterial suspension were transferred to an Eppendorf tube, followed by homogenization on a vortex shaker. Then, 200 µL of this suspension was transferred to Petri dishes containing Mueller Hinton agar, which were sown with the aid of a Drigalsky loop using the spread plate method, followed by incubation at 37°C for 24 hours. Plates only with inoculum were used as a positive control. The tests were performed in triplicate. The inhibitory effect produced by each test solution was calculated according to Eq. 1 [1,6]:

$$\eta = \frac{N_1 - N_2}{N_1} \text{ x } 100\% \qquad \text{Eq.}$$

where η is defined as the inhibitory effect, N_1 is the arithmetic mean of the colony-forming units of the control plates and N_2 is the arithmetic mean of the colony-forming units of each of the tested solutions [20].

2.9 Inhibitory activity of α-glucosidase

The inhibitory activity of α-glucosidase was analyzed as described by Bento et al., 2018 [21]. The solutions were prepared for each sample with KH_2PO_4 buffer solution (10 mM, pH 7). Each well contained 100 µl of 2.5 mM 4-nitrophenyl α-D-glucopyranoside (PNP-G), 150 mL of KH_2PO_4 buffer solution (10 mM, pH 7), and 50 µl of CG, AMCG or acarbose (positive control). The reaction was initiated by the addition of 25 µL of α-glucosidase (0.28 U/mL). The plates were incubated at 37°C for 10 min. The absorbance of 4-nitrophenol released from PNP-G at 405 nm was measured. The increase in absorbance was compared with that of the control (buffer instead of the sample solution) to calculate the inhibitory activity. The experiments were carried out in triplicate.

2.10 Antioxidant capacity

The antioxidant capacity of chicha gum was determined according to the methodology described by Cambrussi et al., 2019 [22]. For the tests, chamber of radiation, provide with a 125 W without bulb. In suspension, 1 mg mL^{-1} catalyst composed of TiO_2 supported on palygorskite incorporated with silver nanoparticles (AgNPs/TiO_2-PAL) was mixed with 0.5 mL of an aqueous solution of acid yellow dye 73 (AY73) at a concentration of $2x10^{-5}$ mol L^{-1}, and 0.5 mL of aqueous CG solution (or AMCG solution) at different concentrations (10 to 400 µg mL^{-1}). The samples were irradiated under UV–Vis radiation for 60 minutes. After the radiation time, samples were centrifuged for 15 minutes at 10,000 rpm to remove the catalyst. Subsequently, the supernatant was transferred to a microplate, and absorbance readings were performed on a microplate reader (Elisa Polaris®) at 492 nm. The absorbance readings of these exact non-irradiated solutions were compared to calculate the discoloration of AY73 as a function of CG concentration (or AMCG concentration).

The discoloration of the solutions was determined by Eq. 2 [23]:

$$Discoloration\ (\%) = \left(\frac{A_0 - A_t}{A_0}\right) x\ 100 = \left(\frac{C_0 - C_t}{C_0}\right) x\ 100 \qquad \text{Eq.}$$

According to the Lambert-Beer law, where A_0 is the initial absorbance of the solution and A_t is the absorbance at time t, which refers to the initial concentrations (C_0) and time (C_t). Considering the values obtained, reaction curves were created to obtain the inhibitory concentration (IC50) in vitro to decrease AY73 discoloration by 50%. Quercetin, a natural compound recognized in the literature for its antioxidant potential, was used as a standard antioxidant for comparison purposes. Linearity was assessed using linear regression analysis, with data adjustment using the least squares method. Each concentration was determined in triplicate (n = 15). The concentration of the AY73 dye in the solution was analyzed by a UV–Vis spectrophotometer (Agilent Technologies spectrophotometer, Cary 60 UV) at its maximum wavelength at 490 nm [22].

2.11 Effect of CG and AMCG gum on the viability of Caco-2 cells by the MTT test

Cell culture conditions and treatments were performed according to the methodology described by Silva & Teixeira, 2015 [24]. The human colorectal adenocarcinoma cell line Caco-2 from the American Type Culture Collection (LGC Standards S.L.U., Spain) was routinely cultured using Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 1% nonessential amino acids, 1% antibiotic, 1% fungizone and 6 μg mL^{-1} transferrin. The cells were incubated at 37°C in 5% CO_2. The cells were washed with HEPES-buffered saline, trypsinized and sub-cultured in 48-well plates at a density of 25000 cells cm^{-2}. All tests were performed after confluence. CG and AMCG gums (62.5-1000 μg mL^{-1}) were dissolved in a medium containing 0.5% (v/v) DMSO. The final concentration of DMSO did not affect cell viability. To determine the effect of gums on cells, viability was assessed 24 h after exposure.

The MTT assay was evaluated by reducing MTT to formazan in viable cells. After discarding the cell culture supernatant, the cells were incubated with 1 mL of MTT solution (0.5 mg ml^{-1} in supplemented DMEM) for 30 min at 37°C. After this period, the supernatant was eliminated, and 1 mL of DMSO was added to each well for complete dissolution of the formazan. The absorbance of the different solutions was then measured using a Multiskan Ascent plate reader (Thermo, Electron Corporation) working at 570 nm. The results were expressed as the percentage of cell viability, which was considered a control group, with 100% viability, the one in which the cells were incubated only with the culture medium, without the presence of any test substance.

2.12. Statistics

Statistical analysis was performed using GraphPad Prism 7.0 software, where the unpaired t test was applied. The results were expressed as the standard deviation of the mean and a p value < 0.05 [6].

3. Results and discussion

3.1 Composition of monosaccharides

The monosaccharide composition has a considerable effect on the rheological and functional properties of the gum. Thus, the monosaccharide composition of the gum obtained from exudates collected from the *Sterculia striata* tree was determined by gas chromatography with a flame ionization detector (CG-FID). The following monosaccharides were found: uronic acid (74.9%), galactose (6.9%), rhamnose (9.1%), xylose (6.3%), and arabinose (2.8%). These results present differences in relation to those reported by previous publications [13,14], where CG was collected from trees native to the city of Fortaleza, Brazil. These observed differences are due to several factors, such as plant age, the edaphoclimatic conditions of the plant, the period of exudate collection, growing conditions, and differences in the methodological processes used in the isolation and determination of the composition of monosaccharides. The high amount of uronic acid in the CG composition is greater than that of commercial gums, indicating that CG gum is a strong polyelectrolyte that has a greater negative charge than these gums [25].

3.2 Viscosity of chicha gum

The rheological properties of diluted and concentrated solutions of CG are represented using the Cross model. Upon lower shear rate, the viscosity of CG gums ranged from 16 to 4060 mPa.s and 0.002 to 0.06 mPa.s. in the higher concentration and diluted gums, respectively. As seen in Fig. 2(a), for diluted solutions, no viscosity change was observed for a concentration of 0.01 % (w/v), whereas the decreasing effect of shear rate on the viscosity of CG at 0.1 % (w/v) is due to the disentanglement of gum polymeric chains during flow. For CG solutions with concentrations from 1.0% to 5.0 % (w/v), a Newtonian plateau was observed at low shear rates, and a pseudoplastic behavior was observed at higher shear rates.

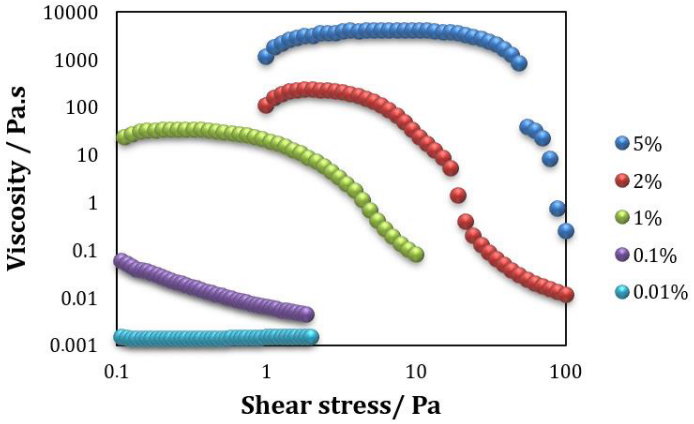
The effect of pH and ionic composition, along with the volume of nasal delivery formulations, have a critical impact on the drug delivery performance of gellan gum [26,27].

Dynamic viscosity tests were carried out at different pH values to evaluate the viscosity of CG. The results are shown in Fig. 2(b). The pH variation did not affect the viscosity of CG linearly. When the pH increased from 1 to 6, there was a significant increase in viscosity. This could be related to the increase in the negative charge of CG observed in the zeta potential analysis (Fig.3), which allowed the expansion of the polysaccharide chain due to intramolecular electrostatic repulsion, thus favoring a very significant increase in the viscosity of CG. Above pH 6, the viscosity of the CG solution decreased with increasing pH. This effect can be attributed to the electrostatic interaction between CG and the Na^+ cations added during pH adjustment, which caused contraction of the macromolecule, favoring a decrease in viscosity.

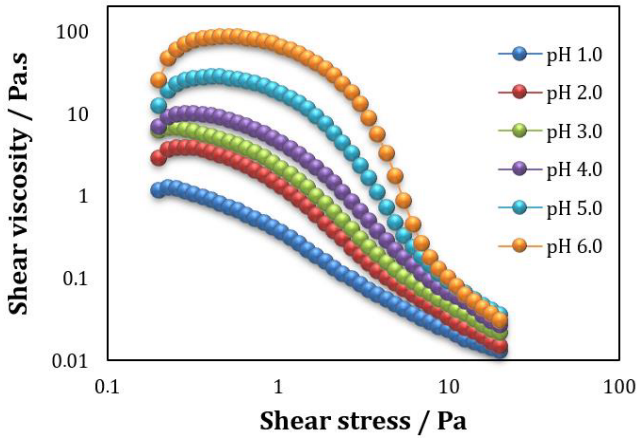
The viscosity measurement has been a useful tool for understanding the sol-gel transition properties of a gum. The effect of pH on the viscosity of diluted solutions of CG is thus relevant because gums' sol-gel transition properties have been a screening tool for biopolymers best fitting

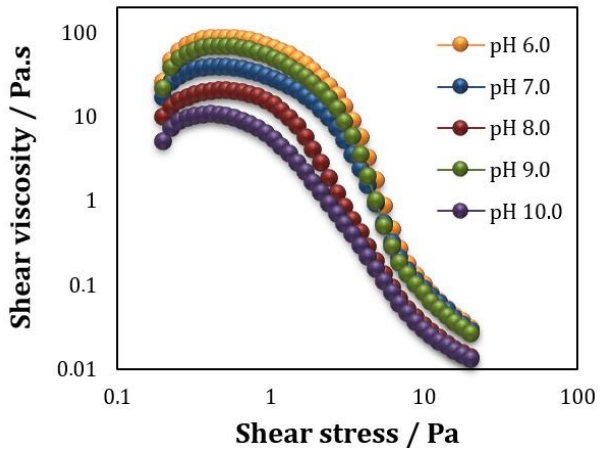
with other biopolymers in the development of new delivery systems such as nanoparticle-based formulations [28]

As the introduction of amino groups in the structure of CG can increase intra- and inter-molecular interactions and consequently impact gum rheological properties and interaction with other biopolymers such as polysaccharides and proteins [29,30] further experiments need to be performed with AMCG to assess its potential regarding role of experimental factors such as pH on AMCG tendency to react with other biopolymers.



(a)





(b)

Fig. 2. Apparent viscosity using the Cross model of CG at different concentrations (a) and influence of pH on viscosity of CG solutions at 0.03% (w/v) (b)

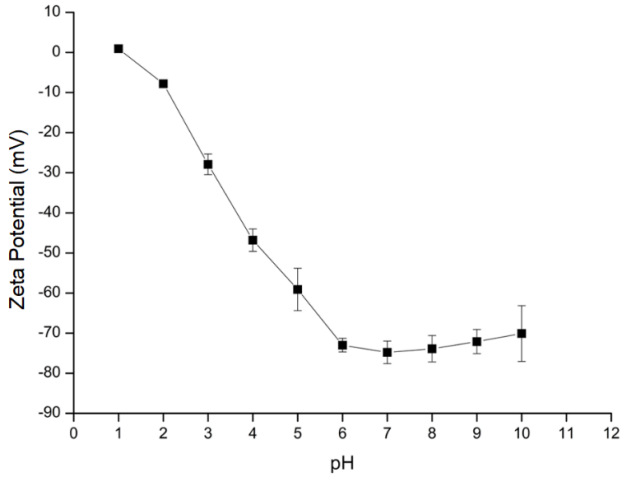


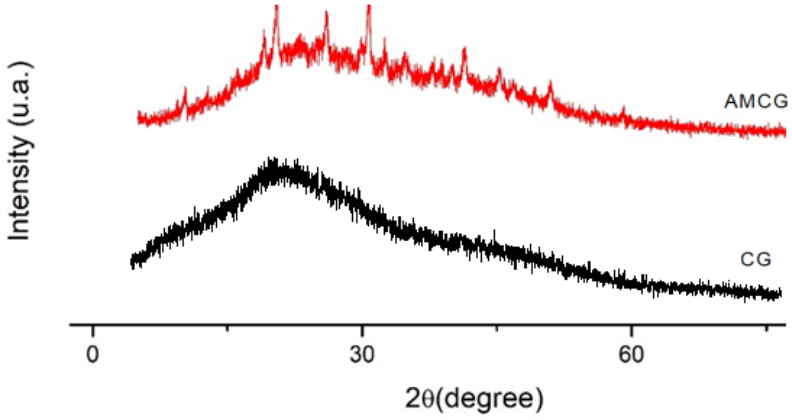
Fig. 3. Zeta potential of CG.

3.3 Physicochemical characterization

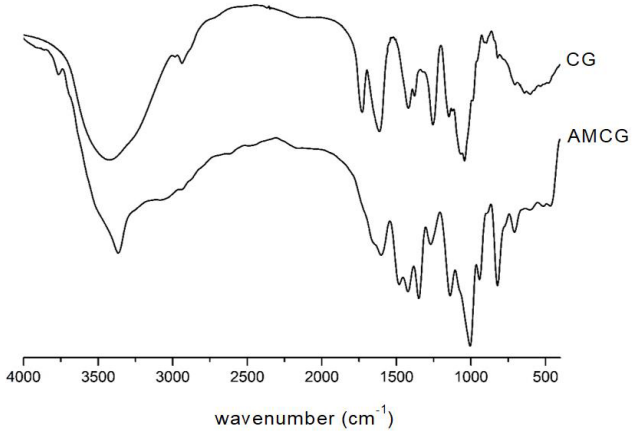
Elemental analysis was used to quantitatively determine the amount of carbon and nitrogen in the modified gum (AMCG). Through this analysis, it can be seen that the composition of carbon was 20.15 mmol g^{-1} and nitrogen was 0.24 mmol g^{-1} in modified gum. X-ray diffraction (XRD) was used to analyze the crystal structure of gum after chemical modification. Fig.4 (a) shows the XRD of the pure gum and the modified gum. From the graph, it can be seen that pure Chicha gum has an amorphous crystallographic structure [6]. After the chemical modification of the chicha gum, new crystallographic peaks can be observed, indicating an increase in the

crystallinity of the compound [31]. The appearance of these new peaks is caused by the surface rearrangement in the gum structure resulting from the incorporation of amino groups [6,31].

Infrared spectroscopy (FTIR) was used to qualitatively assess the incorporation of amino groups on the CG surface, and the results are shown in Fig. 4 (b). From the spectrum of CG, a band can be observed at 3450 cm^{-1} related to the OH stretch of the galactopyranose and glucopyranose rings. The CG spectrum also showed a band at 2933 $cm^{-1,}$ which is attributed to the CH stretch of the aliphatic groups. The 1729 cm^{-1} band is attributed to the C=O stretching vibration of the free carboxylic acid and methyl ester groups of galacturonic acid. The band at 1614 cm^{-1} is related to stretching asymmetrical and symmetrical carboxylate groups. The band at 1419 cm^{-1} represents the deformation of the OH group of the carboxylic acid group. Finally, the band at approximately 1255 cm^{-1} is related to the stretching vibrations of C-O and C-O-C, which are characteristic of natural polysaccharides [1,3,4,6,31,32,33].



(a)



(b)

Fig. 4. XRD (a) and FTIR (b) of CG and AMCG.

After the incorporation of the amino groups on the CG surface, changes in the FTIR spectrum occurred (Fig. 4 (b)). It is possible to observe the appearance of a band at 3750 cm^{-1}, which is related to the N-H stretching vibration of the primary amines [34,35]. Furthermore, the 3450 cm^{-1} band present in the FTIR spectrum of CG changed its shape due to the replacement of OH groups by NH_2 groups on the surface of CG [12,17]. In addition, it is possible to observe the disappearance of the band at 1729 cm^{-1} due to the formation of amide groups in substitution with carboxyl groups. This is confirmed by the presence of the band at 1610 cm^{-1}, which is attributed to the stretching of the C=O group of amides [6]. In the AMCG spectrum, there is also the appearance of a band at approximately 1325 cm^{-1}, which corresponds to the C-N stretching vibration of aliphatic amines [17,36]. Finally, it is possible to observe an increase in intensity in the band of 780 cm^{-1}, caused by the bending of the amine groups (NH_2) [37].

Natural gums are composed of polysaccharides of multiple sugar units linked together to form large molecules; that is, they have a high molecular weight. However, chemical modification may cause a change in the molecular weight of CG, and this change may influence CG physicochemical properties. The molecular weight of gum as well as its distribution may impact the rheological behavior of gum solutions. Thus, SEC analysis was performed to investigate possible changes in the molecular mass of CG after chemical modification through amination. The results of size exclusion chromatography showed that the modification had a decreasing effect on the molar mass of CG, 12 x 10^6 $g.mol^{-1}$, while AMCG showed a molecular mass of 3.7 x 10^6 $g.mol^{-1}$. The results also showed that both gums are polydisperse macromolecules, with polydispersity values (Mw/Mn) of 3.25 and 7.25, respectively, for CG and AMCG. These results corroborate the results obtained by Simi & Abraham (2010) [12], who performed this same synthesis route to obtain the aminated xyloglucan gum, where this amination reaction also promoted a reduction in the molecular mass of the modified polysaccharide.

Changes in the chemical structure of polysaccharide gums can lead not only to changes but also to add of newer properties. The amine modification of polysaccharide gums can create amphiphilicity properties by integration of amine moiety's alkyl chains or create a positive charge upon introduction of a primary amine in the structure. Octylamine-grafted c xanthan gum [38] and cationic charge-bearing aminated gellan gum [39] are examples of these purposes, respectively. The introduction of hydrophobicity in the hydrophilic polysaccharide gum structure imparts amphiphilicity, thus increasing gums' surface properties to the extent that it can improve emulsifying and foaming properties [40].

The zeta potential of gums is critically related to its aqueous solutions' surface charges and mucoadhesive properties. A higher positive charge on the modified gums may indicate an increased potential for gum ionic interactions with the negatively charged domains in the

epithelial protein, mucin [39].

Surface charge is an important parameter to be investigated in the characterization of a modified CG, as this factor can influence its properties involving electrostatic interactions. The results indicate that the CG modification process promoted a change in the polymer surface charge. The CG surface charge is -59.10 ± 0.79 mV, and this negative zeta potential is justified due to the presence of a significant number of uronic acids (74.9%) in the CG composition. After modification, AMCG showed a lower anionic character of -33.10 ± 3.32 mV, and this reduction can also be justified due to the insertion of positively charged amino groups, previously proven by FTIR, into the polymeric chain of CG.

For biopolymer research, thermal analysis is especially suited for studying its thermal stability as well to depict a decomposition pattern. Both CG and AMCG have two stages of decomposition, as shown in Fig. 5 (thermogravimetric analysis (TG), first derivative (DTG) and differential scanning calorimetry (DSC)). These steps are shown in the peaks of the DTG curves (Fig. 5). For CG, a mass loss of 6.77% can be observed in the temperature range of 40-125°C, with a maximum decomposition temperature of 88°C. This step corresponds to water loss. After chemical modification, it can be observed that the first decomposition event of the modified polymer (AMCG) showed a greater loss of mass (13.76%) than the pure polymer (CG), which indicates a greater hydrophilicity of the AMCG polymer due to the presence of high-water absorption affinity amino groups (NH_2). The second step occurs in the temperature range of 230-340°C with a mass loss of 52.44% and a maximum decomposition temperature of 288°C and is related to the decomposition of the polymeric chain of CG. After modification with the amino groups, the maximum decomposition temperature of the second event increased to 302°C, which indicates that the presence of the amino groups on the CG surface increased its degradation temperature. as confirmed by the number of residues generated after 500°C, while CG presented a residue of 28.91%, the modified gum AMCG presents a greater amount of residue of approximately 46.24% [1,6].

The DSC results (Fig. 5) for CG and AMCG showed exothermic and endothermic changes with increasing temperature, corroborating the thermal decomposition events observed in the TG curves. The two biopolymers presented endothermic peaks attributed to water loss as the first event. AMCG had a higher endothermic peak than CG, as it has a greater water absorption capacity. CG and AMCG also showed an exothermic peak that corresponds to thermal decomposition of the polymer. Furthermore, the AMCG exothermic peak at a temperature higher than CG indicates that the modified polymer has a greater thermal stability at higher temperatures compared to the pure polymer, as observed in TG/DTG [1,6].

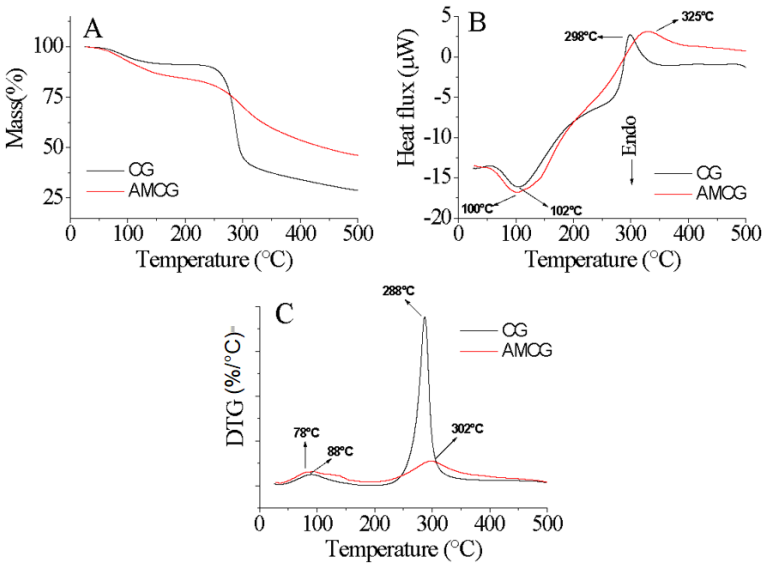


Fig. 5. TG/DTG and DSC curves for CG and AMCG gums obtained by thermogravimetric analysis

Based on the results of the AMCG characterization, a reaction scheme was proposed for the incorporation of amino groups on the CG surface, as shown in Fig. 6.

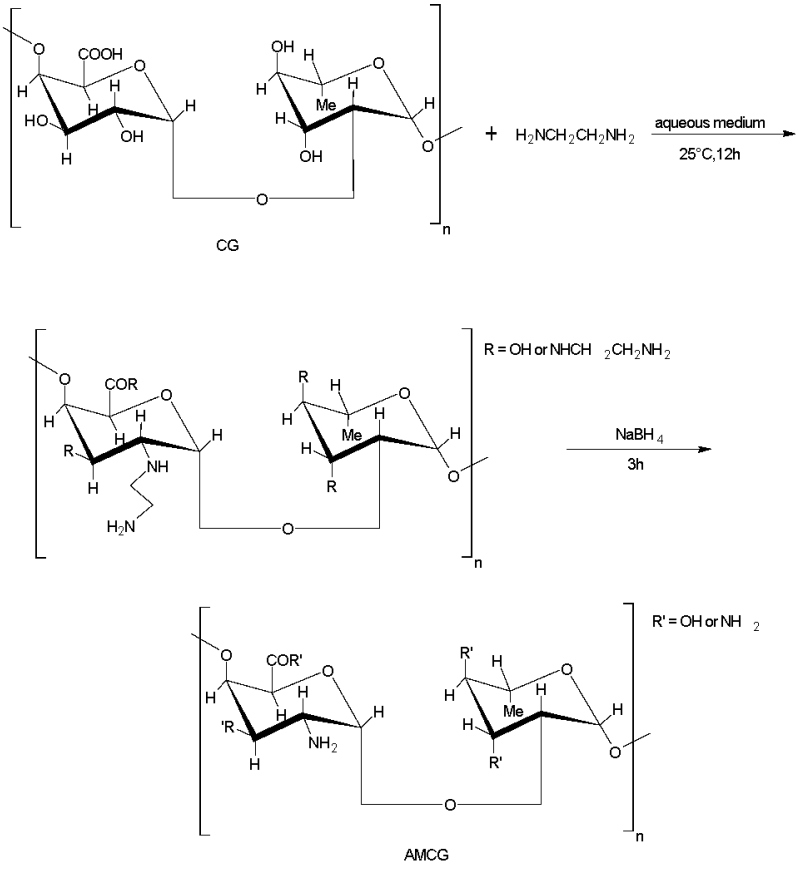


Fig. 6. Scheme of the synthesis process.

3.4 *Ex vivo* mucoadhesion strength

The term bioadhesion refers to the adhesion of two surfaces that occur in a biological environment. This bioadhesion process usually occurs between a biological substrate and a polymer (natural or synthetic). Being that, adhesion between surfaces occurs due to interfacial forces. when the biological surface is a mucosa, the bioadhesion is called mucoadhesion [41,42]. Thus, *ex vivo* mucoadhesion studies were performed to assess the adhesion strength of pure and modified gum. CG and AMCG showed adhesion strengths of 74.78 ± 1.09 mN and 81.85 ± 0.17 mN, respectively. CG mucoadhesiveness is related to the presence of carboxyl and hydroxyl groups in its polymer chain. These groups favor mucoadhesion through hydrogen with the mucosa [43]. The AMCG polymer showed a higher adhesion strength than CG because in addition to carboxyl and hydroxyl groups, the modified polymer has amino groups in its polymeric chain. AMCG can interact with mucin through electrostatic interactions/hydrogen bonding between amino groups of aminated AMCG and glycoproteins of mucin, thus resulting in stronger adhesion [44]. On the other hand, amino groups incorporated onto the CG surface after chemical modification lower the negative charge of the polymer (as shown in the zeta potential) and favor adhesion to the mucosa by electrostatic interactions, since it has a negative character [45, 46]. An enhanced mucoadhesiveness of aminated polysaccharide gums has been previously described for guar gum [47] and gellan gum [39]. AMCG in water can lead to a complexation reaction between amino groups and water molecules conducting NH_3^+-OH^-.This complexation reaction retaining water molecules within the matrix of aminated gum can provide hydration. Therefore, not only a higher electrostatic attraction between the amino groups of the modified gum and mucus but also a benefic hydration must be considered [39].

3.5 Antibacterial activity against the standard strain of *Staphylococcus aureus*

Fig. 7 shows the results of the antibacterial activity against *Staphylococcus aureus* bacteria (ATCC 25923) of CG and AMCG by the direct contact method. CG showed an inhibitory effect against S. aureus of 69.27 ± 1.39%, and after chemical modification, this effect increased to 89.77 ± 2.90%. These results are related to the presence of amine groups in CG and AMCG. Studies with cationic polysaccharides, such as chitosan, showed antimicrobial potential caused by the amino groups present in their structure due to electrostatic interactions with teichoic acids, a major class of bacterial surface glycopolymers. This interaction destabilizes and consequently compromises the functioning of the membrane, promoting a leakage of intracellular components into the extracellular environment that results in cell death [48,49,50]. Thus, it is assumed that the amino groups inserted in the structure of CG, similar to what occurs with chitosan, may have interacted electrostatically with the negative groups present in the membrane of the *S. aureus* bacteria (teichoic acids), which consequently caused a better antibacterial action against a gram-positive bacteria compared to the unmodified CG.

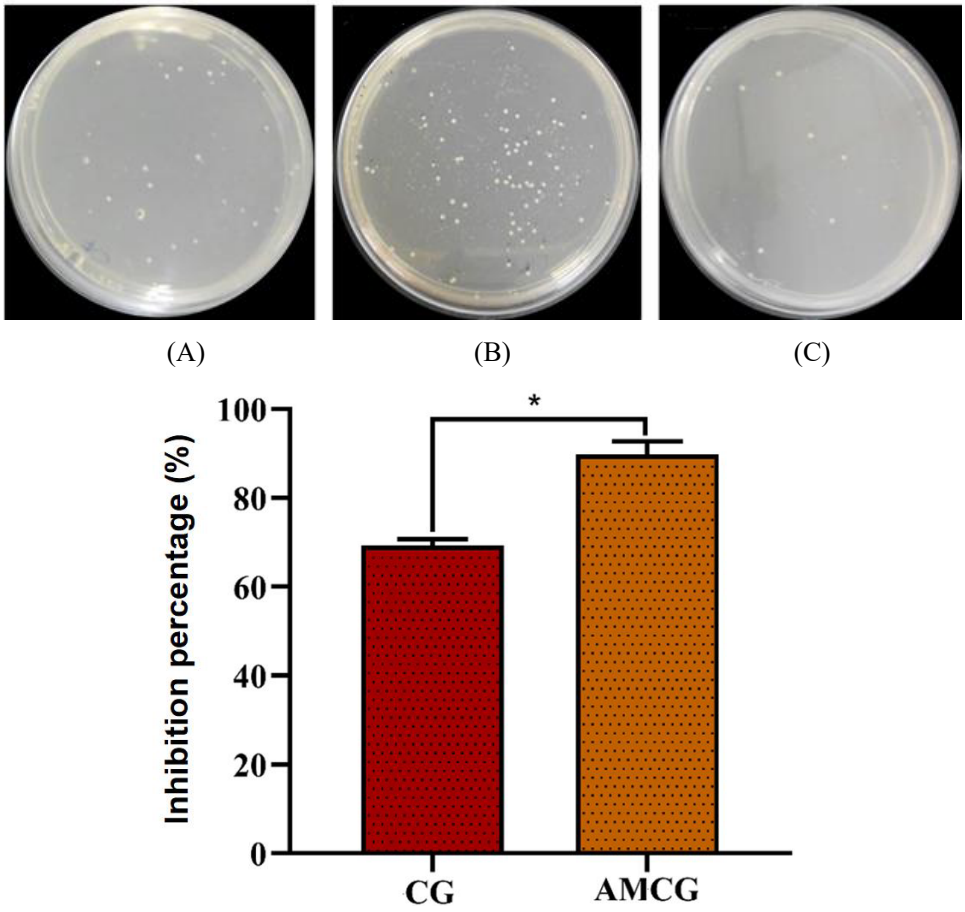


Fig. 7. Direct contact testing for CG and AMCG with the standard strain of *Staphylococcus aureus* bacteria (ATCC 25923), (A = CG, B = Control, and C = AMCG).

3.6 Inhibitory activity of α-glucosidase

α-Glucosidase is an enzyme that hydrolyzes carbohydrates into monosaccharides that are easily absorbed in the gastrointestinal tract and is responsible for the increase in postprandial blood glucose. Thus, inhibition of α-glucosidase is an important factor in digestion/hydrolysis of carbohydrates and has been studied as an alternative to control and reduce blood glucose levels [21,51]. Both unmodified and aminated CG were able to inhibit this enzyme; the maximum inhibition occurred at a concentration of 125 µg/mL, and the highest concentrations tested (250, 500 and 1000 µg/mL) did not cause an increase in inhibition. CG and AMCG presented similar inhibitory results (CG = 9.74% and AMCG = 8.67%), indicating that the chemical modification did not significantly affect the inhibitory effect of the gum against the α-glucosidase enzyme.

3.7 Antioxidant capacity

Antioxidants are bioactive molecules that have the ability to maintain the structure and function of cells through the elimination of free radicals, inhibition of lipid peroxidation reactions

and prevention of other oxidative damage. These also play an important role in the body's defense mechanisms against pathological processes caused by oxidative stress and have been considered important in the prevention of chronic diseases such as cancer, cardiovascular disease and diabetes [52,53,54].

Thus, the antioxidant potential of CG and AMCG gums was evaluated. The gums presented a minimum inhibitory concentration (CG = 302.76 µg/mL and AMCG = 316.80 µg/mL) lower than quercetin (380.00 µg/mL), which is a natural compound recognized in the literature for its antioxidant potential, thus revealing a great antioxidant potential for the studied gums. Gums exuded from plants are raw materials that have been the object of study based on evidence of their bioactive properties. In addition to being low cost, they are widely available in nature and are considered reliable for consumption, as they are nontoxic [52].

3.8 Effect of CG and AMCG gum on the viability of Caco-2 cells by the MTT test

The cytotoxicity of CG and AMCG gums on Caco-2 cells was evaluated by the MTT reduction assay. Aminated Chicha gum (AMCG) had a higher cell viability than Chicha gum (CG) at concentrations of 62.5 and 125.0 µg/mL (Fig. 8). However, at higher concentrations (250.0, 500.0, and 1000.0 µg/mL), the opposite occurred: AMCG cell viability was reduced, and CG had higher cell viability.

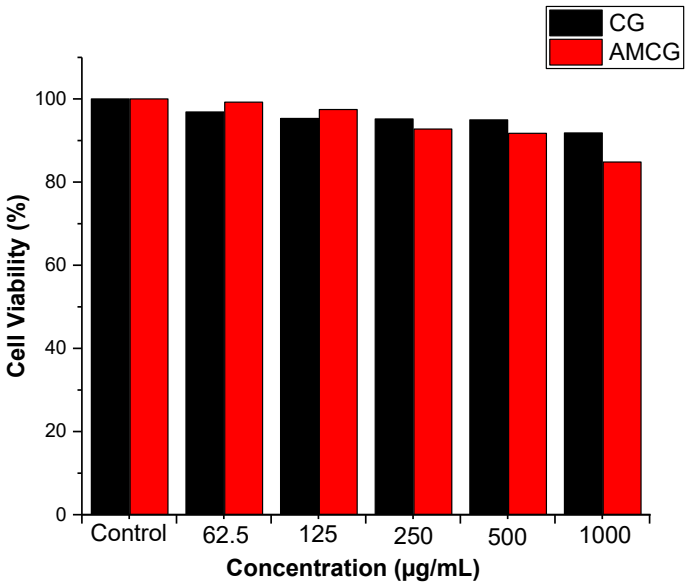


Fig. 8. Cell viability by MTT test.

Cytotoxicity analysis is a prerequisite for assessing the biocompatibility of biomaterials. In accordance with the guidelines of ISO 10993-5, a material is considered toxic when cell viability decreases below 70% of the cell viability of the control group [11,55]. The

experimentally found viability difference values compared to the control were below 10% for all tested CG concentrations. AMCG gum also showed high values of cell viability at the concentrations tested, except at 1000 μg/mL, with cell viability 15.2% lower than that of the control group. Thus, the results obtained indicate that the tested gums do not have cytotoxic potential and are thus a valid and safe alternative for biomedical applications.

4. Conclusions

In this study, the amination of *Sterculia striata* gum was accomplished by an easy and simple synthetic strategy and confirmed by FTIR spectroscopy. Chicha gum viscosity and zeta potential varied as a function of pH. Further characterization results revealed a lower zeta potential and molar mass values for modified AMCG when compared to the native CG. The modification of the polysaccharide increased CG mucoadhesive and antimicrobial properties, thus suggesting their use in the pharmaceutical industry for application in wound dressings, gels and in pharmaceutical formulations as excipients for drugs that have poor permeability and low retention in mucosa. Both native and modified gums showed antioxidant capacity and inhibitory effects against the α-glucosidase enzyme, and no cytotoxic potential was found.

While studies on their complete viscosity characterization including AMCG sol-gel properties still need to be performed toward their use in drug delivery systems, amine-modified CG improved mucoadhesive and antimicrobial properties against *Staphylococcus aureus* and broadened gum application potential in the pharmaceutical field,

These properties can also be attractive for applications related to the food industry as adhesiveness, providing modulation of food organoleptic properties, and as antimicrobial agents in the production of films while protecting food against spoilage and pathogens.

Declaration of Competing Interest

None.

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Modification of chicha gum: antibacterial activity, *ex vivo* mucoadhesion, antioxidant activity and cellular viability

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Abstract

The aim of the present work was to modify the exuded gum of *Sterculia striata* tree by an amination reaction. The viscosity and zero potential of the chicha gum varied as a function of pH. The modification was confirmed by X-ray diffraction (XRD), infrared spectroscopy (FTIR), size exclusion chromatography (SEC), zeta potential, thermogravimetric analysis (TG), and differential scanning calorimetry (DSC). Furthermore, the chemical modification changed the molar mass and surface charge of the chicha gum. In addition, the gums were used in tests for *ex vivo* mucoadhesion strength, antibacterial activity against the standard strain of *Staphylococcus aureus* (ATCC 25923), inhibitory activity of α-glucosidase, antioxidant capacity, and viability of Caco-2 cells. Through these tests, it was found that amination caused an increase in the mucoadhesive and inhibitory activity of chicha gum against the bacterium *Staphylococcus aureus*. In addition, the gums (pure and modified) showed antioxidant capacity and an inhibitory effect against the α-glucosidase enzyme and did not show cytotoxic potential.

Keywords: Chicha gum; Mucoadhesion; Antimicrobial activity; Antioxidant activity; Cell viability.

Highlights

- The exuded gum from the *Sterculia striata* tree was chemically modified by an amination reaction.
- Chemical modification of the gum was confirmed by XRD, FTIR, SEC, zeta potential and thermal analysis (TG/DTG/DSC).
- Chicha gum aminated showed an increase in mucoadhesive and inhibitory activity against *Staphylococcus aureus* bacteria.
- The chicha gum and modified gum showed no cytotoxic potential.

1. Introduction

Natural gums are polysaccharides exhaled from trees that represent an abundant raw material for the most diverse industrial applications, including the food industry and pharmaceutical and cosmetic fields. This wide industrial application of natural gums is because they are chemically inert, biodegradable, non-toxic, cost saving as raw materials and easily available in nature [1,2].

In addition, natural gums have varied compositions and rheological properties that allow their easy use in various biomedical applications, including tissue engineering, biosensors, drug delivery systems, antibacterial activity and cytotoxic properties. In these applications, natural gums are inexpensive and have few side effects [3,4]. In addition, natural gums are preferred over synthetic materials due to their non-toxicity, low cost, abundance, and they are derived from renewable sources [5].

Among the most varied gums found in nature, chicha gum has attracted much attention from researchers for its application in several purposes. Chicha gum is exuded by *Sterculia striata*, an abundant plant in northeastern Brazil. This gum belongs to the family *Sterculiaceae* and has a high molecular weight ($4.2 \times 10^6 - 3.6 \times 10^7$ g mol^{-1}). Moreover, chicha gum is a heteropolymer formed of partially acetylated chains and composed of uronic acid (42.2–49.2%), rhamnose (23.8–28.8%), galactose (19.3–23.4%), xylose (5.6–7.7%), and acetyl groups (9.6–10.7%) [1,3,4,6]. This polysaccharide has polyanionic character due to the presence of the carboxylic groups present in the glucuronic acids [3,4]. This makes chicha gum sensitive to the pH variation of the medium [7].

Further to the carboxylic groups, chicha gum has several hydroxyl groups in its polymer chain that provide the possibility of inserting new functional groups in its structure through various chemical modifications [1,6]. The modified polymers have improved properties relative to crude material in terms of solubility, mucoadhesion, swelling, coagulation and flocculation, i.e., the modification allows obtaining a customized product according to the appropriate characteristics for the desired application [8]. In this respect, the chemical modification of gums has increased their reactivity potential seeking the development of new delivery systems, among which nanoparticles, thus improving their self-assembly capacity [9] and performance in pharmaceutical applications such as swelling, mucoadhesion and drug release [10,11].

Among the various reactions of chemical modification of the gum surface, the incorporation of alkaline sites from nitrogen compounds (amination) can be highlighted. Many natural polymers have been modified with the incorporation of nitrogen groups and have shown improvements in their properties. For example, fenugreek gum modified with amino groups obtained a greater adhesion force than films prepared with gum without modification [11]. The

xyloglucan of tamarind with amino groups obtained better thermal properties in relation to the polymer without modification. In addition, amino gum showed better antimicrobial activity than chitosan [12].

In this context, studies on the properties of pure and modified chicha gum with amino groups are necessary, considering that there are still few reports in the literature about the characteristics of pure chicha gum and no reports on the properties of this modified gum with amino groups.

Thus, the present study aims to chemically modify chicha gum with amino groups through the reaction of this gum with ethylenediamine. The gums (pure and modified) were characterized by elementary analysis, X-ray diffraction (XRD), infrared spectroscopy (FTIR), size exclusion chromatography (SEC), zeta potential, thermogravimetric analysis (TG), first derivative (DTG) and differential scanning calorimetry (DSC). In addition, the gums were assessed for *ex vivo* mucoadhesion strength, antibacterial activity against *Staphylococcus aureus*, inhibitory activity of α-glucosidase, antioxidant capacity, and human cell viability.

2. Materials and methods

2.1 Materials

Sterculia striata gum (Chicha gum) was obtained from exudates collected manually from native *Sterculia striata* trees located in the city of Teresina, Piauí, Brazil, registered at Herbarium Graziela Barroso under the number TEPB: 30418, sodium chloride (Salado *et al*), sodium hydroxide NaOH), and ethanol (C_2H_6O) were purchased from Dinâmica Química Contemporânea Ltda. Acetone (C_3H_6O), sodium borohydride ($NaBH_4$), acetic anhydride ($C_4H_6O_3$), 1-methylimidazole ($C_4H_6N_2$), dichloromethane (CH_2Cl_2), trifluoroacetic acid ($C_2HF_3O_2$), sulfuric acid (H_2SO_4), and quercetin (95% purity) were purchased from Sigma-Aldrich. Nutrient agar (NA, Himedia, India), brain heart infusion broth (BHI, Himedia, India), Müller Hinton agar (Sigma–Aldrich), and MTT (3-[4,5-dimethylthiazol-zyl]-2,5-diphenyletrazolium) (Sigma-Aldrich), were used for the biological assays, as was analytical grade dimethyl sulfoxide (DMSO) (Sigma-Aldrich). The acid yellow 73 dye (AY73) was supplied by Danny Color Dyes and was used without prior purification. (Brazilian SISGEN Registration nº ABD61DA)

2.2 Chicha gum (CG) isolation

Chicha gum (CG) was isolated in salt form using a method described by Brito et al., 2005 [13] and Brito et al., 2004 [14]. *Sterculia striata* exudate (1.0 g) was dissolved in distilled water (100.0 mL) under stirring (2000 rpm/12 h on a mechanical stirrer - Tecnal, model TE-139) at 25°C for 12 h. NaCl (1.0 g) was added and then filtered (porcelain filter with the aid of a vacuum

pump) to solution, and its pH was adjusted to 7.0 with 0.1 mol L^{-1} NaOH solution. The polysaccharide was precipitated with 95% ethanol, washed with acetone, dried at 40°C in a hot air oven for 24 h, and macerated to obtain it in powder form (Fig. 1).

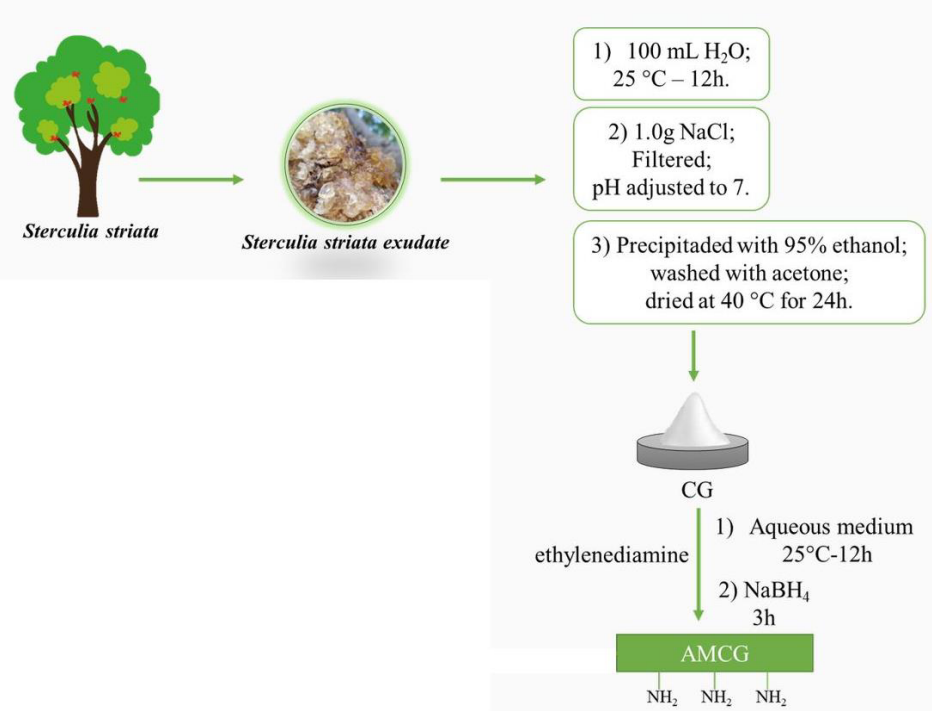


Fig. 1. Isolation scheme and chemical modification of chicha gum (CG).

2.3 Analysis of the composition of monosaccharides

The neutral sugars were determined by gas chromatography as alditol acetates. Hydrolysis was performed with 2 M trifluoroacetic acid (TFA) at 120°C for 1 h. The monosaccharides were reduced with $NaBH_4$ (15% in 3 M NH_3) for 1 h at 30°C and subsequently acetylated with acetic anhydride (3 mL) in the presence of 1-methylimidazole (450 µL) for 30 min at 30°C. Alditol acetate derivatives were separated with dichloromethane and analyzed by gas chromatography with a flame ionization detector (GC-FID) equipped with a 30 m DB-225 column (J & W Scientific, Folsom, CA, USA) with film thicknesses of 0.25 mm and 0.15 µm, respectively. The temperature program of the oven used was as follows: initial temperature 200°C, increase in temperature at a rate of 40°C/min up to 220°C, remaining for 7 min, followed by a rate of 20°C/min to 230°C and maintaining this temperature for 1 min. The injector and detector temperatures were 220 and 230°C, respectively. The flow rate of the carrier gas (H_2) was adjusted to 1.7 mL/min [15].

Uronic acids were determined colorimetrically according to the method of Nunes et al., 2012 [15]. The samples were prepared by pre-hydrolysis in 0.2 mL of 72% H_2SO_4 for 3 h at room

temperature, followed by 1 h of hydrolysis in 1 H_2SO_4 at 100°C. A calibration curve was made with D-galacturonic acid.

2.4 Preparation of CG solutions at different pH values

CG solutions were prepared at a concentration varying from 0.01 % to 5.00 % (w/v) at pH 4.5. To assess the effect of pH of diluted solutions on their sol gel properties, the pH was adjusted to values from 1 to 10 using HCl or NaOH (0.1 and 1.0 mol L^{-1}).

2.4.1 Viscosity

A Thermo Scientific HAAKE MARS III rheometer with a cone geometry (35 mm, 1°). was used to measure the viscosity to assess the effect of pH of diluted solutions on sol-gel properties of CG. Apparent viscosity curves were fitted to Cross model [16].

2.5 Synthesis of aminated chicha gum (AMCG)

The CG was modified by means of an amination reaction using the method previously described in the literature [17]. The synthesis of AMCG was carried out by the addition of ethylenediamine as an aminating agent that replaces the OH group of CG with $-NHCH_2CH_2NH_2$. Then the $-NHCH_2CH_2NH_2$ group was reduced to the $-NH_2$ group by the addition of sodium borohydride ($NaBH_4$) [17]. CG (1.0 g) was dissolved in 250 mL of distilled water under stirring at a temperature of 25°C for 12 h. Subsequently, 25 mL of ethylenediamine was added to the homogeneous solution of CG and allowed to react under continuous stirring for 12 h. Then, 50 mL of sodium borohydride (5%) was added and stirred vigorously for 3 h. After completion of the reaction, the modified polysaccharide was precipitated with ethanol, washed with acetone, dried at 40°C in a hot air oven for 24 h and macerated to obtain it in powder form. The chicha gum amination reaction scheme is shown in Fig. 6 (results section).

2.6 Physicochemical characterization

The elementary analysis of carbon, hydrogen, and nitrogen were evaluated in a PerkinElmer model PE 2400 elemental analyzer. The zeta potential measurements of the gums were performed on Zetasizer Nano ZS equipment (Malvern Instruments). The molar masses of the gums were obtained by size exclusion chromatography (SEC) in a Shimadzu LC-10AD chromatograph with an RID-6A refractive index detector, 7.8x300 mm linear Ultrahydrogel column, with 0.1 mol L-1 NaN3 as the mobile phase. The X-ray diffraction (XRD) patterns of the samples were obtained using an X-ray diffractometer (Shimadzu XR-D600 A) with CuKα as the radiation source and a wavelength of 154 p.m. The infrared spectra (FTIR) were obtained using a Varian 660-IR spectrophotometer by the tablet method in KBr 1% (m/m) of sample in 32 scans

in the region of 400 to 4000 cm-1 with a resolution of 4 cm-1. Thermogravimetric analysis was performed using a DSC-TGA thermogravimetric analyzer (SDT Q600 V20.9) in an atmosphere of inert air (synthetic air). For scanning, the heating rate was between 0°C and 800°C with an interval of 10°C/min.

2.7 *Ex vivo* mucoadhesion strength

The *ex vivo* mucoadhesion strength was assessed on a TA-XT plus texture analyzer (TA Instruments®, UK) using porcine intestinal mucosa as a mucoadhesion model because it resembles the human intestinal mucosa [18]. During the test, CG and AMCG gums were fixed with double-sided tape to a 10 mm cylindrical probe, while sections of mucosa of the porcine small intestine were fixed on a specific lower support for mucoadhesion testing and moistened with pH 7.4 buffer solution preheated to 37°C. The probe was moved perpendicularly toward the mucosa with a constant velocity of 0.1 mm/sec, and contact with the mucosa was maintained for 60 seconds with a compression force of 0.5 N. After the contact time, the probe was removed at a speed of 0.1 mm/sec. The analysis was performed in triplicate [19].

2.8 Antibacterial activity against the standard strain of *Staphylococcus aureus* (ATCC 25923)

2.8.1 Inoculum Preparation

Cultures were obtained by transferring a range of bacterial growth on nutrient agar to a falcon tube containing 3.0 mL of 3% brain heart infusion (BHI) medium, followed by incubation at 37 °C for 24 h. From this culture in BHI, a standard bacterial suspension was prepared to a density equivalent to 0.5 on the Mac Farland scale, approximately 1.5 x 10^8 CFU/mL (colony forming units - CFU) [1,6].

2.8.2 Direct Contact Test

To carry out these tests, 2000 µg of the material to be tested and 2000 µL of bacterial suspension were transferred to an Eppendorf tube, followed by homogenization on a vortex shaker. Then, 200 µL of this suspension was transferred to Petri dishes containing Mueller Hinton agar, which were sown with the aid of a Drigalsky loop using the spread plate method, followed by incubation at 37°C for 24 hours. Plates only with inoculum were used as a positive control. The tests were performed in triplicate. The inhibitory effect produced by each test solution was calculated according to Eq. 1 [1,6]:

$$\eta = \frac{N_1 - N_2}{N_1} \text{ x } 100\% \qquad \text{Eq.}$$

where η is defined as the inhibitory effect, N_1 is the arithmetic mean of the colony-forming units of the control plates and N_2 is the arithmetic mean of the colony-forming units of each of the tested solutions [20].

2.9 Inhibitory activity of α-glucosidase

The inhibitory activity of α-glucosidase was analyzed as described by Bento et al., 2018 [21]. The solutions were prepared for each sample with KH_2PO_4 buffer solution (10 mM, pH 7). Each well contained 100 µl of 2.5 mM 4-nitrophenyl α-D-glucopyranoside (PNP-G), 150 mL of KH_2PO_4 buffer solution (10 mM, pH 7), and 50 µl of CG, AMCG or acarbose (positive control). The reaction was initiated by the addition of 25 µL of α-glucosidase (0.28 U/mL). The plates were incubated at 37°C for 10 min. The absorbance of 4-nitrophenol released from PNP-G at 405 nm was measured. The increase in absorbance was compared with that of the control (buffer instead of the sample solution) to calculate the inhibitory activity. The experiments were carried out in triplicate.

2.10 Antioxidant capacity

The antioxidant capacity of chicha gum was determined according to the methodology described by Cambrussi et al., 2019 [22]. For the tests, chamber of radiation, provide with a 125 W without bulb. In suspension, 1 mg mL^{-1} catalyst composed of TiO_2 supported on palygorskite incorporated with silver nanoparticles (AgNPs/TiO_2-PAL) was mixed with 0.5 mL of an aqueous solution of acid yellow dye 73 (AY73) at a concentration of $2x10^{-5}$ mol L^{-1}, and 0.5 mL of aqueous CG solution (or AMCG solution) at different concentrations (10 to 400 µg mL^{-1}). The samples were irradiated under UV–Vis radiation for 60 minutes. After the radiation time, samples were centrifuged for 15 minutes at 10,000 rpm to remove the catalyst. Subsequently, the supernatant was transferred to a microplate, and absorbance readings were performed on a microplate reader (Elisa Polaris®) at 492 nm. The absorbance readings of these exact non-irradiated solutions were compared to calculate the discoloration of AY73 as a function of CG concentration (or AMCG concentration).

The discoloration of the solutions was determined by Eq. 2 [23]:

$$Discoloration\ (\%) = \left(\frac{A_0 - A_t}{A_0}\right) x\ 100 = \left(\frac{C_0 - C_t}{C_0}\right) x\ 100 \qquad \text{Eq.}$$

According to the Lambert-Beer law, where A_0 is the initial absorbance of the solution and A_t is the absorbance at time t, which refers to the initial concentrations (C_0) and time (C_t). Considering the values obtained, reaction curves were created to obtain the inhibitory concentration (IC50) in vitro to decrease AY73 discoloration by 50%. Quercetin, a natural compound recognized in the literature for its antioxidant potential, was used as a standard antioxidant for comparison purposes. Linearity was assessed using linear regression analysis, with data adjustment using the least squares method. Each concentration was determined in triplicate (n = 15). The concentration of the AY73 dye in the solution was analyzed by a UV–Vis spectrophotometer (Agilent Technologies spectrophotometer, Cary 60 UV) at its maximum wavelength at 490 nm [22].

2.11 Effect of CG and AMCG gum on the viability of Caco-2 cells by the MTT test

Cell culture conditions and treatments were performed according to the methodology described by Silva & Teixeira, 2015 [24]. The human colorectal adenocarcinoma cell line Caco-2 from the American Type Culture Collection (LGC Standards S.L.U., Spain) was routinely cultured using Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 1% nonessential amino acids, 1% antibiotic, 1% fungizone and 6 μg mL^{-1} transferrin. The cells were incubated at 37°C in 5% CO_2. The cells were washed with HEPES-buffered saline, trypsinized and sub-cultured in 48-well plates at a density of 25000 cells cm^{-2}. All tests were performed after confluence. CG and AMCG gums (62.5-1000 μg mL^{-1}) were dissolved in a medium containing 0.5% (v/v) DMSO. The final concentration of DMSO did not affect cell viability. To determine the effect of gums on cells, viability was assessed 24 h after exposure.

The MTT assay was evaluated by reducing MTT to formazan in viable cells. After discarding the cell culture supernatant, the cells were incubated with 1 mL of MTT solution (0.5 mg ml^{-1} in supplemented DMEM) for 30 min at 37°C. After this period, the supernatant was eliminated, and 1 mL of DMSO was added to each well for complete dissolution of the formazan. The absorbance of the different solutions was then measured using a Multiskan Ascent plate reader (Thermo, Electron Corporation) working at 570 nm. The results were expressed as the percentage of cell viability, which was considered a control group, with 100% viability, the one in which the cells were incubated only with the culture medium, without the presence of any test substance.

2.12. Statistics

Statistical analysis was performed using GraphPad Prism 7.0 software, where the unpaired t test was applied. The results were expressed as the standard deviation of the mean and a p value < 0.05 [6].

3. Results and discussion

3.1 Composition of monosaccharides

The monosaccharide composition has a considerable effect on the rheological and functional properties of the gum. Thus, the monosaccharide composition of the gum obtained from exudates collected from the *Sterculia striata* tree was determined by gas chromatography with a flame ionization detector (CG-FID). The following monosaccharides were found: uronic acid (74.9%), galactose (6.9%), rhamnose (9.1%), xylose (6.3%), and arabinose (2.8%). These results present differences in relation to those reported by previous publications [13,14], where CG was collected from trees native to the city of Fortaleza, Brazil. These observed differences are due to several factors, such as plant age, the edaphoclimatic conditions of the plant, the period of exudate collection, growing conditions, and differences in the methodological processes used in the isolation and determination of the composition of monosaccharides. The high amount of uronic acid in the CG composition is greater than that of commercial gums, indicating that CG gum is a strong polyelectrolyte that has a greater negative charge than these gums [25].

3.2 Viscosity of chicha gum

The rheological properties of diluted and concentrated solutions of CG are represented using the Cross model. Upon lower shear rate, the viscosity of CG gums ranged from 16 to 4060 mPa.s and 0.002 to 0.06 mPa.s. in the higher concentration and diluted gums, respectively. As seen in Fig. 2(a), for diluted solutions, no viscosity change was observed for a concentration of 0.01 % (w/v), whereas the decreasing effect of shear rate on the viscosity of CG at 0.1 % (w/v) is due to the disentanglement of gum polymeric chains during flow. For CG solutions with concentrations from 1.0% to 5.0 % (w/v), a Newtonian plateau was observed at low shear rates, and a pseudoplastic behavior was observed at higher shear rates.

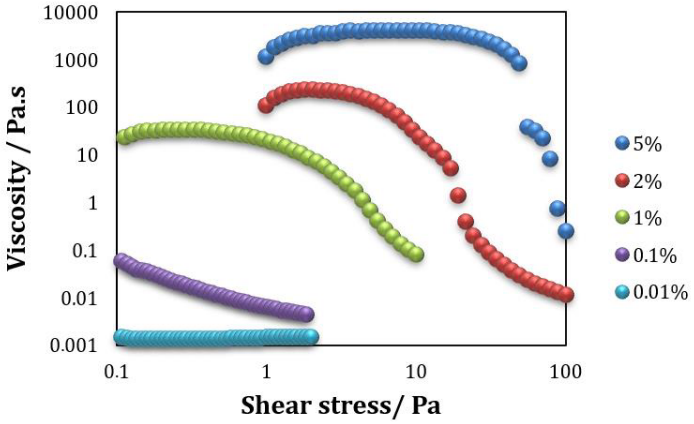
The effect of pH and ionic composition, along with the volume of nasal delivery formulations, have a critical impact on the drug delivery performance of gellan gum [26,27].

Dynamic viscosity tests were carried out at different pH values to evaluate the viscosity of CG. The results are shown in Fig. 2(b). The pH variation did not affect the viscosity of CG linearly. When the pH increased from 1 to 6, there was a significant increase in viscosity. This could be related to the increase in the negative charge of CG observed in the zeta potential analysis (Fig.3), which allowed the expansion of the polysaccharide chain due to intramolecular electrostatic repulsion, thus favoring a very significant increase in the viscosity of CG. Above pH 6, the viscosity of the CG solution decreased with increasing pH. This effect can be attributed to the electrostatic interaction between CG and the Na^+ cations added during pH adjustment, which caused contraction of the macromolecule, favoring a decrease in viscosity.

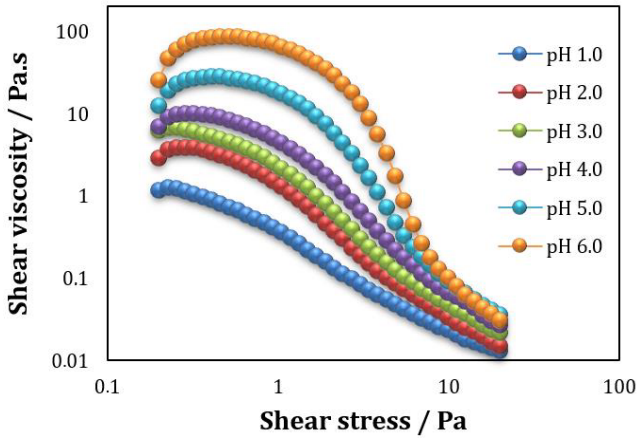
The viscosity measurement has been a useful tool for understanding the sol-gel transition properties of a gum. The effect of pH on the viscosity of diluted solutions of CG is thus relevant because gums' sol-gel transition properties have been a screening tool for biopolymers best fitting

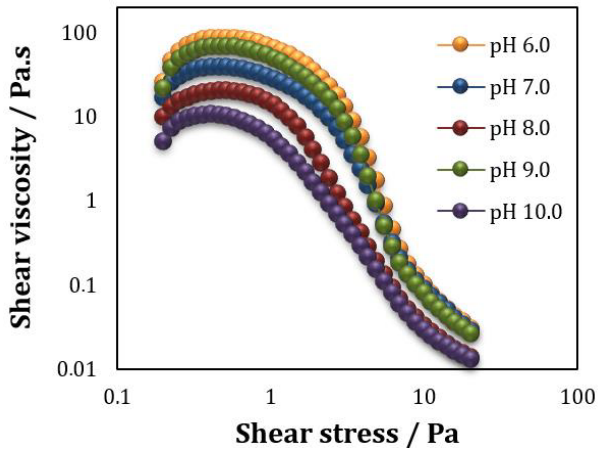
with other biopolymers in the development of new delivery systems such as nanoparticle-based formulations [28]

As the introduction of amino groups in the structure of CG can increase intra- and inter-molecular interactions and consequently impact gum rheological properties and interaction with other biopolymers such as polysaccharides and proteins [29,30] further experiments need to be performed with AMCG to assess its potential regarding role of experimental factors such as pH on AMCG tendency to react with other biopolymers.



(a)





(b)

Fig. 2. Apparent viscosity using the Cross model of CG at different concentrations (a) and influence of pH on viscosity of CG solutions at 0.03% (w/v) (b)

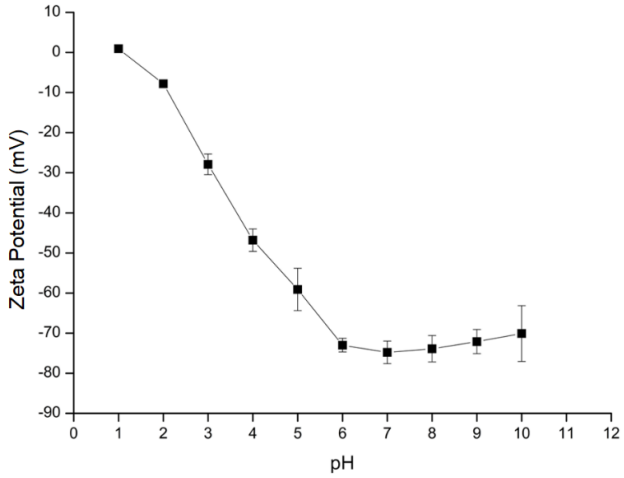


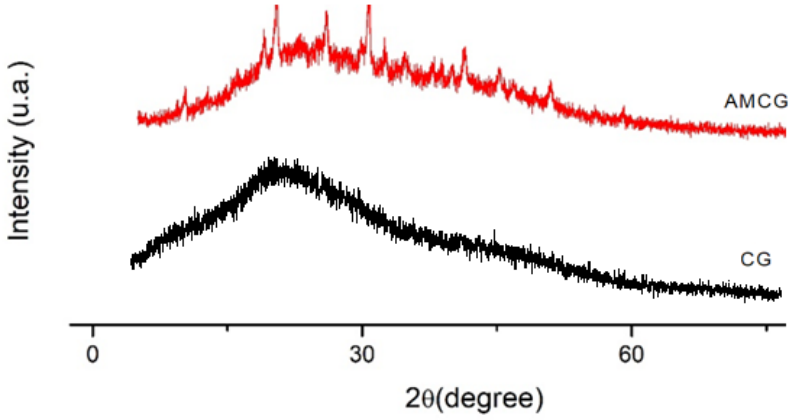
Fig. 3. Zeta potential of CG.

3.3 Physicochemical characterization

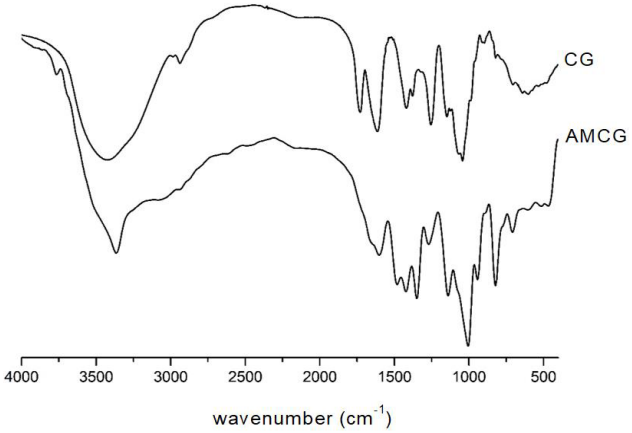
Elemental analysis was used to quantitatively determine the amount of carbon and nitrogen in the modified gum (AMCG). Through this analysis, it can be seen that the composition of carbon was 20.15 mmol g^{-1} and nitrogen was 0.24 mmol g^{-1} in modified gum. X-ray diffraction (XRD) was used to analyze the crystal structure of gum after chemical modification. Fig.4 (a) shows the XRD of the pure gum and the modified gum. From the graph, it can be seen that pure Chicha gum has an amorphous crystallographic structure [6]. After the chemical modification of the chicha gum, new crystallographic peaks can be observed, indicating an increase in the

crystallinity of the compound [31]. The appearance of these new peaks is caused by the surface rearrangement in the gum structure resulting from the incorporation of amino groups [6,31].

Infrared spectroscopy (FTIR) was used to qualitatively assess the incorporation of amino groups on the CG surface, and the results are shown in Fig. 4 (b). From the spectrum of CG, a band can be observed at 3450 cm^{-1} related to the OH stretch of the galactopyranose and glucopyranose rings. The CG spectrum also showed a band at 2933 $cm^{-1,}$ which is attributed to the CH stretch of the aliphatic groups. The 1729 cm^{-1} band is attributed to the C=O stretching vibration of the free carboxylic acid and methyl ester groups of galacturonic acid. The band at 1614 cm^{-1} is related to stretching asymmetrical and symmetrical carboxylate groups. The band at 1419 cm^{-1} represents the deformation of the OH group of the carboxylic acid group. Finally, the band at approximately 1255 cm^{-1} is related to the stretching vibrations of C-O and C-O-C, which are characteristic of natural polysaccharides [1,3,4,6,31,32,33].



(a)



(b)

Fig. 4. XRD (a) and FTIR (b) of CG and AMCG.

After the incorporation of the amino groups on the CG surface, changes in the FTIR spectrum occurred (Fig. 4 (b)). It is possible to observe the appearance of a band at 3750 cm^{-1}, which is related to the N-H stretching vibration of the primary amines [34,35]. Furthermore, the 3450 cm^{-1} band present in the FTIR spectrum of CG changed its shape due to the replacement of OH groups by NH_2 groups on the surface of CG [12,17]. In addition, it is possible to observe the disappearance of the band at 1729 cm^{-1} due to the formation of amide groups in substitution with carboxyl groups. This is confirmed by the presence of the band at 1610 cm^{-1}, which is attributed to the stretching of the C=O group of amides [6]. In the AMCG spectrum, there is also the appearance of a band at approximately 1325 cm^{-1}, which corresponds to the C-N stretching vibration of aliphatic amines [17,36]. Finally, it is possible to observe an increase in intensity in the band of 780 cm^{-1}, caused by the bending of the amine groups (NH_2) [37].

Natural gums are composed of polysaccharides of multiple sugar units linked together to form large molecules; that is, they have a high molecular weight. However, chemical modification may cause a change in the molecular weight of CG, and this change may influence CG physicochemical properties. The molecular weight of gum as well as its distribution may impact the rheological behavior of gum solutions. Thus, SEC analysis was performed to investigate possible changes in the molecular mass of CG after chemical modification through amination. The results of size exclusion chromatography showed that the modification had a decreasing effect on the molar mass of CG, 12 x 10^6 $g.mol^{-1}$, while AMCG showed a molecular mass of 3.7 x 10^6 $g.mol^{-1}$. The results also showed that both gums are polydisperse macromolecules, with polydispersity values (Mw/Mn) of 3.25 and 7.25, respectively, for CG and AMCG. These results corroborate the results obtained by Simi & Abraham (2010) [12], who performed this same synthesis route to obtain the aminated xyloglucan gum, where this amination reaction also promoted a reduction in the molecular mass of the modified polysaccharide.

Changes in the chemical structure of polysaccharide gums can lead not only to changes but also to add of newer properties. The amine modification of polysaccharide gums can create amphiphilicity properties by integration of amine moiety's alkyl chains or create a positive charge upon introduction of a primary amine in the structure. Octylamine-grafted c xanthan gum [38] and cationic charge-bearing aminated gellan gum [39] are examples of these purposes, respectively. The introduction of hydrophobicity in the hydrophilic polysaccharide gum structure imparts amphiphilicity, thus increasing gums' surface properties to the extent that it can improve emulsifying and foaming properties [40].

The zeta potential of gums is critically related to its aqueous solutions' surface charges and mucoadhesive properties. A higher positive charge on the modified gums may indicate an increased potential for gum ionic interactions with the negatively charged domains in the

epithelial protein, mucin [39].

Surface charge is an important parameter to be investigated in the characterization of a modified CG, as this factor can influence its properties involving electrostatic interactions. The results indicate that the CG modification process promoted a change in the polymer surface charge. The CG surface charge is -59.10 ± 0.79 mV, and this negative zeta potential is justified due to the presence of a significant number of uronic acids (74.9%) in the CG composition. After modification, AMCG showed a lower anionic character of -33.10 ± 3.32 mV, and this reduction can also be justified due to the insertion of positively charged amino groups, previously proven by FTIR, into the polymeric chain of CG.

For biopolymer research, thermal analysis is especially suited for studying its thermal stability as well to depict a decomposition pattern. Both CG and AMCG have two stages of decomposition, as shown in Fig. 5 (thermogravimetric analysis (TG), first derivative (DTG) and differential scanning calorimetry (DSC)). These steps are shown in the peaks of the DTG curves (Fig. 5). For CG, a mass loss of 6.77% can be observed in the temperature range of 40-125°C, with a maximum decomposition temperature of 88°C. This step corresponds to water loss. After chemical modification, it can be observed that the first decomposition event of the modified polymer (AMCG) showed a greater loss of mass (13.76%) than the pure polymer (CG), which indicates a greater hydrophilicity of the AMCG polymer due to the presence of high-water absorption affinity amino groups (NH_2). The second step occurs in the temperature range of 230-340°C with a mass loss of 52.44% and a maximum decomposition temperature of 288°C and is related to the decomposition of the polymeric chain of CG. After modification with the amino groups, the maximum decomposition temperature of the second event increased to 302°C, which indicates that the presence of the amino groups on the CG surface increased its degradation temperature. as confirmed by the number of residues generated after 500°C, while CG presented a residue of 28.91%, the modified gum AMCG presents a greater amount of residue of approximately 46.24% [1,6].

The DSC results (Fig. 5) for CG and AMCG showed exothermic and endothermic changes with increasing temperature, corroborating the thermal decomposition events observed in the TG curves. The two biopolymers presented endothermic peaks attributed to water loss as the first event. AMCG had a higher endothermic peak than CG, as it has a greater water absorption capacity. CG and AMCG also showed an exothermic peak that corresponds to thermal decomposition of the polymer. Furthermore, the AMCG exothermic peak at a temperature higher than CG indicates that the modified polymer has a greater thermal stability at higher temperatures compared to the pure polymer, as observed in TG/DTG [1,6].

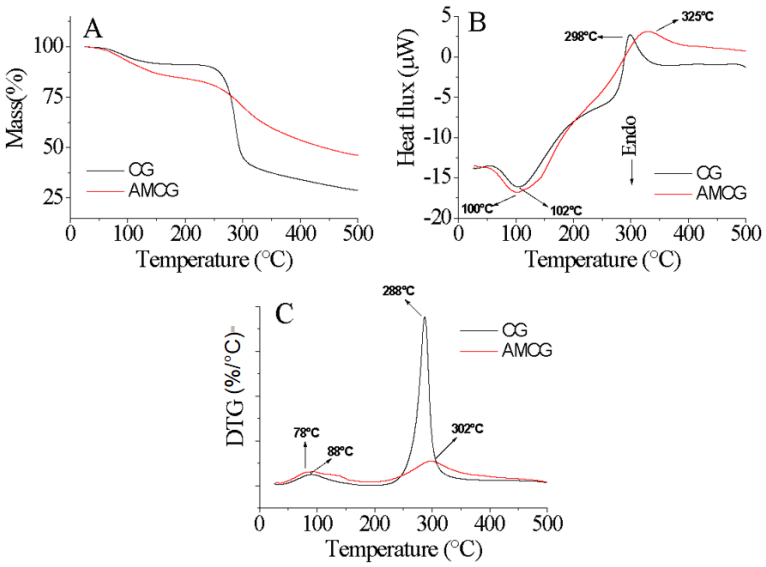


Fig. 5. TG/DTG and DSC curves for CG and AMCG gums obtained by thermogravimetric analysis

Based on the results of the AMCG characterization, a reaction scheme was proposed for the incorporation of amino groups on the CG surface, as shown in Fig. 6.

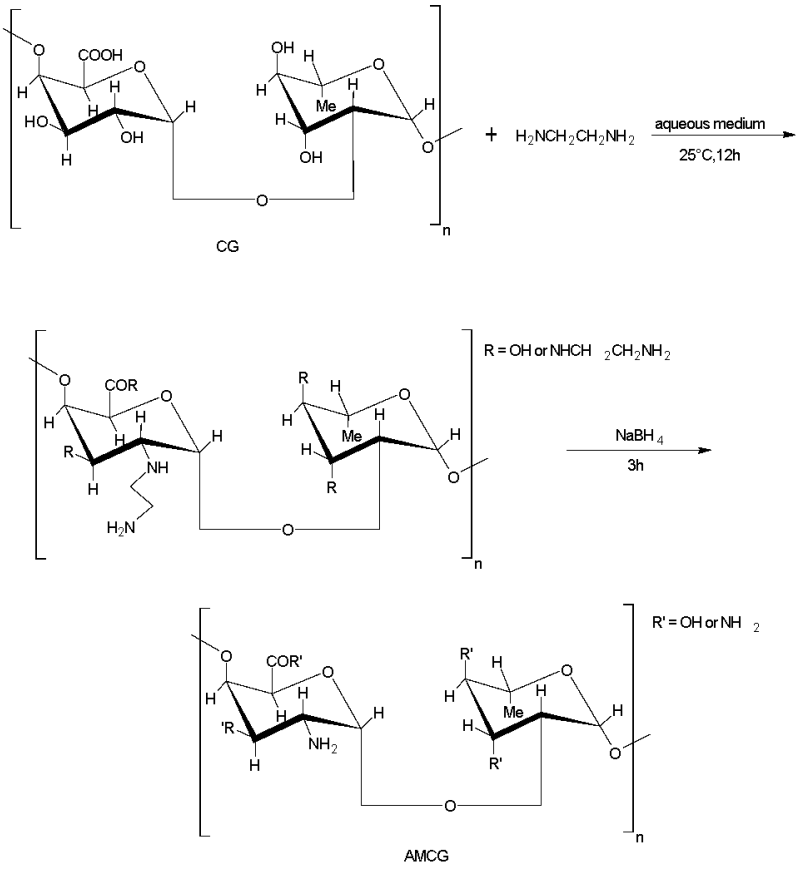


Fig. 6. Scheme of the synthesis process.

3.4 *Ex vivo* mucoadhesion strength

The term bioadhesion refers to the adhesion of two surfaces that occur in a biological environment. This bioadhesion process usually occurs between a biological substrate and a polymer (natural or synthetic). Being that, adhesion between surfaces occurs due to interfacial forces. when the biological surface is a mucosa, the bioadhesion is called mucoadhesion [41,42]. Thus, *ex vivo* mucoadhesion studies were performed to assess the adhesion strength of pure and modified gum. CG and AMCG showed adhesion strengths of 74.78 ± 1.09 mN and 81.85 ± 0.17 mN, respectively. CG mucoadhesiveness is related to the presence of carboxyl and hydroxyl groups in its polymer chain. These groups favor mucoadhesion through hydrogen with the mucosa [43]. The AMCG polymer showed a higher adhesion strength than CG because in addition to carboxyl and hydroxyl groups, the modified polymer has amino groups in its polymeric chain. AMCG can interact with mucin through electrostatic interactions/hydrogen bonding between amino groups of aminated AMCG and glycoproteins of mucin, thus resulting in stronger adhesion [44]. On the other hand, amino groups incorporated onto the CG surface after chemical modification lower the negative charge of the polymer (as shown in the zeta potential) and favor adhesion to the mucosa by electrostatic interactions, since it has a negative character [45, 46]. An enhanced mucoadhesiveness of aminated polysaccharide gums has been previously described for guar gum [47] and gellan gum [39]. AMCG in water can lead to a complexation reaction between amino groups and water molecules conducting NH_3^+-OH^-.This complexation reaction retaining water molecules within the matrix of aminated gum can provide hydration. Therefore, not only a higher electrostatic attraction between the amino groups of the modified gum and mucus but also a benefic hydration must be considered [39].

3.5 Antibacterial activity against the standard strain of *Staphylococcus aureus*

Fig. 7 shows the results of the antibacterial activity against *Staphylococcus aureus* bacteria (ATCC 25923) of CG and AMCG by the direct contact method. CG showed an inhibitory effect against S. aureus of 69.27 ± 1.39%, and after chemical modification, this effect increased to 89.77 ± 2.90%. These results are related to the presence of amine groups in CG and AMCG. Studies with cationic polysaccharides, such as chitosan, showed antimicrobial potential caused by the amino groups present in their structure due to electrostatic interactions with teichoic acids, a major class of bacterial surface glycopolymers. This interaction destabilizes and consequently compromises the functioning of the membrane, promoting a leakage of intracellular components into the extracellular environment that results in cell death [48,49,50]. Thus, it is assumed that the amino groups inserted in the structure of CG, similar to what occurs with chitosan, may have interacted electrostatically with the negative groups present in the membrane of the *S. aureus* bacteria (teichoic acids), which consequently caused a better antibacterial action against a gram-positive bacteria compared to the unmodified CG.

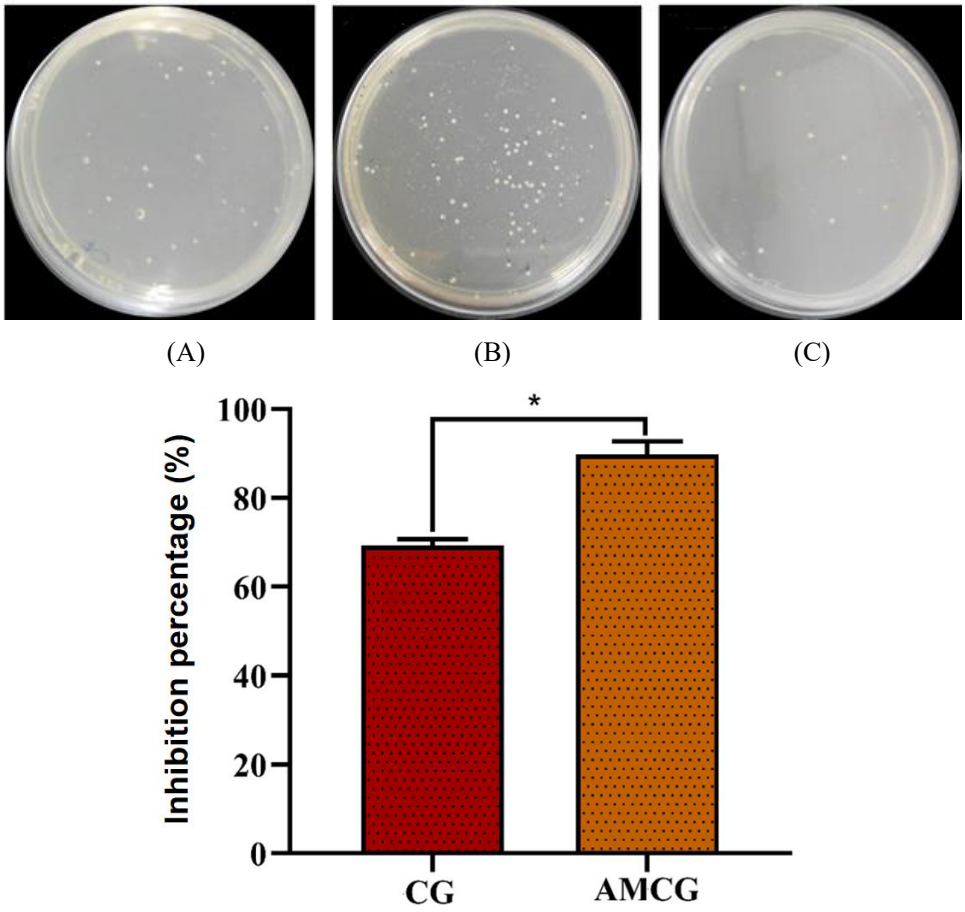


Fig. 7. Direct contact testing for CG and AMCG with the standard strain of *Staphylococcus aureus* bacteria (ATCC 25923), (A = CG, B = Control, and C = AMCG).

3.6 Inhibitory activity of α-glucosidase

α-Glucosidase is an enzyme that hydrolyzes carbohydrates into monosaccharides that are easily absorbed in the gastrointestinal tract and is responsible for the increase in postprandial blood glucose. Thus, inhibition of α-glucosidase is an important factor in digestion/hydrolysis of carbohydrates and has been studied as an alternative to control and reduce blood glucose levels [21,51]. Both unmodified and aminated CG were able to inhibit this enzyme; the maximum inhibition occurred at a concentration of 125 µg/mL, and the highest concentrations tested (250, 500 and 1000 µg/mL) did not cause an increase in inhibition. CG and AMCG presented similar inhibitory results (CG = 9.74% and AMCG = 8.67%), indicating that the chemical modification did not significantly affect the inhibitory effect of the gum against the α-glucosidase enzyme.

3.7 Antioxidant capacity

Antioxidants are bioactive molecules that have the ability to maintain the structure and function of cells through the elimination of free radicals, inhibition of lipid peroxidation reactions

and prevention of other oxidative damage. These also play an important role in the body's defense mechanisms against pathological processes caused by oxidative stress and have been considered important in the prevention of chronic diseases such as cancer, cardiovascular disease and diabetes [52,53,54].

Thus, the antioxidant potential of CG and AMCG gums was evaluated. The gums presented a minimum inhibitory concentration (CG = 302.76 µg/mL and AMCG = 316.80 µg/mL) lower than quercetin (380.00 µg/mL), which is a natural compound recognized in the literature for its antioxidant potential, thus revealing a great antioxidant potential for the studied gums. Gums exuded from plants are raw materials that have been the object of study based on evidence of their bioactive properties. In addition to being low cost, they are widely available in nature and are considered reliable for consumption, as they are nontoxic [52].

3.8 Effect of CG and AMCG gum on the viability of Caco-2 cells by the MTT test

The cytotoxicity of CG and AMCG gums on Caco-2 cells was evaluated by the MTT reduction assay. Aminated Chicha gum (AMCG) had a higher cell viability than Chicha gum (CG) at concentrations of 62.5 and 125.0 µg/mL (Fig. 8). However, at higher concentrations (250.0, 500.0, and 1000.0 µg/mL), the opposite occurred: AMCG cell viability was reduced, and CG had higher cell viability.

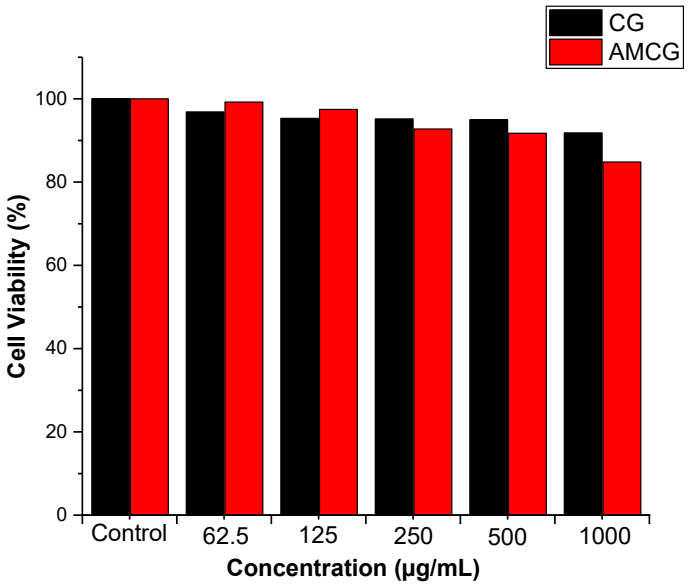


Fig. 8. Cell viability by MTT test.

Cytotoxicity analysis is a prerequisite for assessing the biocompatibility of biomaterials. In accordance with the guidelines of ISO 10993-5, a material is considered toxic when cell viability decreases below 70% of the cell viability of the control group [11,55]. The

experimentally found viability difference values compared to the control were below 10% for all tested CG concentrations. AMCG gum also showed high values of cell viability at the concentrations tested, except at 1000 μg/mL, with cell viability 15.2% lower than that of the control group. Thus, the results obtained indicate that the tested gums do not have cytotoxic potential and are thus a valid and safe alternative for biomedical applications.

4. Conclusions

In this study, the amination of *Sterculia striata* gum was accomplished by an easy and simple synthetic strategy and confirmed by FTIR spectroscopy. Chicha gum viscosity and zeta potential varied as a function of pH. Further characterization results revealed a lower zeta potential and molar mass values for modified AMCG when compared to the native CG. The modification of the polysaccharide increased CG mucoadhesive and antimicrobial properties, thus suggesting their use in the pharmaceutical industry for application in wound dressings, gels and in pharmaceutical formulations as excipients for drugs that have poor permeability and low retention in mucosa. Both native and modified gums showed antioxidant capacity and inhibitory effects against the α-glucosidase enzyme, and no cytotoxic potential was found.

While studies on their complete viscosity characterization including AMCG sol-gel properties still need to be performed toward their use in drug delivery systems, amine-modified CG improved mucoadhesive and antimicrobial properties against *Staphylococcus aureus* and broadened gum application potential in the pharmaceutical field,

These properties can also be attractive for applications related to the food industry as adhesiveness, providing modulation of food organoleptic properties, and as antimicrobial agents in the production of films while protecting food against spoilage and pathogens.

Declaration of Competing Interest

None.

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