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Opuntia ficus-indica mucilage reduces wheat starch *in vitro* digestibility

El mucílago de *Opuntia ficus-indica* reduce la digestibilidad *in vitro* del almidón de trigo

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Abstract

Nopal (*Opuntia ficus-indica*) is traditionally used in Mexico for the treatment of diabetes mellitus. Several clinical studies have reported an anti-hyperglycemic effect from the plant, but the underlying mechanisms are still controversial. In this study, the potential beneficial effects of using nopal juice for controlling glucose release during the hydrolysis of starch molecules, and the possible mechanisms of action involved, were explored using *in vitro* testing. To this end, wheat starch dispersions (3 g/100 g water) were gelatinized, subjected to either hydrochloric acidic (pH 2.0) or α-amylase (pH 6.9) hydrolysis, and glucose production was monitored. Nopal mucilage (NM) extracted from nopal cladodes was added to starch dispersions which were completely gelatinized. NM reduced the acidic hydrolysis of starch by about 50%, although NM also contributed to glucose production. Some insights pointed out to a mechanism involving the formation of electrostatic complexes between starch and nopal polysaccharides, which are resistant to acidic hydrolysis. On the other hand, nopal hydrolysis also reduced the amylolytic starch hydrolysis by 40%, possibly due to the formation of enzyme-polyphenol complexes which hindered the action of the enzymes upon the starch molecules, plus the fact that the α-amylase was unable to hydrolyze the nopal mucilage. Thus, the formation of different types of complexes produced mechanical hurdles that hindered starch hydrolysis and glucose production.

Keywords: *Opuntia ficus-indica*; *in vitro* digestion; hydrolysis kinetics; amylolytic enzymes.

Resumen

El nopal (*Opuntia ficus-indica*) es tradicionalmente utilizado en México para el tratamiento de la diabetes mellitus. Varios estudios clínicos han informado un efecto antihiperglucémico de la planta, pero los mecanismos subyacentes aún son controvertidos. Este estudio utilizó pruebas *in vitro* para explorar el posible mecanismo de acción del nopal para controlar la liberación de glucosa por hidrólisis de moléculas de almidón. Para esto, se hicieron dispersiones de almidón de trigo (3 g/100 g de agua) que se gelatinizaron, y se sometieron a hidrólisis con HCl (pH 2.0) o con α-amilasa (pH 6.9), monitoreando la producción de glucosa. Se agregó jugo extraído de cladodios de nopal para evaluar sus efectos en la digestibilidad de almidón. El jugo de nopal redujo la hidrólisis ácida del almidón en aproximadamente un 50 %, aunque también contribuyó a la producción de glucosa. El mecanismo responsable de esto parece ser la posible formación de complejos electrostáticos entre el almidón y los polisacáridos de nopal que son resistentes a la hidrólisis. Por otro lado, También se redujo la hidrólisis amilolítica del almidón en un 40%, careciendo la α-amilasa de capacidad para hidrolizar el jugo de nopal. Se sugiere que en este caso el mecanismo de inhibición de la hidrólisis es debido a la formación de complejos enzimáticos con polifenoles que actúan como una barrera mecánica a la acción de la enzima, resultando en una menor producción de glucosa.

Palabras clave: *Opuntia ficus-indica*, digestión *in vitro*, cinética de la hidrólisis, enzimas amilolíticas.

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1 Introduction

Nopals (*Opuntia ficus-indica*) are a family of plants belonging to the Cactaceae, which grow in semi-arid and arid environments by forming large cladodes. Nopal has diverse uses in traditional gastronomy (Valencia-Sandoval *et al*., 2010), animal forage (Torres-Ponce *et al*., 2015), and as building material (Ventolà *et al*., 2011). For instance, nopal juice is known to have used in stucco formulations by ancient Meso-American civilizations (Montes *et al*., 2005). Besides, recent proposed applications include inhibition of metal corrosion (Torres-Acosta, 2007), edible coating for food (Dominguez-Martinez *et al*., 2017), wall material for spray-drying microencapsulation (Cortés-Camargo *et al*., 2017) and remover of heavy metals (Tejada-Tovar *et al*., 2021). The most interesting attractiveness of nopal is perhaps its use as a traditional remedy to deal with the adverse effects of Type-2 diabetes. In fact, the anti-hyperglycemic action of nopal in ancient Mexico was documented in the 16th-century Florentine Codex by F. Hernandez. Nopal has been reported as a source of bioactive compounds for nutrition improving or protecting health and treating diseases (Espinosa-Solares and Domínguez-Puerto, 2023). Nopal juice and dried extracts are commonly recommended by nutritionists and naturists as a complement to deal with nutrition problems and diabetes symptoms. Clinical studies have been conducted to elucidate the ability of nopal to treat Type-2 diabetes patients (Shane-McWhorter, 2001, 2005). For instance, some results have shown that extracts of *Opuntia streptacantha* cladodes administered to STZ-diabetic rats did not produce a significant hypoglycemic effect, while the same extracts produced an anti-hyperglycemic effect (Andrade-Cetto and Wiedenfeld, 2011). It has been also found that flours obtained from small- and medium-size cladodes of *Opuntia ficus-indica* reduced postprandial blood glucose in 46.0 and 23.6%, respectively (Nuñez-Lopez *et al*., 2013). The mechanisms behind these effects are not clear, although it was postulated that the reduction of glucose in the plasma is likely caused by entrapment of amylolytic enzymes and delay of intestinal absorption. In this context, the viscosity is apparently a prominent factor directly affecting the antidiabetic potential based entrapment mechanisms where polysaccharides isolated from *Opuntia* sp. showed discrete antihyperglycemic effects attributable to a fiber and the viscosity that contribute in the medium (Nuñez-López *et al*., 2013). Many researchers have described the dietary factors that can mitigate the symptoms of diabetes. Of these, dietary fibers and resistant starch have been reported to have beneficial effects in lowering the glycemic level in serum by increasing the growth and function of the upper gastrointestinal tract as well as the plasma levels of the intestinotrophic factor, glucagon-like peptide, lowering the insulin response, and slowing glucose absorption through an effect on gastric emptying and/or entrapment of materials in the viscous digesta (Ou *et al*., 2001). According to Benítez *et al*. (2017) the inhibition of alpha-amylase activity and the decrease of starch digestibility might be attributed to several factors, such as fiber concentration, starch, or enzyme entrapment by fibers, thereby reducing the accessibility of the enzyme to starch, presence of inhibitors in fiber matrix, or direct adsorption of alpha amylase onto fiber, the entrapment of the enzyme reduced overall enzymatic activity and consequently reduced starch hydrolysis (Colosimo *et al*., 2020). Results based on monitoring the α-glucosidase activity showed that the total extract and the juice of the nopal produced an anti-hyperglycemic effect (Becerra-Jiménez and Andrade-Cetto, 2012). It was suggested that viscosity is an important factor directly affecting the anti-diabetic potential by acting as a barrier for the enzymatic action (Nuñez-López *et al*., 2013). The mechanisms underlying the anti-hyperglycemic ability of extracts (either flour or juice) of nopal cladodes have not been clarified (Becerra-Jiménez and Andrade-Cetto, 2012). Information in this line should provide valuable insights for designing products oriented to deal with the adverse nutritional effects of metabolic syndrome in general, and diabetes disease. To this end, the interactions between amylolytic enzymes and starch chains in the presence of nopal mucilage could provide some guidelines for elucidating the inhibitory effects of opuntia extracts in amylolytic hydrolysis. In this regard, functional groups in the mucilage as hydroxyl (-OH) groups are essential for the inhibitory activity of non-hydrolysable polysaccharides as occurring in other inhibitory compounds like flavonoid compounds against α-amylase, where the inhibition is likely to depend on the formation of hydrogen bonds between -OH groups and side chains of amino acids (such as Asp197 and Glu233) at the active site of α-amylase (Kawamura-Konishi *et al*., 2012; Lo Piparo *et al*., 2008; Sun *et al*., 2016). Medina Torres *et al*. (2011) reported the presence of polyphenols in nopal, which may display competitive and non-competitive inhibitory effects with α-amylase. The aim of this work was to explore link between physicochemical properties of cladode juice and the *in vitro* digestibility properties of starch hydrogels. The motivation relies on the fact that a large fraction of human dietary is composed by cooked starch from a given botanical source. The study explores the hypothesis that both physical and chemical mechanisms are involved in the effects of opuntia juice on the starch digestion. Relative to previous reports, our work provides insights on the mechanisms involved in the inhibitory effect of opuntia juice in the acidic and amylolytic hydrolysis of starch chains.

2 Materials and methods

2.1 Materials

Opuntia ficus-indica cactus cladodes were purchased from a local market (WalMart, Mexico City, Mexico). Native wheat starch was acquired from Sigma-Aldrich (CAS 9005-25-8, amylose 28.4%, St. Louis, MO, USA). Deionized water was used for all experiments.

2.2 Mucilage extraction

Nopal cladodes were washed with distilled water and cut in small pieces of 1×1 cm. A fraction of mucilage was extracted following a protocol previously reported by León-Martínez *et al*. (2010), with slight modifications. Briefly, cladodes were weighed and put into a container with distilled water in the ratio of 1:2 at 80 °C for 3 hours with gentle agitation. Then, the mixture was filtered through a 200-mesh plastic sieve. Additionally, centrifugation at 6,000 rpm for 10 min was applied to obtain a homogeneous extract (supernatant). Finally, the extracted mucilage (NM) reached a concentration of 2° Brix (2g/100 g of solution) determined with a refractometer. A fraction of the mucilage was air-dried at 30 °C for 24 h for analysis requiring dried samples (FTIR).

2.3 Gelatinized starch dispersions

In order to elucidate the effects of NM, wheat starch (3% w/w) was dispersed either in water or in a mixture of nopal mucilage + water, and the mixtures were heated at 92 °C under gentle stirring for 25 min for allowing a complete gelatinization of starch granules, to yield wheat starch gels coded SG and NM+SG, respectively. The gelatinized starch dispersions were let to rest for one hour at room temperature, before their use for analysis to minimize possible adverse effects caused by starch retrogradation.

2.4 Average molecular weight

The average molecular weight of the mucilage was determined by static light scattering (SLS) using a Zetasizer Nano ZS (Malvern Instruments, UK). Samples at different concentrations of mucilage were studied using the Rayleigh equation, which provides a relation between intensity of scattered light and molecular weight, given by

$$\frac{KC}{R_\theta} = \frac{1}{M} + 2A_2C \tag{1}$$

Here, M is the weight-averaged molecular weight, A_2 is the second virial coefficient, is the sample concentration, K is the optical constant and R_θ is the Rayleigh ratio (www.materials-talks.com). Toluene was used as reference. The weight-average molecular weight was expressed in Daltons.

2.5 Thermogravimetric analysis (TGA)

Dried mucilage samples (4.5±0.05 mg) were placed on the pan of a TGA 2950 (TA Instruments, New Castle, USA) and scanned from 0 to 600 °C at a 10 °C/min scanning rate. Results were used to obtain the rate weight loss (W/W_0) and derivative of weight loss as a function of temperature. All experiments were replicated two times for each formulation and averages were used.

2.6 Images and optical microscopy

The nopal mucilage was insolubilized with acetone in a ratio 1:2 and was observed with an optical microscope (Olympus BX45, Tokyo, Japan) coupled to an image analyzer system (digital Olympus camera C3030 and Image Pro-Plus 4.5 software, Media Cybernetics, Inc., Rockville, MD, USA). Selected micrographs at 4, 10 and 40× are used for illustration purposes.

2.7 Apparent viscosity

The apparent viscosity as function of the shear-rate for mucilage and gelatinized starch dispersions were determined with a Kinexus Pro rheometer (Malvern Instruments Ltd., UK) equipped with a double gap cylindrical geometry (Couette, DG25), by applying an increasing logarithmic shear rate from 0.01 to 1000 s^{-1}. All measurements were conducted at 37 °C (i.e., conditions for hydrolysis tests). The samples were equilibrated for 5 min for structure recovery.

2.8 Particle size distribution

The particle size distribution of mucilage and gelatinized starch dispersions were determined with a laser light scattering analyzer Mastersizer 2000 (Malvern Instruments Ltd., Malvern, UK). Samples were dispersed using HYDRO 2000MU unit at obscuration 10-20%. Measurements were carried out at room temperature. Absorption parameter and refractive index were set to 1.331 and 1.337 respectively. The mean particle size was expressed as the volume weighted mean diameter, D[4,3]. These parameters were determined at 1, 2, 4 and 6 hours after preparations for evaluating the retrogradation effects (García-de la Rosa *et al*., 2023).

2.9 ζ-potential

The electrophoretic mobility was measured with a Zetasizer Nano ZS (Malvern Instrument, UK) by laser Doppler electrophoresis. The ζ-potential was obtained from the electrophoretic mobility by the Smoluchowski approximation. One milliliter of gelatinized starch dispersions was placed in a dip-cell. Measurements were carried out as function of pH for nopal mucilage, wheat starch gel and the blend of them. Measurements were conducted after 5 h of preparation of dispersions for allowing equilibration (García-de la Rosa *et al*., 2023).

2.10 *ATR-FTIR spectroscopy*

Nopal mucilage and gelatinized starch dispersion samples were characterized by ATR-FTIR spectroscopy according to the methodology reported by van Soest *et al*. (1995). The spectra of gelatinized dispersions were measured at 1, 2, 4 and 6 hours after preparations for evaluating the effects on 1047/1022 and 995/1022 ratio. FTIR spectra were recorded on a Perkin Elmer spectrophotometer (Spectrum 100, Perkin Elmer, Waltham, MA, USA) equipped with a crystal diamond universal ATR sampling accessory. Each spectrum represented an average of four scans. All spectra were deconvoluted using Gaussian and Lorentzian functions. In this case, the assumed line shape was Lorentzian with a halfwidth of 15 cm^{-1}. The resolution enhancement factor was chosen as 1.5 (García-de la Rosa *et al*., 2023).

2.11 *Acidic hydrolysis*

Acidic hydrolysis tests were carried out on mucilage and gelatinized starch dispersions. Samples were dispersed in an aqueous hydrochloric acid solution (50 mL, pH 2) by stirring and keeping at 37 °C for 120 min. The degree of hydrolysis was related to the amount of glucose released at different times.

2.12 *Enzymatic hydrolysis*

Enzymatic hydrolysis of the nopal mucilage and gelatinized starch dispersions was performed as follows. Samples were put in 50 ml of phosphate buffer (pH 6.9) and incubated with 0.16 mg of porcine pancreatic α-amylase (A3176, Sigma Aldrich, St. Louis, MO, USA) enzyme at 37 °C for 120 min. The hydrolysis advance was determined measuring the reducing sugar content released by DNS technique at different times.

2.13 *Statistical analyses*

The experimental data were analyzed by means of one-way analysis of variance (ANOVA) with the SPSS Statistics 19.0 package.

3 Results and discussion

3.1 *Nopal mucilage characteristics*

Optical images are used to illustrate the morphology of the solids (e.g., polysaccharides) contained in the nopal juice. Figures 1.a-1.c show images of three different magnifications (10-40×) of fresh nopal juice. Transparent large, entangled fibers can be observed for all magnifications. Nopal juice contains a relatively low amount of lignin (less than 4%), and the main fractions are polysaccharides (~70%), including cellulose (~20%) (Malainine *et al*., 2003). Fibers observed in images can be ascribed to cellulose and high-molecular weight polysaccharides, including cellulosic material. In fact, Felkai-Haddache *et al*. (2016) reported that the polymers composing the nopal mucilage for Opuntia from Algeria were high molecular weight polymers ranging from 15.3 to 15.7×10^6 g/mol, and the rest are low molecular weight polymers that can act as a polyelectrolyte. The mean molecular weight estimated in this work by SLS was $7.41\pm1.23\times10^6$ g/mol, a result that is in line with reports for mucilage extracted from Opuntia commercialized in Mexico City (Medina-Torres *et al*., 2011). The mean molecular weight of NM depends strongly on the geographical region, the harvesting conditions, and the age of the cladodes. Additionally, TGA pattern of the solids obtained from the nopal mucilage is presented in Figure S.1 of the Supplementary Material (Appendix A). Following Rodríguez-González *et al*. (2014) a more accurate characterization of the peaks in the first-derivative curve can be obtained by means of deconvolution methods. The corresponding first-derivative is presented in Figure S.1b. The first-derivative curve presented several well-defined peaks, which can be ascribed to water vaporization and decomposition of several polysaccharides associated to reported decomposition temperatures, thus galactose and arabinose (Ramos-Sanchez *et al*. 1998), xylose (Werner *et al*., 2014), galacturonic acid and β-glucans (Morina *et al*., 2014) were identified. On the other hand, Medina-Torres *et al*. (2011) reported that nopal mucilage contained 6.0, 2.08, 0.89 and 0.12 g/100 g db. of polyphenols, flavonoids, flavonols and β-carotenes, respectively. Flavonols are composed mainly by quercetin, kaempferol and isorhamnetin.

3.2 *Apparent viscosity*

The behavior of the apparent viscosity with the shear-rate of the nopal mucilage is presented in Figure 2.a. For low shear-rate values (<0.05 s^{-1}), the viscosity follows a shear-thinning behavior, while for higher shear rate values the behavior tends to become Newtonian. The case of the starch dispersion is similar, with an apparent Newtonian behavior starting at about 0.35 s^{-1}. The combination of NM+GS for preparing the dispersion for hydrolysis led to a shear-thinning behavior with three distinct regions. The faster decay of the apparent viscosity was observed for high shear-rate values (>100 s^{-1}). The combination of nopal mucilage and gelatinized starch increased the apparent viscosity of the dispersion, and the increase was about two times relative to the viscosity of the starch dispersion. The increase of

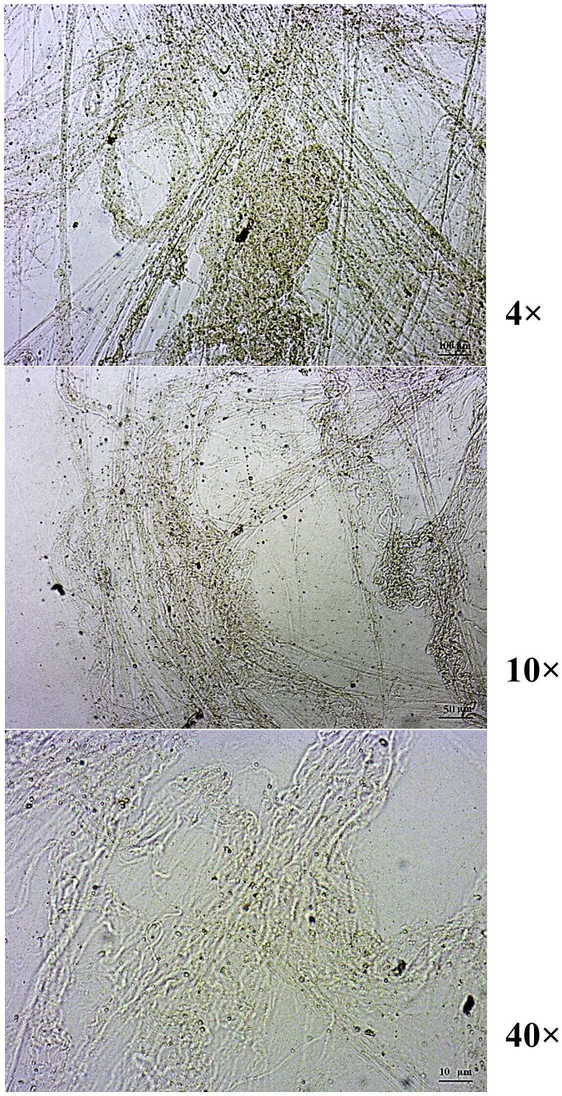

Figure 1. Optical images of nopal mucilage for three different magnifications: (a) 4×, (b) 10×, and (c) 40×.

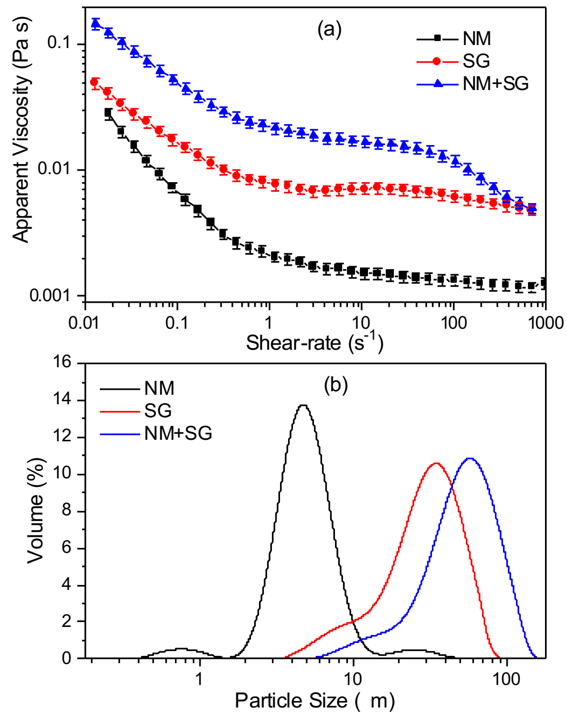

Figure 2. (a) Apparent viscosity, and (b) particle-size distribution of nopal mucilage (NM), gelatinized starch dispersion (SG) and the blend of nopal mucilage with gelatinized starch (NM+SG).

viscosity can be ascribed to the increase of total solids after the addition of nopal mucilage and to the entanglement of starch insoluble remnants by effect of nopal mucilage fibers (see Figure 1). Apar and Özbek (2005) showed that viscosity has an important effect in the hydrolysis of starch dispersions. In fact, an increase in viscosity of about five times led to a huge decrease of the starch advance, from about 80% to about 20%.

3.3 Particle-size distribution

Figure 2.b displays the PSD of the fresh nopal mucilage, showing multimodality with a large peak at about 4.92 μm, and two small peaks at about 0.72 and 26.3 μm. In contrast, the starch gel showed a shoulder at about 8.5 μm, and a large peak at about 35.0 μm. The large peak can be ascribed to insoluble remnants, also known as ghosts, corresponding to complex structures found after gelatinization (Debet and Gidley, 2007). The incorporation of the nopal mucilage into the starch gel shifted the particle size distribution to the right as the large peak was exhibited at about 58.74 μm. It is suggested that the increased size can be explained by the entanglement between the starch insoluble remnants and the hemi-cellulose fibers of the nopal mucilage. In turn, the formation of a complex network between the starch structures and the nopal mucilage fibers induced the increased viscosity observed in Figure 2.a.

3.4 FTIR

Figure 3.a presented the FTIR spectra of the lyophilized nopal mucilage, with some distinctive bands highlighted. The bands at 1462 and 1153 cm^{-1} are ascribed to C-H alkenes and -OH aromatics and have been linked to the presence of catechin residues (Haruenkit *et al*., 2010). On the other hand, the large band at 1618 cm^{-1} together with the smaller bands at 1625 and 1242 cm^{-1} has been related with the presence of gallic acid (Haruenkit *et al*., 2010). The band at 1327 cm^{-1} has been found for cellulose and hemi-cellulose fractions (Yang *et al*, 2007). The FTIR spectra of aqueous dispersions are exhibited in Figure 3.b.

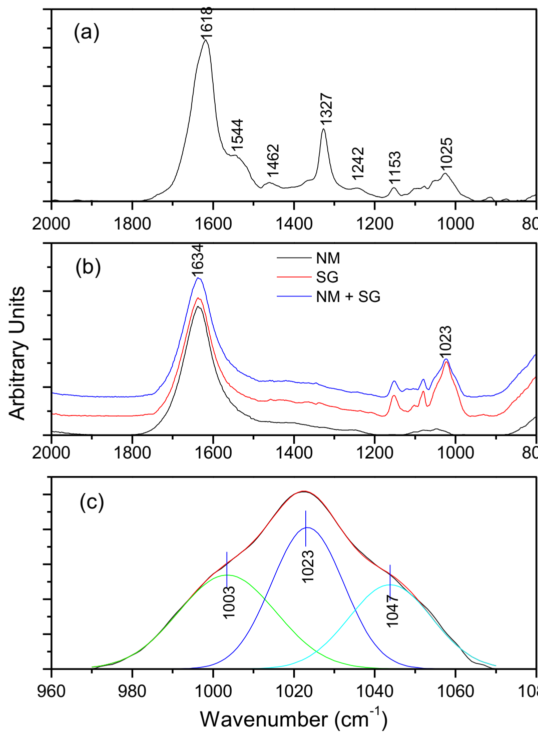

Figure 3. (a) FTIR spectrum of lyophilized nopal mucilage. (b) FTIR spectrum of fresh nopal mucilage (NM), starch gel (SG) and the combination of them (NM + SG). (c) Illustration of deconvolution of the region 960-1080 cm^{-1}.

The wide band at 1634-1650 cm^{-1} corresponds to free water (-OH scissors) (), reflecting the large amount of water contained in all dispersions. The nopal mucilage dispersion exhibited a wide band in 1120-1000 cm^{-1}, reflecting the presence of gallic acid and hemi-cellulose. The gelatinized starch dispersion exhibited the fingerprint region (∼950-1100 cm^{-1}, C-O-C interactions) peaking at about 1023 cm^{-1}, which also appeared under the presence of the nopal mucilage. The gelatinization of the starch granules induced swelling and eventually leaching out of amylose chains, which tend to re-crystallize to form double- and triple-helix structures (Wang *et al*, 2015). The spectrum at this region is composed of several overlapping effects, which were studied by van Soest *et al*. (1995). In fact, it was found that the band at about 1048 cm^{-1} is sensitive to ordering content, while the band at about 1022 cm^{-1} can be attributed to vibration of amorphous components. In this regard, the ratio R1047/1022 was considered as indication of the short-range ordering of starch chains dispersed into the aqueous dispersion. The spectrum band can be deconvoluted to extract the individual contributions, as shown in Figure 3.c. The band at about 1005 cm^{-1} reflects the presence of hydrated (i.e., bonded water) structures. The deconvolution of the starch gel spectrum gave R1047/1022=0.68±0.03, while the deconvolution with the presence of nopal mucilage gave R1047/1022=0.43±0.04. This means that the nopal mucilage obstructed partially the formation of crystalline structures. This result is not surprising at all since several studies have reported that hydrocolloids have the ability of retarding the retrogradation process of starch chains (He *et al*., 2015).

3.5 ζ-potential

The ζ-potential provides the net surface charge of a particle, as well as being an important factor in determining the grade of interaction (repulsion or attraction) between polymers or colloidal materials (García-de la Rosa *et al*., 2023). The ζ-potential as function of the pH was measured for nopal mucilage and starch gel. Nopal mucilage exhibited negative charge over the whole pH range. In contrast, the wheat starch dispersion showed iso-electric point at about 3.2 mV, which is in line with previous report by Marsh and Waight (1982). It is known that wheat starch granules carry out lipids and proteins on the surface. After gelatinization, lipids and proteins are retained by insoluble remnants, conferring structural stability to ghosts (Debet and Gidley, 2007). The positive charge below pH 3.2 could be due to amino groups in the surface lipids and proteins. Marsh and Waight (1982) found that the treatment of wheat starch granules with $CHCl_3$/MeOH reduced the positive charge, postulating that lipid carboxyl, phosphate and amine-groups are responsible of the positive charge. The blending of nopal mucilage and gelatinized wheat starch led to a behavior between the two components, with negative charge over the whole pH range. This behavior suggests some interactions between fractions of nopal mucilage and wheat starch. For pH 2.0 (condition for acidic hydrolysis), interactions between nopal mucilage with negative charge and wheat starch with positive charge are likely to occur via electrostatic interactions. Complex coacervation between some fractions of the nopal mucilage and proteins and lipids present on the surface of insoluble remnants (Debet and Gidley, 2007) can be found when the two components are blended (Espinosa-Andrews *et al*., 2007). In contrast, nopal mucilage and wheat starch exhibited anionic function for pH 6.9 (condition for enzymatic hydrolysis), such that the formation of complexes is unlikely to occur.

3.6 Hydrolysis

The effect of the addition of nopal mucilage in the hydrolysis of gelatinized wheat starch was assessed for acidic and enzymatic conditions. Figure 5.a presents the kinetics for nopal mucilage, showing that hydrochloric acid is able to hydrolyze the polysaccharides contained in the mucilage. That is, the action of the hydrochloric acid also contributed to de-polymerization of the starch chains, and hence to the production of glucose. Also is shown the case for gelatinized wheat starch. The combination of nopal mucilage and gelatinized starch produced more glucose than the individual cases. However, the sum of the individual hydrolysis

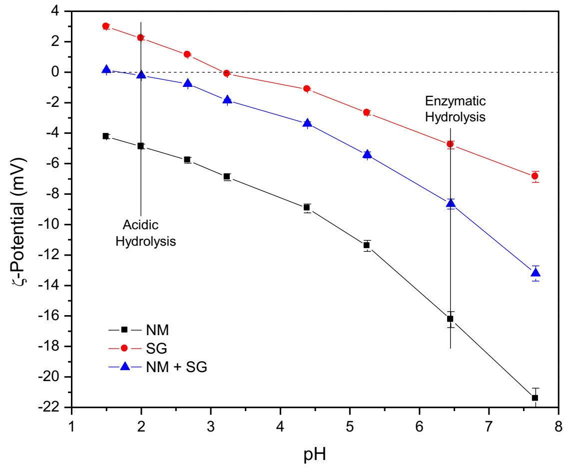

Figure 4. ζ-potential pattern as function of pH for nopal mucilage (NM), starch gel (SG) and the combination of nopal mucilage and starch gel (NM + SG).

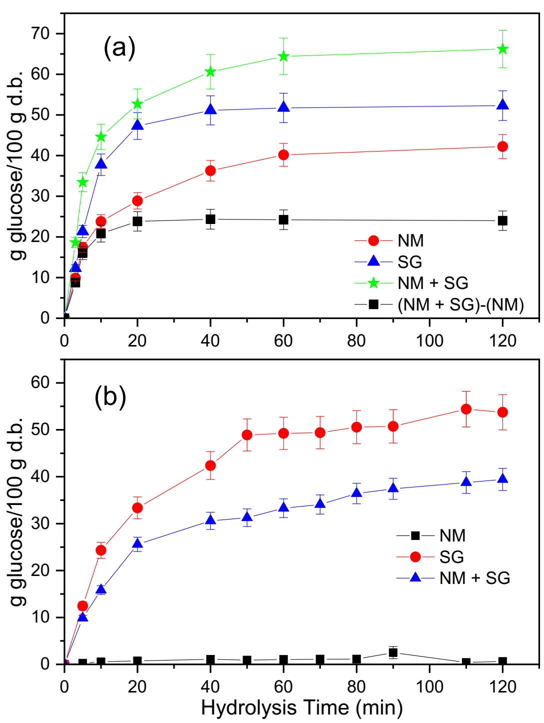

Figure 5. (a) Acidic hydrolysis (pH 2.0), and (b) enzymatic hydrolysis (pH 6.9) for nopal mucilage (NM), starch gel (SG), blend of NM and SG (NM+SG). The notation (NM+SG)-(NM) denotes that the result of NM was subtracted from the NM+SG case.

responses was higher than the glucose production by the combination of nopal mucilage and starch gel, indicating that the incorporation of the nopal mucilage hindered in some extent the hydrolysis of starch chains. The results in Figure 4 suggest the formation of complexes between the starch components and the nopal mucilage fractions, resulting in the presence of hydrolysis resistant starch fractions. The increased viscosity of the nopal mucilage and starch gel blend (Figure 2.a) can also affect the mobility of hydrogen ions, limiting the transport to the glycosidic sites of the starch chains.

Figure 5.b presents the results of the enzymatic kinetics. The nopal mucilage was poorly hydrolyzed by amylolytic enzymes, leading only to marginal production of glucose. As expected, amylolytic enzymes were effective hydrolyzing starch chains as reflected by the large production of glucose. However, the incorporation of nopal mucilage reduced the production of glucose by about 40% after 120 min. This result suggests that nopal mucilage may have an anti-hyperglycemic effect during the metabolism of wheat starch chains (Andrade-Cetto and Wiedenfeld, 2011). The reduction in glucose formation cannot be ascribed completely to the formation of complex coacervates (via electrostatic interaction) since both, the nopal mucilage and the starch gel exhibited negative charge in hydrolysis conditions (pH 6.9). On the other hand, for such conditions the α-amylase exhibits negative charge (Salgin *et al*., 2012), such that the formation of complexes between the enzyme and polysaccharides of nopal mucilage can be discarded. The results in Figure 2 suggest that increased particle size and apparent viscosity could obstruct partially the transport of enzyme molecules to the binding sites in the starch chains (Campos-Montiel *et al*., 2021). However, Sasaki and Kohyama (2012) found that the starch digestion rate was hardly altered by increasing viscosity above a critical value. Hence, one can suppose that hydrogen bonding and/or other interactions between the enzymes and some fractions of the nopal mucilage are a major determinant for the decreased starch digestion rate. Bai *et al*. (2017) reported that amyloglucosidase can interact with pectin via electrostatic forces or hydrogen bonding, an effect that limited the digestibility rate of starch. Wu *et al*. (2017) reported green tea polysaccharides can inhibit the activity of α-amylase via the formation of complexes with acidic polysaccharides. According to such findings, the hydroxyl and carboxylic acid groups in the branched chains of tea polysaccharides would generate strong hydrogen bonding interaction with the amino acid residues of α-amylase, resulting in the formation of polysaccharide/α-amylase complexes. Given the diversity of non-starch polysaccharides contained in the nopal mucilage (Table S.1), it is possible that the inhibitory effect presented in the enzymatic digestion of starch can be caused by the formation of complex species with the α-amylase.

Conclusions

Nopal mucilage (*Opuntia ficus-indica*) exhibited inhibition of wheat starch hydrolysis for acidic and enzymatic conditions. Interaction between some nopal mucilage fractions and starch for pH 2 led probably to the formation of complexes via electrostatic attraction, yielding resistant starch that withstood the acidic attack. On the other hand, the formation of complexes between α-amylase and polysaccharides from nopal mucilage could be behind the enzymatic inhibition, and hence the reduction of glucose production at pH 6.9. The increased particle size and viscosity of starch gel/nopal mucilage blends had also some effect in the inhibitory effects as mobility of the hydrolysis agent (either hydrogen ions or enzymes) is negatively affected. The results of the present study have provided interesting insights into the interaction of nopal mucilage with wheat starch and could be used for improving the understanding of the mechanisms responsible for the inhibitory activity of nopal mucilage.

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