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Abstract: The edible leathers, produced by puree of fruits or vegetables, stand out for their nutritional and functional properties. This study aimed to produce and characterize *leather* made from *Pereskia aculeata* Mill. (ora-pro-nóbis) leaves, with different concentrations of carrageenan gum, to evaluate its potential as a functional leather and the impact of carrageenan gum on product properties. The *leathers* were produced by *tape-casting* using a fixed puree concentration (40 g/100 g) and different carrageenan gum concentrations (1.0, 1.5, and 2.0 g/100 g), then dried at 40 °C for 7 hours. The products were characterized for centesimal composition, total phenolic compounds, antioxidant capacity, physico-chemical properties, mineral composition, chlorophyll content, carotenoids, cytotoxicity, and stability of bioactive compounds during storage. Additionally, the product's sensory analysis was conducted. The leather has high nutritional value, particularly in protein and fiber content. No significant variation was observed in pH, Aw, or leather thickness with the addition of carrageenan gum. The formulation with a minor carrageenan gum concentration showed the best antioxidant properties and the greatest preservation of bioactive compounds during the stability evaluation. The sensorial analysis indicated potential consumer acceptance, with intermediate values for the evaluated attributes. Overall, incorporating carrageenan gum contributes to developing leathers that are nutritious, safe, and nutritionally promising, highlighting ora-pro-nóbis as a promising raw material.

Keywords: Bioactive compounds; functional food; health snacks; protein.

1. INTRODUCTION

Hunger is a major global challenge and is related to food insecurity and malnutrition caused by unbalanced nutrient intake. According to the World Food Program [1], eradicating hunger remains a distant global challenge to be achieved by 2030. Despite the growth in food production, a large portion of the world's population cannot meet their basic micronutrient needs. This reality highlights not only unequal access to nutritious foods, but also the expansion of low-quality diets that promote deficiencies.

Micronutrient deficiencies and low-quality diets do not occur in isolation; several factors drive them, including population growth, economic crises, and extreme weather events [2] According to the United Nations Department of Economic and Social Affairs, Population Division [3], the population is estimated to reach 9.7 billion people by 2050, creating a challenging scenario for sustainable development, especially in relation to the Sustainable Development Goals. This increase also requires transforming global food systems to ensure the production and consumption of healthy, safe, and nutritious food [4].

Therefore, governments, community organizations, the food industry, and the distribution sector are working to promote foods with better nutritional profiles, reduce meat and sugar consumption, and incorporate more functional foods [5]. In this context, consumers have increasingly favored products made with ingredients from fruits, vegetables, and other plant sources because of concerns about diet and its impact on health [6]. As nutritional awareness grows, demand for healthy snack options is also rising, leading people to change their eating habits and prioritize more nutritious food. This scenario has driven the development of innovative, healthy products that meet new consumer demands [7].

Although snacks were traditionally associated with ultra-processed foods (low nutritional value), they have transformed with the incorporation of fruits and vegetables [8]. Among these innovations, edible vegetable leathers are processed foods made from dried fruits, vegetables, or other plant sources, transformed into thin, chewable sheets [9] that offer greater durability, easier transport, and convenient consumption [9]. These products, made with functional ingredients, may be a promising way to incorporate protein, fiber, and bioactive compounds into the daily diet [10].

In developing these products, methods to improve the sensory experience of plant-based foods include using polymers as an alternative to create texture and improve sensory properties [11]. Natural polymers play a fundamental role, especially polysaccharides such as carrageenan gum, a sulfated polysaccharide extracted from seaweed that is biodegradable, non-toxic, and water soluble, making it a versatile material for different applications [12]. Furthermore, the Food and Drug Administration (FDA) recognizes carrageenan gum as Generally Recognized as Safe (GRAS) under Section 21 CFR 172.620 [13]. Given the benefits of using hydrocolloids in edible leather production, there is also growing interest in incorporating ingredients with high nutritional and functional value.

Among the available ingredients, we highlight ora-pro-nobis (OPN), or *Pereskia Aculeata* Mill., an unconventional food plant with high nutritional value and great interest to the general population. Studies report that its leaves contain 19.97% to 26.96% protein, [14,15]6.65% to 31.90% fiber [14,15], minerals (potassium, magnesium, phosphorus, iron, manganese, calcium), vitamins [15,16], and phenolic compounds content (696.37 mg EAG/g to 8.57 mg EAG/g) [17,18], in addition to exhibiting high antioxidant capacity [19–21].

Beyond its nutritional profile, OPN is a hardy species that grows vegetatively year-round and adapts to a variety of soils and edaphoclimatic environments [15].

Incorporating it into products such as edible leather is an innovative strategy for adding healthy, practical products. Pereira et al. (2025) [18] used ora-pro-nóbis leaves to produce edible leather by adding agar-agar and varying concentrations of ora-pro-nóbis puree, resulting in a product with nutritional value and high antioxidant capacity.

In this context, this work aimed to investigate the impact of incorporating different concentrations of carrageenan gum (CG) in producing edible leathers from ora-pro-nóbis (POR) leaf puree, and to characterize in relation to centesimal composition, antioxidant compounds and total phenolic compound content, physico-structural parameters, sensory analysis and stability evaluation.

2. MATERIALS AND METHODS

2.1 Materials

The ora-pro-nobis plants (*Pereskia aculeata* Mill.) were cultivated at the Experimental Farm of São Paulo State University (UNESP), Botucatu campus, São Paulo, Brazil (22°50'32.5"S, 48°25'59.4"W). The leaves were harvested from these cultivated plants for the preparation of the edible leathers. The exsicata is deposited in the Irina Delanova De Gemtchujnicov Herbarium (BOTU 35880), UNESP, Botucatu, São Paulo, Brazil. Food-grade carrageenan gum was obtained from Adicel LTDA (Belo Horizonte, Brazil). Petroleum ether, sodium hydroxide, sulfuric acid, Folin-Ciocalteu reagent, gallic acid, acetone, and pure ethyl alcohol were obtained from Dinâmica Química Contemporânea LTDA (Indaiatuba, São Paulo, Brazil). Anhydrous sodium carbonate, 2,2-diphenyl- 1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic) (ABTS) were obtained from Sigma-Aldrich (Missouri, EUA). 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) was obtained from Sigma-Aldrich (Buchs, Switzerland). For cytotoxicity assessment, Dulbeccos's Modified Eagle Medium (DMEM, Gibco, Paisley, UK), fetal bovine serum (FBS) and streptomycin from Gibco (São Paulo, Brazil), penicillin (Nova Biotecnlogia, São Paulo, Brazil), and 3-(4,5-dimethyltiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich) were used. Ready-to-use plates for detecting *Salmonella* spp. and *Escherichia coli* (Shimadzu Corporation, Japan) were used.

2.2 Production of ora-pro-nóbis leaves puree

Ora-pro-nóbis leaves were manually separated, washed under running water, and sanitized with a 10% sodium hypochlorite solution for 10 minutes. To produce the puree, the leaves were steamed (Electrolux, ECC20) for 10 minutes. The leaves were then processed in a blender (Oster, Superchef) until a homogeneous puree was obtained. The single batch of ora-pro-nóbis puree (POR) was packaged in plastic bags (Grespan, Zip bags, 24X34 cm) and frozen in a freezer at -20°C (Consul, CRM54, SP)

2.3 Production of edible leathers

The edible leather was produced with a fixed amount of 40 g ora-pro-nóbis (POR) puree for the three formulations (determined by preliminary tests). The variable component was carrageenan gum (CG), added at three concentrations: 1.0 g, 1.5 g, and 2.0 g/100 g leather forming mass (MFLC). To produce edible leather forming mass (MFLC), the carrageenan gum was solubilized in water at 100 °C using a heating plate with magnetic stirring at 400 rpm (IKA, RCT Basic, Brazil) for 15 minutes. Then the

temperature was reduced to ~60 °C, and the previously heated OPN puree (40 °C) was added in a water bath (Catel, Piracicaba, Brazil). The mixture was stirred magnetically (800 rpm) for 10 minutes. After mass correction, the MFLC was spread into Teflon plates using a ZAA 2300 spreader (Zehntner Testing Instruments, Sissach, Switzerland) at a constant speed of 20 mm/s. The universal applicator (TBK Erichsen) was used to control the edible leather thickness to 4000 µm. The edible leather was dried in an oven with an air circulation system (Marconi, MA035/5, Piracicaba, Brazil) at 40 °C for 7 hours, as determined by preliminary assessments.

2.4 Characterization of edible leathers

2.4.1 Visual evaluation and color parameters

The edible leather was visually evaluated for leather-forming capacity, homogeneity, and manageability. Color parameters (Luminosity, chroma a*, and chroma b*) were evaluated using a colorimeter (CR-410, Minolta, Osaka, Japan). The browning index (BI) was evaluated according to equations (1) and (2) proposed by [22]

$$IE = 100(X - 0{,}31)/0{,}172 \quad \text{Eq. (1)}$$

$$X = (a^* + 1{,}75L^*)/(5{,}64L^* + a^* - 3{,}012b^*) \quad \text{Eq. (2)}$$

2.4.2 Centesimal composition and caloric value

The moisture content of leathers was determined by a gravimetric method of Association of Official Agricultural Chemists (AOAC, 2005) using a drying oven (Fanem, 315SE, São Paulo, Brazil) at 105 °C for 24 hours. The ash content was quantified by the dry incineration method, using a muffle furnace (Quimis, Q318M24, Diadema, Brazil). The lipid content was determined by the Soxhlet method (Tecnal, TE-1881/6, Piracicaba, Brazil), according to AOAC (2005). Protein content was determined by the Kjeldahl method using a nitrogen-to-protein conversion factor of 6.25, using a digestion system (Tecnal, TE-036, Piracicaba, Brazil). Total fiber was quantified by chemical methods based on acid and alkaline digestions, as described by (AOAC, 2005). The carbohydrate content was estimated by difference, following AOAC (2005). The caloric value was calculated according to Equation 3 established by Regulation (EU) N° 1669/2011 of the European Parliament and of the Council [23] using the Atwater factors.

$$Caloric\ values\ (kcal) = (\%\ carbohydrates\ 4) + (\%\ protein * 4) + (\%lipids * 9) \quad \text{Eq. (3)}$$

2.4.3 pH, thickness and water activity

The pH of the leathers was determined according to AOAC 14.022 (2005). Leather samples (1 g) were homogenized in 10 mL of distilled water (98°C ±2) and kept under a magnetic stirrer (Tecnal, TE-0851, Piracicaba, Brazil) for 15 minutes at 75 rpm. After stirring, the samples were left to stand at room temperature (25±2°C), and the pH was determined with a digital pH meter (CBLab, Brazil).

The leather's thickness was determined using a digital micrometer (Mitutoyo, Digital Coolant Proof, Japan). For each formulation, six distinct samples were analyzed, with ten measurements taken at different points. Water activity was determined using a water activity analyzer (Aqualab, Meter Group, EUA).

188

189 **2.4.4 Antioxidant activity and total phenolic compounds**

190 To determine antioxidant capacity and total phenolic content, edible leather samples
191 (0.150 g) were added to 10 mL of 70% ethanol (v/v), and the extraction was performed in
192 an ultrasonic bath (Limatec, LT-210, South Korea) for 20 minutes. Subsequently, the
193 samples were centrifuged at 3250 rpm for 15 minutes (Centurion, Laborline, São Paulo,
194 Brazil).
195 The antioxidant capacity was determined by the $ABTS^{\bullet+}$ method according to Re
196 et al. [24]. Samples (30 µL) were added to 3 mL of $ABTS^{\bullet+}$ solution (0.7 Abs). The mixture
197 was homogenized and left in the dark for 6 minutes. Then, the absorbance was
198 determined at 734 nm using a UV-Vis spectrophotometer (BEL Engineering, model 1105,
199 Monza, Italy). Results are reported in µMol of Trolox equivalent/g of dry *leather*.
200 For antioxidant capacity by $DPPH^{\bullet}$ assay (EC_{50}), initially, a $DPPH^{\bullet}$ solution at 0.6
201 mM was prepared; then samples (0.5 g) were solubilized in 70% ethanol (v/v). From this
202 solution, dilutions (0.001 to 0.007 g/ml) were prepared, of 100 µL aliquots from these
203 dilutions were added to 3.9 mL of $DPPH^{\bullet}$. The samples were maintained at rest, protected
204 fro light, for 65 minutes. Antioxidant capacity was expressed as the extract concentration
205 required to reduce 50% of the initial DPPH radical concentration, determined from the
206 linear equation obtained from the relationship between the extract concentration and the
207 percentage of $DPPH^{\bullet}$ inhibition.
208 In the FRAP assay, the reagent was prepared from a mixture of 25 mL of 300 mM
209 sodium acetate, 2.5 mL of TPTZ (2,4,6-tris(2-pyridyl) -s-triazine) solution, and 2.5 mL of
210 20 mM ferric chloride[25]. Samples (150 µL) were added to 2850 µL of FRAP reagent,
211 homogenized in a tube shaker (Marconi, MA 162, Piracicaba, Brazil) and incubated in a
212 water bath (Catel, Piracicaba, Brazil) at 37 °C for 30 minutes. The absorbance was
213 measured using a spectrophotometer UV-Vis (BEL Engineering, model 1105, Monza,
214 Italy) at 593 nm. The results were expressed as µMol of Trolox equivalent/g of dry leather.
215 The content of total phenolic compounds was determined by Folin-Ciocalteu
216 method, as described for Singleton and Rossi [26]. Samples (500 µL) were added to 2.5
217 mL of Folin-Ciocalteu reagent at 10% (v/v) reagent, the mixture was agitated for 30
218 seconds in tube shaker (Marconi, MA 162, Piracicaba, Brazil). After 5 minutes, 2 mL of
219 sodium carbonate solution at 7,5% (p/v) was added, and the mixture was kept at rest,
220 protected from light, for 2 hours. The absorbance was measured at 740 nm using a UV-
221 Vis spectrophotometer (BEL Engineering, model 1105, Monza, Italy). The results are
222 expressed in mg GAE/g of dry leather.
223

224 **2.4.5 Determination of chlorophyll A and B, carotenoids**

225 Pigment evaluation was realized according to Lichtenthaler and Wellburn [27].
226 Initially, 1.5 mL of acetone at 80% (v/v) was added to edible leather (30 mg). The mixture
227 was placed in an ultrasonic bath (Limatec, LT-210, South Korea) for 10 minutes and an
228 extract aliquot (1 mL) was added in acetone at 80% (1 mL). The samples were then stored
229 under refrigeration (Electrolux, Curitiba, Brazil) for 48 hours. After this period, the
230 absorbance was measured using a UV-Vis spectrophotometer (BEL Engineering, model
231 1105, Monza, Italy) at 663, 647, and 470 nm. The concentrations of chlorophyll a (C_a),
232 chlorophyll b (C_b), and total carotenoids (C_t) were calculated according to equations Eq.
233 4, Eq. 5, and Eq. 6, respectively.
234

235 $C_a = 12{,}25 * A_{663{,}2} - 2{,}79 * A_{647}$ Eq. (4)

$$C_b = 221{,}50*A_{647}\text{-}5{,}10*A_{6632} \quad \text{Eq. (5)}$$

$$C_t = 1000*A_{470}\text{-}182*C_a*85{,}02*C_b \quad \text{Eq. (6)}$$

2.4.6 Scanning electron microscopy (MEV)

Edible leather morphology (internal and surface structure) was evaluated using scanning electron microscopy (Tabletop Hitachi TM-3000, Hitachi High-Technology Corporation, Tokyo, Japan). Previously, the samples were stored in a desiccator with silica gel for 10 days. Images were obtained at an accelerating voltage of 15 kV and a magnification of 500 k. To evaluate the internal structure, the samples were fractured with liquid nitrogen.

2.4.7 Mineral determination

The content of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), boron (B), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) was measured in the samples. The total nitrogen (N) was measured by the semi-micro Kjeldahl method after sulfuric digestion. The elements P, K, Ca, Mg, S, Cu, Fe, Mn, and Zn were measured after nitro-perchloric digestion, following the methods described by Malavolta, Vitti, and Oliveira [28].

Phosphorus (P) was measured by metavanadate colorimetry using a spectrophotometer (Fento, 600 Plus, São Paulo, Brazil). Sulfur (S) was determined by turbidimetry of barium sulfate in a spectrophotometer (Fento, 600 Plus, São Paulo, Brazil). The elements K, Ca, Mg, Cu, Fe, Mn, and Zn were measured by atomic absorption spectrophotometry (Perkin Elmer, model 2380, Illinois, USA). The boron (B) content was determined by dry digestion (incineration) and colorimetric reading with azomethine H in a spectrophotometer (Vis, Fento, 600 Plus, São Paulo, Brazil). The results were shown in g/Kg (N, P, K, Ca, Mg and S) and mg/Kg (B, Cu, Fe, Mn and Zn)

2.4.8 Cytotoxicity assessment

Fibroblasts (3T3-E1) were cultured in culture flasks containing RPMI culture medium (Gibco, Paisley, United Kingdom) supplemented with 10% fetal bovine serum (FCS; Gibco) and 100 IU/mL of penicillin and 100 µg/mL of streptomycin (Gibco) to evaluate the effects of leathers on cell viability. The cells were incubated at 37°C in a humidified atmosphere with 5% CO_2 until they reached 80% confluence. Next, the cell samples were seeded in a 96-well plate at a density of 1000 cells per well. After cell adhesion (~4 hours), samples of the edible leather were previously prepared by dissolving 0.150 g of sample in 10 mL of 70% ethanol, resulting in an extract concentration of 15 mg/mL, diluted in the culture medium and incubated on a shaker for 24 and 48 hours (at room temperature). Then, 200µL of this solution was added to each well and kept in contact with the leather for 24 hours. Subsequently, the culture medium was removed, MTT solution (5 mg/mL) was added, and the presence of formazan crystals was evaluated using a microplate reader (Epoch) at 500 nm. The experimental group results were calculated relative to the control group. For the negative control group, cells were seeded in culture medium with a 70% alcohol solution.

2.5 Stability evaluation of edible leathers

For the stability evaluation of edible leathers, samples were stored in desiccators

with saline solution (relative humidity of 75%), maintained in a BOD incubator (MARCONI, MA 415, Piracicaba, Brazil) at 40°C, for 40 days with a frequency of analysis (1, 20, and 40 days). The samples were evaluated for color parameters and browning index (item 2.4.1).

During the assessment, stability was evaluated by antioxidant capacity using the $ABTS^{\bullet+}$ method (item 2.4.4) and the DPPH• method. The content of total phenolic compounds was determined according to item (2.4.4).

Antioxidant capacity by the DPPH radical method: DPPH• was prepared according to Brand-Williams, Cuvelier, and Berset (1995)[29]. Subsequently, 2 mL of diluted samples were mixed with 2 mL of radical DPPH• solution, corresponding to sample absorbances (A_1). In parallel, the control (Ac) was prepared by mixing 2 mL of DPPH • solution with 2 mL of ethanol to 70% (v/v). Additionally, 2 mL of diluted extract was combined with 2 mL of distilled water to prepare the blank (A2). The absorbances (A_1, A_2, A_c) were measured in a spectrophotometer at 517 nm after 1 hour of reaction. The antioxidant capacity was determined according to Li et al. (2014) [30] following Eq. (7)

$$Antioxidant\ capacity = \left[\left(1 - \frac{A1-A2}{AC}\right) * 100\%\right] \quad \text{Eq. (7)}$$

2.6 Sensorial analysis and antimicrobial activity

The sensorial analysis of edible leathers was carried out according to the ethical principles of São Paulo State University "Júlio de Mesquita Filho" and approved by the Ethics Committee CAAE 83179824.6.0000.5411, opinion number 7.231.533. Previously, sensorial analysis, the microbial analysis of edible leather was performed according to the manufacturer's methodology (Shimadzu, 2024). Initially, samples (1 g) were added to 9 mL of peptone water and subjected to agitation (10 minutes) in a shaker (Marconi, Brazil). Next, 1 mL of the inoculated extract was plated on commercial plates (Shimadzu Corporation, Japan) in a laminar flow hood (Pachane, Montevidéu, Uruguay) to detect *Salmonella spp.* (code 54058) and *Escherichia coli* (code 5402). The plates were incubated at 35 °C for 24 hours in BOD (MARCONI, MA 415, Piracicaba, Brazil).

The acceptance test of edible leathers was conducted by 100 untrained tasters aged 18 to 60, of both sexes, who participated in the study. Each participant received three formulations, randomly coded with three digits. Untrained evaluators assigned scores based on aroma, color, flavor, texture, and overall appearance using a 9-point hedonic scale (1 = hated, 9 = liked very much). Purchase intent was also assessed.

Even for the hedonic scale, a qualitative analysis was conducted by creating a word cloud using the Sensorial Online platform, approved by the Ethics Committee of São Paulo State University "Júlio de Mesquita Filho" (CAAE: 83179824.6.0000.5411 - Opinion Number: 7.231.533). Evaluators aged 18-65 years were invited to freely describe their perceptions of leather-type products, considering general sensory aspects. The platform automatically compiled the words, and their frequency of occurrence was graphically represented as a word cloud, in which the most frequently cited words appear more prominently.

2.7 Statical analysis

Data were analyzed using analysis of variance (ANOVA). When statistically significant differences were found, Duncan's multiple range test ($p < 0.05$) was performed using the InfoStat software (Version 2018d).

3. RESULTS AND DISCUSSION

3.1 Visual assessment and color parameters

Visually, edible leather made with carrageenan gum (CG) and added ora-pro-nobis puree (POR) presents a rough and heterogeneous surface, regardless of the formulation, possibly due to the presence of fibers in the puree (**Fig. 1**). The production of edible leathers with CG is still incipient in literature. However, some studies reported the use of carrageenan gum as a gelling agent, contributing to improved firmness in edible films made from chayote, pitaya peel and kepop banana peel [31] to increase tensile strength in edible films made with red pitaya puree and watermelon peel [32], and to increase firmness and texture of melon leathers supplemented with pineapple, sorbitol and carrageenan gum [33].

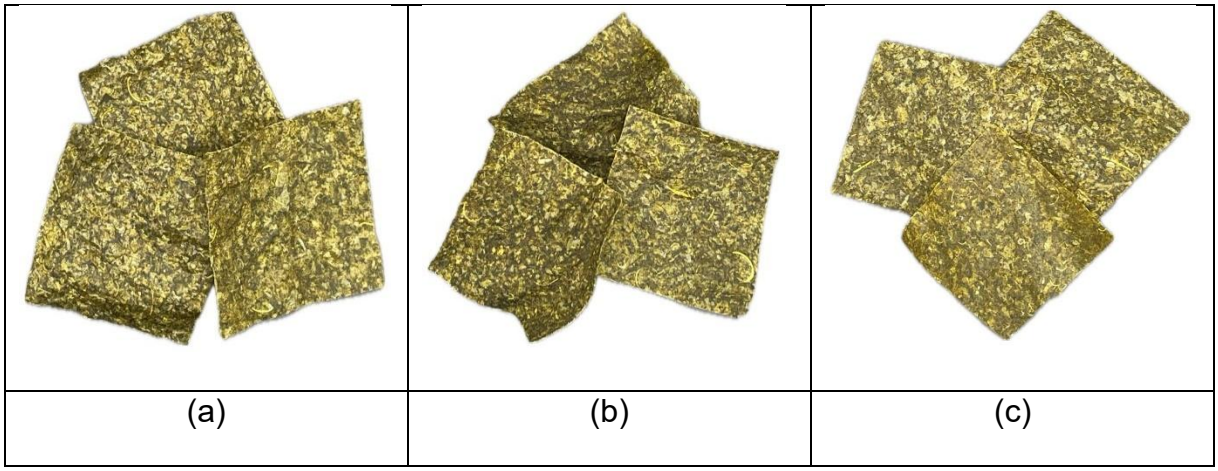


Fig. 1 Visual assessment of edible leathers made of ora-pro-nobis puree leaves (40 g/ 100 g of leather forming mass) with different concentrations of carrageenan gum: (a) 1.0 g of carrageenan gum, (b) 1.5 g of carrageenan gum, and (c) 2.0 g of carrageenan gum

The addition of CG significantly affected color parameters and the browning index of the edible leathers (Table 1). The formulations with higher CG concentrations (1.5 g and 2.0 g) showed a significant increase in the L* (luminosity) value. The gum may make the product appear lighter, possibly by forming a gelled network with high water-retention capacity that promotes better dispersion of solid compounds [34] . Similarly, increased luminosity may relate to the formation of colloidal structures that bind and disperse more effectively with added gum, improving light scattering [35].

Chroma a* was significantly reduced in the 1.5 g and 2.0 g CG formulations compared to the 1.0 g CG formulation. The reduced chroma a* values may be associated with green coloration of ora-pro-nóbis. This reduction may be attributed to the CG effect, which may have affected pigment dispersion or caused interactions with components that reduced color saturation. Being a plant material, ora-pro-nóbis is a source of chlorophyll, the pigment responsible for the green coloration of most vegetables [15]. Pereira et al. [18] also reported that chlorophyll is responsible for green coloration in edible leathers made of ora-pro-nóbis and agar-agar.

Table 1 Color parameters (L*, chroma a* and chroma b*) and Browning index (BI) of edible leather produced with puree of ora-pro-nóbis leaves (POR) (40 g / 100 g of leather forming mass) with different concentrations of carrageenan gum (CG).

Analysis	Leather formulations		
	1.0 g CG	1.5 g CG	2.0 g CG
L*	37.77 ± 1.21^{b}	50.58 ± 1.21^{a}	47.07 ± 2.57^{a}
Chroma a*	2.14 ± 0.00^{a}	1.32 ± 0.13^{b}	1.31 ± 0.67^{b}
Chroma b*	3.86 ± 0.67^{b}	8.48 ± 0.75^{a}	9.47 ± 1.61^{a}
Browning index	0.16 ± 0.02^{b}	0.19 ± 0.01^{b}	0.23 ± 0.04^{a}

*Means with the same letter in different columns did not indicate a significant difference ($p<0.05$).

Chroma b*values were significantly higher at 1.5 g and 2.0 g CG, indicating a tendency toward yellowish hues at higher CG concentrations. Although CG is considered colorless, it can form gelled structures that trap water, air, and solids, affecting light scattering within the product and favoring reflection of yellow wavelengths [36].

The Browning index (BI) was higher in leather with 2.0 g CG compared to1.0 g and 1.5 g CG ($p<0.05$). Higher gum concentration is associated with greater water retention and a more viscous, structured matrix; these conditions may favor the concentration of reducing sugars and proteins during heat treatment, increasing the incidence of Maillard reactions and, consequently, the browning index values [37,38]. Rodrigues et al. [39] also attributed the increase in the Browning index to the Maillard reaction during the drying of agar-agar leathers with the addition of chayote puree, observing an increase in BI (75.37 to 80.98).

3.2 Centesimal composition and caloric value

No significant differences were observed for the moisture and fiber content of the edible leather (Table 2), possibly due to the thickening properties and water-retaining capacity of carrageenan, which favors the formation of a gelled network capable of trapping water within the food matrix [40,41].

Regarding fiber content, this may be because the total fiber procedure primarily quantifies insoluble fiber, while soluble fibers, such as carrageenan gum, are solubilized and eliminated during the analytical process; therefore, they did not remain as the residue quantified as crude fiber [42]. However, the value is similar to that reported for ora-pro-nobis leaves [14].

Table 2 Centesimal composition and caloric value of edible leathers produced with puree of ora-pro-nóbis leaves (POR) (40 g / 100 g of leather forming mass) with different concentrations of carrageenan gum (CG).

Centesimal composition	Leather formulations		
	1.0 g CG	1.5 g CG	2.0 g CG
Moisture	15.70±0.26[a]	15.57±0.70[a]	15.41±0.14[a]
Fibers[1]	9.51±0.70[a]	9.70±0.86[a]	9.15±0.62[a]
Ash[1]	19.4±0.21[c]	20.12±0.14[b]	22.45±0.27[a]
Proteins[1]	13.88±0.39[a]	13.26±0.54[a]	12.11±0.13[b]
Lipíds[1]	9.15±0.56[a]	8.65±0.76[a]	6.32±0.61[b]
Carbohydrates[1]	31.23±1.23[b]	31.87±0.51[ab]	33.72±0.46[a]
Caloric value (Kcal/100g)	105.10±0.60[a]	103.32±3.46[a]	96.09±1.81[b]

*Means with the same letter in different columns did not indicate a significant difference ($p<0.05$).
[1]Results expressed in (g/100g) wet basis.

The addition of CG resulted in a significant increase ($p<0.05$) in the ash content of edible leathers (Table 2). This increase may be associated with higher CG concentration, as it is a hydrocolloid rich in minerals such as iron, magnesium, potassium, sodium, zinc, iodine, and calcium [32]. The addition of *Kappaphycus alvarezii* (red seaweed used in the production of carrageenan) also resulted in an increase in ash content in pitaya and watermelon leathers [32].

Protein content in edible leather ranged from 12.11% to 13.88%, indicating a significant protein content that could contribute to daily protein intake. According to the Food and Agriculture Organization of the United Nations [43] food is a good source of protein when it provides 10% to 20% of the recommended daily value per serving. Based on these criteria, the leathers developed in this study can be classified as an adequate protein source, reinforcing their potential as a functional and nutritious product.

Lipid content decreased significantly as CG concentration increased in the formulations. Overall, carrageenan gum is not considered a significant lipid source, which may have contributed to the reduction in lipid content as formulation concentration increased. According to Naseri et al. [44], red algae used for CG production have reduced amounts of lipids (0.4-0.07%). This result is relevant from a nutritional standpoint, as it shows that the developed product has low lipid content. This characteristic supports healthy food production, contributes to a balanced nutritional profile, and may attract consumers seeking lower-fat options.

The carbohydrate content ranged from 31.23% to 33.72% (Table 2). The 2.0 g CG formulations showed a significant increase, which may be due to reduced protein and lipid content in the samples. These results are consistent with those reported in the literature by Almeida et al. [45] and Ciriaco et al. [46]. They reported that ora-pro-nobis flour contains 29.50% to 48.04% carbohydrates on average.

Edible leathers with 2.0 g CG showed the lowest energy value (Table 2). Although this formulation increased carbohydrate content, total caloric value was significantly reduced because lipids and protein, macronutrients that contribute considerably to energy intake, especially lipids (9 kcal/g), were reduced. Because CG provides low energy, it dilutes energy nutrients, resulting in a formulation with lower caloric intake. Das et al.

[47]reported similar values for red pitaya and mango (98.14 ± 1.93 kcal/100 g and 97.76 ± 1.8 kcal/100 g). Mphaphuli et al. [48] reported higher values (270 kcal/100g) for mango leathers with Natal plum (*Carissa macrocarpa*).

3.3 pH, thickness and water activity

The pH of edible leathers differed significantly ($p<0.05$) among all formulations, indicating that increasing CG concentration reduced pH. This may be associated with sulfated groups in carrageenan gum, which can release protons (H^+) and influence the medium's ionic balance, creating a more acidic environment [49]. Similar values (4.75 ± 0.01 – 4.82 ± 0.03) were reported for ora-pro-nobis puree leathers with the addition of agar-agar [18].

Table 3. pH, thickness, and water activity (Aw) of edible leathers produced with puree of ora-pro-nóbis leaves (POR) (40 g / 100 g of leather-forming mass) with different concentrations of carrageenan gum (CG).

Analysis	Leather formulations		
	1.0 g CG	1.5 g CG	2.0 g CG
pH	5.50±0.14[a]	5.21±0.03[b]	4.98±0.04[c]
Thickness (mm)	0.35±0.02[a]	0.35±0.03[a]	0.35±0.03[a]
Aw	0.59±0.01[a]	0.59±0.02[a]	0.57±0.03[a]

*Means with the same letter in different columns did not indicate a significant difference ($p<0.05$)

In relation to thickness, no significant differences were observed ($p<0.05$), indicating that the increase in CG concentration didn't influence the thickness of the edible leathers, which may indicate efficient spreading control (tape-casting). Water activity (Aw) didn't show a significant difference ($p<0.05$) (Table 3) in edible leathers with different CG concentrations. In general, products with Aw below 0.65, such as dried products, snacks, and dehydrated products, show a lower susceptibility to microbial growth [50]. Kurniadi et al. [40] reported values of 0.57 to 0.63 for guava and banana leather with added CG.

3.4 Antioxidant activity and total phenolic compounds

In general, edible leather made with lower carrageenan concentrations (1.0 and 1.5 g/100 of LFM) showed higher antioxidant activity ($ABTS^{\bullet+}$, EC_{50}, and FRAP) and total phenolic compounds, which differed significantly from formulations with 2.0 g of carrageenan gum (Table 4). This was expected, since CG isn't considered a source of active compounds, and increasing its concentration dilutes the active compounds present in the sample.

According to Ramadas et al. [51], CG has low or no antioxidant capacity and, therefore, instead of contributing to the formulation's antioxidant power, it traps and reduces the availability of antioxidant compounds in the matrix.

Pereira et al. [18] produced edible leathers made of agar-agar with the addition of ora-pro-nobis leaf puree and reported valued ranged in entre 12.72 ± 111.27 e 14.99 ± 74.28 µmol TE eq/g (evaluated by the ABTS method) and between 680.35 ± 65.14 e 830.57 ± 91.71 mg GAE/g for the total phenolic compounds content, indicating that the

addition of different concentrations of puree increased the concentration of active compounds. Therefore, although the incorporation of CG may cause a partial trapping effect of bioactive compounds in the gelled matrix, this occurs because the three-dimensional polysaccharide network of CG retains these compounds, limiting their availability [52]; ora-pro-nobis remains the main contributor to antioxidant capacity in formulations, highlighting its potential as a functional ingredient. Several studies report high concentrations of antioxidants and total phenolic compounds in ora-pro-nobis leaves [15,53].

Table 4. Antioxidant activity ($ABTS^{\bullet+}$, EC_{50} and FRAP) and total phenolic compounds (TPC) of edible leather produced with puree of ora-pro-nóbis leaves (POR) (40 g / 100 g of leather forming mass) with different concentrations of carrageenan gum (CG).

Analyses	Leather formulations		
	1.0 g CG	1.5 g CG	2.0 g CG
$ABTS^{\bullet+}$ (µmol TE/g)	2.08±0.05[a]	1.96±0.05[b]	1.92±0.02[b]
EC_{50} (g/mL)	0.0074±0.0001[a]	0.0076±0.0003[a]	0.0078±0.0004[b]
FRAP (µmol TE/g)	132.7±1.50[a]	109.3±1.52[b]	100.1±1.42[c]
TPC (mg GAE/g)	86.0±3.98[a]	81.0±3,98[a]	64.3±4.01[b]

*Means with the same letter in different columns did not indicate a significant difference ($p<0.05$)

Silva et al. [15] reported a value of 4.48 µmol Teg for ora-pro-nobis dry leaves. Massocatto et al. [19] and Amaral et al. [54] reported EC_{50} concentrations of 0.003351 g/mL and 0.000060 g/mL for OPN leaf extracts. For FRAP, ora-pro-nobis leaves showed results between 0.909±0.026 and 132.0±17.0 µmol Te Eq/g [17,20,55]. Variations between studies can be attributed to intrinsic plant factors, such as growing conditions, maturation stage, harvest time, and climate, as well as differences in sample processing, solvent type, and assay parameters [21,22,54]. However, these results reinforce that ora-pro-nobis has antioxidant capacity, highlighting its potential to reduce bioactive compounds and justifying its use in functional food formulations.

3.5 Chlorophyll A, B and carotenoids

Photosynthetic pigments (chlorophyll + carotenoids) are closely associated with the plant's antioxidant system in general physiology [56]. Chlorophyll A and B contents did not differ significantly ($p>0.05$) between formulations, indicating that the variation in carrageenan concentration did not influence the concentration of chlorophyll pigments. Although chlorophyll is known to be sensitive to thermal processes and can undergo structural manipulation and conversion into pheophytins with a consequent reduction in its bioactive activity [57], the results suggest that the conditions employed in the production and drying of the leathers were sufficiently mild to preserve these compounds. This behavior may relate to interactions between the polymeric matrix and the pigments, which may act as a protective barrier against chemical and oxidative manipulation.

Table 5 Chlorophyll A, Chlorophyll B and total carotenoids of edible leathers produced with puree of ora-pro-nóbis leaves (POR) (40 g / 100 g of leather forming mass) with different concentrations of carrageenan gum (CG).

Analyses	Leather formulations		
	1.0 g CG	1.5 g CG	2.0 g CG
Chlorophyll A (µg/g)	984.43±14.30[a]	969.57±11.71[a]	964.60±8.81[a]
Chlorophyll B (µg/g)	100.88±15.26[a]	85.60±9.28[a]	66.71±8.38[a]
Carotenoids (µg/g)	234.21±8.19[b]	242.40±3.40[ab]	254.57±0.39[a]

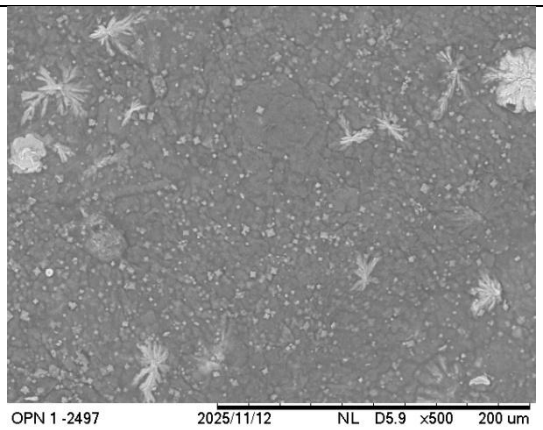
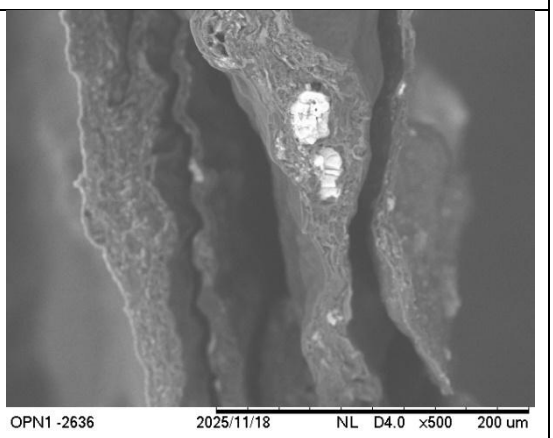
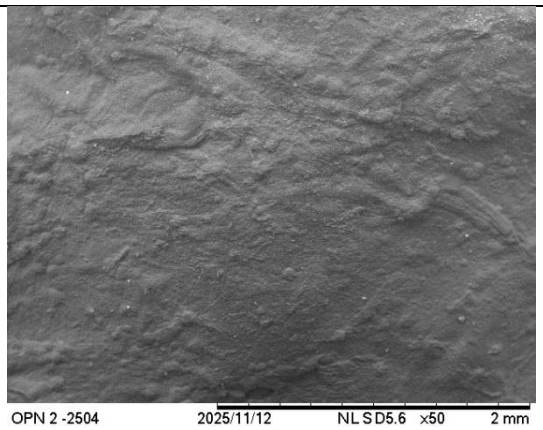
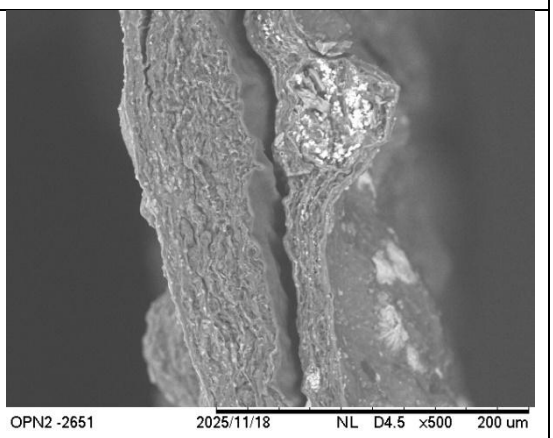
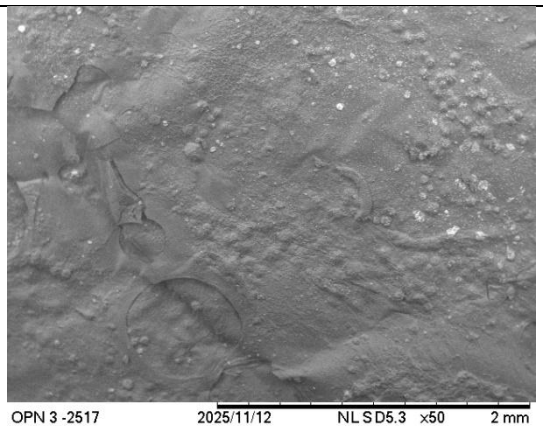
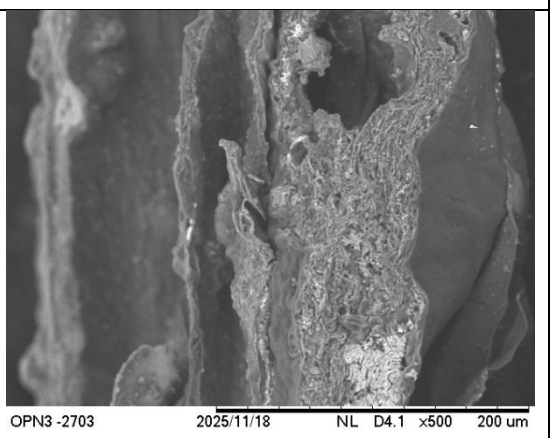
*Means with the same letter in different columns did not indicate a significant difference ($p<0.05$)

Carotenoids ranged from 234.21 to 254.57 µg/g (Table 5), with the highest concentration in the formulation containing 2.0 g CG and significantly higher than the edible leathers with the lowest CG concentration. This higher value may reflect a higher proportion of CG, which reduces carotenoid exposure to oxidation by acting as a physical barrier for these hydrophobic pigments [58]. According to Divya et al. (2025) [59], non-covalent interactions between hydrocolloids and pigments contribute to maintaining the integrity of carotenoids in food systems. Several studies report high carotenoid content in ora-pro-nobis leaves [15,16,60].

3.6 Scanning electron microscopy (SEM)

All formulations showed a rough, heterogeneous surface (**Fig. 2**), with plant fibers or filaments, likely derived from the filaments in ora-pro-nobis leaves. In the micrographs of the internal structure (**Fig. 2**), discontinuous areas with pores and the presence of crystals were observed; this behavior agrees with com Pereira et al. [18], who observed internal and structurally discontinuous particles in OPN leather with agar-agar, attributed to the presence of not completely solubilized gum fractions.

533

	SEM image of the surface	SEM image of a cross section
(a)		
(b)		
(c)		

534 **Fig. 2** Scanning electron microscopy (SEM) of the surface and of edible leather produced
535 with puree of ora-pro-nóbis leaves (POR) (40 g / 100 g of leather forming mass) with
536 different concentrations of carrageenan gum (CG). (a) 1.0 CG (b) 1.5 CG (c) 2.0 CG
537

538 **3.7 Mineral's content**

The macro minerals (phosphorus, nitrogen, potassium, calcium, magnesium, sulfur) and the microminerals (boron, copper, iron and zinc) don't show significant differences except for manganese (Table 6). The mineral contents of the 1.0g CG, 1.5g CG, and 2.0g GC formulations generally did not differ significantly. Several studies report macro- and micronutrient values for OPN leaves, such as nitrogen in the range of 33.35 – 37.61 g/kg [61]. For phosphorus, Souza et al. [60], Botrel et al. [61], and Fioroto et al. [62] reported values from 0.17 g/kg to 5.6 g/kg. Regarding potassium levels, Conceição et al. and Fioroto et al. [62] reported values ranging from 30 to 50.96 g/kg. Calcium was reported in the range of 28 to 50 g/kg [62,63]. In the case of magnesium, (7 g/kg–14.32 g/kg) by Berto et al. [53]; Fioroto et al. [62]. Souza et al.[61] and Sato et al. [63] reported sulfur (2.49 g/kg and 9.8 mg/g).

For microminerals such as boron, Silva et al. (2024)[64] reported values between 0.10 and 0.23 mg/kg. Although recent literature presents a limited number of studies reporting boron levels in ora-pro-nóbis leaves, the values found here are close to those reported in the U.S. Borax [65] technical leaflet "Boron in vegetables" for widely consumed green leafy vegetables such as lettuce, celery, cabbage, kale, and mustard (25–60 mg/kg). Copper remained close to values reported in the literature (10–20 mg/kg) by Fioroto et al. [62] and Berto et al. [53]. Iron is an essential micronutrient that supports various enzymatic and non-enzymatic proteins and plays critical roles in erythropoiesis, oxygen transport, cellular respiration, and immune response. Fioroto et al. [62] and Berto et al. [53].

Another mineral evaluated in this study was manganese; the literature reports values ranging from 73.1 to 160 mg/kg [61]; Berto et al. [51]; and Fioroto et al.[62]. Finally, zinc values range between 2.8 and 34.98 mg/kg, as reported by Souza et al. [61]; Botrel et al. [66]; Berto et al. [53] and Fioroto et al. [62].

Overall, the results in this study remain within the ranges reported by other studies analyzing the mineral content of OPN, in both flour and leaves. Thus, it can be concluded that the presence of carrageenan gum didn't interfere with the mineral content of OPN, preserving the characteristic mineral profile of this raw material in the development formulations.

Table 6 Mineral composition of edible leathers produced with puree of ora-pro-nóbis leaves (POR) (40 g / 100 g of leather forming mass) with different concentrations of carrageenan gum (CG).

Minerals	Formulations		
	1.0 g CG	1.5 g CG	2.0 g CG
N- Nitrogen[1]	20.0 ±1.33[a]	19.0±0.70[a]	18.0±0.32[a]
P- Phosphorus[1]	1.03±0.03[a]	0.98±0.00[a]	0.95±0.00[a]
K- Potassium[1]	40.6±2.3[a]	45.0±0.35[a]	48.0±145[a]
Ca- Calcium[1]	32.0±1.75[a]	35.0±0.45[a]	36.0±0.10[a]
Mg- Magnesium[1]	10.05±0.05[a]	10.0±0.05[a]	9.6±0.05[a]
S- Sulfur[1]	20.1±1.75[a]	23.6±0.35[a]	24.4±0.28[a]
B- Boron[2]	42.0±1.35[a]	41.0±1.43[a]	40.0±4.14[a]
Cu- Copper[2]	17.0±4.0[a]	34.0±9.0[a]	17.0±3.0[a]
Fe- Iron[2]	104.0±2.5[a]	133±8.5[a]	91±6.0[a]
Mn- Manganese[2]	140±0.5[a]	138±0.5[a]	127±1.5[b]
Zn- Zinc[2]	41.0±2.5[a]	42.0±1.0[a]	37.0±1.0[a]

[1] g/kg; [2] mg/Kg

*Means with the same letter in different columns did not indicate a significant difference ($p<0.05$)

3.8 Cytotoxicity assessment

In general, all leather formulations showed cell viability above 70% after 24 and 48 hours of exposure (**Fig. 3**), indicating a significant absence of cytotoxicity. According to ISO 10993-5 [67], materials that maintain cell viability above 70% are considered non-cytotoxic. Similar results were reported by Pereira et al. [18], who observed results above 70% in leathers with different concentrations of ora-pro-nobis after 24 and 48 hours, and Cruz et al. [17], who reported cell viability above 70% for lyophilized extracts of ora-pro-nobis leaves.

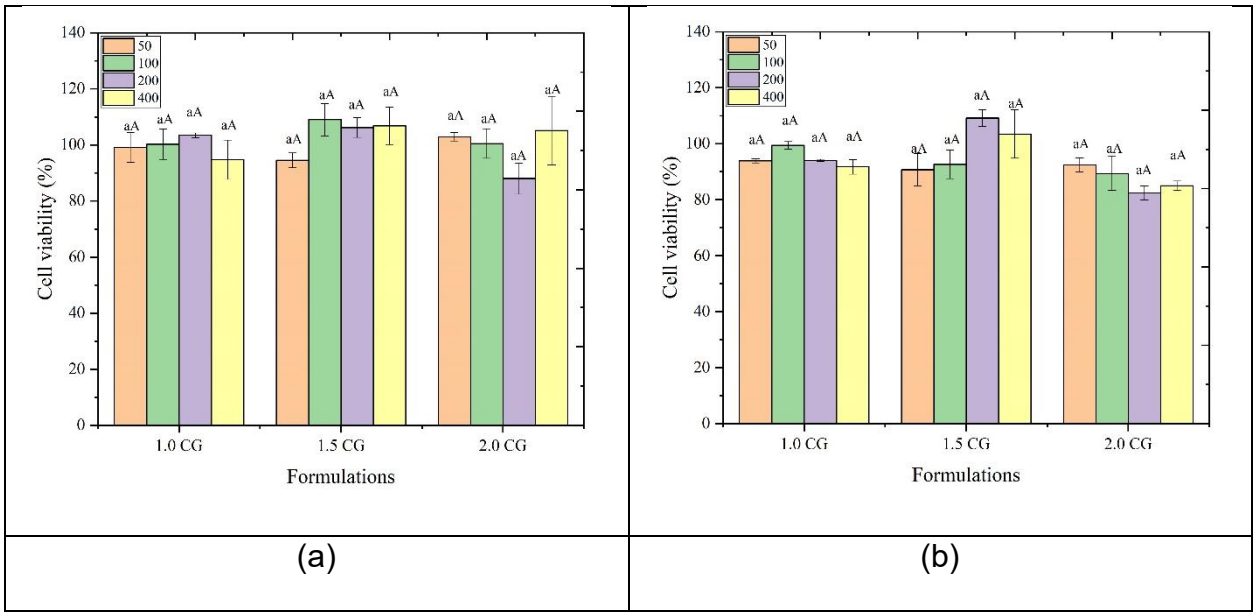


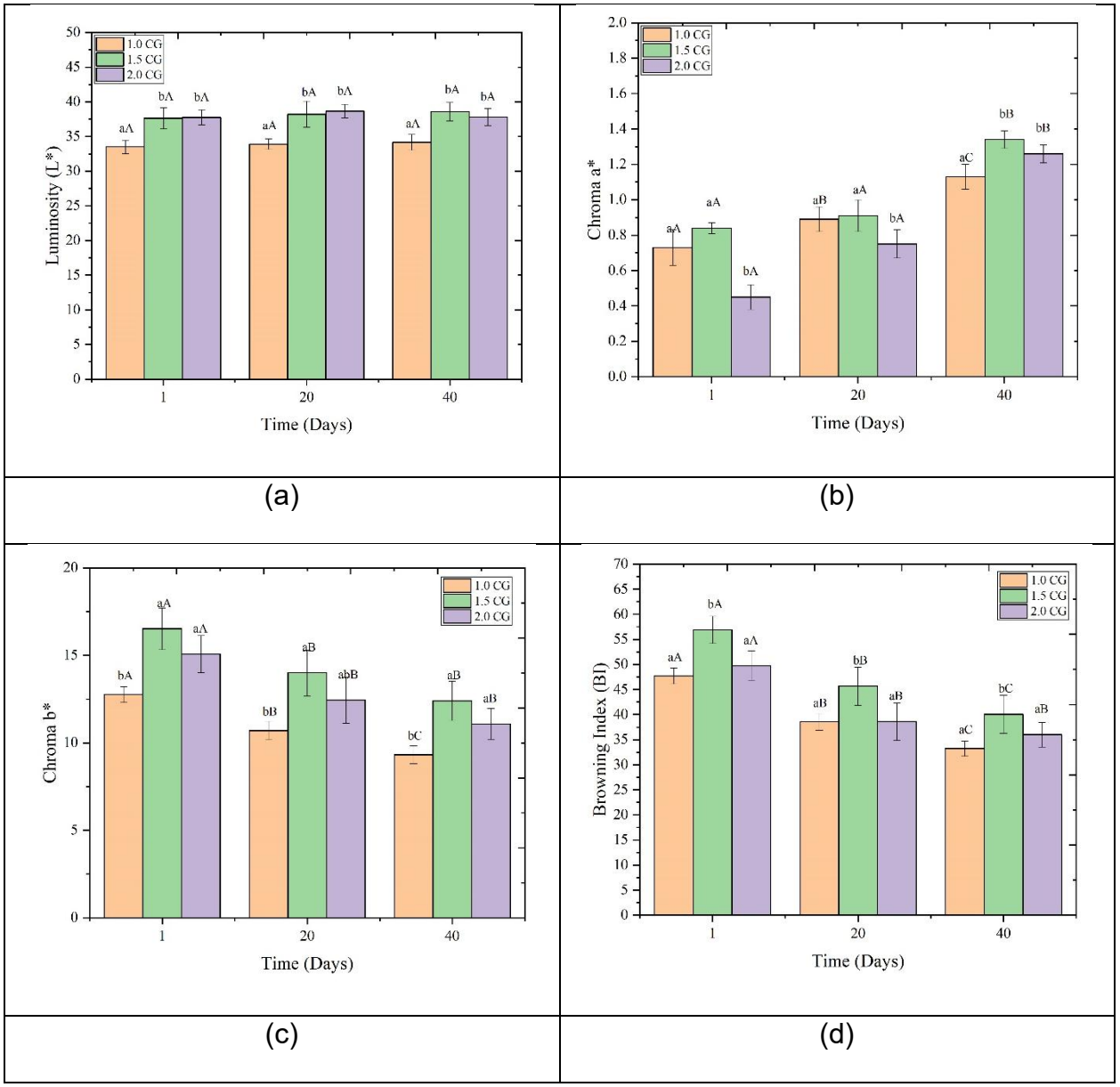
Fig. 3 Evaluation of cytotoxicity of edible leathers produced with puree of ora-pro-nóbis leaves (POR) (40 g / 100 g of leather forming mass) with different concentrations of carrageenan gum (CG), where: (a) 24 h and (b) 48 h. ** At the same exposure time, significant differences between formulations and control (p < 0,05) are represented by distinct minuscule letters. Significant differences (p< 0.05) between exposure times for the same formulation are indicated by distinct capital letters*

3.9 Stability Assessment during storage of edible leathers

Color parameters L*, a*, b* and browning index (BI) were significantly influenced by storage time and CG concentrations (p< 0.05). Studies indicate that color parameters can change during storage because physicochemical processes affect the food's pigments and matrix, and these changes often correlate with storage time or other factors such as temperature, humidity, and oxidation [68].

Regarding temporal variation within each formulation, we observed no significant differences (p>0.05) (**Fig. 4**), indicating adequate stability of the L* parameter over time between days 1, 20, and 40 of storage. However, on each day of analysis, the 1.0 g CG showed significantly lower L* values (p<0.05) than the other formulations, which did not differ significantly from one another.

606



607 **Fig. 4** Evaluation of color stability parameters of edible leathers produced with puree of
608 ora-pro-nóbis leaves (POR) (40 g / 100 g of leather forming mass) with different
609 concentrations of carrageenan gum (CG), where: (a) Luminosity (L*), (b) Chroma a*, (c)
610 Chroma b*, and (d) Browning Index (BI). * *Significant differences ($p<0.05$) between*
611 *formulations at the same storage time are represented by distinct lowercase letters, while*
612 *differences between storage times for the same concentration are indicated by uppercase*
613 *letters.*
614
615 This behavior may be attributed to lower CG content, which results in a matrix with
616 less capacity to disperse and reflect light uniformly. Furthermore, the lower proportion of
617 CG may increase the apparent concentration of natural pigments, reducing the system's
618 luminosity. In contrast, formulations with higher CG content exhibit a denser matrix that
619 acts as a physical barrier, increasing light reflection and, consequently, L* values.
620 Similarly, increased luminosity may relate to the formation of colloidal structures that bind
621 and disperse more effectively with the addition of gum, improving light scattering [35].
622 The a* parameter (**Fig. 4**) increased significantly during storage, reflecting a

contribution of reddish hues to the product. This behavior can be associated with oxidation processes and non-enzymatic browning reactions that occur slowly during storage [69]. Rodrigues et al. [37] reported a similar result in a study of accelerated stability of chayote and cucumber skin leather under comparable conditions. The authors observed that, on day 1, the parameter a* averaged 1.9 for chayote, while on day 40 of storage, it increased to 5.81. Similarly, for cucumber, the average a* increased from 8.93 on day 1 to 9.15 on day 40, consistent with these findings.

In contrast, chroma b* decreased over storage time for all formulations evaluated (Fig. 4), indicating a loss of yellowish tones, possibly associated with pigment degradation and chemical transformations induced by environmental factors. From the start of storage, the formulations differed significantly, especially in 1.0 CG, where 1.5 CG and 2.0 CG showed the highest b* values. This behavior aligns with literature on color changes during storage, where reductions in b* values have been associated with the degradation of natural pigments (carotenoids and flavonoids) responsible for yellow coloration in food matrices [69,70]. Rodrigues et al. [39] reported a similar behavior in the b* parameter for cucumber leather, observing a significant reduction during storage, from approximately 40 days (19.81). The behavior observed in this study suggests that higher CG concentrations contribute to greater retention of yellow color.

Regarding the Browning index (BI) in **Fig. 4**, the figure shows the evolution of BI in samples with different CG concentrations (1.0, 1.5, and 2.0 g) over storage (0, 20, and 40 days). All formulations show a progressive decrease in BI over time, indicating darkening during storage. Among the concentrations, the 1.5 CG sample consistently maintained higher BI values, significantly different from 1.0 and 2.0 CG. The 1.0 CG concentration showed the lowest values at the end of storage (day 40), while 2.0 CG showed intermediate values. These results suggest that CG concentration influences color stability during accelerated storage, indicating changes in the compounds responsible for light absorption and confirming important visual changes in the product. Concha-Meyer et al. [71] reported that different processes can cause color changes in plant matrices, for example, thermal destruction of pigments, especially carotenoids and chlorophyll.

The observed reduction aligns with Rodrigues et al. [37], who evaluated the browning index's stability under similar conditions and reported a reduction from 73.29 (day 1) to 59.21 (day 40) in cucumber leathers. Similar behavior was observed by Giacalone et al. [72], who observed a BI reduction on Actinidia arguta leather, reporting values of 55 on day 1 and 29 on day 21. They attributed this reduction to the degradation of ascorbic acid in the raw material. Overall, these results show that storage causes physicochemical transformations that directly affect the product's chromatic stability.

In **Fig. 5,** it can be observed that the antioxidant capacity of extracts of edible leathers was evaluated by the $ABTS^{\bullet+}$ method. In general, antioxidant capacity remained relatively stable during storage, with similar values among the treatments evaluated. However, small variations were observed with storage time and CG concentration. The statistical differences, indicated by distinct letters ($p<0.05$), show that both concentration and time influenced the antioxidant potential measured by the $ABTS^{\bullet+}$ method.

The $ABTS^{\bullet+}$ results followed a trend similar to that observed for o DPPH•, with a gradual reduction in antioxidant activity during storage. However, differences between the formulations were less pronounced in the $ABTS^{\bullet+}$ assay; the stability observed in formulations with higher CG content may relate to the hydrophilic nature of the $ABTS^{\bullet+}$ radical, which favors detection of aqueous-soluble antioxidant compounds. Carrageenan gum, a sulfated polysaccharide, has negatively charged groups that can interact with hydrophilic phenolic compounds, helping stabilize it during storage. These interactions can preserve the reducing capacity of bioactive compounds, resulting in less loss of

antioxidant capacity as assessed by ABTS$^{\bullet+}$ [24,73].

The DPPH● radical scavenging capacity showed distinct behavior among the formulations (**Fig. 5**). The 1.0 g CG formulation exhibited high initial antioxidant activity, followed by a more pronounced reduction during storage, indicating lower stability of the compounds responsible for antioxidant activity. In contrast, the 1.5 and 2.0 CG formulations showed less variation over time, suggesting greater resistance to degradation. The 2.0 CG formulation also showed greater antioxidant stability, maintaining relatively constant values until day 20, with a moderate reduction on day 40.

The lower degradation observed in samples with higher gum content may be linked to polysaccharides' ability to form a protective matrix around antioxidant compounds, reducing their exposure to oxidative processes and delaying degradation [51]. A study on microcapsules containing alginate and carrageenan demonstrated that, after storage, the antioxidant capacity was better preserved in capsules with carrageenan compared to non-encapsulated ones, indicating the protective function of this polysaccharide on antioxidant activity [74].

The total phenolic compounds content (**Fig. 5**) showed non-linear behavior during storage. It increased on day 20, then decreased on day 40. This behavior may be attributed to polymerization during initial storage, which can form derivatives with greater antioxidant activity than the original components [39,75].

A similar behavior in cucumber and chayote leather was reported by Rodrigues et al. [39], who evaluated an increase in the phenolic compounds after 40 days, which may be attributed to the Maillard reaction during storage, resulting in interactions between reducing sugars and amino acids and, consequently, an increase in antioxidant activity. In this study, the decrease in phenolic compounds observed on day 40 can be attributed to chemical degradation, reducing their activity and quantification. Arisut et al. [10] observed similar behavior, reporting a 34% reduction in phenolic compounds in apple leathers and artichoke powder compared to day 1 and attributing it to the degradation of polyphenols and other antioxidant molecules during drying.

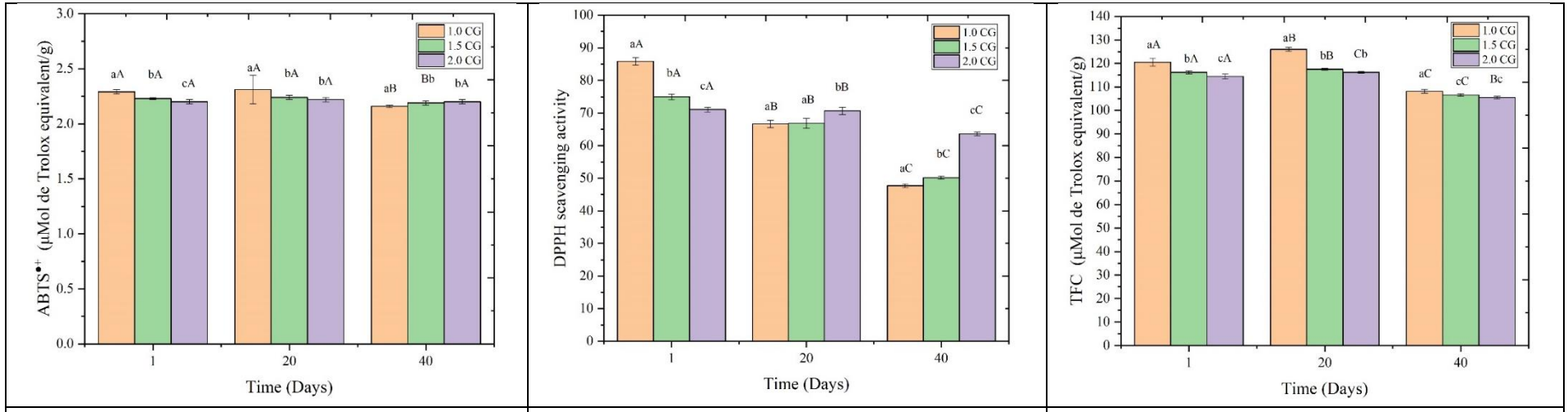


Fig. 5 Evaluation of antioxidant potential stability using: (a) ABTS•+, (b) DPPH● method and (c) Total phenolic compounds (TPC) of edible leathers produced with puree of ora-pro-nóbis leaves (POR) (40 g / 100 g of leather forming mass) with different concentrations of carrageenan gum (CG). **Significant differences ($p<0.05$) between formulations at the same storage time are represented by distinct lowercase letters, while differences between storage times for the same concentration are indicated by uppercase letters.*

3.10 Sensorial analysis and antimicrobial activity

Before the sensorial analysis, a microbial safety analysis was performed on the edible leather samples, which were evaluated for *Salmonella spp.* and *Escherichia coli* using commercial ready-to-use plates. All results were negative, indicating these bacteria were absent. Furthermore, the samples had a slightly acidic pH (5.50-4.98) and moderate water activity (0.59-0.57), conditions that make the environment less favorable for microbial growth and reinforce the microbiological test results.

Sensory evaluation is a crucial approach to ensure consumer acceptance of new products by assessing reactions to food stimuli [76]. The sensory evaluation results for edible leathers with different concentrations of CG (**Fig. 6**) generally showed no significant differences in the evaluated attributes: aroma, color, flavor, texture, and overall appearance.

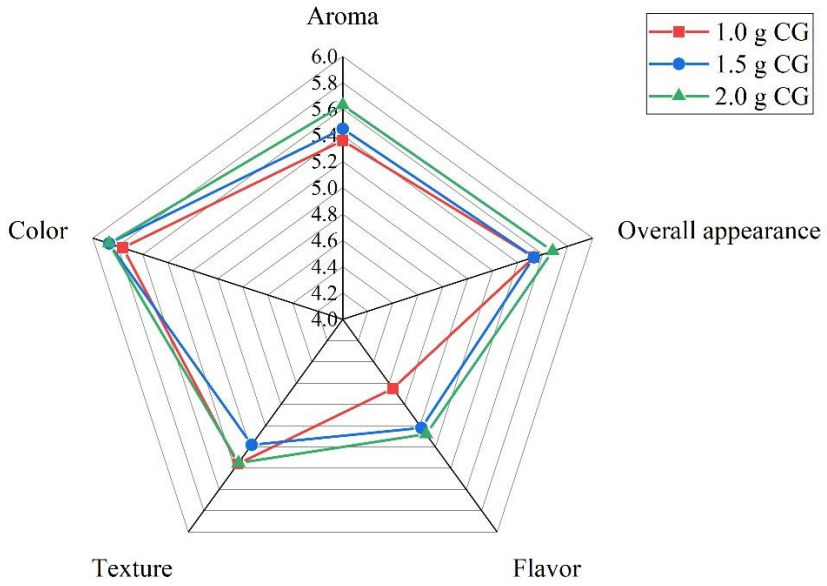


Fig. 6 Sensory evaluation of edible leathers produced with puree of ora-pro-nóbis leaves (POR) (40 g / 100 g of leather forming mass) with different concentrations of carrageenan gum (CG): (a) aroma, (b) color (c) flavor, (d) texture, (e) overall appearance

As part of the sensory analysis, a question about purchase intention was also included to determine whether sensory perception translated into willingness to purchase the product, an important indicator of consumer acceptance. Most evaluators (70%) stated they would buy the product, suggesting good overall acceptance and purchase intention.

Regarding the online sensorial analysis, the word cloud analysis (**Fig. 7**) showed that the terms most frequently mentioned by the evaluators were “natural”, “organic”, “fresh”, “nutritious”, and “interesting”, indicating a positive sensory perception of leather-type products



Fig. 7 Word cloud of consumer perceptions about edible leathers produced with puree of ora-pro-nóbis leaves (POR) (40 g / 100 g of leather forming mass) with different concentrations of carrageenan gum (CG).

Although the hedonic scale results showed moderate sensory acceptance of the leather-type products made with ora-pro-nóbis, the complementary word cloud analysis revealed predominantly positive terms and significant evaluator interest. This behavior may be partly because these products are little known to most consumers, suggesting that familiarity with this type of formulation may have influenced the sensory evaluation. Nevertheless, responses reflected a positive perception of the product as a healthy and nutritious option, indicating potential for acceptance that could expand with greater dissemination.

4. CONCLUSIONS

The edible leathers made from ora-pro-nobis leaf puree and different concentrations of carrageenan gum proved to be functional products with attractive nutritional and sensory potential. Carrageenan gum significantly influenced physicochemical properties, improving luminosity, modifying color tone, and promoting a stable, flexible, uniform matrix. In relation to centesimal composition, it was observed that incorporation of carrageenan gum increased the ash content and reduced lipids, while protein and fiber levels remained adequate to be considered a relevant source of vegetable protein. Furthermore, the leathers showed remarkable antioxidant capacity and significant levels of phenolic compounds and minerals, indicating that they retain the functional benefits of ora-pro-nobis. The cytotoxicity assessment showed that leather was not cytotoxic. During storage, the addition of CG improved the preservation of bioactive compounds. These results suggest that combining ora-pro-nobis puree with carrageenan gum enables the production of healthy, convenient, plant-based snacks that meet demand for functional foods with added value, offering an innovative alternative to traditional ultra-processed products. In combination, this study demonstrates that ora-pro-nobis leathers with

carrageenan gum are viable functional food products, offering nutritional benefits, antioxidant properties, and favorable physical sensory characteristics, positioning them as a healthy and sustainable option.

Ethics approval and consent to participate

The sensorial analyze was approved by the Research Ethics Committee of São Paulo State University (UNESP) (CAAE: 83179824.6.0000.5411; approval number: 7.231.533). All procedures involving human participants were conducted in accordance with the ethical principles and relevant institutional guidelines and regulations of São Paulo State University (UNESP). Informed consent was obtained from all participants prior to their participation in the sensory evaluation.

Data Availability Statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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