

Reformulating gummy candies with carob flour: A nutritional strategy to enhance bioactive compounds and functional properties

Héctor Gómez-Llorrente, María Dolores Ortolá, Isabel Fernández-Segovia, José Manuel Barat, Édgar Pérez-Esteve^{*}

Instituto Universitario de Ingeniería de Alimentos Food-UPV, Universitat Politècnica de València, C/ Camf de Vera s/n, Valencia, Spain

ARTICLE INFO

Keywords:

Confectionery
Ceratonia L. siliqua
 D-pinitol
 Postprandial glycemia modulation
 Antioxidant activity

ABSTRACT

The growing demand for healthier foods has driven the development of functional products in the confectionery sector. In this context, carob (*Ceratonia siliqua* L.), a Mediterranean legume naturally rich in fiber, polyphenols, and D-pinitol, was incorporated into gelatin-based gummies to enhance their nutritional and functional properties. The incorporation of carob flours with different roasting degrees and milling sizes had a significant impact on fiber, D-pinitol, and phenolic content, as well as on antioxidant activity and α -glucosidase inhibitory capacity. Among the tested conditions, the intermediately roasted flour (R2) and industrial milling (R2IM) achieved the best balance between functional, sensory, and safety attributes, showing low levels of hydroxymethylfurfural (HMF) and absence of acrylamide. In vitro digestion assays of the gummies, compared with sugar solutions containing equivalent amounts of carob, revealed that polyphenols were more stable under both gastric and intestinal conditions, whereas D-pinitol exhibited a delayed but more sustained release. These findings would indicate that the protective and modulating effects observed are mainly attributed to the interactions between carob components and the gelatin matrix, which enhance gastrointestinal stability and enable controlled bioactive delivery. Overall, these results highlight the potential of carob as a multifunctional ingredient in gelatin-based confectionery, supporting the development of health-oriented sweets with improved nutritional profile, antioxidant stability, and potential to modulate postprandial glycemia through gradual compound release.

1. Introduction

Consumption of confectionery products, especially gummy-type candies, has experienced sustained growth at an international level in recent years. In countries such as the United States, it is estimated that around 97 % of the population consumes them at least once a year (Hornick et al., 2014), while in Europe, the annual growth rate of Grand View Research, a based market research and consulting company, estimates a growth of 12.5 % between 2025 y 2030 (Grand View Research, 2024). This global increase in demand is mainly attributed to the sensory pleasure these products provide, their wide availability, and the influence of marketing strategies targeting diverse audiences, especially children and young people. Additionally, their ease of consumption, extended shelf life, and relatively low price compared to other snack categories contribute to their growing popularity across various social and cultural contexts (Roudbari et al., 2024; Tarahi et al., 2023).

Traditional gummy candies are primarily made from simple sugars such as sucrose and glucose, along with gelling agents, such as gelatin, resulting in products with limited nutritional value and high levels of "empty calories" without any health-beneficial nutrients (Bernier et al., 2024; Ergun et al., 2010; Hinkova et al., 2015), which only add to the overall caloric intake, thereby contributing to the development of overweight and obesity (Caballero et al., 2023; Galvez Espinoza et al., 2022).

In this context, the development of functional gummies becomes necessary products designed not only to meet the sensory expectations of consumers but also to offer nutritional and functional benefits. In recent years, considerable efforts have been made to design specific new products that help provide advantages such as immune support, digestive health, or stress management (Briceño et al., 2024; Pienihäkkinen et al., 2024; Rubio-Arrea et al., 2016; Wickham & Spriet, 2018). Nevertheless, there remains a pressing need to identify functional

^{*} Corresponding author.

E-mail address: edpees@upv.es (É. Pérez-Esteve).

<https://doi.org/10.1016/j.fhfh.2025.100262>

Received 2 September 2025; Received in revised form 30 October 2025; Accepted 11 December 2025

Available online 11 December 2025

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ingredients capable of replacing sugars, thereby improving the nutritional profile without compromising sensory acceptability.

In this regard, carob (*Ceratonia siliqua* L.), the fruit of the carob tree, emerges as a promising candidate. Traditionally used in animal feed, it has recently gained interest in the human food industry due to its nutritional value and sensory profile similar to cocoa (Loullis et al., 2018). Unlike cocoa, it contains no stimulants such as caffeine or theobromine and has a lower caloric value, making it suitable for children (Fatima et al., 2024). Carob flour is notable for its high fiber content (up to 40 %, mostly insoluble), low fat content, and presence of minerals such as calcium, potassium, and magnesium (Castillejo et al., 2025). Moreover, it contains bioactive compounds, primarily polyphenols like gallic acid, tannins, various flavonoids, and D-pinitol, which have been associated with antioxidant, glycemia modulation, hypolipidemic and hepatoprotective effects (Kong et al., 2024; Kim et al., 2007; Mithul Aravind et al., 2021; Zunft et al., 2003).

To develop its characteristic organoleptic profile, carob pulp must undergo a thermal roasting process. This treatment improves microbiological stability and enhances sensory properties through non-enzymatic browning reactions such as the Maillard reaction and caramelization (Rodríguez-Solana et al., 2019). These transformations not only affect color, aroma, and flavor but also influence the final chemical composition. During roasting, melanoidins and volatile compounds may form, generating sensory notes reminiscent of cocoa and coffee (Debona et al., 2025). Functionally, roasting also impacts the polyphenol content, which can degrade or even increase antioxidant or glycemia modulation if the roasting is light or medium (Cegledi et al., 2024). In the case of D-pinitol, its thermal stability has been demonstrated, allowing its functionality to be preserved after heat treatment. In conclusion, carob flour appears to be an ideal functional ingredient for the formulation of healthier gummies.

Additionally, gelatin has been shown to be effective in the controlled release of molecules within a food matrix (Young et al., 2005). In this context, the incorporation of carob flour emerges as a particularly interesting strategy, since it contains a wide array of bioactive compounds with health-promoting properties, as previously discussed. Thus, combining gelatin with carob in gummy formulations would not only preserve desirable texture and sensory qualities but also create a system capable of delivering carob-derived bioactives in a controlled manner. Such an approach could result in a product with enhanced nutritional value, offering sustained energy release, antioxidant potential, and additional benefits related to glycemic regulation.

In this context, the aim of the present work is to evaluate the impact of using different types of carob flour, varying in roasting degree and milling, on the nutritional, functional, and sensory properties of gummy-type candies. This research seeks to provide scientific evidence on the potential of carob as a functional ingredient and to support the development of value-added confectionery products that better meet current consumer demands, including those of specific populations such as sportspersons (i.e. provide rapid but sustained energy release during training or competition, support glycemic control, and supply bioactive compounds with antioxidant or anti-inflammatory properties to aid recovery).

2. Materials and methods

2.1. Materials

The carob pods utilized in this study were supplied by GA Torres S.A. (Quart de Poblet, Valencia, Spain). Sucrose was purchased from a local supermarket, while gelatin was sourced from Junca Gelatines, S.L. (Girona, Spain).

Acetonitrile, acetone, ammonium carbonate, ascorbic acid, calcium chloride dihydrate, ethanol, hydrochloric acid, magnesium chloride hexahydrate, methanol, pentaerythritol, phosphoric acid, potassium chloride, monopotassium phosphate, pyridine, sodium bicarbonate,

sodium carbonate, sodium chloride, and sodium 1-heptanesulfonate, sodium hydroxide were supplied by Scharlab S.L. (Barcelona, Spain). On the other hand, α -amylase, protease, amyloglucosidase 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH), 4-nitrophenol- α -D-glucopyranoside (PNG), 5-(Hydroxymethyl)furfural, acrylamide, Folin-Ciocalteu reagent, fluorescein, gallic acid, Pepsin (P7012) from gastric porcine mucosa, pancreatin from porcine pancreas and porcine bile extract, pefabloc®, pepsin from gastric porcine mucosa, porcine bile extract (B8631), p-toluenesulfonyl chloride (TsCl), and Trolox were purchased from Sigma-Aldrich (Darmstadt, Germany).

2.2. Preparation and characterization of the flours

To evaluate the effect of roasting and milling degree on the nutritional, functional, and sensory properties of the gummy candies, four types of carob flours were prepared.

Natural carob flour (N) was obtained by milling unroasted carob pods using an HC-2500Y grinder (Vevor, Shanghai, China). For the roasted flours, the pods were subjected to thermal treatments in a SelfCookingCenter® oven (Rational, Landsberg am Lech, Germany) prior to milling under the same conditions. Roasting at 200 °C for 195 min yielded flour R1; at 240 °C for 195 min, flour R2 was obtained; and at 280 °C for the same duration, flour R3 was produced. The time and temperature parameters were established based on typical industrial practices and corroborated by studies reporting roasting temperature ranges between 150 and 450 °C (Eldeeb & Mosilhey, 2022; Yousif & Alghzawi, 2000). After milling, all flours were sieved, and only the fraction smaller than 200 μ m was retained for use in the gummy formulations. To further assess the influence of particle size on carob flour and gummy candies properties, the medium-roasted flour (R2) underwent an additional industrial milling process to obtain finer particles, below 75 μ m, referred to as R2IM, with a particle size more comparable to commercially available flours.

Once flours were obtained, a comprehensive physicochemical characterization was carried out, including the determination of pH, particle size, intrinsic color, water swelling capacity, and water holding capacity.

2.2.1. Extractable pH

To determine the extractable pH, a standardized 12 % (w/v) aqueous suspension of carob flour was prepared in distilled water, thoroughly homogenized, and analyzed using a calibrated SevenEasy pH meter (Mettler Toledo, Barcelona, Spain). Assay was carried out in triplicate ($n = 3$).

2.2.2. Intrinsic color

The intrinsic color of the carob flours was objectively quantified using a CM-3600d spectrophotometer (Konica Minolta, Tokyo, Japan), following the methodology described by Vekiari et al. (2011) in triplicate ($n = 3$). A standardized 5 % (w/v) aqueous suspension of carob flour was prepared in distilled water. The mixture was thoroughly homogenized and transferred into a standard 1 cm optical path glass cuvette. Measurements were performed by reflectance, using a standard D65 illuminant and a 10° standard observer angle. Chroma (C^*ab) which express the color saturation and hue angle (h^*ab) which indicates the color tone, were calculated using following Eqs. (1) and (2) (Wibowo et al., 2015):

$$C^*ab = \sqrt{(a^*)^2 + (b^*)^2} \quad (1)$$

$$h^*ab = \arctan\left(\frac{b^*}{a^*}\right) \quad (2)$$

2.2.3. Particle size characterization

Particle size characterization was performed following the methodology described by Tsatsaragkou et al. (2023), using laser diffraction with a Mastersizer 2000 particle size analyzer (Malvern Instruments, UK). Measurements were conducted in wet mode using the Hydro 2000S liquid dispersion unit. The instrument was set to perform 10 measurement cycles, each lasting 10 s, with a sample refractive index of 1.52 and an absorption index of 0.1. Distilled water, with a refractive index of 1.33, was used as the dispersant. Assay was conducted in triplicate ($n = 3$).

2.2.4. Water holding capacity

Water holding capacity was determined using the method proposed by Sosulski et al. (1976). One gram of the carob flour was mixed with 10 mL of distilled water and allowed to hydrate for 30 min at room temperature ($30 \pm 2^\circ\text{C}$). The mixture was then centrifuged at $2000 \times g$ for 30 min and supernatant was removed. The result was expressed as the percentage of water retained by the flour per unit of mass. Assay was performed in triplicate ($n = 3$).

2.2.5. Swelling capacity

Swelling capacity was quantified according to the method described by Okaka and Potter (1977). Briefly, a volume of 10 mL of flour was hydrated with distilled water to reach a final volume of 50 mL in a graduated cylinder. Then, the mixture was inverted to mix, allowed to stand for 2 min, inverted again, and left to rest for an additional 8 min. Subsequently, the volume of the swollen sample was recorded. Swelling capacity was calculated as the volume of distilled water retained per gram of flour (mL/g). Assay was carried out in triplicate ($n = 3$).

2.3. Preparation of carob gummy candies

Prior to the initiation of the study, a comprehensive evaluation of various water and carob flour levels and binding agents, including gelatin and saccharose, was conducted to obtain the standardized formula. The selection of the standard formula was made after considering the texture evaluation by a panel of 10 tasters involved in the investigation group. The control formulation (C) ultimately selected consisted of 59 g/100 g of sucrose 5 g/100 g of gelatin, and 36 g/100 g water. Afterwards, the ratio of sucrose and carob flour that provides acceptable texture attributes was determined by same panel in 9:1. Therefore, the optimized formulation for the carob-enriched candies contained 53.1 g/100 g sucrose, 5.9 g/100 g carob flour, 5 g/100 g gelatin, and 36 g/100 g water. Efforts to increase the carob flour content were unsuccessful because the gelling process did not result in a gummy with a cohesive structure.

Preparation of carob candies involved a multistep process. First, tap water (20 % w/w) was added together with sucrose (51.9 % w/w) and carob flour (5.9 % w/w) to the Thermomix® (TM31, Vorwerk, Germany) for **N**, **R1**, **R2**, **R3**, **R2IM**; while sucrose (59 % w/w) was used for **C** formulation. Second, mixture was left at a temperature of 100°C (Varoma function) for 5 min at a speed setting of 2, achieving a proper dissolution and integration of the components. In parallel, gelatin (5 % w/w) was dissolved in tap water (16 % w/w) previously heated at 60°C . Once the first cooking phase was completed, the third step entailed the incorporation of a gelatin solution into the initial mixing phase, followed by a 5 min stirring process at a temperature of 60°C and a speed setting of 2. This phase facilitated the integration of the homogeneous phase without compromising the integrity of the gelatin. Finally, uniform and lump-free mixture was poured into cylindrical moulds, previously impregnated with a layer of sunflower oil, with a diameter of 28 mm and a height of 20 mm. After filling, the moulds were covered with cling film to prevent air contact and placed in refrigeration chambers at 20°C for 24 h, allowing the gummy candies to solidify completely.

Subsequent to the preparation of control and carob gummy candies, a series of physical-chemical, technological, functional, and sensory

analyses were conducted, as delineated in the following sections.

2.4. Fiber content

Dietary fiber content was quantified following the standardized protocol 991.43 established by the Association of Official Analytical Collaboration (AOAC, 2016). Briefly, samples were sequentially digested with heat-stable α -amylase, protease, and amyloglucosidase to remove digestible starch and protein. After enzymatic digestion, soluble dietary fiber was precipitated by the addition of 95 % ethanol. The total dietary fiber, including both insoluble and precipitated soluble fractions, was collected by filtration, washed with ethanol and acetone, dried, and weighed gravimetrically. Analysis was carried out in triplicate ($n = 3$).

2.5. Physicochemical analysis

2.5.1. Determination of pH, moisture content, and water activity

The pH was measured using a pH meter equipped with a penetration probe for foodstuffs (SevenEasy, Mettler Toledo, Barcelona, Spain). Moisture content was determined using the gravimetric method AOAC 925.09 (AOAC, 2016), and water activity (a_w) was assessed following the protocol described by Tan et al. (2025), using an Aqualab 4TE dew point hygrometer (Pullman, Washington, USA). To ensure accuracy and consistency, each formulation was analyzed in triplicate ($n = 3$).

2.5.2. Color analysis

Instrumental color measurements were conducted employing the same spectrophotometer and settings as detailed in Section 2.2.3. In this case, an 8 mm aperture diaphragm was utilized for data acquisition. The results of the chroma and tone calculations are detailed in Section 2.2.3. Each formulation was analyzed in triplicate ($n = 3$).

2.5.3. Texture profile analysis (TPA)

The textural properties of the gummy candies were evaluated using a Texture Profile Analysis (TPA), following the methodology described by Rubio-Arreaz et al. (2016), with a texture analyzer (TA.XTplus, Stable Micro Systems, Godalming, UK). The device was equipped with a 50 kg load cell. A cylindrical probe with a diameter of 45 mm was used to apply the compression force to the gummy samples. Each sample underwent two consecutive compression cycles. The deformation applied in each cycle corresponded to 50 % of the initial sample height, at a speed of 1 mm/s, with a 15-second interval between the two cycles. Subsequently, the following parameters were determined in accordance with Bourne (1978): hardness, adhesiveness, springiness, cohesiveness, chewiness, and resilience. To ensure accuracy and reliability of the findings, each gummy formulation was analyzed in sextuplicate ($n = 6$).

2.6. Chemical analysis

2.6.1. Extracts obtention

To determine the functional properties of the **C**, **N**, **R1**, **R2**, **R3** and **R2IM** gummy candies, a hydroalcoholic extract was first prepared following the procedure described by Ribes et al. (2025) with some modifications. In this method, 5 g of sample were mixed with 15 mL of a 50:50 (v/v) ethanol-water solution in 50 mL Falcon tubes. The mixture was dispersed using an Ultraturrax® (Z732443, Sigma-Aldrich, MilliporeSigma, Massachusetts, USA), operating for 1 min at 9800 rpm and then for 1 min at 11,500 rpm. After homogenization, samples underwent 10 min of ultrasound treatment at room temperature (RT) to enhance analyte mass transfer into the solvent. The liquid phase was separated from solid residues by centrifugation at 8500 rpm at RT. The supernatant was collected. This extraction process was repeated twice more on the solid residue. For the final cycle, 15 mL of an acetone-water mixture (70:30; v/v) was used. All extracts were combined, stored at -18°C until analyses of 5-hydroxymethylfurfural (HMF) and acrylamide, total phenolic content and antioxidant activity, while liquid were removed

under vacuum and lyophilization for D-pinitol and α glucosidase activity quantification.

2.6.2. 5- hydroxymethylfurfural and acrylamide content

The quantification of HMF and acrylamide in gummy extracts was carried out following the methodologies described by [Martin-Perez et al. \(2025\)](#) and [Michalak et al. \(2013\)](#), respectively. Both analyses were performed using the same chromatographic equipment detailed in [Section 2.6.4](#), with an injection volume of 10 μ L.

For HMF, an isocratic mobile phase of deionized water–acetonitrile (80:20, v/v) was used at a flow rate of 1.0 mL/min, with UV detection at 285 nm. For acrylamide, the mobile phase consisted of 5 mM sodium 1-heptanesulfonate in water–acetonitrile (97:3, v/v), under isocratic conditions, with a flow rate of 1.0 mL/min and detection wavelength fixed at 200 nm.

Calibration curves for both compounds were established using standard solutions, enabling accurate quantification based on extract concentrations.

2.6.3. Total phenolic content

The quantification of Total Phenolic Content (TPC) was performed using the Folin–Ciocalteu method as described by [Ribes et al. \(2025\)](#). For each assay, a volume of 15.8 μ L of each sample (diluted 1:2, 1:3 or 1:4 in the extraction solution, containing 57 % v/v of water, 33 % v/v of ethanol and 10 % v/v of acetone) was transferred into a 96-well microplate. Subsequently, 158 μ L of Folin–Ciocalteu reagent diluted 1:10 in double-distilled water were added and incubated for 2 min. Afterwards, a volume of 126 μ L sodium carbonate solution previously dissolved in distilled water at a concentration of 1 M were added to each well. After 1 h of incubation in darkness at RT, the absorbance of the mixture reagents was measured at 765 nm using a multimode plate reader (VANTASTAR, BMG Labtech, Ortenberg, Germany). Absorbance values were interpolated from a standard calibration curve prepared with gallic acid in the extraction solution. Results were expressed as milligrams of gallic acid equivalents per 100 g of gummy sample (mg Trolox E/100 g). Analysis was conducted three times in triplicate ($n = 9$).

2.6.4. Antioxidant capacity

Antioxidant activity was determined by two methods. On the one hand, the method based on the ability of the samples to scavenge the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), following the method by [Shahidi et al. \(2006\)](#). A total of 30 μ L of diluted gummy extract (1:3 and 1:2 in the extraction solution) was mixed with 270 μ L of DPPH solution previously dissolved in methanol at a concentration of 64.9 μ M. After 1 h of incubation darkness at RT, the absorbance was measured at 517 nm using a VANTASTAR microplate reader. Absorbance values were interpolated from a standard calibration curve prepared with Trolox in the extraction solution. Results were expressed as milligrams of Trolox equivalents per 100 g of gummy sample (mg Trolox E/100 g). Analysis was conducted three times in triplicate ($n = 9$).

On the other hand, antioxidant capacity was also evaluated by oxygen radical absorbance capacity (ORAC) assay. For this aim, a methodology described by [Cao et al. \(1993\)](#) with slight modifications was performed. Briefly, a volume of 20 μ L of diluted gummy extract (1:100 and 1:300 in 75 mM phosphate buffer; pH 7.4) was mixed with 70 μ L of 200 nM fluorescein, previously diluted in same buffer, and incubated in darkness at 37 °C for 15 min. Subsequent to the incubation period, a volume of 110 μ L of 250 mM AAPH, previously diluted in the buffer, was added, and fluorescence (excitation wavelength 485 nm, emission wavelength 520 nm, at a controlled temperature of 37 °C) was measured at 1-minute intervals for a duration of 110 min using a VANTASTAR microplate reader. Calibration curve was prepared with Trolox at different concentrations in 75 mM phosphate buffer at pH 7.4. Results expressed as milligrams of Trolox equivalents per 100 g of sample (mg Trolox E/100 g) were calculated by normalized areas under the

fluorescence decay (AUC). Analysis was conducted three times in triplicate ($n = 9$).

2.6.5. D- pinitol quantification

The quantification of D-pinitol of samples was performed using High-Performance Liquid Chromatography (HPLC) with a UV detector, following the study of [Pereira et al. \(2012\)](#) with minor modifications. A total of 10 mg of lyophilized gummy was resuspended in 150 μ L of pyridine, sonicated, and centrifuged. A volume of 90 μ L of supernatant was combined with 50 mg of TsCl and an internal standard (pentaerythritol at a concentration of 1 mg/mL) and incubated at 50 °C for 3 h. Afterwards, a volume of 500 μ L of acetonitrile was added, and the mixture was sonicated before being filtered through a 0.45 μ m membrane for HPLC analysis.

Chromatographic separation was performed on a Hitachi LaChrom Elite HPLC system equipped with a UV detector, utilizing an InfinityLab Poroshell 120 EC—C18 column (Agilent, Barcelona, Spain). Mobile phase, flow, injection volume and detection length were retrieved from [Pereira et al. \(2012\)](#). Each sample was analyzed in triplicate ($n = 3$) and the results were expressed as mg pinitol per 100 g of gummy sample (mg Pinitol/100 g).

2.6.6. Determination of α -glucosidase activity

The effect of the carob gelatin-based gummies to modify the postprandial glycemia was measured by their capacity to inhibit the α -glucosidase activity. To this end, the materials and methodology previously documented in the study of [Cardullo et al. \(2020\)](#) were employed with minor modifications. Briefly, a volume of 25 μ L of α -glucosidase solution (1 U/mL in 0.1 M phosphate buffer; pH 6.8) was mixed with 0.5 mg/mL of gummy extracts dissolved in same buffer and incubated at 37 °C for 10 min under shaking at 400 g in a 96-well microplate. Subsequently, a volume of 25 μ L of the PNG substrate (5 mM in 0.1 M phosphate buffer, pH 6.8) was added, and the microplate was incubated at 37 °C for 5 min under shaking at 400 g. The reaction was stopped by the addition of 75 μ L 1 M sodium carbonate buffered solution and was subsequently measured at 405 nm using microplate reader. Acarbose (0.5 mg/mL in 0.1 M phosphate buffer, pH 6.8) was utilized as the reference standard.

The calculation of the inhibition percentage was performed using the following [Eq. \(3\)](#):

$$\text{Inhibition (\%)} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100 \quad (3)$$

where Abs control is the absorbance measured for the mixture enzyme/substrate (without gummy extract); Abs sample is the absorbance measured in the same conditions and in the presence of the gummy extracts. The percentage of enzymatic activity inhibition was compared to those calculated in the reference standard.

2.7. Bioaccessibility of functional compounds

The bioaccessibility of polyphenols and D-pinitol throughout the gastrointestinal tract was evaluated in **R2IM** carob gelatin-based gummies, as well as in a corresponding sugar–carob solution prepared without gelatin but subjected to the same thermal treatments described in [Section 2.3](#). Both samples were subjected to in vitro gastrointestinal digestion following the standardized INFOGEST 2.0 protocol ([Ribes et al., 2025](#)).

Briefly, samples were subjected to the chew-and-spit method, performed by a healthy young male volunteer with excellent oral health. Each sample was chewed until the natural urge to swallow occurred, then transferred to a centrifuge tube and immediately placed on ice.

Before initiating the gastric phase, an initial aliquot (t_0) was collected from the supernatant and enzymatic activity was halted using 6 M HCl. Simulated gastric digestion was performed by mixing the oral bolus with

simulated gastric fluid and pepsin (2000 U/mL) in a 1:1 (v/v) ratio, adjusting the pH to 3 with 6 M HCl, and incubating for 120 min. Samples were collected every 15 min and enzymatic activity was stopped by neutralizing to pH 7 with 1 M NaOH.

For the intestinal phase, the gastric chyme was mixed with simulated intestinal fluid, pancreatin (adjusted to 100 U/mL trypsin activity), and bile salts (10 mM) in a 1:2 (v/v) ratio. The pH was raised to 7 using 1 M NaOH, and digestion continued for 120 min. Samples were collected every 15 min and treated with Pefabloc® (1 µL, 500 mM) to inhibit proteolysis.

Blank controls (containing enzymes and bile only) and undigested controls (sample and water, 1:1 w/v) were processed under identical conditions. The entire digestion procedure was performed in triplicate at 37 °C with continuous gentle mixing using an Intelli-Mixer™ RM-2 placed inside a Selecta incubator chamber. All samples were centrifuged (8000 × g, 10 min, 4 °C). Supernatants were used for analyzing total polyphenols and D-pinitol content as described in Sections 2.6.3 and 2.6.5, respectively.

2.8. Sensory analysis

The sensory evaluation of carob-based products was conducted in a dedicated sensory laboratory equipped with individual booths, designed in accordance with the UNE-EN ISO, 8589:2010 standard to minimize bias and ensure consistent testing conditions. A total of 100 untrained consumers, aged between 20 and 55 years, participated in the study. The evaluation was carried out through an anonymous survey developed using Google Forms, which participants accessed via a QR code using their mobile devices.

Prior to beginning the testing, participants were provided with detailed information about the project and the nature of the test, followed by a request for informed consent. This protocol adhered to the principles of the Helsinki Declaration and was approved by the Ethics Committee of the Universitat Politècnica de València (Reference Number: P01_30-06-2025). Participants were then asked to provide demographic data, including gender, age, frequency of gummy consumption and knowledge of carob.

The samples prepared according to standardized procedures (see Section 2.3) and served monadically, each marked with randomly assigned three-digit codes.

Sensory evaluation was performed using three types of scales. First, a 9-point hedonic scale (UNE-ISO, 4121:2006) was used to assess overall liking, texture, and flavor, where 1 indicated “Dislike extremely” and 9 “Like extremely.” Second, a 5-point Just-About-Right (JAR) scale was used to evaluate specific attributes such as color, gloss, aroma, texture, sweetness, and flavour. A score of 3 represented “Just about right,” while scores of 1–2 and 4–5 indicated insufficient or excessive intensity, respectively. This allowed for the identification of attributes requiring adjustment. Based on the acceptability data, penalty analysis was performed to determine the impact of non-optimized attribute levels on overall liking (Fernández-Segovia et al., 2018). Finally, a 5-point purchase intention scale was included, where 1 meant “Definitely would not buy” and 5 “Definitely would buy.” At the end of each sample evaluation, participants were invited to leave additional comments in a free-text section. To minimize residual effects between samples, participants were instructed to rinse their mouths with water before each tasting.

2.9. Statistical analysis

Statistical analyses were conducted using Statgraphics Centurion software (versions XVII and XIX; Statpoint Technologies, Warrenton, VA, USA) to evaluate the effect of formulation type on nutritional, technological, physicochemical, and functional properties. A one-way analysis of variance (ANOVA) was applied, followed by the least significant difference (LSD) test, to identify statistically significant

differences among formulations at a 95 % confidence level ($p < 0.05$). To perform the association between variables, Spearman's rank correlations were performed (Gómez-Llorente et al., 2025).

Sensory evaluation data, including overall liking and purchase intention, were processed using XLSTAT 2020 software (Long Island, NY, USA). To determine whether formulation influenced consumer perception, a Kruskal–Wallis test was performed, followed by Dunn's post hoc test with Bonferroni correction ($p < 0.05$). Additionally, data from the Just-About-Right (JAR) scale were simplified from a 5-point to a 3-point format to avoid sparsely populated categories, as recommended in previous studies (Palma-Morales et al., 2024). Penalty analysis was further applied to assess how deviations from optimal sensory attributes impacted on overall liking (Fernández-Segovia et al., 2018). Differences in penalty analysis across JAR categories were further examined using ANOVA-based followed by Tukey's HSD test to determine the significance of intergroup variations.

3. Results and discussion

3.1. Characterization of the carob flours

Table 1 presents the physicochemical properties of carob flours subjected to different roasting levels and milling conditions, including pH, color parameters, particle size distribution, swelling capacity, and water holding capacity.

The pH of the samples ranged from 4.45 to 4.97, with **R1** flour showing the highest (least acidic) value and **R3** the lowest, reflecting a clear decrease in pH with increased roasting intensity. This acidification is mainly driven by caramelization and Maillard reactions, which generate acidic intermediates like pyruvic, acetic, lactic, and formic acids, as well as furan and pyrrole compounds (e.g., HMF and furfural) that contribute indirectly to acidity through further reactions (Reyes Suárez et al., 2025). Additionally, the formation of melanoidins in advanced Maillard stages releases carbonyl and carboxyl-containing molecules, explaining the progressive pH drop as roasting and milling intensify (Boublenza et al., 2017; Jaeger et al., 2010). **R2IM** also had a lower pH than **R2**, highlighting the influence of particle size on acid solubilization.

When evaluating the color parameters of the different carob flours, **R1** flour showed the highest lightness (ca. $L^* = 56$), while **R3** had the lowest (ca. $L^* = 33$), indicating a general decrease in lightness with increased roasting intensity. The significantly higher L^* of **R1** suggests mild thermal treatment without strong pigmentation. The observed trends are attributed to intensified non-enzymatic browning, primarily Maillard reactions and caramelization. As roasting time and temperature increase, the product darkens, confirming that the roasting treatments were suitable for producing distinct carob flour types. **R2IM** displayed lower lightness (ca. $L^* = 38$) than **R2**, likely due to the effect of particle size on color perception. Regarding chroma (C^*) and tone (h^*ab), all flours differed significantly; chroma decreased consistently with roasting (from 26.6 in **N** carob flour to 15 in **R3**), while tone was more affected by milling intensity than roasting level (Eldeeb & Mosilhey, 2022).

Regarding the particle size distribution, $D[4,3]$ values were highest in the unroasted carob flour (**N**) and progressively decreased with increasing roasting intensity, reaching significantly lower levels in sample **R3**. This downward trend was also evident in the $d(0.9)$, suggesting that higher roasting promotes greater particle breakdown during water dispersion. According to different studies, this phenomenon could be attributed to tissue structure weakening caused by roasting, such as cell wall rupture and protein denaturation (Macku Villa & Rodriguez, 2022), making roasted flours more prone to disintegration when stirred in water (Belorio et al., 2019). Additionally, significant differences in particle size parameters between **R2** and industrially milled **R2IM** samples, confirm the effectiveness of industrial milling in achieving finer particle sizes.

Table 1

Physicochemical properties of carob flours subjected to different roasting levels and milling conditions: pH, colour, WHC (water holding capacity) and swelling capacity. Values were expressed as mean (3) $\pm$ SD.

	pH	Colour			Particle Size Distribution (μ m)				WHC (mL/100 g)	Swelling capacity (mL/100 g)
		L*	C*	h*	D [4, 3]	d (0.1)	d (0.5)	d (0.9)		
N	4.68 $\pm 0.01^c$	49.5 $\pm$ 0.3 ^d	26.6 $\pm$ 0.2 ^e	65.7 $\pm$ 0.2 ^b	85 $\pm$ 5 ^e	7,74 $\pm$ 0,17 ^e	63 $\pm$ 2 ^e	195 $\pm$ 13 ^{cd}	50.4 $\pm$ 0.1 ^d	10.56 $\pm$ 0.01 ^c
R1	4.96 $\pm 0.01^e$	56.8 $\pm$ 0.2 ^e	22.7 $\pm$ 0.2 ^d	66.6 $\pm$ 0.1 ^e	77,0 $\pm$ 1,8 ^d	5,29 $\pm$ 0,09 ^d	44,7 $\pm$ 1,1 ^d	199 $\pm$ 6 ^d	50.1 $\pm$ 0.1 ^d	10.54 $\pm$ 0.01 ^c
R2	4.76 $\pm 0.01^d$	46.2 $\pm$ 0.1 ^c	21.9 $\pm$ 0.0 ^c	66.0 $\pm$ 0.1 ^c	74 $\pm$ 3 ^c	5,16 $\pm$ 0,06 ^c	43,0 $\pm$ 1,2 ^c	190 $\pm$ 8 ^c	45.4 $\pm$ 0.6 ^b	10.13 $\pm$ 0.01 ^b
R2IM	4.63 $\pm 0.01^b$	38.0 $\pm$ 0.1 ^b	21.3 $\pm$ 0.2 ^b	61.4 $\pm$ 0.1 ^a	28,04 $\pm$ 0,12 ^a	4,16 $\pm$ 0,03 ^b	20,63 $\pm$ 0,09 ^a	63,7 $\pm$ 0,3 ^a	47.8 $\pm$ 0.4 ^c	10.11 $\pm$ 0.01 ^b
R3	4.45 $\pm 0.01^a$	33.3 $\pm$ 0.1 ^a	15.1 $\pm$ 0.0 ^a	66.3 $\pm$ 0.1 ^d	36,2 $\pm$ 0,4 ^b	3,49 $\pm$ 0,02 ^a	22,99 $\pm$ 0,16 ^b	89,4 $\pm$ 1,1 ^b	39.6 $\pm$ 0.2 ^a	9.89 $\pm$ 0.02 ^a

The capacity for swelling, a functional property defined by the ability of flour particles to absorb and retain water, resulting in an increase in volume, was observed to be maximal in **N** flour, while **R3** exhibited the minimal capacity. **R2** and **R2IM** exhibited intermediate and similar values, indicating no significant difference between them and suggesting that particle size did not influence the swelling capacity of the flours. Regarding water holding capacity, which, unlike swelling, refers to the flour's ability to retain water under external force (centrifugation), **R3** again showed the lowest value, whereas the highest values were observed in **N** and **R1** flour. **R2** and **R2IM** flours had intermediate values, but in this case, significant differences were found between them ($p < 0.05$), indicating that particle size did have an effect on water retention capacity.

In short, the roasting level of carob flour influences both swelling capacity and water holding capacity, with both parameters decreasing as roasting intensity increases. This is attributed to the effect of roasting on protein denaturation, polysaccharide degradation, moisture loss, Maillard reactions, and the decrease in the matrix hydrophilicity (Adimas & Abera, 2025). These functional changes could affect the properties of gummy products: flours with high water retention capacity, such as **N** and **R1**, may lead to firmer, more chewable gummies by forming a denser and more resistant structure. This could also impact the balance between free water and solids, potentially increasing adhesiveness and reducing cohesiveness, while influencing elasticity and resilience. Conversely, flours with lower water retention (like **R3**) would likely result in softer, and less chewable gummies due to reduced interaction with the aqueous phase.

Altogether, the findings of this study demonstrate that the implementation of disparate roasting treatments yielded the successful production of distinct types of carob flour, each exhibiting unique physicochemical and functional profiles. These differences are not only relevant from a technological standpoint but also suggest a strong potential to tailor the textural and sensory attributes of derived products such as gummy candies.

3.2. Physico-chemical characterization of candies

3.2.1. Fiber content

Dietary fiber is a key nutritional component linked to improved metabolic regulation, gastrointestinal function, and chronic disease prevention (Anderson et al., 2009). Carob flour, naturally rich in insoluble fiber and bioactive polyphenols, represents an attractive ingredient for the development of functional confectionery (Castillejo et al., 2025).

As shown in Table 2, the total dietary fiber content of carob-enriched gummies ranged from 2.7 to 2.9 g/100 g gummy across all formulations. Although no statistically significant differences were detected ($p > 0.05$), the values consistently approximated 2.7 g/100 g gummy, indicating that all formulations provide a meaningful contribution of fiber regardless of the roasting and grinding process.

Table 2

Total dietary fiber content of gummy candies (g/100 g). Values were expressed as mean (3) $\pm$ SD.

Sample	C	N	R1	R2	R2IM	R3
Total dietary fiber	n. d. ^a	2.9 $\pm$ 0.3 ^b	2.6 $\pm$ 0.4 ^b	2.7 $\pm$ 0.5 ^b	2.7 $\pm$ 0.5 ^b	2.8 $\pm$ 0.4 ^b

Carob gummies were formulated with different roasting carob flours; N: natural carob flour; R1–R3: roasted flours; R2IM: roasted flour finely milled at industrial scale. Different lowercase letters indicate statistically significant differences between samples ($p < 0.05$).

Beyond the quantitative values, the physiological implications of carob fiber are particularly relevant. Indeed, its fermentation in the colon leads to the production of short-chain fatty acids, which support epithelial integrity, strengthen the intestinal barrier, and exert anti-inflammatory effects (Liu et al., 2022). In addition, dietary fiber from carob acts as a reservoir of non-extractable polyphenols. These compounds are not readily available in the upper gastrointestinal tract but can be partially released through microbial fermentation in the colon. Once liberated, they may exert antioxidant, anti-inflammatory, and metabolic benefits, thereby contributing to the overall health-promoting effects of the fiber fraction. (Pérez-Jiménez et al., 2013). Finally, carob fiber may modulate postprandial glucose metabolism and appetite regulation through its effects on hormonal pathways, including the reduction of circulating ghrelin, modulation of insulin responses, and, indirectly, the stimulation of gut hormones such as GLP-1 and PYY (Gruendel et al., 2006, 2007).

Taken together, these attributes would highlight that carob-enriched gummies could be considered functional foods with added value for health promotion.

3.2.2. pH, moisture content, and water activity

The incorporation of carob flour, particularly in its roasted forms, significantly influences the physicochemical properties of gummy candies (Table 3). The pH values showed a decreasing trend as the

Table 3

pH, moisture content, and water activity of gummy candies. Values were expressed as mean (3) $\pm$ SD.

Sample	pH	Moisture (g water/100 g)	a _w
C	5.52 $\pm$ 0.04 ^f	27.5 $\pm$ 1.5 ^a	0.781 $\pm$ 0.005 ^a
N	5.26 $\pm$ 0.03 ^d	32 $\pm$ 2 ^b	0.8444 $\pm$ 0.0015 ^b
R1	5.33 $\pm$ 0.03 ^e	32.3 $\pm$ 1.7 ^b	0.842 $\pm$ 0.004 ^b
R2	5.21 $\pm$ 0.03 ^c	33 $\pm$ 2 ^{bc}	0.844 $\pm$ 0.004 ^b
R2IM	5.07 $\pm$ 0.02 ^b	35.1 $\pm$ 0.3 ^c	0.8464 $\pm$ 0.0007 ^b
R3	4.86 $\pm$ 0.06 ^a	34.7 $\pm$ 0.9 ^c	0.855 $\pm$ 0.002 ^c

N: natural carob flour; R1–R3: roasted flours; R2IM: roasted flour finely milled at industrial scale. Different lowercase letters indicate statistically significant differences between samples ($p < 0.05$).

roasting level of carob flour increased, in accordance with the different pH levels of the flours (see Section 3.1). This decrease may be attributed to the formation of acidic compounds during the roasting process, such as sugar degradation products and Maillard reaction intermediates. An increase in moisture content was observed in samples that underwent more intense roasting (R2 and R3) ($p < 0.05$). This behavior may be attributed to structural modifications after roasting (Gu et al., 2022). R2IM flour, which was subjected to industrial milling, demonstrated the highest moisture content. However, this variation was not statistically significant when compared to the same roasting flour milled in a laboratory setting (R2), suggesting that particle size would be not the key role in water interaction within the gummy matrix.

The water activity (a_w) followed a similar trend to moisture, increasing with the degree of roasting ($p < 0.05$). The lower a_w observed in the control gummies (C) is since sucrose is a highly hydrophilic solute that readily binds to water molecules through hydrogen bonding, thereby reducing the amount of free water available in the food matrix (Zuorro, 2021). On the other hand, the higher a_w measured in the gummies R3 may be related to the partial gelatinization of starch, which results in a reduced capacity to retain water in a bound and stable form (Tako et al., 2014). Additionally, while light roasting may cause controlled protein denaturation and expose more hydrophilic sites, moderate roasting leads to irreversible protein aggregation, reducing the surface area available for water interaction and leaving more water in its free state (Stone et al., 2021).

3.2.3. Color properties

The color parameters of the gummies formulated with carob flour under different roasting conditions and particle sizes are shown in Fig. 1.

Luminosity values (L^*) decreased progressively from N to R3, indicating a darkening of the gummies with increasing roasting intensity.

This trend is consistent with the formation of melanoidins and other brown pigments through Maillard reactions and caramelization during roasting (Bresciani et al., 2020). R3The gummies made with N flour showed the highest values for both the a^* and b^* coordinates. As the degree of roasting increases, these coordinates decrease, shifting the samples toward the origin of the plane, indicating a loss of red and yellow tones and exhibiting less vivid, more brownish colors characteristic of roasted products. Gummy candies made with R1 flour exhibited the highest C^* value, corresponding to the most intense and vivid color. In contrast, color intensity decreased dramatically in the gummies made with more roasted flour, thus reaching the lowest value in the sample R3. This loss of saturation is consistent with the darkening and appearance of more neutral tones (browns/grays) due to more severe toasting, as mentioned above. In terms of hue angle (h^*ab), statistically significant differences ($p < 0.05$) were only observed in the R3 gummies compared to the rest, which are located at the same hue angle, indicating a change in color hue towards a more reddish or brownish shade.

3.2.4. Mechanical properties

A texture profile analysis (TPA) was conducted to assess the impact of incorporating carob flour with varying degrees of roasting and milling on the mechanical properties of gummy candies. This analysis was conducted in comparison to a control sample (C) that did not utilize sucrose replacement by carob flour.

As can be seen in Fig. 2A, the hardness of the control samples was 27 ± 2 N. This hardness increased significantly ($p < 0.05$) when 10 % of the sucrose was replaced by carob flour with different degrees of roasting, reaching the maximum value when R1 flour was incorporated (55.6 ± 1.7 N). This increase can be attributed to the ability of the insoluble fiber (cellulose, hemicellulose, and lignin) in carob flour to retain water and

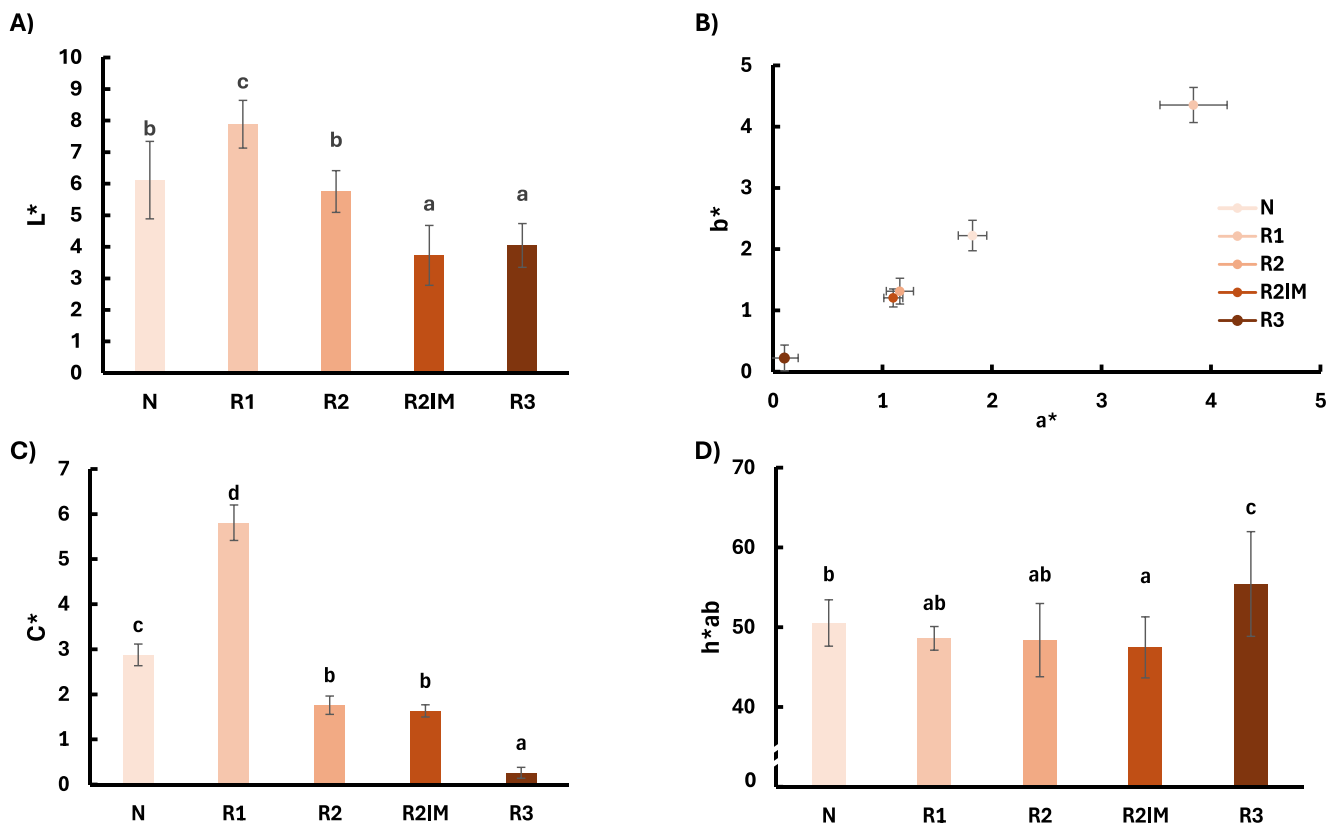


Fig. 1. Colour attributes of gummy candies: luminosity; L^* (A); a^* and b^* coordinates (B); chroma; C^* (C); hue angle; h^*ab (D). Values were expressed as mean (3) $\pm$ SD. Lowercase letters denote significant ($p < 0.05$) differences among formulations. Carob gummies were formulated with different roasting carob flours; N: natural carob flour; R1–R3: roasted flours; R2IM: roasted flour finely milled at industrial scale.

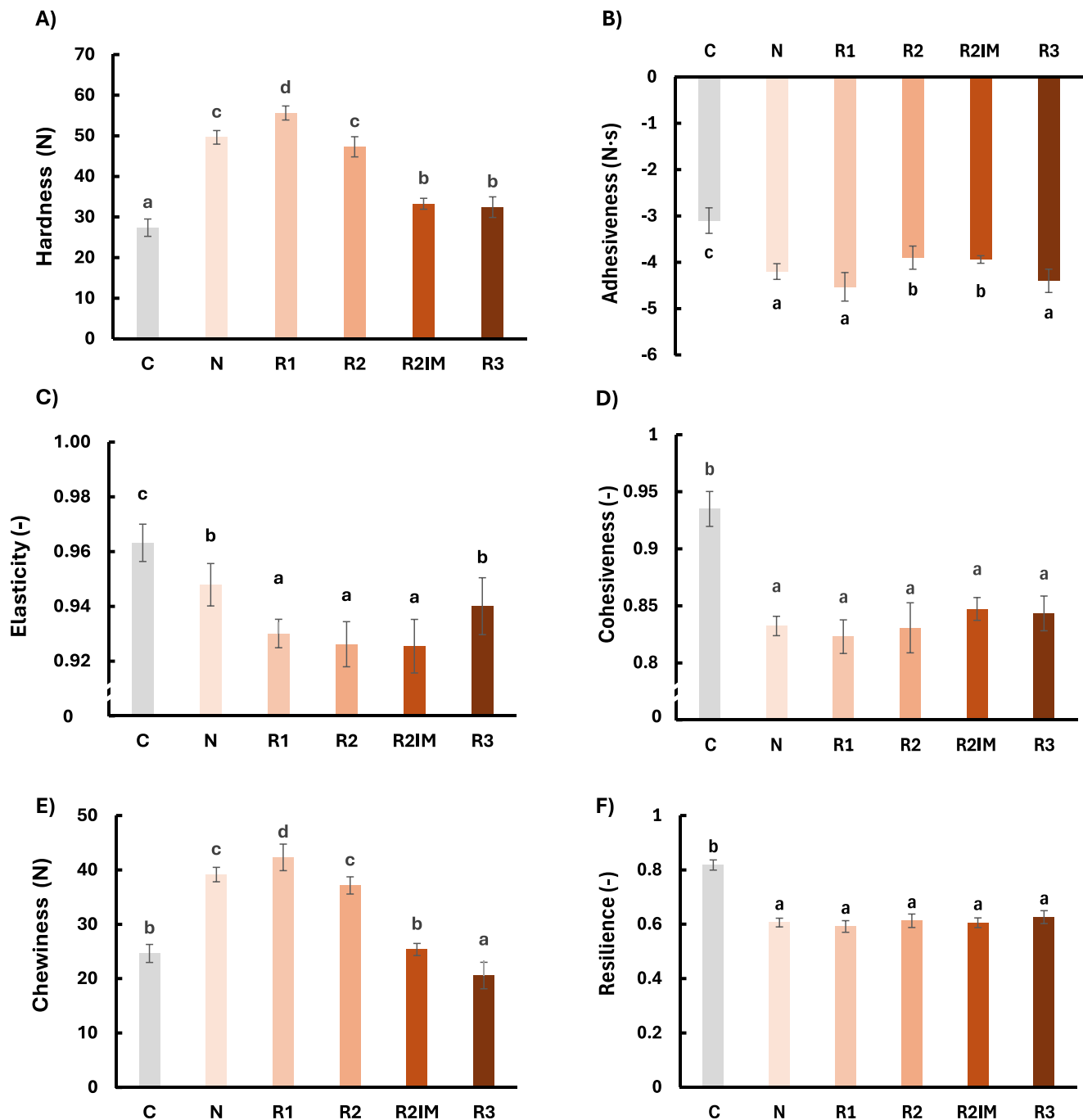


Fig. 2. Mechanical properties of gummy candies: hardness; N (A), adhesiveness; N-s (B), elasticity (C), cohesiveness (D), chewiness; N (E) and resilience (F). Values were expressed as mean (6) $\pm$ SD. Lowercase letters denote significant ($p < 0.05$) differences among formulations. Carob gummies were formulated with different roasting carob flours; N: natural carob flour; R1–R3: roasted flours; R2IM: roasted flour finely milled at industrial scale.

reinforce the gelatin matrix (Wang et al., 2017). However, at higher roasting levels, hardness decreased significantly, possibly due to thermal degradation of structural polysaccharides or a lower hydration capacity of the roasted flour (Román et al., 2017). On the other hand, the effect of milling process was also significant ($p < 0.05$). Indeed, R2IM gummies were less hard than R2 gummies, which could be attributed to better dispersion of the flour in the matrix at a smaller particle size, contributing to a less dense and firm structure (Pang et al., 2021). Chewiness (Fig. 2E) followed the same trend as hardness, which is consistent with its derivation from hardness, elasticity and cohesiveness, both of which were near 1 in all samples. Concretely, elasticity (Fig. 2C) ranged from

0.92 to 0.96, and cohesiveness (Fig. 2D) from 0.82 to 0.93, with the highest values in gummies made with unroasted flour (N). Although statistically significant differences were observed for hardness and chewiness among control and carob-enriched gummies ($p < 0.05$), as it can be observed, the magnitude of change between formulations was small.

Adhesiveness (Fig. 2B) was significantly more negative for carob-containing gummies compared to the control (C) one, without a clear trend related to flour roasting degree or milling conditions. This increased stickiness is likely due to the lower free sugar content and the higher polysaccharide content, which would provide more accessible

hydroxyl groups for interactions with surfaces, as fewer sugars are present in crystalline or soluble forms to occupy these binding sites.

Finally, the resilience of the control (C) gummies was significantly higher ($p < 0.05$) than that of the carob-containing samples (Fig. 2F). This indicates a reduced ability to recover their shape after deformation, likely due to disruption of the gel structure caused by the presence of insoluble fibers or the small fat fraction contributed by carob (Koç et al., 2019).

3.2.5. 5-hydroxymethylfurfural and acrylamide content

After assessing the impact of roasting on the physical properties, its effect on the formation of 5-hydroxymethylfurfural (HMF) and acrylamide was also evaluated. These compounds are typically formed during roasting processes and, if generated under the evaluated carob flour roasting conditions, could be transferred to the gummies, thereby representing a potential dietary source of exposure to these substances with genotoxic and carcinogenic potential (Kaur & Halford, 2023).

As shown in Table 4, C and N gummies contained HMF levels below the limit of detection (< 0.1 mg/L or 0.04 mg HMF/100 g gummy). In contrast, HMF was detected in gummies prepared with thermally treated carob, with significantly higher concentrations observed in those exposed to more intense roasting conditions ($p < 0.05$). Nonetheless, the applied treatments did not yield HMF concentrations of concern in most samples, taking as a reference the regulatory limit established for tropical honey (8 mg/100 g) (Council of the European Union, 2001). However, sample R3 exhibited a markedly elevated value, approximately three times above this threshold, suggesting that more severe heat exposure may increase precursor availability and promote HMF formation (Choudhary et al., 2021). Previous studies have reported HMF concentrations ranging from 4.5 to over 26 mg/100 g in carob syrups, which is consistent with the ranges found in our samples (Toufeili et al., 2022).

In contrast, acrylamide was not detected in any of the formulations, as its concentration remained below the limit of detection (< 0.02 µg/L or 0.008 mg/100 g gummy). This absence is a favorable outcome from a food safety perspective, considering the potential health risks associated with acrylamide formation during the thermal processing of both flour and gummies.

Based on these results, only sample R3 would present potential limitations from a food safety standpoint.

3.3. Functional characterization of carob gelatin-based gummies

3.3.1. Phenolic content

Polyphenols are bioactive compounds with well-documented antioxidant and health-promoting properties (Castillejo et al., 2025). Their concentration can be significantly influenced by processing conditions such as roasting and milling. Therefore, measuring total phenolic content (TPC) provides valuable insight into how these processes affect the potential health benefits of food products.

Fig. 3A shows the TPC of the different gummy formulations. As expected, samples without carob (C) exhibited negligible antioxidant activity. In contrast, the incorporation of carob flour significantly

increased TPC values, with statistically significant differences among groups ($p < 0.05$). Gummies prepared with unroasted carob (N) showed the lowest TPC (101 ± 9 mg gallic acid equivalents/100 g gummy). A marked increase was observed in formulations R1 and R2, roasted at 200 °C and 240 °C for 195 min, reaching 153 ± 9 and 157 ± 12 mg GAE/100 g, respectively. This trend aligns with findings by Mohamed Ahmed et al. (2024), who reported a 14 % increase in phenolic concentration after roasting carob at 150 °C for 15 min. Similar behavior has been observed in coffee, where roasting between 210 °C and 240 °C did not degrade key phenolic compounds such as chlorogenic acid and tannins (Van Cuong et al., 2014). However, in the sample with the highest roasting temperatures (280 °C, R3), a significant reduction in TPC was observed ($p < 0.05$), consistent with the observed thermal degradation of phenolic compounds presented in carob like gallic acid, ferulic acid, and syringic acid at temperatures above 250 °C (Castillejo et al., 2025; Khuwijtjaru et al., 2014). A comparison of the values obtained for gummy candies in this study with those reported in other studies that evaluated TPC using the same method revealed that the values for chocolate powder were similar (147 mg gallic acid/100 g product) (Ibrić & Čavar, 2014). This is especially relevant as in our formulation all polyphenol provides of the carob, which represents 4.5 % of total formula (see Section 2.3).

Despite the roasting effect on phenolic content, comparison of laboratory (R2) and industrial milling (R2IM) did not find significant reduction of values ($p > 0.05$). This suggests that the obtention of flours with lower particles size would not compromise the phenolic components.

3.3.2. Antioxidant activity

Measuring antioxidant activity in carbohydrate-based products such as gummies is essential for determining whether these compounds remain active after formulation and processing, and whether they can contribute to reducing oxidative stress in consumers. Additionally, antioxidant capacity can influence sensory stability, and nutritional value (Castillejo et al., 2025). In matrices rich in sugars and gelling agents, interactions with antioxidants might affect their bioavailability and efficacy (Sobaszek et al., 2020). This information supports health claims and enhances the appeal of gummies as convenient, health-promoting foods. Results of the antioxidant capacity of the carob gummies, evaluated by the DPPH and ORAC assay, are presented in Fig. 3B and C, respectively.

As expected, the control gummies (without carob flour) showed no antioxidant activity in either assay. In contrast, the functional gummies exhibited significant antioxidant activity, with notable differences depending on the degree of roasting of the carob flour used. According to the DPPH assay, the highest antioxidant activity was observed in samples made with R2 and R1 gummies (158 ± 12 and 154 ± 9 mg E Trolox/100 g gummy, respectively), followed by R2IM (149 ± 16 mg E Trolox/100 g gummy), and R3 (123 ± 12 mg E Trolox/100 g gummy) and N (101 ± 10 mg E Trolox/100 g gummy). These results suggest that moderate roasting (R1 and R2) enhances antioxidant capacity, possibly due to the formation of Maillard reaction products such as melanoidins, which possess antioxidant properties (Delgado-Andrade et al., 2005). On the other hand, excessive roasting (R3) would lead to degradation of thermolabile polyphenols, explaining the reduction of antioxidant activity ($p < 0.05$). Conversely, the ORAC assay yielded a divergent pattern of results. All functional gummies exhibited substantial antioxidant capacity (ca. 1.1 g Trolox E/100 gummy), except for the R3, which demonstrated values of 0.92 ± 0.23 g Trolox E/100 gummy ($p < 0.05$).

DPPH and ORAC assays demonstrated statistical differences of samples with R3, suggesting that the antioxidants present following roasting reduce their biological activity and capacity to scavenge peroxyl radicals. This would indicate that roasting may modify the mechanism of action or chemical structure of antioxidant compounds. Consistent findings with our study have been documented by Barba et al. (2013), who reported a magnitude of reduction reaching up to 10 times

Table 4

5-Hydroxymethylfurfural and acrylamide content of gummy candies (mg/100 g). Values were expressed as mean (3) $\pm$ SD.

Sample	C	N	R1	R2	R2IM	R3
HMF	n. d. ^a	n. d. ^a	$2.8 \pm$ 0.02^b	$5.0 \pm$ 0.02^c	$5.4 \pm$ 0.03^d	$22.8 \pm$ 0.02^e
Acrylamide	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Carob gummies were formulated with different roasting carob flours; N: natural carob flour; R1–R3: roasted flours; R2IM: roasted flour finely milled at industrial scale. Different lowercase letters indicate statistically significant differences between samples ($p < 0.05$).

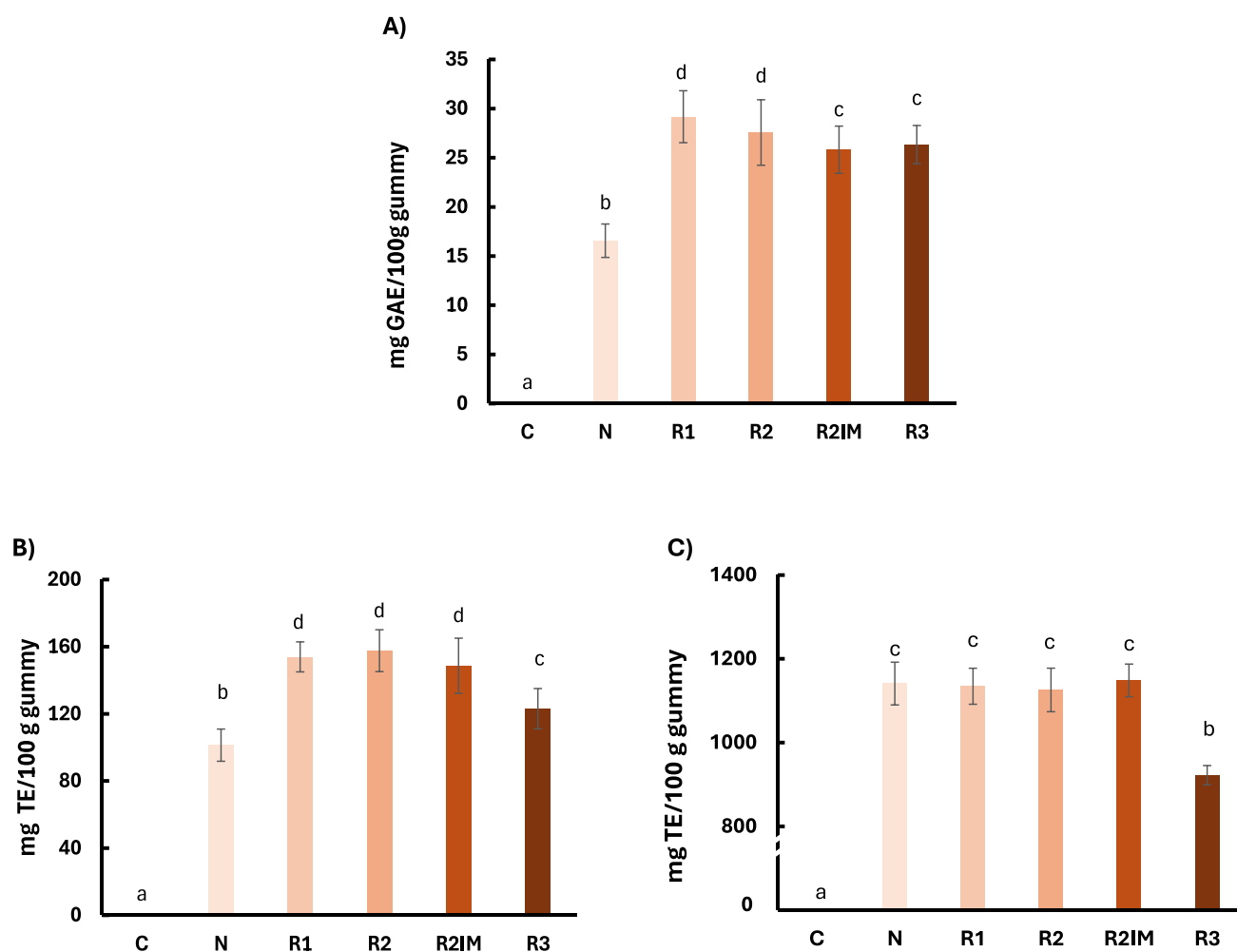


Fig. 3. Total phenolic content (A), mg gallic acid/ 100 g, and antioxidant properties of gummy candies using DPPH assay (B) and ORAC (C) assay, mg trolox E/ 100 g. Values were expressed as mean (3) $\pm$ SD. Lowercase letters denote significant ($p < 0.05$) differences among formulations. Carob gummies were formulated without carob (C) with different roasting carob flours; N: natural carob flour; R1–R3: roasted flours; R2IM: roasted flour finely milled at industrial scale.

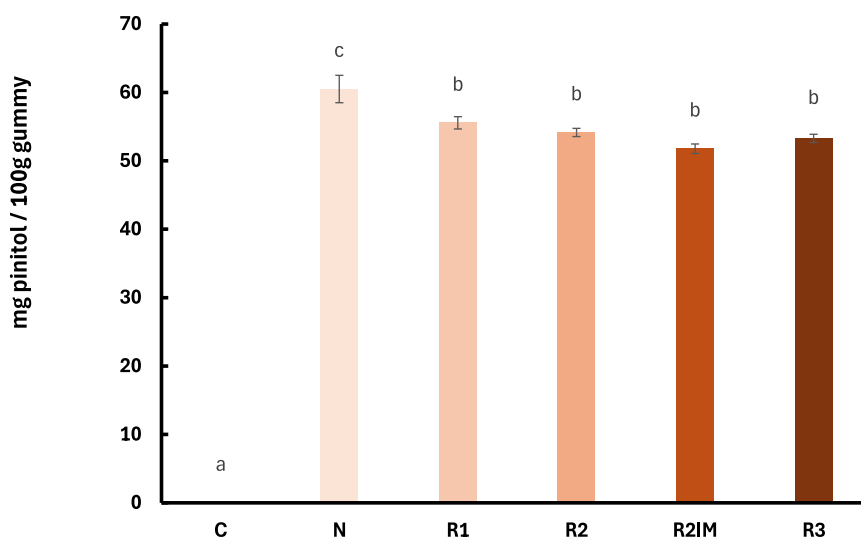


Fig. 4. D-pinitol content, mg D-pinitol/100, of gummy candies. Values were expressed as mean (3) $\pm$ SD. Lowercase letters denote significant ($p < 0.05$) differences among formulations. Carob gummies were formulated without carob (C) with different roasting carob flours; N: natural carob flour; R1–R3: roasted flours; R2IM: roasted flour finely milled at industrial scale.

higher in ORAC testing compared to DPPH testing for diverse liquid foods. Furthermore, different studies published by the U.S. government and the Journal of the American Chemical Society revealed that the ORAC values of carob gummies were comparable to those reported for prunes (1.4 g Trolox E/100 g product) and significantly higher than those for raisins (0.7 g Trolox E/100 g product), blueberries (0.6 g Trolox E/100 g product), and blackberries (0.51 g Trolox E/100 g product) (Carlsen et al., 2010; Wang & Lin, 2000). These findings contextualize and highlight a high level of antioxidant activity in carob gelatin-based gummies.

3.3.3. D-pinitol content

D-pinitol is a naturally occurring cyclitol found in carob, known for its antioxidant and anti-inflammatory and glycemic modulation activities. Fig. 4A illustrates the D-pinitol content among formulations. Logically, the control formulation did not show D-pinitol values, since it did not contain carob flour. After the incorporation of carob flour, N gummies showed statistically higher D-pinitol values when compared with the rest of the formulations made with flours subjected to different degrees of roasting (R1, R2, R3). However, in this case, the different heat treatments applied during the roasting process were not aggressive enough to cause high degradation of D-pinitol (the range between the highest and lowest formulation levels is <11 %). Furthermore, the industrial grinding of flour (R2IM) did not reduce the D-pinitol content compared to those achieved in laboratory, suggesting finer flours could be created without comprising the bioactive molecule. This trend aligns with the fact that D-pinitol possesses some thermal stability, although it might be subject to modifications when subjected to intense heat treatments (Jaendl & Popp, 2006).

Presence of D-pinitol in gummy candies is an advantage for the confectionery sector. It mimics insulin action by enhancing glucose uptake and regulating blood sugar levels. Additionally, D-pinitol has shown potential in protecting against oxidative stress, reinforcing its value as a bioactive compound in carob-based formulations (Castillejo et al., 2025).

3.3.4. Bioaccessibility of functional compounds

To evaluate whether the incorporation of carob into a gelatin-based matrix could influence the bioaccessibility of its main functional compounds (D-pinitol and polyphenols), an in vitro gastrointestinal digestion (including oral, gastric, and intestinal phases) was performed. Two samples were compared: a carob-based gummy candy and a control

solution containing the same ingredients but without gelatin, which was subjected to the same thermal treatments. For both digestion experiments, the R2IM carob variety was selected, as it was considered the optimal sample based on its functional and sensory characterization results (see Sections 3.3.1, 3.3.2, 3.3.3 and 3.4).

The total polyphenol content (Fig. 5A) was quantified throughout the in vitro digestion of the gummies and sugar-based solutions. During the gastric phase, both formulations exhibited a progressive increase in soluble polyphenol content, reaching a relatively stable plateau toward the end of this stage. This initial rise is likely associated with the release of phenolic compounds previously bound to the food matrix, promoted by the acidic conditions and the enzymatic activity of pepsin.

At the onset of the intestinal phase (120–150 min), a marked increase in polyphenol content was detected in both samples. This surge can be attributed to neutralization of pH and the synergistic action of pancreatic enzymes and bile salts, which are known to disrupt interactions between phenolics, carbohydrates, and proteins, thus liberating matrix-bound polyphenols. However, at later time points (≥ 150 min), a gradual decline was observed in both formulations. This trend aligns with the well-documented instability of phenolic compounds under alkaline conditions, where oxidation, polymerization, or structural rearrangements may occur, thereby diminishing detectability (Baeza et al., 2018).

Notably, the formulation containing gelatin consistently exhibited higher polyphenol levels than the sample without gelatin, suggesting that the protein matrix contributes to the stabilization of phenolic compounds (McClements, 2021). During the gastric phase, gelatin may interact with polyphenols through hydrogen bonding and hydrophobic interactions, providing a protective environment that mitigates acid-induced degradation and modulates compound solubility. In the intestinal phase, the less pronounced decline in polyphenol content observed for the gelatin formulation further supports the hypothesis that the protein matrix acts as a physical and chemical barrier, preserving polyphenolic integrity during digestion.

Similarly, Fig. 5B illustrates the evolution of D-pinitol content across formulations during in vitro digestion. In the sugar solution containing carob, it can be observed that after the oral phase (5 min of digestion), 24 mg of D-pinitol per 100 g of sample were detected. This suggests that a portion of the compound exists in a free, non-covalently bound state within the carob flour matrix (Ersan et al., 2020). After the addition of gastric fluids, the amount slightly increased approximately a 10 %, remaining constant throughout this phase. After the addition of intestinal fluids (120 min), a more pronounced increase of approximately 20

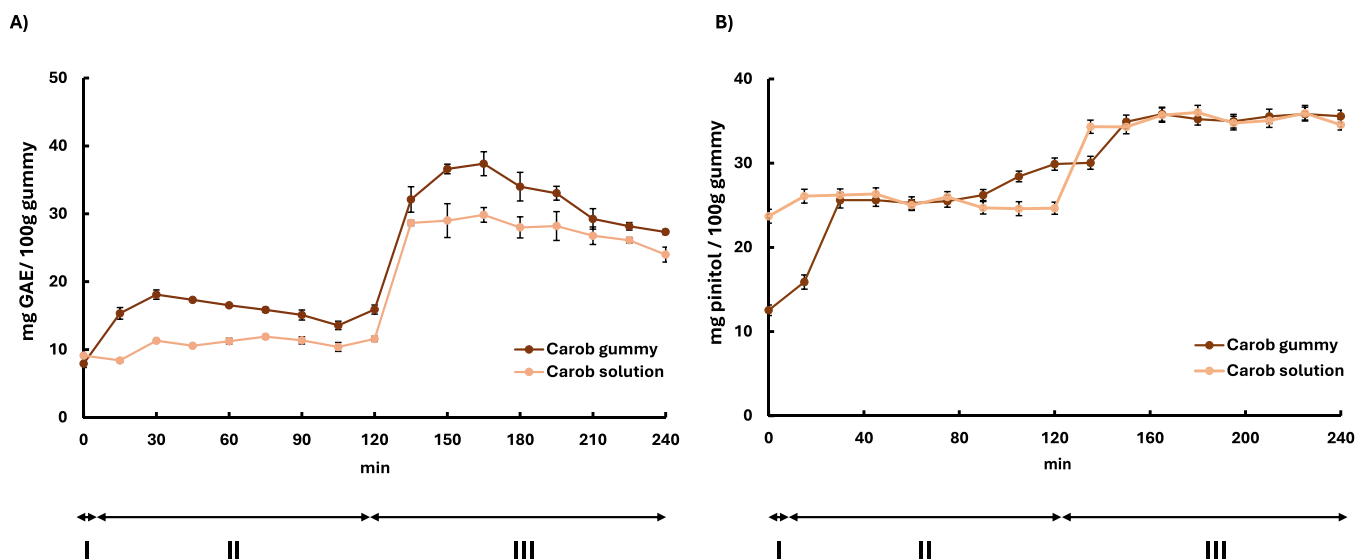


Fig. 5. Total phenolic (A), mg gallic acid/100 g, and D-pinitol content (B), mg pinitol/100 g gummy candies, during in vitro digestion of gummy candy. Values were expressed as mean (3) $\pm$ SD. Carob gummy was formulated with R2IM carob flour.

% in the bioaccessible fraction was observed (34 mg/100 g), suggesting that intestinal conditions, particularly the action of pancreatic enzymes, disrupt physical interactions between D-pinitol and dietary components (Anandakumar et al., 2018; Navarro et al., 2022).

A different pattern was observed for the carob incorporated into the gelatin-based gummy. In this case, after the oral phase, only 12.5 mg/100 g were bioaccessible, likely because the gelatin matrix entrapped part of the compound. Upon addition of gastric fluids, a delayed release of D-pinitol was observed overall during the first 30 min of the gastric phase, followed by a sustained release up to 150 min. This behavior is consistent with the digestion profile of gelatin, a collagen-derived protein. As reported by Sousa et al. (2020), gelatin undergoes partial hydrolysis by pepsin in the gastric phase, while complete degradation occurs during the intestinal phase due to pancreatic proteases, as evidenced by the disappearance of intact protein bands in SDS-PAGE analyses after intestinal digestion.

The delayed and sustained release observed in the gelatin-based gummy matrix is consistent with the behavior of hydrocolloid networks that modulate nutrient bioaccessibility. As reported by McClements (2021), protein-based hydrogels such as gelatin would entrap bioactive compounds within their polymeric network, limiting their diffusion and enzymatic accessibility. This behavior has been also tested in resistant starch type 5 (RS5) inclusion complexes, which exhibited controlled release and reduced glycemic response due to their stable crystalline structure (Guo et al., 2023). Therefore, the gelatin matrix may act as a functional carrier for sustained delivery of D-pinitol, potentially contributing to improved glycemic control.

Overall, these results demonstrate that incorporating carob into gummies promotes gelatin–carob interactions, which could serve as an effective strategy to enhance gastrointestinal stability and enable controlled release of these key bioactive compounds.

3.3.5. Postprandial glycemia regulation

Following the characterization of their functional properties and bioactive composition, the α -glucosidase inhibitory activity of the carob gelatin-based gummies was evaluated to assess the potential of carob to modulate postprandial glycemia induced by free sugar consumption in comparison with acarbose, a well-established α -glucosidase inhibitor used in the management of type 2 diabetes. This enzyme catalyzes the

final step in the hydrolysis of starches and disaccharides, and its inhibition is a well-established strategy for attenuating postprandial glycemia (Li et al., 2022). As expected, acarbose exhibited significant inhibitory activity at a concentration of 0.5 mg/mL, resulting in a reduction of 39 ± 4 % (See Fig. 6). Interestingly, carob gelatin-based gummies at the same concentration (0.5 mg/mL) showed even greater inhibitory effects than the reference compound. Among samples, those prepared with natural carob flour (N) and mildly to intermediately roasted powders (R1, R2, R2IM) achieved approximately 50–55 % inhibition, whereas strong roasting (R3) led to a decrease to around 45 % ($p < 0.05$).

According to the available evidence, the most likely α -glucosidase inhibitors present in carob are those belonging to the polyphenol family, including flavonoids and condensed tannins (proanthocyanidins) as catechin and epicatechin, quercetin and kaempferol; phenolic acids such as gallic, protocatechuic, and ferulic acids; and, on the other hand, certain oligosaccharides (Castillejo et al., 2025).

To better interpret the results and strengthen the discussion, correlation analyses were performed between α -glucosidase inhibitory activity and three key compositional parameters: total polyphenols, as the most frequently reported inhibitors; D-pinitol, to assess complementary glycemia modulation effects; and fiber content, given its role in enzyme–substrate interactions (Li et al., 2023).

Regarding total polyphenols, a lack of significant correlation with α -glucosidase inhibition across all formulations ($p = 0.2571$, $p = 0.5653$) was found. These patterns suggest that inhibitory activity would depend more on phenolic profile than on total content, and that roasting may alter phenolic structures or generate Maillard reaction products that modulate enzyme interaction. Similar observations have been reported in coffee (Alongi & Anese, 2018) and botanical extracts (Tripathi, 2018). For D-pinitol, Spearman correlation also showed no statistically significant association with α -glucosidase inhibition ($p = 0.8286$, $p = 0.0639$). This outcome would indicate its distinct mode of action. As previously discussed, D-pinitol exerts glycemia modulation effects via insulin-mimetic and insulin-sensitizing pathways, rather than directly inhibiting digestive enzymes. A different pattern emerged in case of correlating the enzyme inhibition with fiber content with values of $\rho = 0.9274$ and $p = 0.0425$. This positive correlation suggests that carob fiber may contribute to α -glucosidase inhibition by enhancing the

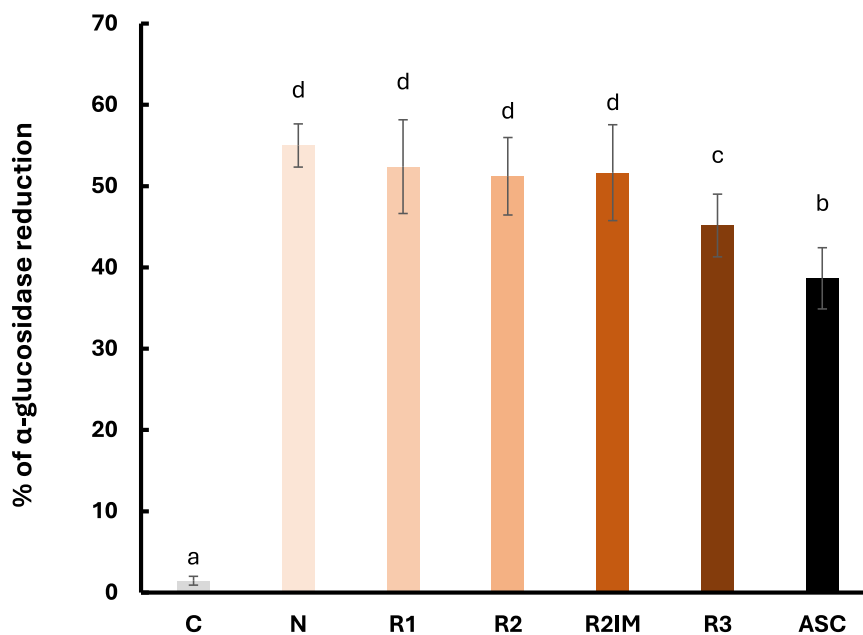


Fig. 6. Reduction of α -glucosidase of gummy candies, %. Values were expressed as mean (3) $\pm$ SD. Lowercase letters denote significant ($p < 0.05$) differences among formulations. Carob gummies were formulated without carob (C) with different roasting carob flours; N: natural carob flour; R1–R3: roasted flours; R2IM: roasted flour finely milled at industrial scale.

stability of bioactives, or physically hinder enzyme–substrate interactions, thereby amplifying the inhibitory effect of other biomolecules (Li et al., 2023).

Taken together, the α -glucosidase inhibition provided by carob's polyphenols and fiber, combined with the insulin-mimetic and insulin-sensitizing actions of D-pinitol, may promote a more gradual release and utilization of glucose, supporting sustained energy supply and a controlled postprandial glucose response. Additionally, recent evidence indicates that D-pinitol activates AMP-activated protein kinase (AMPK), a key cellular energy sensor involved in metabolic stress regulation (Azzini et al., 2023). AMPK activation can trigger several adaptations relevant to metabolic health and physical performance: (i) increased glucose uptake into muscle cells via GLUT4 translocation, (ii) enhanced fatty acid oxidation and mitochondrial biogenesis, improving metabolic efficiency and endurance, and (iii) preservation of ATP levels, thereby reducing fatigue (Jäger et al., 2007; Shirwany et al., 2014).

3.4. Sensory evaluation of the carob gelatin-based gummies

Sensory analysis is imperative for evaluating the quality of final food products, as consumer acceptance ultimately dictates market success. The sensory panel consisted of 100 participants with a balanced gender distribution (53 % female and 47 % male). The majority of the panelists were young adults, with 65 % aged 18–30, 14 % aged 31–45, and 21 % aged 46–69. Regarding gummy consumption habits, only 7 % of participants reported consuming gummies once a week, 24 % consumed them two to three times per month, 30 % once a month, and 39 % less than once a month. Knowledge of carob products was limited, as 65 % of participants indicated that they were unfamiliar with them.

Table 5 presents the values obtained in terms of acceptance (9-point scale) and purchase intention (5-point scale) following sensory analysis conducted by 100 participants. The gummies obtained acceptance values higher than 5 in all the attributes evaluated (texture, flavor, overall acceptance), with no significant differences among them, except for sample R3, which presented an average overall acceptance value significantly lower than the rest, making it the worst rated formulation. In the remaining samples, despite all exhibiting acceptance values above 5, none demonstrate adequate acceptance, particularly when values exceed 7 (Yang & Lee, 2018). This is probably explained by infrequent consumption of gummy products by the participants.

With respect to purchase intention, it should be noted that intermediate roasting, both grinded in laboratory (R2) and industrially (R2IM), were the best rated samples, receiving a score of 3.2–3.7 on a 5-point scale. Furthermore, gummy made with unroasted carob (N) received similar score that intermediate roasting (R2). The worst purchase intention was for R3, consistent with its overall acceptance.

To determine which attributes were optimally balanced and how they influenced overall consumer acceptance, JAR (Just-About-Right) scales were employed. Fig. 7A–E present the percentages of consumers rating each sample, N, R1, R2, R2IM, and R3, across three levels: too low, JAR, and too high. Samples N and R2IM exhibited the highest proportion of JAR responses, indicating that their sensory attributes

were the most optimally balanced.

Complementary penalty analysis was conducted to quantify the impact of non-optimized attributes on overall acceptance, Fig. 7F–J. The results revealed that sample R3, formulated with more intensely roasted carob flour, exhibited the least optimized attributes. Specifically, 78 % of participants perceived its carob flavor as excessively intense, which resulted in a decrease of 3 points in overall acceptance. Additionally, excessive aroma and disproportionate sweetness contributed to further reductions of approximately 2 points. This perception aligns with the elevated levels of 5-hydroxymethylfurfural (ca. 22.8 mg/100 g gummy, see Section 3.2.4), a compound known to contribute to burnt or acidic flavor notes in thermally processed foods (Reyes Suárez et al., 2025).

Samples R1 and R2 showed improved balance compared to R3; however, approximately 50 % of consumers still reported excessive sweetness and flavor intensity, leading to mean drops of 1–2 points in acceptance. In contrast, sample N, prepared with unroasted carob flour, exhibited lower negative impacts, although 31 % of participants noted excessive sweetness and 43 % reported a soft texture, resulting in modest decreases of 1.2–1.5 points.

Comparison of R2 and R2IM demonstrated that the use of finer flour increased the number of attributes perceived as JAR and eliminated the excessive hardness observed in R2, thus improving texture and overall acceptance. This was reflected in the purchase intention, with a median score of 4 out of 5, confirming the high consumer acceptance and attractiveness of this sample.

In summary, the sensory analysis results show that carob-based gummies were well accepted by consumers, as all formulations obtained scores above 5 for texture, flavor, and overall acceptance. This indicates that the product is generally appealing, even though most participants are not frequent gummy consumers and are unfamiliar with carob products.

4. Conclusions

This study demonstrates that the incorporation of carob flour, varying in roasting degree and milling process, significantly influences the nutritional, functional, and sensory properties of carob gelatin-based gummies. The presence of high phenolic contents and D-pinitol across formulations, together with notable fiber content and α -glucosidase inhibitory activity, underscores the potential of carob-based gummies as functional foods with antioxidant and glycemia modulation properties, promoting a more controlled glycemic response compared to conventional high-sugar gummies. Moreover, the colloidal nature of gummies may facilitate the controlled delivery of nutrients and functional compounds present as D-pinitol and polyphenols in the formulations. This property could make these gummies suitable as functional nutritional supplements, particularly for sports nutrition, where sustained energy, improved insulin sensitivity, and reduced oxidative stress are desirable. Finally, sensory analysis confirmed that the products were generally well accepted, particularly those formulated with unroasted or moderately roasted flours, highlighting the importance of optimizing processing conditions to balance health-promoting attributes with

Table 5

Mean and median acceptance in relation to texture, flavor and overall acceptance (9-point scale) and purchase intention (5-point scale) of gummy candies. Values were expressed as mean (100) $\pm$ SD.

Sample	Texture acceptance		Flavor acceptance		Overall acceptance		Purchase intention	
	Average $\pm$ SD	Median	Average $\pm$ SD	Median	Average $\pm$ SD	Median	Average $\pm$ SD	Median
N	6.4 $\pm$ 1.9 ^{bc}	7	6.7 $\pm$ 1.7 ^b	7	6.6 $\pm$ 1.6 ^b	7	3.3 $\pm$ 0.8 ^{bc}	3
R1	5.9 $\pm$ 1.8 ^{ab}	6	5.6 $\pm$ 1.9 ^b	6	5.5 $\pm$ 1.7 ^b	6	2.9 $\pm$ 1.0 ^{ab}	3
R2	6.4 $\pm$ 1.8 ^{abc}	7	6 $\pm$ 2 ^b	6	5.7 $\pm$ 1.9 ^b	6	3.2 $\pm$ 1.1 ^{bc}	3
R2IM	6.9 $\pm$ 1.8 ^c	8	6 $\pm$ 2 ^b	7	6.2 $\pm$ 1.9 ^b	7	3.7 $\pm$ 1.1 ^c	4
R3	5 $\pm$ 2 ^a	5	4 $\pm$ 2 ^a	4	4 $\pm$ 2 ^a	4	2.4 $\pm$ 1.2 ^a	3

Different lowercase letters indicate statistically significant differences between samples ($p < 0.05$). Carob gummies were formulated with different roasting carob flours; N: natural carob flour; R1–R3: roasted flours; R2IM: roasted flour finely milled at industrial scale.

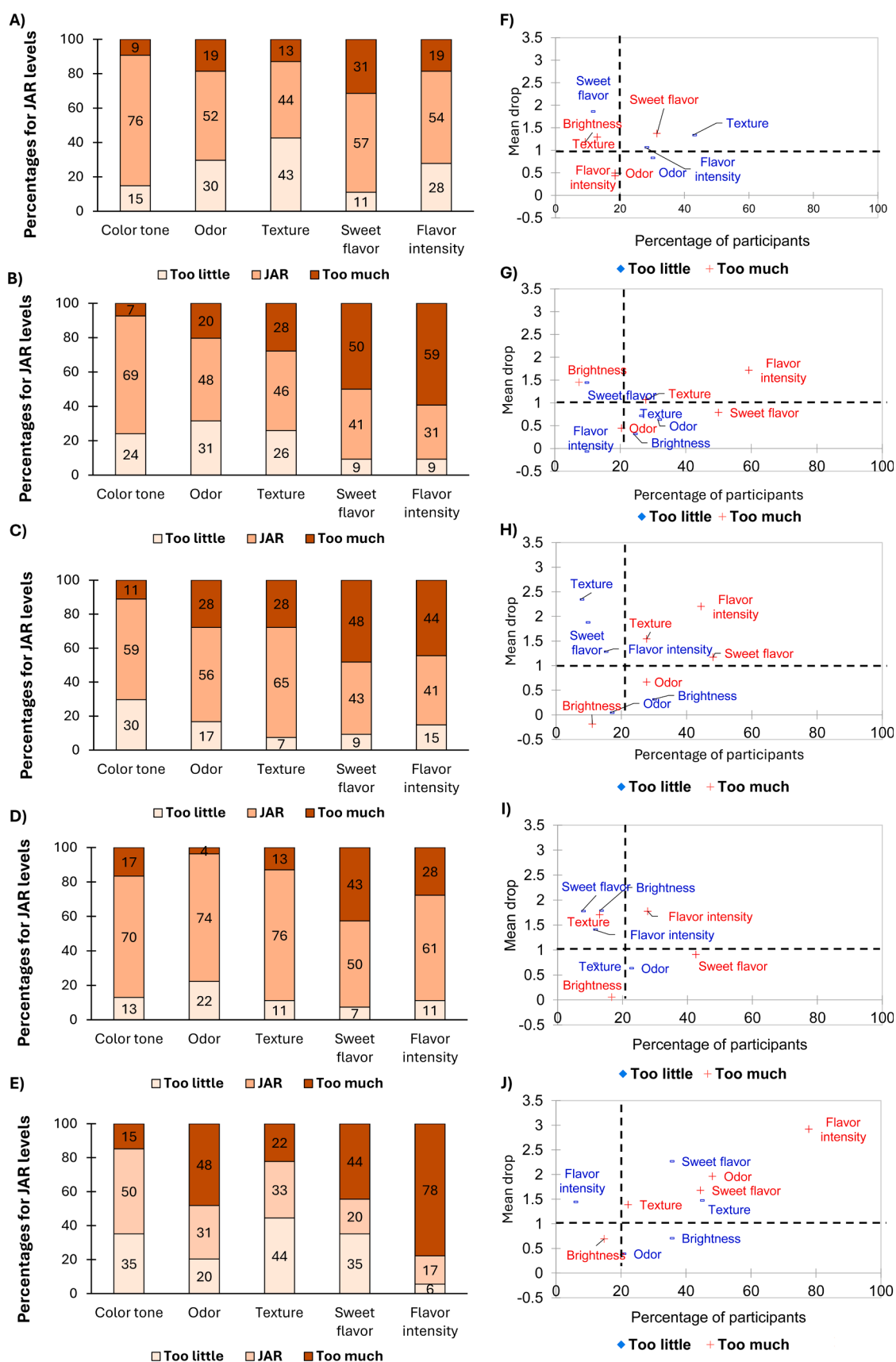


Fig. 7. Percentages for the JAR levels for N (A), R1 (B), R2 (C), R2MI (D), R3 (E), and penalty analysis for N (F), R1 (G), R2 (H), R2MI (I), R3 (J). Values were expressed as mean (100) $\pm$ SD. Carob gummies were formulated with different roasting carob flours; N: natural carob flour; R1–R3: roasted flours; R2IM: roasted flour finely milled at industrial scale.

consumer appeal.

Nevertheless, human intervention studies would be necessary to confirm the potential of these gummy formulations to modulate postprandial glycemia and to evaluate their capacity to promote a gradual release of energy.

Ethical statement

This study was conducted in accordance with the principles of the Declaration of Helsinki for research involving human participants. All sensory evaluations were performed with informed consent obtained from participants prior to their involvement. The study protocol was reviewed and approved by the Ethics Committee of the Universitat Politècnica de València (Reference Number: P01_30-06-2025).

CRediT authorship contribution statement

Héctor Gómez-Llrente: Writing – original draft, Investigation, Data curation. **María Dolores Ortolá:** Writing – review & editing, Writing – original draft, Methodology, Data curation, Conceptualization. **Isabel Fernández-Segovia:** Writing – original draft, Methodology, Data curation. **José Manuel Barat:** Supervision, Project administration, Funding acquisition. **Édgar Pérez-Esteve:** Writing – review & editing, Methodology, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This study forms part of the AGROALNEXT programme (AGRO-ALNEXT 2022/002) and was supported by MCIN with funding from European Union NextGenerationEU (PRTR-C17.11) and by Generalitat Valenciana. It was also funded by the PROMETEO-GVA grant for Excellence Research Groups (CIPROM/2024/17) given by the Conselleria de Educación, Cultura y Universidades, Generalitat Valenciana (GVA).

Data availability

Data will be made available on request.

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