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Research Article

Simulated Digestion and Bioaccessibility of Cookies Enriched with Microencapsulated Polyphenols from Habanero Pepper Leaves Extracted Using NADES

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Abstract: This study presents the formulation of a cookie enriched with microencapsulated polyphenols from *Capsicum chinense* (Habanero pepper) leaves, extracted using a green ultrasound-assisted Natural Deep Eutectic Solvent (NADES) of choline chloride and glucose (1 : 0.8 mol/mol) with 68% water. For microencapsulation, the extract was spray-dried with maltodextrin, modified starch, and guar gum, and the powder with the highest Total Polyphenol Content (TPC) was used to replace 20% (w/w) of common wheat flour. Digestion was simulated under fasted and postprandial conditions across gastric and intestinal phases. The enriched cookie showed a maximum TPC release of 273.6 ± 12.2 mg Gallic Acid Equivalent (GAE)/100 g at the intermediate intestinal stage (fasted), and antioxidant capacity peaked at 84.4 ± 1.5% inhibition in the final intestinal phase (postprandial). High-Performance Liquid Chromatography (HPLC)-Diode-Array Detector (DAD) identified protocatechuic acid (3.86 ± 0.23 mg/100 g), catechin (6.99 ± 0.42 mg/100 g), and rutin (1.79 ± 0.06 mg/100 g) in the undigested cookie. After digestion, their concentrations increased at the intestinal stage (fasted): protocatechuic acid by 101.3%, catechin by 828.76%, and rutin by 1,481.82%. Statistical analysis ($p < 0.05$) showed significant effects of cookie type, digestion condition, phase, time, and their interactions. These findings support NADES-based microencapsulation as a promising strategy for enhancing polyphenol bioaccessibility in functional bakery products.

***Keywords*:** simulated digestion, polyphenols, Habanero pepper leaf, enriched food, Natural Deep Eutectic Solvent (NADES)

1. Introduction

The growing demand for functional foods has led to increased attention on agro-industrial by-products as promising sources of bioactive compounds [1, 2]. Among these, phenolic compounds have attracted attention because of their antioxidant and anti-inflammatory activities, as well as their beneficial effects on human health, positioning

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them as prime candidates for the development of nutraceuticals and functional food ingredients [3]. In this context, the Capsicum genus stands out not only for its culinary and economic importance, but also for its rich phytochemical profile, including polyphenols, capsaicinoids, and carotenoids [4]. In particular, *Capsicum chinense* Jacq. commonly known as the Habanero pepper and native to the Yucatán Peninsula, has gained international recognition. Its fruit has been granted a designation of origin "Chile habanero de la Península de Yucatán" [5]. Although fruit is the primary commercial product, its cultivation generates a substantial amount of residues such as stems, peduncles, and leaves, which are frequently discarded despite being rich sources of phenolic compounds and other bioactive molecules [6].

Antioxidant and anti-inflammatory bioactivities are not only well documented, but they also underpin the growing interest in deploying phenolics as nutraceuticals and functional food ingredients [1]. Among these phenolics, catechin, a flavan-3-ol abundant in green tea and various fruits, has demonstrated hypoglycemic effects by lowering fasting blood glucose in diabetic models, along with protective roles against oxidative stress and mitochondrial damage [7, 8]. Moreover, quercetin exhibits a broad spectrum of physiological benefits, including antioxidant, anti-inflammatory, and antidiabetic actions, as well as anticancer potential; a substantial proportion of studies have focused on its role in cancer prevention and metabolic regulation [9, 10]. Among these, rutin stands out due to its extensive pharmacological profile. Clinical evidence demonstrates that rutin supplementation in individuals with type 2 diabetes significantly reduces systolic and diastolic blood pressure, enhances the activity of antioxidant enzymes (Superoxide Dismutase, Catalase, Glutathione Peroxidase), and improves overall quality of life [11]. Preclinical studies further show that rutin strengthens vascular tissues, promotes healthy blood flow, and contributes to the prevention of clot formation [12].

Given the remarkable health-promoting potential of phenolic compounds, their effective application in food and nutraceutical products largely depends on developing extraction processes that are not only efficient but also safe and environmentally sustainable. Although conventional extraction methods employing organic solvents such as methanol, ethanol, acetone, chloroform, or hexane have been widely used to recover phenolic compounds from plant matrices, these methods are associated with several limitations. They are often toxic, volatile, and environmentally harmful, making them incompatible with food-grade applications while raising concerns over human and ecological safety [13, 14]. Consequently, the push for replacing such methods with green technologies that combine high efficiency, safety, and environmental responsibility has become increasingly important. In recent years, green extraction strategies have become a priority, and among them, Natural Deep Eutectic Solvents (NADES) stand out as eco-friendly alternatives. NADES are biodegradable, non-volatile, and exhibit low toxicity, while achieving high extraction yields of phenolic compounds from agro-industrial matrices [15]. When combined with Ultrasound-Assisted Extraction (UAE), the cavitation effects significantly increase solvent penetration and recovery yield of phenolic compounds [16].

However, despite efficient extraction, phenolic compounds may lose their bioactivity unless adequately protected during processing and storage, phenolic compounds can still lose their bioactivity unless they are properly protected during and after processing, as well as during storage. This vulnerability stems from their sensitivity to environmental stressors such as pH changes, temperature fluctuations, and enzymatic degradation [17]. Spray-drying microencapsulation provides an effective solution to these challenges, safeguarding phenolic compounds through the formation of protective Microcapsules. This technique significantly enhances stability and preserves functionality in food systems exposed to adverse conditions [18]. While spray drying has been extensively applied to conventional plant extracts, the microencapsulation of NADES-based extracts is still an innovative and emerging approach, as the physicochemical properties of NADES (e.g., viscosity, polarity, hygroscopicity) can influence drying performance and the interaction with carrier agents. Recent work has demonstrated that optimizing spray-drying conditions for NADES-derived extracts of *Capsicum chinense* leaves is feasible and effective for improving encapsulation efficiency and ensuring physicochemical stability [19]. This highlights spray drying as a promising strategy for extending the applicability of NADES in the formulation of sustainable functional ingredients.

An equally important step for translating these advances into consumer-oriented products is the identification of suitable food matrices for delivery. Bakery products, such as cookies, serve as practical and versatile vehicles for functional ingredients due to their widespread consumption, long shelf-life, and flexible formulations. Importantly, microencapsulated plant extracts have been shown to enhance both the nutritional profile and functional properties of baked goods. For example, in the development of functional cookies enriched with microencapsulated pomegranate peel extract, significant increases were observed in total phenolic content, from approximately 2.5 to 74.7 mg Gallic Acid Equivalent (GAE)/100 g, together with improved antioxidant capacity compared to control cookies [20]. However, an

increased concentration of phenolic compounds in the final product does not necessarily guarantee their physiological effectiveness, as their health benefits depend on their bioaccessibility, defined as the fraction of a compound that is released from the food matrix during gastrointestinal digestion and becomes available for intestinal absorption [21]. Despite the promising enrichment strategies reported to date, the fate and bioaccessibility of polyphenols during digestion, particularly those derived from Habanero pepper leaves and incorporated into baked systems, remain underexplored. Therefore, this study addresses this gap by evaluating the simulated digestive behavior and bioaccessibility of total and individual polyphenols in a cookie enriched with microencapsulated *Capsicum chinense* leaf extract obtained through NADES-assisted extraction and spray-drying microencapsulation. This work proposes a novel functional bakery product that advances the valorization of agro-industrial by-products through sustainable green technologies.

2. Materials and methods

2.1 *Plant material*

Leaves from the Jaguar variety of Habanero pepper (*Capsicum chinense* Jacq.) were used. Plants were grown under greenhouse conditions in black soil, locally referred to as Boox Lu'um in the Mayan language, in Chablekal, Yucatán (21°06′02.3″ N, 89°33′40.5″ W). Leaf samples were collected during the first fruiting cycle, occurring 120 days after transplanting.

2.2 *Habanero pepper leaf polyphenol extraction by NADES*

2.2.1 *Habanero pepper leaf pretreatment*

Pretreatment was adapted from Chel-Guerrero et al. [22] with minor modifications. Habanero pepper leaves were first manually separated and dried in a stainless-steel tray dryer (model HS60-AID) at 44 °C for 48 h, until reaching a moisture content of less than 5%. The dried leaves were subsequently ground using a Braun® grinder (model KSM-2, Treviso, Italy), and the resulting powder sieved through a 500 µm mesh sieve (#35, Fisher Scientific, Boston, MA, USA) to obtain a uniform particle size. This powder was then used for polyphenol extraction.

2.2.2 *Polyphenol extraction using an optimized NADES*

Following the methodology described by Avilés-Betanzos et al. [23], a polyphenol-rich extract from Habanero Pepper Leaves (HPRE) was prepared using a NADES formulated with choline chloride and glucose in a molar ratio of 1 : 0.8 mol/mol, containing 68% added water. The solvent was combined with the leaf powder at a 1 : 10 w/v ratio and subjected to ultrasonic-assisted extraction with a Sonics Vibra-Cell® probe (model CV 505, Sonics®, New York, NY, USA) operating at 750 W, 20 kHz, and 30% amplitude for 5 min. The resulting mixture was centrifuged at 4,700 rpm for 30 min at 4 °C, and the supernatant was collected and stored under refrigeration at temperatures below 4 °C until further use. The extract obtained was named NADES-68.

2.3 *Microencapsulation of the Habanero pepper extract obtained by NADES*

Microencapsulated powders from NADES-68 were obtained under optimized spray-drying conditions to produce two variants: one formulated for high polyphenol content (inlet temperature of 89.4 °C, 7.8% Guar gum) and another for enhanced antioxidant capacity (inlet temperature of 104.1 °C, 8.06% Guar gum). The encapsulating system comprised maltodextrin (Dextrose Equivalent (DE) 17-20), modified starch, and Guar gum [19].

For microencapsulation, HPRE was blended with the encapsulating agents at a weight ratio of 1 : 3 w/w (HPRE: encapsulant), yielding a 5% solution. Mixture was processed in a spray dryer under the previously described optimized conditions [19], with additional fixed parameters including a feed rate of 10 mL/min, atomization pressure of 3.5 bar, and air flow of 80 kg/h. The dried powder was recovered using a cyclone separator, packaged in aluminum-lined plastic bags, weighed, and stored at room temperature (30 °C) until analysis.

2.4 *Enriched cookie with microencapsulated Habanero pepper leaf extract*

2.4.1 *Preparation of the control and enriched Cookies*

The control cookie (The control cookies were not supplemented with microencapsulated material or encapsulating agents) was prepared according to the method reported by Flores-Balcázar [24]. Wheat flour (500 g, ≤ 500 µm) was first sieved and mixed with salt (4.1 g) and sugar (48 g). Subsequently, butter (193.8 g, at room temperature) was added, and the mixture was kneaded until a sandy texture was achieved. At this stage, vanilla extract (2.05 mL) and water (82.5 mL) were incorporated. The resulting dough was kneaded until a cohesive, moldable texture was obtained. Then it was rolled out and cut using a flower-shaped cookie cutter (47.5 mm diameter). The cut cookies were placed on trays lined with wax paper and baked in a preheated oven (20 min) at 180 °C for 30 minutes. For the enriched cookies, 10% of a polyphenol-rich microencapsulated extract and 10% of a high antioxidant capacity microencapsulated extract (based on the weight of wheat flour) were substituted. These were mixed with the remaining wheat flour (400 g), salt, and sugar. The subsequent preparation steps were identical to those used for the control cookies.

The baked cookies were stored in resealable bags lined with aluminum foil and kept at room temperature (30 °C) until further use.

2.4.2 *Colorimetry of cookies enriched with microencapsulated Habanero pepper leaf extract*

Color of dough and cookie samples was evaluated using a Konica Minolta CM-5 spectrophotometer (Konica Minolta, Inc., Tokyo, Japan), based on the methodology described by Singh et al. [25]. Approximately 20 grams of the dough sample and a piece of baked cookie were placed in a quartz cell to completely cover the reading area. Prior to measurement, the equipment was calibrated according to the manufacturer's instructions. Each sample was subjected to three consecutive readings, and the results were reported using the International Commission on Illumination 1976 L*a*b* color system (CIELAB). Additionally, chroma and hue angle values were determined using Equations (1) and (2).

$$\text{Chroma} = \sqrt{a^{*2} \times b^{*2}} \tag{1}$$

$$\text{Hue}° = \arctan\left(\frac{b^*}{a^*}\right). \tag{2}$$

2.4.3 *Moisture determination of the dough and the cookie enriched with microencapsulated extract*

Dough and cookies' moisture were determined using a modified procedure based on the method of Tolun et al. [26]. For this, a Thermobalance (OHAUS® MB90, Parsippany, NJ, USA) was employed to measure the moisture by heating 0.5 g of each sample at 105 °C until a constant weight was reached, defined as a weight variation of less than 1 mg over 90 seconds.

2.4.4 *TPA of the baked enriched cookie*

Texture analyzer model EZ-SX (Shimadzu Corporation, Japan) equipped with the Trapezium X® Material Testing Operation Software was used to perform the Texture Profile Analysis (TPA) of cookies (control and enriched with microencapsulated Habanero pepper leaf extract). The test was conducted using a three-point bending fixture to evaluate mechanical resistance under penetration conditions according to Delgado-Andrade et al. [27] with some modifications. Each cookie was placed across two supporting points, and a central probe applied a downward force until reaching a penetration depth of 10 mm at a speed of 1 mm/s with a displacement limit of 20 mm, by using two compression cycles to obtain parameters such as hardness and fracturability. All analytical measurements were performed in triplicate ($n = 3$).

2.4.5 *Determination of total polyphenol content and antioxidant capacity of the baked enriched cookie*

The total polyphenol content of the baked cookie was determined following the method described by Johnson et al. [28]. Briefly, 36 g of the sample were ground using a Braun® grinder (model KSM-2, Treviso, Italy), and the

resulting powder was sieved (≤ 500 µm), and 0.5 g was extracted with 2.5 mL of 80% methanol (w/v). The mixture was sonicated (20 min, 120 W, 40 KHz) and subsequently centrifuged at 7,000 rpm for 30 minutes at 4 °C. A volume of 50 µL of the supernatant was transferred to a microplate well, followed by the addition of 400 µL of distilled water and 50 µL of Folin-Ciocalteu reagent (previously diluted 1 : 3 v/v with distilled water). After a reaction time of 5 minutes, 50 µL of 10% sodium carbonate solution was added. The plate was incubated at room temperature for 60 minutes, and the absorbance was measured at 764 nm using a microplate reader (Thermo Scientific Multiskan FC, Massachusetts, United States).

To determine Total Polyphenol Content (TPC) concentration, a calibration curve was prepared using gallic acid as the standard (1 mg/mL stock solution). Serial dilutions were made to obtain the following concentrations: 5, 10, 25, 50, 75, 100, 125, 150, 175, and 200 µg/mL (Figure S1).

To determine Antioxidant capacity (A_x), 20 µL of the cookie supernatant (previously sonicated, centrifuged, and filtered) was added to 140 µL of an adjusted 2,2-diphenyl-1-picrylhydrazyl (DPPH) (A_{adj}) solution (4 mg of 2,2-diphenyl-1-picrylhydrazyl in 100 mL methanol) calibrated to an absorbance of 0.700 ± 0.002 at 517 nm, using a microplate spectrophotometer (Thermo Scientific Multiskan FC). The absorbance of the sample (A_{sp}), measured after 30 minutes of incubation, was used in Equation (3) to calculate the percentage of DPPH radical inhibition [28].

$$\% \text{ Inhibition} = \left(\frac{A_{adj} - A_{sp}}{A_{adj}} \right) \times 100 \,. \tag{3}$$

2.5 *Simulated digestion of a cookie enriched with microencapsulated Habanero pepper leaf extract*

2.5.1 *Experimental design*

To evaluate the effect of simulated digestion on the total polyphenol content, polyphenol profile, and antioxidant capacity of a cookie enriched with microencapsulated Habanero pepper leaf extract, a $2^3 \times 3$ factorial design was employed. The two-level factors were: cookie type (Control = -1, Enriched = 1), digestion condition (Fasted = -1, Postprandial = 1), and digestive phase (Gastric = -1, Intestinal = 1). The fourth factor, with three levels, corresponded to sampling time (Early = -1, Middle = 0, Final = 1).

Response variables included were TPC, antioxidant capacity (% inhibition), and the polyphenol profile (gallic acid, protocatechuic acid, p-coumaric acid, cinnamic acid, chlorogenic acid, rutin, quercetin + luteolin, kaempferol, and hesperidin). The experimental design is summarized in Table 1.

Table 1. Factorial design $2^3 \times 3$ for the evaluation of the effect of simulated digestion on a cookie enriched with microencapsulated Habanero pepper leaf

# Exp	Main factors				Variables response	
	Cookie type	Digestion type	Digestive phase	Sampling time	TPC	A_x
1	-1	-1	-1	-1	$Y_{1,1}$	$W_{1,1}$
2	-1	-1	-1	0	$Y_{1,2}$	$W_{1,2}$
3	-1	-1	-1	1	$Y_{1,3}$	$W_{1,3}$
4	-1	-1	1	-1	$Y_{1,4}$	$W_{1,4}$
5	-1	-1	1	0	$Y_{1,5}$	$W_{1,5}$
6	-1	-1	1	1	$Y_{1,6}$	$W_{1,6}$
7	-1	1	-1	-1	$Y_{1,7}$	$W_{1,7}$
8	-1	1	-1	0	$Y_{1,8}$	$W_{1,8}$

Table 1. (cont.)

# Exp	Main factors				Variables response	
	Cookie type	Digestion type	Digestive phase	Sampling time	TPC	A_x
9	-1	1	-1	1	$Y_{1,9}$	$W_{1,9}$
10	-1	1	1	-1	$Y_{1,10}$	$W_{1,10}$
11	-1	1	1	0	$Y_{1,11}$	$W_{1,11}$
12	-1	1	1	1	$Y_{1,12}$	$W_{1,12}$
13	1	-1	-1	-1	$Y_{1,13}$	$W_{1,13}$
14	1	-1	-1	0	$Y_{1,14}$	$W_{1,14}$
15	1	-1	-1	1	$Y_{1,15}$	$W_{1,15}$
16	1	-1	1	-1	$Y_{1,16}$	$W_{1,16}$
17	1	-1	1	0	$Y_{1,17}$	$W_{1,17}$
18	1	-1	1	1	$Y_{1,18}$	$W_{1,18}$
19	1	1	-1	-1	$Y_{1,19}$	$W_{1,19}$
20	1	1	-1	0	$Y_{1,20}$	$W_{1,20}$
21	1	1	-1	1	$Y_{1,21}$	$W_{1,21}$
22	1	1	1	-1	$Y_{1,22}$	$W_{1,22}$
23	1	1	1	0	$Y_{1,23}$	$W_{1,23}$
24	1	1	1	1	$Y_{1,24}$	$W_{1,24}$

Abbreviations: TPC = Total polyphenol content (mg Gallic acid Equivalent/100 g cookie); Ax = Antioxidant capacity (% Inhibition); Control Cookie = -1; Enriched Cookie = 1; Digestion condition Fasted = -1; Digestion condition Postprandial = 1; Digestive phase Gastric = -1; Digestive phase Intestinal = 1; Sampling time Early = -1; Sampling time Middle = 0, Sampling time Final = 1
Source: Authors' own elaboration

2.5.2 *Standard food preparation for postprandial simulated digestion of a cookie enriched with microencapsulated Habanero pepper leaf extract*

The use of a standardized food model to mimic human dietary intake is a well-recognized strategy for evaluating the behavior of nutrients, bioactive molecules, or contaminants under controlled laboratory settings. This approach improves reproducibility and facilitates direct comparison among experimental treatments by minimizing the variability typically associated with real diets [28].

To reproduce postprandial conditions, a specific formulation was established to represent a 2,000 kcal diet, providing 60% of energy from carbohydrates, 25% from lipids, and 15% from proteins, which corresponds to the usual dietary pattern of the Mexican population [29]. This formulation was obtained by dissolving in 1,000 mL of drinking water the following components: 24.5 mL of pure corn cooking oil (Cristal®), 155.4 g of nixtamalized corn flour (MASECA®), 16.1 g of refined white sugar (Aurrera®), 36.4 g of meat peptone of bacteriological grade (Merck, Darmstadt, Germany), and 7.7 g of casein peptone (Becton Dickinson de México S.A. de C.V.).

Subsequently, the mixture was heated to 75 °C with continuous stirring for 15 minutes to guarantee proper homogenization and to accomplish pasteurization. The resulting product (Standard Food, StF) was refrigerated and exhibited a shelf life of up to 76 hours.

2.5.3 *Simulated digestion of the cookie enriched with microencapsulated Habanero pepper leaf extract*

The simulated digestion procedure followed the methodology described by Torres-Martínez et al. [30], with modifications that included an oral digestion protocol for the cookie. A quantity of 7.2 g of either the enriched or control cookie was subjected to simulated mastication for 30 s in the presence of artificial saliva (1 mL/g cookie) and salivary amylase (800 µg). The resulting bolus was mixed with 0.33 g of pepsin and transferred to a screw-cap flask containing 200 mL of either distilled water or basal diet medium, previously equilibrated at 37 °C and adjusted to pH 2.0-2.5 with 5 M HCl. This mixture was maintained under constant stirring (150 rpm) for 2 h to simulate gastric digestion.

Following the gastric phase, the pH was raised to 5.0-5.5 using 3 M NaOH to initiate the intestinal phase, after which pancreatin (0.19 g), lipase (1 mg), and bile salts (1 g, Oxgall) were added. Intestinal digestion proceeded for 4 h under the same agitation and temperature conditions.

All digestions were conducted in triplicate, and samples were collected (sampling time) at the initial, intermediate, and final time points of both gastric and intestinal phases. The collected samples were centrifuged (13,000 rpm, 4 °C, 30 min), and supernatants were transferred to chromatographic vials and stored under refrigeration until analysis.

2.5.4 *Determination of total polyphenol content and antioxidant capacity from simulated digestion stages of enriched cookie*

Throughout the gastric and intestinal stages of digestion, samples of the enriched cookie were collected at three different times: T_0 (initial point), T_i (midpoint), and T_f (final point). This allowed monitoring of changes in Total Polyphenol Content (TPC) and antioxidant capacity under both fasting and postprandial conditions. Following collection, the samples were centrifuged at 7,000 rpm for 30 minutes at 4 °C, after which the supernatants were filtered through a 0.22 µm membrane. Aliquots of 50 µL were taken for TPC determination according to Section 2.4.5, while 20 µL of supernatant from each digestion stage was analyzed in parallel to assess antioxidant capacity, using the method described in Section 2.4.5.

2.5.5 *Determination and quantification of individual polyphenols in a cookie enriched with microencapsulated Habanero pepper leaf during simulated digestion*

Individual polyphenols were identified and quantified using an Acquity H Class High-Performance Liquid Chromatography (HPLC) system (Waters, Milford, MA, USA) equipped with a reverse-phase Nova-Pak C18 column (Waters, Milford, MA, USA) and a Diode-Array Detector (DAD). Chromatographic separation was performed at a constant flow rate of 0.8 mL/min, with the mobile phase consisting of water containing 0.2% formic acid (Phase A) and acetonitrile containing 0.1% formic acid (Phase B).

Quantification was based on calibration curve prepared from ten polyphenol standards, gallic, acid, protocatechuic acid, catechin, chlorogenic acid, coumaric acid, cinnamic acid, rutin, quercetin, luteolin and kaempferol, these were initially dissolved at 1 mg/mL and diluted to a working range of 1-75 µg/mL, following the procedure reported by Chel-Guerrero et al. [22]. Polyphenols in the microcapsules, both before and after simulated digestion, were identified by matching their retention times to those of the standards. Results were expressed as milligrams per 100 g of cookie. Quercetin and luteolin were reported as their combined sum due to peak overlap during detection (similar retention times).

2.6 *Total polyphenol content bioaccessibility during in vitro digestion of an enriched cookie*

Bioaccessibility of individual polyphenols throughout the simulated digestion was evaluated following the procedure described by Aguilera-Chávez et al. [31], applying Equation (4) to determine the intestinal phase Bioaccessibility Index (%BII):

$$\%\mathrm{BII} = \left(\frac{PhI_{\mathrm{In}}}{PhI_{\mathrm{bf}}} \times 100 \right) \tag{4}$$

where PhI_{bf} denotes the individual polyphenol content measured before the digestion process, and PhI_{bf} refers to the individual polyphenol content determined at the end of the simulated digestion for the intestinal phase. Only the concentration identified at the end of the intestinal phase of the simulated digestion was reported for individual polyphenols whose bioaccessibility could not be calculated because the compound was not detected prior to digestion, only the concentration identified at the end of the intestinal phase of the simulated digestion was reported.

2.7 *Statistical analysis*

Experimental procedures were conducted in random order using a $2^3 \times 3$ factorial design. For each cookie sample derived from the experimental setup, triplicate analytical measurements were conducted to determine total and individual polyphenols, antioxidant capacity by DPPH, color, moisture content, texture, and sampling time from simulated digestion. Results are reported as mean values with their corresponding standard deviations. Data from the factorial design analyses, Analysis of Variance (ANOVA), post hoc comparisons, and other statistical tests were processed using the Statgraphics Centurion XVII. II X64 software (Statgraphics Technologies Inc., Warrenton, VA, USA).

3. Results

3.1 *Physical properties of cookies enriched with microencapsulated Habanero pepper leaf extracts*

Moisture and color results according to the CIEL*a*b* scale for the control and enriched cookies, before and after baking, are shown in Table 2.

Moisture content of the evaluated cookies showed a significant difference between the samples before and after baking, in both control and enriched cookies ($p < 0.05$). However, a difference was observed ($p < 0.05$, Least Significant Difference (LSD) Test, limit ± 2.0747) in cookies prior to baking, between Raw Control Cookie (RCC) (19.30 ± 1.40%) and Raw Enriched Cookie (REC) (15.21 ± 1.13%), whereas after baking, the moisture contents were similar ($p > 0.05$, LSD Test, limit ± 2.0747) between Baked Control Cookie (BCC) (0.78 ± 0.07%) and Baked Enriched Cookie (BEC) (1.72 ± 0.11%).

Table 2. Moisture and color parameters of the control and the cookie enriched with microencapsulated Habanero pepper leaf extract

Type of cookie	Moisture (%)	Color parameters (CIEL*a*b*)					Visual interpretation**
		*L**	*a**	*b**	Chroma	Hue°	
RCC	19.30 ± 1.40^c	63.39 ± 2.82^b	4.90 ± 0.27^b	24.30 ± 1.65^b	24.79 ± 1.67^b	1.37± 0.01^c	
BCC	0.78 ± 0.07^a	60.32 ± 0.60^a	12.87 ± 0.04^d	33.01 ± 0.12^d	35.43 ± 1.09^d	1.20 ± 0.00^a	
REC	15.70 ± 1.34^b	64.54 ± 1.19^b	3.65± 0.14^a	20.73± 1.12^a	21.05 ± 0.38^a	1.4 ± 0.01^d	
BEC	1.72 ± 0.11^a	65.36 ± 0.46^b	10.38± 0.33^c	31.35± 0.33^c	33.02 ± 0.24^c	1.25 ± 0.01^b	

Abbreviations: RCC = Raw control cookie; BCC = Baked control cookie; REC = Raw enriched cookie; BEC = Baked enriched cookie; ** the CIELAB scale color parameters conversion to images was performed with the e-paint converter: https://www.e-paint.co.uk/convert-lab.asp (accessed on 18 August 2025). Different letters in the same column indicate statistical differences ($p < 0.05$, LSD)
Source: Authors' own elaboration

For color parameters, the L^* value or lightness, there was no difference ($p > 0.05$) in the RCC, REC, and BEC. The lowest L^* value was obtained from BCC.

For the a^* parameter, which represents the green-red axis, all samples showed a tendency to redness coloration (positive values), with the BCC exhibiting the highest value (12.87 ± 0.04, $p < 0.05$), while the REC showed the least

reddish value (3.65 ± 0.14). On the other hand, for the *b** values, which represent the blue-yellow axis, a tendency toward the yellow color was observed, as indicated by the positive values. The BBC (33.01 ± 0.12) exhibited a more yellowish coloration, while the REC sample showed the lowest *b** value (21.05 ± 0.38, $p < 0.05$).

Accordingly, the analysis of Chroma* values revealed differences in color saturation among samples, with higher chroma indicating more vivid coloration, particularly in BCC (35.43 ± 1.09) and REC (21.05 ± 0.38), showing the lowest chroma. Similarly, variations in *h*° indicated shifts in color tone, allowing the discrimination between samples tending toward a more red-yellowish hue and those closer to less saturated yellow or greenish tones.

Figure 1 shows the hardness of both the control and enriched cookies. A statistically significant difference ($p < 0.05$) was observed between the hardness of the BEC (62.77 ± 0.01 N) and BCC, with the BEC exhibiting the highest hardness (73.63 ± 0.82 N).

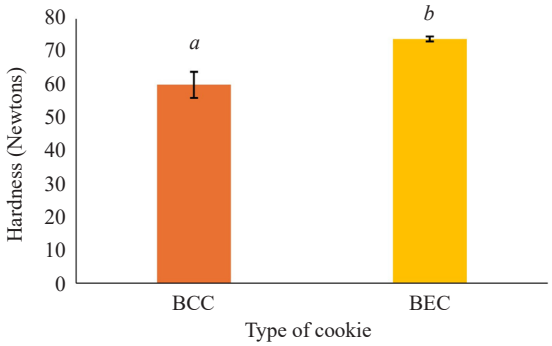

Figure 1. Hardness of the control and enriched cookies after baking for 30 min at 180 °C, where BCC = Baked control cookie; BEC = Baked enriched cookie
Source: Authors' own elaboration

Table S1 presents the TPA results for hardness, brittleness, adhesiveness, and elastic modulus of baked control and enriched cookies. The BEC exhibited a higher elastic modulus value (2.48 ± 0.24 N/mm^2), without adhesiveness (0.00 ± 0.00 N), and lower brittleness (0 ± 0.00 N) compared ($p < 0.05$) to the BCC.

3.2 *Total polyphenol content and antioxidant capacity in a cookie enriched with microencapsulated Habanero pepper leaf extract during simulated digestion*

Table S2 presents the results for TPC and Ax of both the control cookie and the enriched cookie containing microencapsulated Habanero pepper leaf extract, following simulated digestion. Within the experimental design, the highest concentration ($p < 0.05$) of total polyphenols was observed in the simulated digestion samples of the enriched cookie under fasting conditions during the intermediate (270.55 ± 4.51 mg GAE/100 g cookie, experiment #18) and final (273.64 ± 12.21 mg GAE/100 g cookie, experiment #17) stages of the intestinal phase. Conversely, the lowest concentration (74.69 ± 4.01 mg GAE/100 g cookie, experiment #1) was recorded at the initial stage of the gastric phase during the simulated digestion of the control cookie under fasting conditions. However, when comparing the total polyphenol content of the extracts obtained by ultrasonic bath from the control cookie (10.41 ± 0.27 mg GAE/100 g cookie, Control Cookie Extract (CCExt)) and the Enriched Cookie Extract (ECExt) (31.11 ± 1.06 mg GAE/100 g cookie), both values were markedly lower ($p < 0.05$) than those observed in the samples obtained through simulated digestion.

It should be noted that the behavior of the total polyphenols in the cookie during simulated digestion was markedly different under fasting and postprandial conditions. When the cookie was digested under fasting conditions, maintenance of total polyphenol concentration was observed during the gastric phase in both the enriched and control cookies, followed by an increase in polyphenol concentration during the intestinal phase. The enriched cookie maintained a higher TPC concentration at the end of the intestinal phase (273.64 ± 12.21 mg GAE/100 g cookie) compared to the control cookie (239.36 ± 1.82 mg GAE/100 g cookie) and even under postprandial conditions compared to both, the enriched (182.91 ± 5.01 mg GAE/100 g cookie) and control (176.46 ± 7.74 mg GAE/100 g cookie) cookies as well as

the standard food (Figure 2).

On the other hand, antioxidant capacity exhibited a different pattern compared to the TPC results of the digested cookie samples. Within the experimental design, the highest antioxidant capacity was consistently observed in all samples from the intestinal phase, in all three stages (initial, intermediate, and final), for both the control and enriched cookies under postprandial conditions. For example, the antioxidant capacity of the simulated digested enriched cookie under postprandial conditions in the final stage of the intestinal phase was 84.41 ± 1.45% inhibition, a value not significantly different ($p > 0.05$) from that of the control cookie sample, which recorded 81.10 ± 2.88% inhibition under the same condition, stage, and phase.

In Figure 3, the behavior of the antioxidant capacity during the simulated digestion of the enriched cookie and the control cookie under fasting and postprandial conditions is shown throughout the gastric and intestinal digestive phases. Findings show that Habanero pepper leaf extract microencapsulated favors the maintenance of antioxidant capacity during the gastric phase and promotes an increase during the intestinal phase, under both fasting and postprandial conditions, compared to the control cookie and the standard food.

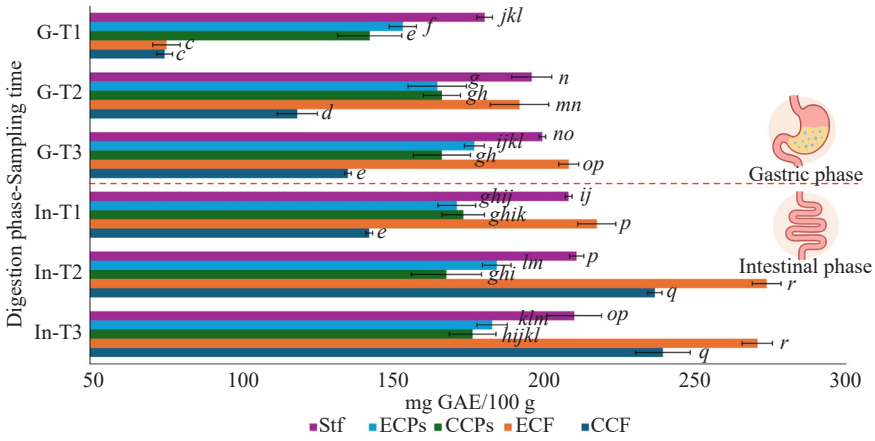

Figure 2. Behavior of total phenolic content during the simulated digestion of the cookie enriched with microencapsulated Habanero pepper leaf extract. G = Gastric, In = Intestinal; T1 = early time, T2 = middle time, and T3 = final time sampling; CC = Control cookie; EC = Enriched cookie, StF = Standard Food; F = fasting, Ps = postprandial condition. Different letters indicate significant differences (LSD, $p < 0.05$, $n = 3$)
Source: Authors' own elaboration

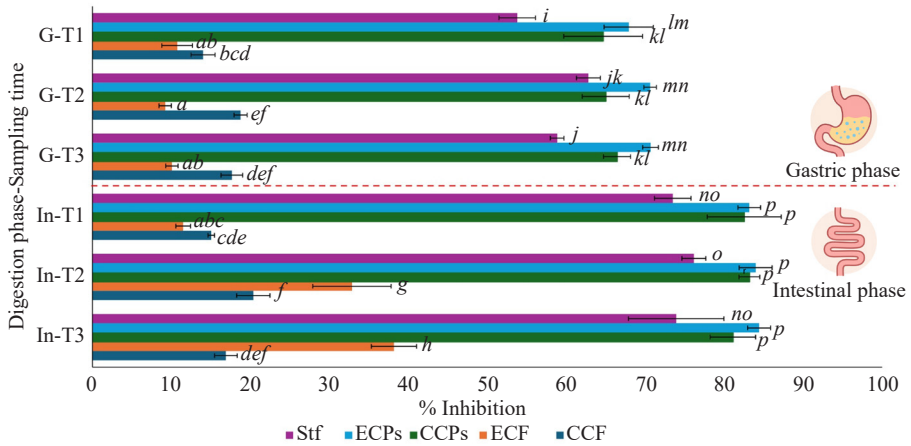

Figure 3. Behavior of antioxidant capacity during the simulated digestion of the cookie enriched with microencapsulated Habanero pepper leaf extract. G = Gastric, In = Intestinal; T1 = early time, T2 = middle time, and T3 = final time sampling; CC = Control cookie; EC = Enriched cookie, StF = Standard Food; F = fasting, Ps = postprandial condition. Different letters indicate significant differences (LSD, $p < 0.05$, $n = 3$)
Source: Authors' own elaboration

Unlike total polyphenols, the extract obtained by ultrasonic bath from the enriched cookie exhibited the highest ($p < 0.05$) antioxidant capacity (91.08 ± 0.08% inhibition) when compared with the samples obtained from simulated digestion. Finally, the sample with the lowest ($p < 0.05$) antioxidant capacity was the simulated digested enriched cookie under fasting conditions during the gastric phase at the intermediate stage, with an antioxidant capacity of 9.21 ± 0.78% inhibition.

Multivariate ANOVA of TPC and Ax revealed that the evaluated factors of the experimental design exerted significant effects on the concentration of polyphenols and antioxidant capacity during the simulated digestion of the cookie enriched with microencapsulated Habanero leaf extract.

Pareto chart of TPC (Figure 4) shows that the triple interaction Type of cookie (A) × Digestive phase (C) × Sampling time (D) had a significant effect ($p < 0.05$) on the polyphenol concentration of the cookie enriched with microencapsulated Habanero leaf extract during its simulated digestion.

It was also observed that the double interactions B × C, B × D, and A × B exerted a significant effect ($p < 0.05$), whereas all main factors exhibited a significant effect ($p < 0.05$) on TPC.

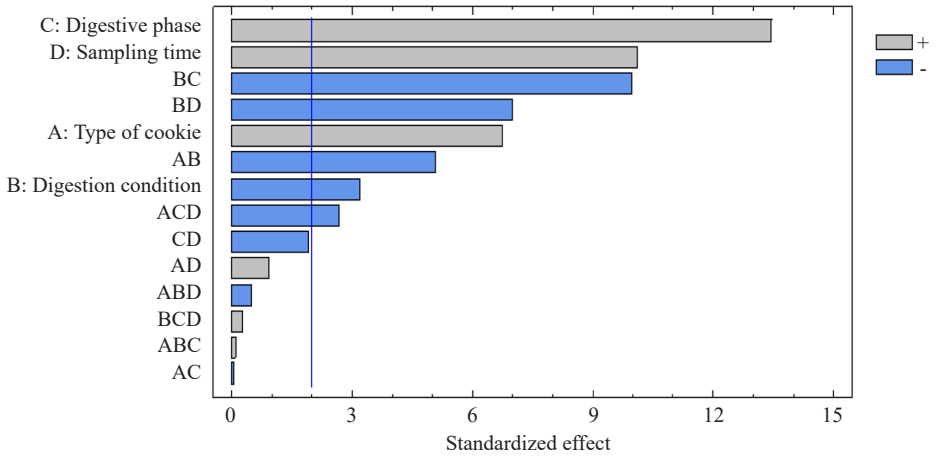

Figure 4. Pareto chart for the total polyphenol content of the cookie enriched with microencapsulated Habanero leaf extract during its simulated digestion
Source: Authors' own elaboration

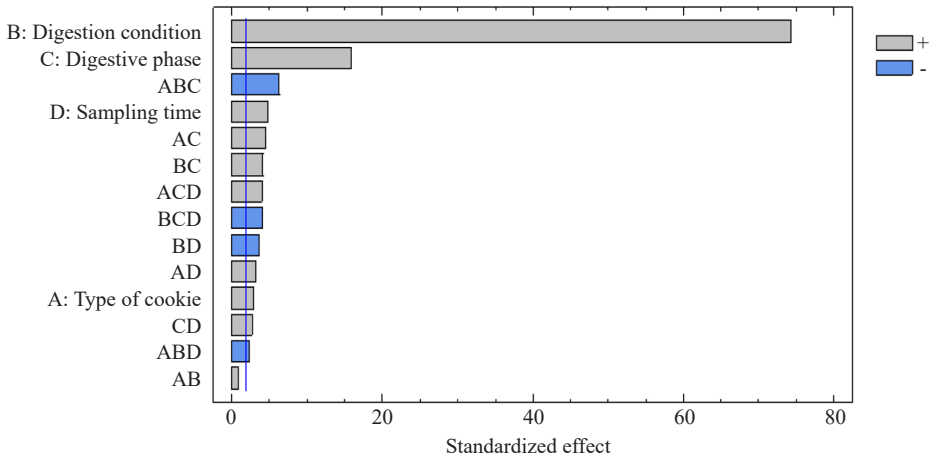

Figure 5. Pareto chart for the antioxidant capacity of the cookie enriched with microencapsulated Habanero leaf extract during its simulated digestion
Source: Authors' own elaboration

Figure 5 also presents the Pareto chart for antioxidant capacity. A significant effect ($p < 0.0001$) on the antioxidant capacity of the cookie during simulated digestion was observed from the triple interaction A × B × C, in comparison with the triple interactions A × C × D, B × C × D, and A × B × D, which also showed an effect ($p < 0.05$) on the Ax.

In contrast with TPC, antioxidant capacity during simulated digestion was affected by a combination of triple interactions from the main factors. This difference shows that Ax is not related to TPC bioaccessibility during simulated digestion.

3.3 *Polyphenol profile and bioaccessibility of cookie enriched with microencapsulated Habanero pepper leaf extract during simulated digestion*

3.3.1 *Identification and distribution of individual polyphenols during simulated digestion*

From both analyzed samples, the enriched and control cookies, under fasting and postprandial conditions in the two digestive phases (gastric and intestinal), the presence of protocatechuic acid, catechin, chlorogenic acid, coumaric acid, cinnamic acid, rutin, quercetin + luteolin, and hesperidin was detected (Table S3) and its bioaccessibility calculated (Table S4).

Catechin (Ctc), Rutin (Rt), and Quercetin + Luteolin (Q + L) were the individual polyphenols found at the highest concentrations throughout the simulated digestion at the intestinal phase and were detected mainly in the enriched cookies (Figure S2). Ctc showed (Figure 6) a concentration of 64.90 ± 0.00 mg/100 g cookie in the enriched cookie during the intermediate stage of the intestinal phase under fasting conditions (EFInT2), the highest among all samples in the experimental design. In contrast, the lowest Ctc concentration was detected in the enriched cookie during the initial stage of the intestinal phase under postprandial conditions (EPsInT1), with 1.57 ± 0.10 mg/100 g cookie.

Rt also exhibited a high concentration in the enriched cookies under fasting conditions; however, whereas Ctc reached its maximum during the intestinal phase, Rt showed its highest concentration (26.62 ± 0.02 mg/100 g cookie) in the intermediate stage of the gastric phase (EFGT2). The lowest Rt concentration (1.88 ± 0.00 mg/100 g cookie) was recorded in the enriched cookie digested under postprandial conditions during the initial stage of the gastric phase (EPsGT1). The behavior of rutin during simulated digestion is shown in Figure S3.

Another polyphenol detected at high concentrations in enriched cookie samples under fasting conditions was Q + L (Figure 7), with 25.51 ± 0.02 mg/100 g cookie in the intermediate stage of the intestinal phase (EFInT3). Under postprandial conditions, the lowest Q + L concentration (3.13 ± 0.00 mg/100 g cookie) was found in the enriched cookie during the initial stage of the gastric phase (EFGT1) in the simulated digestion.

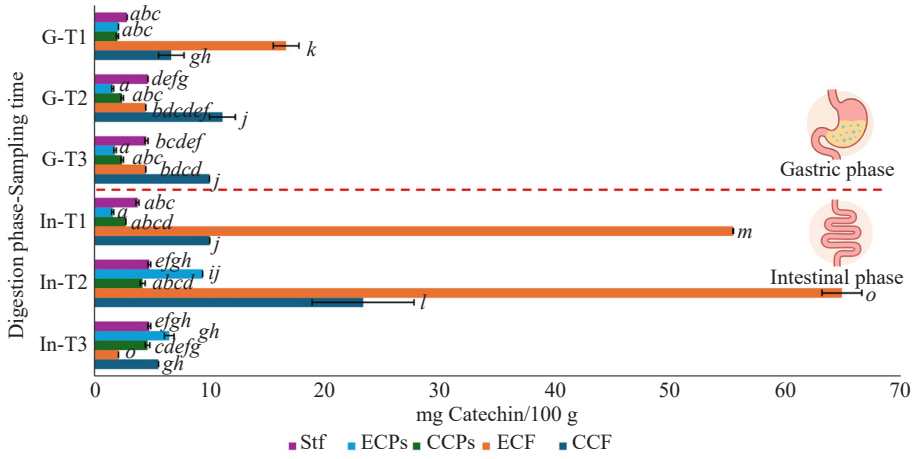

Figure 6. Behavior of the catechin during the simulated digestion of the cookie enriched with microencapsulated Habanero pepper leaf extract. G = Gastric, In = Intestinal; T1 = early time, T2 = middle time, and T3 = final time sampling; CC = Control cookie; EC = Enriched cookie, StF = Standard Food; F = fasting, Ps = postprandial condition. Different letters indicate significant differences (LSD, $p < 0.05$, $n = 3$)
Source: Authors' own elaboration

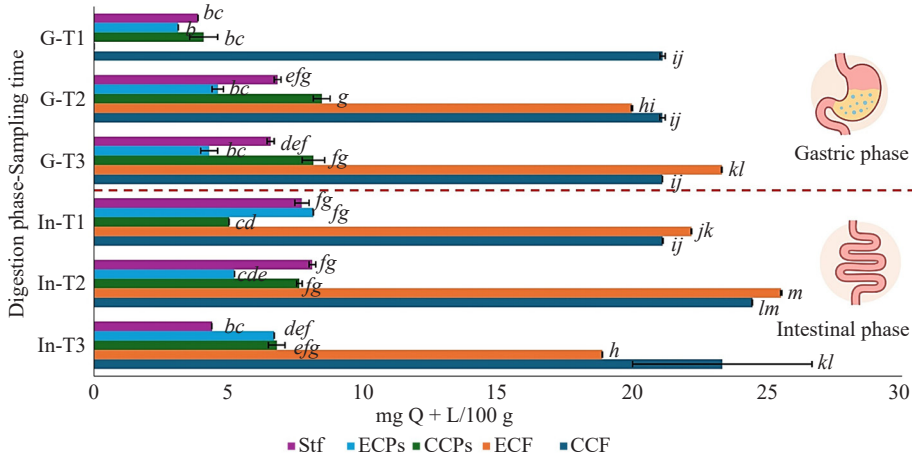

Figure 7. Behavior of the quercetin + luteolin during the simulated digestion of the cookie enriched with microencapsulated Habanero pepper leaf extract. G = Gastric, In = Intestinal; T1 = early time, T2 = middle time, and T3 = final time sampling; CC = Control cookie; EC = Enriched cookie, StF = Standard Food; F = fasting, Ps = postprandial condition. Different letters indicate significant differences (LSD, $p < 0.05$, $n = 3$)
Source: Authors' own elaboration

3.3.2 *Heatmap analysis of polyphenol distribution under fasting and postprandial conditions*

Figure 8 shows the heatmap representing the distribution of individual polyphenols quantified in control and enriched cookie samples during the simulated digestion under fasting and postprandial conditions at the end of both gastric and intestinal phases. The intensity scale ranged from red (highest concentration) to green (lowest concentration), numerical values are presented in Table S3.

Protocatechuic Acid (PtAc) exhibited higher concentrations in the enriched cookie under postprandial conditions during the intestinal phase (EPsIn). In contrast, the lower concentration (green coloration) was observed in the enriched and control cookies, both under postprandial conditions (EPsG), indicating that this compound remained stable under intestinal conditions ($p < 0.05$).

Regarding Catechin (Ctc), high concentrations were detected in both the gastric (EFG) and intestinal (EFIn) phases under fasting conditions during the simulated digestion of the enriched cookie, confirming the potential release of catechins during digestion. In contrast, the control cookie samples showed lower Ctc levels, particularly in the Baked Enriched Cookie (BEC, undigested sample) and control cookie during fasting condition at gastric phase (CFG), consistent with their lower initial polyphenol content ($p < 0.05$).

Chlorogenic Acid (ChAc) remained relatively stable during most treatments, with slight increases in the simulated digestion of the control cookie, under both fasting and postprandial conditions, and in both the gastric (CFG, CPsG) and intestinal phases (CFIn, CPsIn), compared with the enriched cookie at the corresponding digestive stages and digestion conditions (EFG, EPsG, EFIn, EPsIn).

Coumaric acid (CuAc) reached its highest concentration in the enriched cookies under fasting conditions during the gastric phase (EFG), followed by a decreasing trend throughout the intestinal phase. A similar behavior was observed in the control cookie under both fasting and postprandial conditions.

Cinnamic Acid (CiAc) was detected predominantly under postprandial conditions in both the enriched and control cookies, regardless of the digestive phase (EPsG, EPsIn, CPsG, CPsIn). In contrast, very low levels were observed in the baked cookies (BEC, BCC; undigested samples) and during simulated digestion under fasting conditions (EFG, EFIn, CFG, CFIn). Rutin concentrations were highly variable, showing a higher concentration in the enriched cookies during the gastric phase under fasting conditions (EFG), with pronounced decreases (green zones) in most samples. This behavior was like that already presented in Q + L, except that this polyphenol was found at high concentrations in the enriched cookie samples during the intestinal phase under postprandial conditions (EPsIn).

Finally, Hesperidin (Hpn) exhibited high concentrations in both the gastric and intestinal phases under fasting conditions in the enriched (EFG, EFIn) and control cookies (CFG, CFIn). Conversely, during the intestinal phase under postprandial conditions, the enriched cookie showed a slightly higher Hpn concentration than the control cookie.

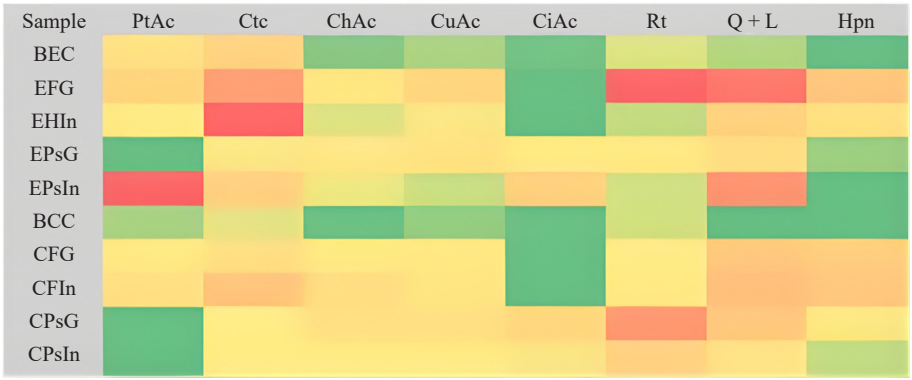

Figure 8. Heatmap representing the distribution of individual polyphenols quantified in control and enriched cookie samples during the simulated digestion under fasting and postprandial conditions. BEC = Baked enriched cookie; EFG = Enriched cookie under fasting conditions during the gastric phase; EFIn = Enriched cookie under fasting conditions during the intestinal phase; EPsG = Enriched cookie under postprandial conditions during the gastric phase; EPsIn = Enriched cookie under postprandial conditions during the intestinal phase; BCC = Baked control cookie; CFG = Control cookie under fasting conditions during the gastric phase; CFIn = Control cookie under fasting conditions during the intestinal phase; CPsG = Control cookie under postprandial conditions during the gastric phase; and CPsIn = Control cookie under postprandial conditions during the intestinal phase. The evaluated individual polyphenols were PtAc = protocatechuic acid; Ctc = catechin; ChAc = chlorogenic acid; CuAc = coumaric acid; CiAc = cinnamic acid; Rt = rutin; Q + L = quercetin + luteolin; and Hpn = hesperidin. The color scale indicates relative concentrations, where red denotes the highest and green the lowest values detected for each compound
Source: Authors' own elaboration

3.3.3 *Bioaccessibility of individual polyphenols during simulated digestion*

Regarding the individual bioaccessibility of these polyphenols, distinct behaviors were observed throughout the simulated digestion, revealing compound-dependent stability and release patterns; however, only catechin, cinnamic acid, and Q + L were related to the enriched cookies.

Table S4 shows the bioaccessibility of the different individual polyphenols detected during the simulated digestion. Figure 9 illustrates the bioaccessibility of catechin during the simulated digestion of the enriched and control cookies under fasting and postprandial conditions.

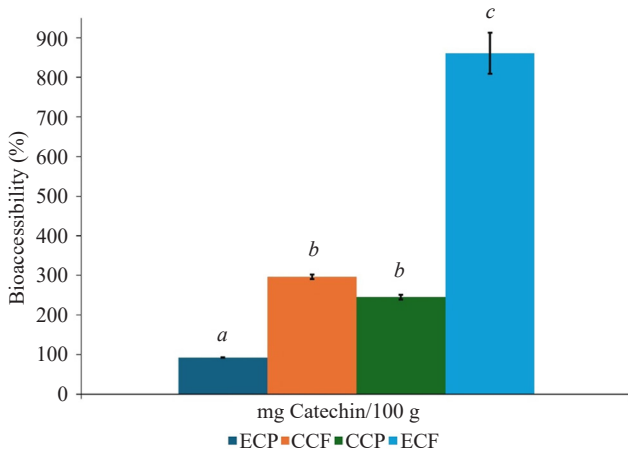

Figure 9. Bioaccessibility of catechin during the simulated digestion of the cookie enriched with microencapsulated Habanero pepper leaf extract. ECP = Enriched cookie under postprandial condition; CCF = Control cookie under fasting condition; CCP = Control cookie under postprandial condition; ECF = Enriched cookie under fasting condition. Different letters indicate statistical differences (LSD, $p < 0.05$)
Source: Authors' own elaboration

High bioaccessibility ($p < 0.05$) was observed for the individual polyphenols cinnamic acid, catechin, and Q + L, the latter two are phenolic compounds characteristic of the leaves of Habanero pepper. The bioaccessibility of cinnamic

acid reached 1,909.07 ± 271.18% under postprandial conditions, while catechin and Q + L achieved bioaccessibility values of 860.52 ± 51.98% and 1,642.00 ± 0.00%, respectively, under fasting conditions (Table S4).

On the other hand, protocatechuic acid (764.39 ± 2.53%), chlorogenic acid (3,932.58 ± 449.79%), coumaric acid (913.16 ± 36.69%), and rutin (335.99 ± 1.11%) exhibited high bioaccessibility ($p < 0.05$) in the control cookie under fasting conditions during the simulated digestion.

3.3.4 *Multivariate analysis of factors affecting individual polyphenols*

Data obtained from the polyphenol profile were analyzed by multivariate ANOVA to identify the effects of the factors and their interactions. Table 3 shows the *p*-values for each main factor and their interactions with respect to each individual polyphenol.

Table 3. *p*-values for the effects and interactions of experimental design $2^3 \times 3$ factors on polyphenol profile of an enriched cookie with microencapsulated Habanero pepper leaf extract

Factors	*p*-value							
	PtAc	Ctc	ChAc	CuAc	CiAc	Rt	Q + L	Hpn
	Main factors							
A	0.0328	< 0.0001	0.0006	0.1476	0.3250	0.9052	0.0047	0.0211
B	< 0.0001	< 0.0001	0.0489	0.0003	< 0.0001	0.0529	< 0.0001	< 0.0001
C	0.0850	< 0.0001	0.0024	0.0378	0.4682	< 0.0001	0.0012	0.3273
D	0.9990	0.8736	0.6861	< 0.0001	0.0048	0.0705	0.0018	0.0749
	Interactions							
AB	0.0328	< 0.0001	0.0013	0.1231	0.3250	< 0.0001	0.1638	0.1446
AC	0.3803	< 0.0001	0.7711	0.8170	< 0.0001	0.0446	0.0109	0.4020
AD	0.5926	0.7238	0.3046	0.0073	0.0006	0.1273	0.1643	0.8094
BC	0.0850	< 0.0001	0.0452	0.3694	0.4682	0.0102	0.0349	0.3823
BD	0.9990	0.2003	0.2854	0.0001	0.0048	0.0765	0.0497	0.0569
CD	0.0026	0.2037	0.7701	0.0099	0.6327	0.0001	0.0011	0.0375
ABC	0.3803	< 0.0001	0.8419	0.0076	< 0.0001	< 0.0001	0.4061	0.2482
ABD	0.5926	0.4654	0.3046	0.3711	0.0006	0.1476	0.0062	0.7084
ACD	0.0006	0.0252	0.7078	0.0391	0.4947	0.5388	0.0011	0.4581
BCD	0.0026	0.8541	0.2391	0.3113	0.6327	0.3960	0.0232	0.1519

Abbreviations: A = Cookie type; B = Type of digestion; C = Digestive phase; D = Sampling time; PtAc = Protocatechuic acid; Ctc = Catechin; ChAc = chlorogenic acid; CuAc = coumaric acid; CiAc = cinnamic acid; Rt = rutin; Q + L = quercetin + luteolin; Hpn = hesperidin
Source: Authors' own elaboration

The polyphenol profile data were analyzed by multivariate ANOVA to assess the effect of the main factors, cookie type (A), digestion condition (B), digestive phase (C), and sampling time (D), and their interactions on the individual polyphenols. The corresponding levels of statistical significance are presented in Table 3.

In general, the results showed that the response of individual polyphenols during simulated digestion depended on

the specific compound and on the combined action of the evaluated factors. Quercetin + luteolin was the most affected polyphenol, showing significant changes in response to all main factors, while catechin and chlorogenic acid were mainly influenced by cookie type, digestion condition, and digestive phase. The remaining compounds exhibited more limited or selective responses, suggesting differences in stability and release within the food matrix.

Furthermore, several significant interaction effects were detected, indicating that polyphenol concentrations during digestion are influenced by the combined effects of formulation, digestion conditions, and sampling time rather than by single factors alone. This behavior highlights the complexity of polyphenol dynamics in fortified food systems and supports the use of a multivariate statistical approach for their evaluation.

4. Discussion

To the best of our knowledge, this work represents the first comprehensive study integrating a previously optimized NADES-based extraction of polyphenols from Habanero pepper leaves with the optimization of spray-drying microencapsulation conditions to obtain a stable, polyphenol-rich powder. This powder was subsequently incorporated into the formulation of a cookie as a food model, and the bioaccessibility of polyphenols was finally evaluated through simulated digestion. In this way, our study shows a new way for the potential application of agricultural by-product extracts in the formulation of functional foods, developed through green technologies that can be considered as Generally Recognized As Safe (GRAS).

Although the extract has beneficial effects on human health, the extracted compounds, mainly polyphenols, are highly sensitive to environmental conditions. Therefore, spray-drying microencapsulation was implemented to protect these metabolites of interest, yielding a stable, easy-to-handle product with potential applications as an ingredient in functional foods.

The application of this technology prior to incorporation into food matrices has resulted in products with high polyphenol content and enhanced antioxidant capacity. For example, Grassia et al. [32] microencapsulated cocoa shell ethanolic extract (50%) by spray drying using maltodextrin (DE 17-19.9) as the encapsulating agent (core : coating, 1 : 5 w/w ratio) at 120 °C as the inlet temperature. The resulting powder was incorporated into a chocolate bar, leading to a 35.57% increase in phenolic compounds compared with the chocolate control bar.

Ranasinghe et al. provide an example of the incorporation of a polyphenol extract obtained using water as an aqueous solvent, combined with a green-assisted extraction technique (ultrasonic probe), for the formulation of enriched cookies [33]. To obtain their extract, defatted date seed powder was mixed with water at a 1 : 25 (w/v) ratio and sonicated for 8 min. The supernatant was subsequently microencapsulated (encapsulating agents: soy protein and gum arabic) and added to the formula. The enriched cookie containing the microencapsulated extract (19 g powder) presented a total polyphenol content of 260 mg GAE/100 g, whereas the control cookie (without microencapsulation) contained 83.85 mg GAE/100 g before an in vitro digestion, whereas at the end of this in vitro digestion process, the enriched food exhibited only 127 mg GAE/100 g of cookie. Although the polyphenol concentration of our cookie prior to digestion was relatively low (31.11 ± 1.06 mg GAE/100 g of cookie), a markedly higher concentration (239.36 ± 1.82 mg GAE/100 g of cookie) was recorded at the end of the intestinal digestion phase under fasting conditions. These results indicate that extracts obtained using NADES and subsequently microencapsulated by spray drying may provide enhanced protection of phenolic compounds and, consequently, improved bioaccessibility, compared with microencapsulated extracts derived from conventional organic solvents incorporated into food matrices.

Although total polyphenol content and antioxidant capacity are often discussed together, they could evolve differently during digestion. Total polyphenol content reflects the concentration of extractable phenolic compounds, whereas antioxidant capacity represents the cumulative redox activity of the whole digestion matrix [34]. During gastrointestinal digestion, antioxidant capacity may be maintained or even enhanced despite a decrease in free phenolics due to the formation of protein-phenolic complexes, the release of antioxidant peptides during proteolysis, lipid-phenolic interactions, and the contribution of Maillard reaction products [35-37]. These processes can stabilize phenoxyl radicals or generate new radical-scavenging species, thereby decoupling antioxidant capacity from total polyphenol content values.

Furthermore, antioxidant peptides generated during gastrointestinal proteolysis have contributed to the overall

antioxidant profile of digested food matrices [38]. In addition, Maillard reaction products formed during thermal processing and subsequently released or transformed under digestive conditions exhibit significant antioxidant capacity through electron donation, transition-metal chelation, and inhibition of lipid peroxidation pathways [35]. Finally, several studies have observed weak correlations between total polyphenol content and antioxidant capacity after intestinal digestion, suggesting that compounds other than extractable polyphenols play important roles in modulating redox activity during digestion [39]. Consequently, antioxidant capacity reflects the integrated redox behavior of phenolics, peptides, Maillard-derived compounds, and other digestion-related species within the matrix, rather than being solely dependent on total polyphenol concentration.

Our cookie formulated with microencapsulated Habanero leaf extract obtained using a deep eutectic solvent exhibited a 198.84% increase in polyphenol content compared to the control cookie. These results are consistent with previous reports employing traditional solvents for obtaining polyphenol-rich extracts. Although the application of spray-drying as a microencapsulation technique is not new [19], the use of NADES in this context remains in early stages of research. Nevertheless, the performance observed in this study reflects results typically achieved with conventional organic solvents, suggesting that NADES-based extracts can be efficiently protected and stabilized through microencapsulation. This effect can be attributed to the well-documented role of microencapsulation in preserving and enhancing polyphenols within food matrices, by providing a protective barrier against environmental stressors such as light, temperature fluctuations, pH changes, and oxygen exposure, i.e., microencapsulation enhances the stability and bioactivity of phenolic compounds throughout processing and storage [17, 40-42]. Moreover, it can mask undesirable flavors, improve sensory acceptance, and enable controlled release with enhanced bioavailability during digestion [43]. In bakery applications, for instance, spray-dried polyphenol microcapsules have been shown to maintain antioxidant capacity after baking while minimizing adverse effects on product taste and texture [17, 34, 44].

At the molecular level, carrier agents such as maltodextrin, Guar gum, and modified starches protect polyphenols through multiple complementary mechanisms. Maltodextrin forms a semi-crystalline or amorphous matrix around phenolic molecules via hydrogen bonding, which effectively reduces exposure to oxygen, light, and heat-driven degradation [45]. Also, Guar gum has been recognized as a highly effective encapsulating agent for the stabilization of polyphenolic compounds. Its galactomannan structure, characterized by a high molecular weight and branched morphology, facilitates extensive hydrogen bonding with phenolic hydroxyl groups and water molecules, thereby creating a viscous and dense protective matrix [46]. This molecular arrangement immobilizes bioactive compounds within the encapsulation system and reduces their direct exposure to environmental stressors such as oxygen, light, and moisture, which trigger oxidative and thermal degradation. Steric hindrance provided by its branched polysaccharide chains further limits molecular diffusion, enhancing the physical stability of encapsulated polyphenols [47]. Collectively, these properties promote the positive and protective role of Guar gum in maintaining both the stability and antioxidant potential of polyphenol-rich extracts obtained through green extraction methods such as NADES.

Modified starches can establish hydrogen bonds and hydrophobic interactions with the hydroxyl groups and aromatic rings of polyphenols, leading to the formation of inclusion and non-inclusion complexes [48-50]. These molecular interactions result in altered starch microstructures and crystalline arrangements, which in turn affect the physicochemical stability of the polyphenol-starch complexes [49, 51]. Notably, the presence of polyphenol-starch complexes enhance the thermal stability and antioxidant efficacy of phenolic compounds under processing and storage conditions. This stabilization emerges from specific molecular interactions, hydrogen bonding between hydroxyl groups of polyphenols and the hydroxyl moieties of starch, as well as hydrophobic stacking of aromatic rings within starch helices. These interactions restrict the mobility of phenolic molecules, thereby reducing their susceptibility to thermal degradation and oxidative activity. Furthermore, the confinement of polyphenols within the starch matrix shields reactive hydroxyl groups from pro-oxidant species, effectively limiting radical propagation pathways and enzymatic hydrolysis [51, 52].

The protective role of microencapsulation in preserving the antioxidant potential of phenolic compounds has been consistently studied in food applications, where enriched products exhibit markedly higher antioxidant capacity compared to their non-encapsulated counterparts. A representative example can be found in the work of Kalajahi et al. [53], who evaluated an extract from sour tea leaves (*Hibiscus sabdariffa* L.) obtained through ethanol-hydrochloric acid (0.1%) maceration and subsequently microencapsulated with maltodextrin, mucilage, and inulin by spray drying. When incorporated into a cake formulation (0.7% w/w), the enriched product showed a remarkable increase in antioxidant

capacity, with DPPH inhibition rising from 4.63% in the control to 72.24% in the fortified cake. This effect was attributed to the presence of phenolic compounds such as protocatechuic acid, quercetin, and luteolin.

Papillo et al. [44] reported a polyphenol-rich extract from cocoa peel using water : methanol (50 : 50 v/v) as solvent in a ratio of 1 : 10 w/v, through agitation (15 min) and sonication (15 min). The extract was microencapsulated by spray drying with maltodextrin (DE 16-20) at an extract-to-carrier ratio of 80 : 20 w/w at an inlet temperature of 150 °C. The obtained powder was added (0.32%) to the cookie formula, which exhibited an antioxidant capacity of 108 mg Trolox Equivalents (TE)/g compared to the cookie with the non-microencapsulated extract (53.9 mg TE/g), showing a 200% increase in antioxidant capacity. In the present study, the enriched cookies prior to the digestion process achieved up to 91.08 ± 0.08% inhibition, indicating that microencapsulates obtained from NADES-based extracts can preserve antioxidant capacity at higher levels than those reported for microencapsulated extracts produced using conventional organic solvents. Consistently, enrichment with the microencapsulated *Capsicum chinense* (Habanero pepper) leaf extract resulted in a clear improvement to the control cookie before simulated digestion, with an antioxidant capacity increase of 23.21% inhibition, supporting the suitability of NADES-derived microencapsulates for functional bakery applications.

Microencapsulation effectively enhances the antioxidant capacity of fortified food products by providing both chemical and physical protection to polyphenols within encapsulating walls. This protective effect is achieved through molecular and physicochemical interactions, where carrier agents such as polysaccharides and proteins establish hydrogen bonds and hydrophobic interactions with the hydroxyl groups and aromatic rings of phenolic compounds. These interactions restrict their exposure to pro-oxidant species, reduce electron transfer and radical propagation, and limit enzymatic hydrolysis and thermal degradation. In addition, the steric confinement within polymeric networks of encapsulants such as maltodextrin, gums, and modified starches creates semi-permeable barriers that regulate oxygen and moisture diffusion, stabilizing redox-active sites in polyphenols. As a result, encapsulated extracts consistently retain higher antioxidant capacity during processing and storage compared to their non-encapsulated counterparts. Beyond stabilization, microencapsulation facilitates more efficient integration of bioactive compounds into complex food matrices, ensuring their functional efficacy throughout the product's shelf life [54-57].

During simulated digestion, foods enriched with microencapsulated extracts exhibit distinct behaviors in terms of total polyphenol content, antioxidant capacity, and the final stage of individual metabolites. This analytical approach provides valuable insights into how encapsulated compounds withstand gastric and intestinal environments, as well as fasting and postprandial conditions, which ultimately determine their bioaccessibility and potential absorption. As highlighted by Qazi et al. [56], encapsulation significantly influences the release dynamics and stability of polyphenols during gastrointestinal transit, while Rezagholizadeh-Shirvan et al. [55] emphasized that simulated digestion models are essential for assessing the functional performance of encapsulated bioactives under physiologically relevant conditions. Together, these findings underscore the importance of simulated digestion studies for linking the protective effects of encapsulation within the food matrix to their actual nutritional and functional impact.

The effect of simulated digestion was evident in the behavior of our enriched cookies, which exhibited higher concentrations of total polyphenols under fasting conditions, exceeding the concentrations measured in both the control and enriched cookies digested under postprandial conditions. Higher polyphenol content detected in the enriched cookie under fasting conditions compared to the postprandial state can be attributed to two complementary mechanisms. First, protein-phenolic interactions occurring in the postprandial state reduce the measurable free fraction of polyphenols. These include covalent linkages formed between oxidized polyphenols (e.g. quinone intermediates) and nucleophilic amino acid residues (e.g. lysine, tyrosine, methionine, cysteine) and non-covalent associations, including hydrogen bonds formed between hydroxyl groups of polyphenols and electronegative atoms (Oxygen, Nitrogen) of proteins, as well as hydrophobic and electrostatic interactions, which can reduce polyphenol solubility and limit their bioavailability [58]. Second, the structural characteristics of solid food matrices also play a crucial role: as highlighted by Qazi et al. [56], semi-crystalline and compact structures slow down disintegration in the gastric phase, delaying the release of bioactive compounds. This observation is consistent with our TPA results, where the enriched cookies showed a significant increase in hardness ($p < 0.05$) compared to the control. Such higher firmness reflects a more compact and cohesive microstructure, likely due to the interaction between the encapsulating agents and the dough components. The resulting structure may hinder water penetration and enzymatic access, thus contributing to slow disintegration and delayed release of bioactive compounds observed during the digestion assays. In the fasting state, where fewer additional food

components are present, the cookie matrix disintegrates more efficiently, facilitating polyphenol release and explaining the higher content measured.

On the other hand, the cookie enriched with microencapsulated Habanero pepper leaf under postprandial conditions exhibited the highest antioxidant capacity compared to both samples under fasting conditions, particularly at the final stage of the intestinal phase. In addition to the protective effect of microencapsulation, the higher antioxidant capacity observed under postprandial conditions can be attributed to interactions in the food matrix during digestion: (a) release of peptides, (b) modulation of polyphenols, and (c) products generated. (a) The proteolysis releases peptides with radical-scavenging and metal-chelating activities, which increase the antioxidant readouts of the digesta and at the same time, protein-polyphenol complexes, may stabilize phenoxyl radicals and protect redox-active sites, thereby prolonging antioxidant capacity even when the fraction of "free" polyphenols quantified by TPC is reduced [37, 59]. (b) The phospholipids and emulsified oil-water interfaces modulate the distribution of polyphenols toward interfacial regions, which are the primary sites of lipid oxidation. The review conducted by Costa et al. [36] highlights that the interfacial region constitutes the microenvironment where peroxyl radicals are generated, and chain-propagating reactions occur, making the localization of antioxidants at these sites crucial for their efficacy. Phospholipids and bile-derived surfactants adsorb at the oil-water boundary, reduce interfacial tension, and create an amphiphilic coating that stabilizes the emulsion droplets and increases the probability of polyphenols partitioning into this reactive zone. From a molecular perspective, polyphenols interact with lipid interfaces through hydrogen bonding, π-π stacking (aromatic compounds) and hydrophobic interactions, which enhance their residence time and effective concentration at the interface. This interfacial enrichment allows polyphenols to efficiently scavenge lipid-derived peroxyl radicals (ROO•) and terminate radical chain reactions, protecting membrane lipids from peroxidation. Moreover, polyphenols may act as co-antioxidants by regenerating chain-breaking antioxidants such as α-tocopherol, extending the duration of oxidative protection. Although the fraction of "free" polyphenols detected in the aqueous phase may appear reduced, their enhanced interfacial activity within emulsified systems explains the higher functional efficacy observed in lipid digesta microenvironments [36]. (c) Maillard reaction products and protein-polysaccharide conjugates generated or restructured during processing and digestion can further contribute to reducing capacity and complementary radical-scavenging pathways, raising the overall antioxidant capacity measured in the postprandial system [35, 60]. Altogether, these contributions explain why antioxidant functionality depends on the concentration of free polyphenols, but also on the kinetics of release and the microenvironment created by proteins, lipids, and other standard food components within the digesta.

Regarding the individual polyphenols' behavior, catechin and quercetin + luteolin were predominantly detected in the intestinal phase during the simulated digestion of the enriched cookie. These flavonoids are recognized as characteristic constituents of Habanero pepper leaves [61]. The high increase in catechin during the intestinal phase, observed in the cookie enriched with microencapsulated Habanero pepper leaf extract, highlights the ability of microencapsulation to provide protection against the acidic gastric environment. The structure of catechin, characterized by multiple hydroxyl groups and the flavan ring typical of flavan-3-ols, confers a degree of tolerance to gastric acidity, which explains the ability of these compounds to pass through the stomach without undergoing significant degradation, according to Wojtunik-Kulesza et al. [34]. Nevertheless, in the absence of an encapsulating agent, catechin is highly susceptible to oxidation, epimerization, or interactions with proteins and enzymes that reduce its free fraction. Encapsulation within polysaccharide-based matrices such as maltodextrin creates a protective barrier that limits protonation of phenolic hydroxyl groups and their exposure to oxygen, preserving redox activity throughout digestion. Studies on green tea extracts encapsulated with maltodextrin have confirmed that this carrier enhances thermal and pH stability, while allowing controlled release with higher retention of polyphenols and high antioxidant capacity in intestinal fluids compared with gastric conditions [62, 63]. Once in the intestine, the rise in pH and the action of bile salts and pancreatic enzymes promote capsule disintegration and hydroxyl group ionization, which increases catechin solubility and facilitates absorption [64].

Like catechin, quercetin appeared at higher concentrations exclusively in the intestinal phase of cookie enriched with microencapsulated Habanero pepper leaf extract, highlighting the protective role of encapsulation against its rapid degradation in the gastric environment. The molecular structure of quercetin, defined by five hydroxyl groups and a conjugated double bond system with a 4-keto group in the C-ring, provides strong radical-scavenging capacity but also makes it highly sensitive to acidic pH, oxidative conditions, and enzymatic conjugation [65]. These structural features

explain why quercetin is typically unstable in the stomach yet becomes more soluble and bioaccessible once exposed to the neutral to slightly alkaline conditions of the small intestine, where ionization of hydroxyl groups enhances its aqueous dispersibility and interaction with bile salt micelles [66].

Encapsulation in carbohydrate-based matrices delays this transition, limiting premature oxidation and enabling controlled release at the intestinal level [67]. Evidence from simulated digestion studies on fortified cookies has shown that quercetin glycosides resist early losses but may also display "expanded bioaccessibility," where intestinal concentrations exceed initial extract levels due to release from bound forms within the matrix [68]. These findings support that microencapsulation, combined with bakery systems, can effectively modulate quercetin delivery, enhancing its stability and functional contribution in the post-gastric phase.

5. Conclusions

The present study demonstrates that the integration of NADES extraction with spray-drying microencapsulation constitutes a robust and sustainable strategy for the valorization of *Capsicum chinense* leaf by-products as functional food ingredients. While microencapsulation is the primary mechanism responsible for the physical protection and controlled gastrointestinal release of phenolic compounds within the bakery matrix, the use of NADES contributes by enabling the recovery of polyphenol-rich extracts with preserved chemical integrity and high compatibility with food-grade encapsulating systems. Together, these complementary effects support NADES-based extracts as a viable green alternative to conventional organic solvent-derived systems within a circular bioeconomy framework.

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Conflicts of interest

The authors declare no conflicts of interest.

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Appendix

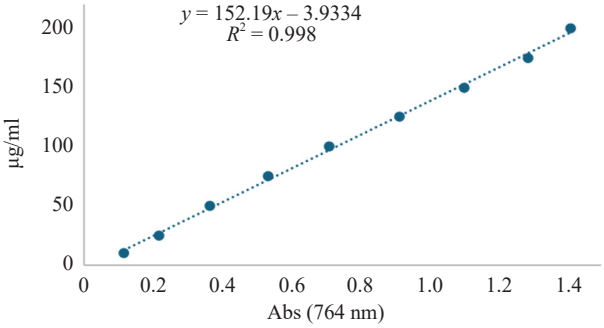

Figure S1. Calibration curve for the determination of total polyphenol content by UV-Vis spectrophotometry. Calibration curve showed a R^2 of 0.9980

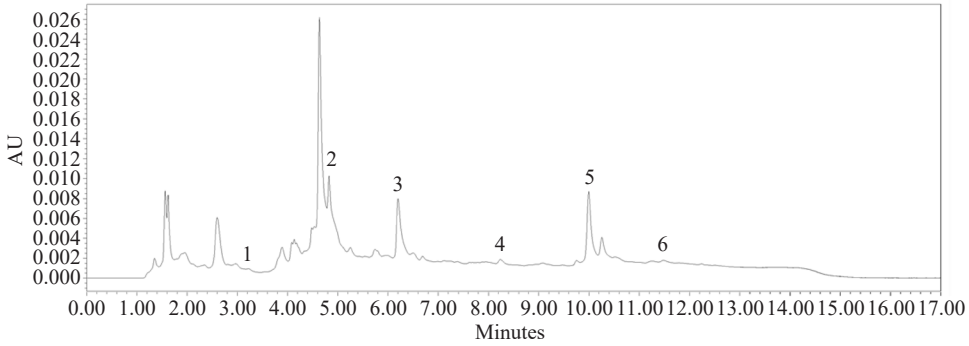

Figure S2. Chromatogram of the enriched cookie sample with microencapsulated habanero pepper extract during the intermediate stage of the intestinal phase of in vitro digestion (EFInT2). 1 = Protocatechuic acid; 2 = Catechin; 3 = Chlorogenic acid; 4 = Cinnamic acid; 5 = Rutin; 6 = Quercetin + Luteolin

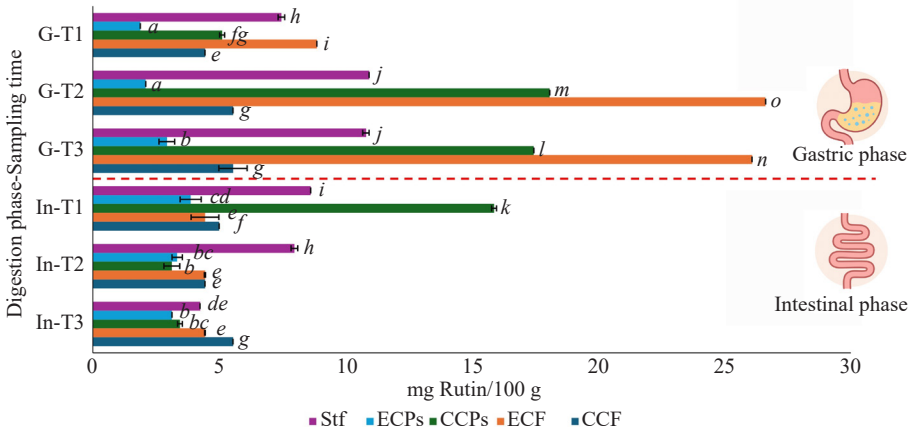

Figure S3. Behavior of rutin during the simulated digestion of the cookie enriched with microencapsulated Habanero pepper leaf extract. G = Gastric, In = Intestinal; T1 = early time, T2 = middle time, and T3 = final time sampling; CC = Control cookie; EC = Enriched cookie, StF = Standard Food; F = fasting, Ps = postprandial condition. Different letters indicate significant differences (LSD, $p < 0.05$, $n = 3$)
Source: Authors' owns elaboration

Table S1. Results of the texture profile analysis of the control cookie and the cookie enriched with microencapsulated habanero pepper leaf after baking

Cookie type	Hardness	Brittleness	Adhesiveness	Elastic module
BEC	73.63 ± 0.82^{b}	0 ± 0.00^{a}	0 ± 0.00^{a}	2.48 ± 0.24^{b}
BCC	62.77 ± 0.01^{a}	7.25 ± 0.74^{b}	0 ± 0.00^{a}	1.75 ± 0.24^{b}

Note: BCC = Baked control cookie; BEC = Baked enriched cookie. Different letters at the same column indicate statistical significance differences (LSD, $p < 0.05$)

Table S2. Results of total polyphenol content and antioxidant capacity from factorial Design $2^3 \times 3$ for the evaluation of the Effect of simluated digestion on a cookie enriched with microencapsulated Habanero pepper leaf

# Exp	Main factors				Variables response	
	Cookie type	Digestion type	Digestive phase	Sampling time	TPC	A_x
1	-1	-1	-1	-1	74.69 ± 4.01^{c}	14.01 ± 1.52^{bcd}
2	-1	-1	-1	0	118.53 ± 4.81^{d}	18.76 ± 0.83^{ef}
3	-1	-1	-1	1	135.25 ± 13.60^{e}	17.66 ± 1.37^{def}
4	-1	-1	1	-1	142.27 ± 3.83^{e}	15.05 ± 0.41^{cde}
5	-1	-1	1	0	236.69 ± 3.87^{q}	20.37 ± 2.11^{f}
6	-1	-1	1	1	239.36 ± 1.82^{q}	16.90 ± 1.43^{def}
7	-1	1	-1	-1	142.47 ± 10.59^{e}	64.67 ± 4.99^{kl}
8	-1	1	-1	0	166.31 ± 6.13^{gh}	65.00 ± 2.97^{kl}
9	-1	1	-1	1	166.31 ± 9.43^{gh}	66.43 ± 1.72^{kl}
10	-1	1	1	-1	173.40 ± 7.03^{ghijk}	82.53 ± 4.68^{p}
11	-1	1	1	0	167.79 ± 11.60^{ghi}	83.19 ± 1.31^{p}
12	-1	1	1	1	176.46 ± 7.74^{hijkl}	81.10 ± 2.88^{p}
13	1	-1	-1	-1	75.31 ± 4.56^{c}	10.73 ± 1.94^{ab}
14	1	-1	-1	0	191.95 ± 8.64^{mn}	9.21 ± 0.78^{a}
15	1	-1	-1	1	208.23 ± 3.15^{op}	10.16 ± 0.78^{ab}
16	1	-1	1	-1	217.50 ± 10.84^{p}	11.49 ± 0.93^{abc}
17	1	-1	1	0	273.64 ± 12.21^{r}	32.86 ± 4.96^{g}
18	1	-1	1	1	270.55 ± 4.51^{r}	38.18 ± 2.86^{h}
19	1	1	-1	-1	153.41 ± 1.76^{f}	67.91 ± 3.12^{lm}
20	1	1	-1	0	164.78 ± 9.67^{g}	70.62 ± 0.79^{mn}
21	1	1	-1	1	177.04 ± 3.29^{ijkl}	70.67 ± 0.99^{mn}
22	1	1	1	-1	171.23 ± 6.27^{ghij}	83.17 ± 1.43^{p}
23	1	1	1	0	184.45 ± 4.75^{lm}	83.98 ± 2.08^{p}
24	1	1	1	1	182.91 ± 5.01^{klm}	84.41 ± 1.45^{p}
25	Stf	-	-1	-1	180.44 ± 2.58^{jkl}	53.79 ± 2.30^{i}
26	Stf	-	-1	0	196.00 ± 6.63^{n}	62.80 ± 1.51^{jk}
27	Stf	-	-1	1	199.52 ± 1.09^{no}	58.85 ± 0.86^{j}
28	Stf	-	1	-1	208.18 ± 1.14^{ij}	73.49 ± 2.31^{no}
29	Stf	-	1	0	210.85 ± 2.37^{p}	76.16 ± 1.52^{o}
30	Stf	-	1	1	210.07 ± 9.01^{op}	73.92 ± 6.04^{no}
25	CCExt	-	-	-	10.41 ± 0.27^{a}	73.92 ± 2.92^{no}
26	ECExt	-	-	-	31.11 ± 1.06^{b}	91.08 ± 0.08^{q}

Note: TPC = Total polyphenol content (mg Gallic acid Equivalent/100g cookie); Ax = Antioxidant capacity (% DPPH Inhibition); Control Cookie = -1; Enriched Cookie = 1; Digestion condition Fasted = -1; Digestion condition Postprandial = 1; Digestive phase Gastric = -1; Digestive phase Intestinal = 1; Sampling time Initial = -1; Sampling time Intermediate = 0, Sampling time Final = 1; Stf = Standard food; CCExt = Control cookie extract (undigested) obtained by ultrasonic bath (20 min, 120 W, 40 kHz); ECExt = Enriched cookie extract (undigested) obtained by ultrasonic bath (20 min, 120 W, 40 kHz); Each treatment in the experimental design was performed in triplicate. Different letters indicate significant differences (LSD, $p < 0.05$)

Table S3. Polyphenol profile results from experimental design $2^3 \times 3$ of an enriched cookie with microencapsulated habanero pepper leaf extract during *in vitro* digestion

Sample	Individual polyphenols (mg/100 g Cookie)							
	PtAc	Ctc	ChAc	CuAc	CiAc	Rt	Q + L	Hpn
CFGT1	5.55 ± 0.00^{c}	6.66 ± 1.11^{gh}	4.44 ± 0.00^{fghi}	ND	ND	4.44 ± 0.00^{e}	21.11 ± 0.01^{ij}	5.00 ± 0.55^{d}
CFGT2	5.55 ± 0.00^{c}	11.11 ± 1.11^{j}	5.55 ± 0.10^{k}	6.66 ± 0.00^{l}	ND	5.55 ± 0.00^{g}	21.10 ± 0.01^{ij}	17.77 ± 0.01^{h}
CFGT3	5.55 ± 0.00^{c}	9.99 ± 0.00^{j}	5.55 ± 0.00^{k}	6.66 ± 0.00^{l}	ND	5.55 ± 0.00^{g}	21.10 ± 0.00^{ij}	18.88 ± 0.00^{i}
CFInT1	6.66 ± 0.00^{d}	10.00 ± 0.00^{j}	5.55 ± 0.00^{k}	ND	ND	5.00 ± 0.56^{f}	21.11 ± 0.01^{ij}	20.55 ± 0.55^{j}
CFInT2	8.89 ± 0.00^{f}	23.32 ± 4.43^{l}	11.66 ± 0.56^{l}	6.66 ± 0.00^{l}	ND	4.44 ± 0.00^{e}	24.44 ± 0.01^{lm}	ND
CFInT3	7.77 ± 0.00^{e}	5.55 ± 0.00^{fgh}	5.00 ± 0.00^{ijk}	6.66 ± 0.00^{l}	ND	5.55 ± 0.00^{g}	23.32 ± 3.33^{kl}	20.54 ± 0.55^{j}
CPsGT1	ND	1.99 ± 0.10^{abc}	3.76 ± 0.55^{cd}	1.88 ± 0.21^{b}	6.37 ± 0.10^{k}	5.12 ± 0.10^{fg}	4.07 ± 0.52^{bc}	0.42 ± 0.00^{ab}
CPsGT2	ND	2.40 ± 0.10^{abcde}	4.18 ± 0.00^{defg}	2.51 ± 0.21^{c}	3.55 ± 0.00^{gh}	18.08 ± 0.31^{m}	8.46 ± 0.31^{g}	0.52 ± 0.10^{ab}
CPsGT3	ND	2.40 ± 0.10^{abcde}	3.87 ± 0.10^{cde}	4.18 ± 0.00^{gh}	2.93 ± 0.00^{def}	17.45 ± 0.10^{l}	8.15 ± 0.42^{fg}	2.19 ± 0.10^{c}
CPsInT1	ND	2.72 ± 0.00^{abcde}	4.18 ± 0.21^{defg}	3.24 ± 0.10^{de}	3.24 ± 0.52^{fg}	15.88 ± 0.00^{k}	5.02 ± 0.00^{cd}	2.61 ± 0.10^{c}
CPsInT2	ND	4.18 ± 0.21^{abcdefg}	4.39 ± 0.00^{efgh}	5.02 ± 0.00^{ijk}	3.87 ± 0.10^{hi}	3.13 ± 0.00^{b}	7.63 ± 0.10^{fg}	2.19 ± 0.10^{c}
CPsInT3	ND	4.60 ± 0.21^{cdefgh}	4.70 ± 0.10^{ghij}	4.49 ± 0.10^{hi}	ND	3.45 ± 0.10^{bc}	6.79 ± 0.31^{efg}	ND
EFGT1	ND	16.64 ± 1.12^{k}	3.33 ± 0.00^{c}	ND	ND	8.87 ± 0.01^{i}	ND	ND
EFGT2	6.66 ± 0.01^{d}	4.44 ± 0.00^{bcdefgh}	2.22 ± 0.00^{b}	6.66 ± 0.01^{l}	ND	26.62 ± 0.02^{o}	19.97 ± 0.02^{hi}	9.43 ± 0.56^{e}
EFGT3	6.66 ± 0.00^{d}	4.44 ± 0.00^{bcdefgh}	2.22 ± 0.00^{b}	6.66 ± 0.00^{l}	ND	26.09 ± 0.55^{n}	23.31 ± 0.01^{kl}	9.44 ± 0.56^{e}
EFInT1	7.76 ± 0.00^{e}	55.45 ± 0.00^{m}	5.55 ± 0.00^{k}	6.65 ± 0.00^{l}	ND	4.44 ± 0.00^{e}	22.18 ± 0.01^{jk}	10.54 ± 0.56^{f}
EFInT2	7.77 ± 0.01^{e}	64.90 ± 0.00^{o}	4.44 ± 0.00^{fghi}	6.66 ± 0.01^{l}	ND	4.44 ± 0.00^{e}	25.51 ± 0.02^{m}	12.20 ± 0.01^{g}
EFInT3	ND	59.94 ± 0.00^{n}	3.33 ± 0.00^{c}	6.66 ± 0.00^{l}	ND	4.44 ± 0.00^{e}	18.87 ± 0.01^{h}	11.10 ± 0.00^{f}
EPsGT1	ND	2.09 ± 0.00^{abcd}	4.07 ± 0.10^{def}	3.13 ± 0.21^{de}	1.88 ± 0.00^{c}	1.88 ± 0.00^{a}	3.13 ± 0.00^{b}	ND
EPsGT2	ND	1.57 ± 0.10^{a}	3.76 ± 0.00^{cd}	3.97 ± 0.00^{fg}	2.72 ± 0.00^{de}	2.09 ± 0.21^{a}	4.60 ± 0.2^{bc}	0.84 ± 0.21^{b}
EPsGT3	ND	1.78 ± 0.10^{a}	3.76 ± 0.00^{cd}	4.18 ± 0.00^{gh}	2.82 ± 0.10^{def}	2.93 ± 0.41^{b}	4.28 ± 0.3^{bc}	ND
EPsInT1	ND	1.57 ± 0.10^{a}	3.76 ± 0.21^{cd}	3.66 ± 0.31^{ef}	4.39 ± 0.63^{j}	3.87 ± 0.31^{cd}	8.15 ± 0.00^{fg}	ND
EPsInT2	ND	9.40 ± 0.00^{ij}	4.39 ± 0.00^{efgh}	3.45 ± 0.31^{e}	6.37 ± 0.10^{k}	3.34 ± 0.00^{bc}	5.22 ± 0.00^{cde}	ND
EPsInT3	ND	6.48 ± 0.42^{gh}	4.70 ± 0.10^{ghij}	2.72 ± 0.00^{cd}	4.18 ± 0.00^{ij}	3.13 ± 0.00^{b}	6.69 ± 0.00^{def}	ND
StFGT1	ND	2.83 ± 0.00^{abcde}	4.25 ± 0.39^{defg}	3.60 ± 0.00^{ef}	3.09 ± 0.26^{efg}	7.46 ± 0.00^{h}	3.86 ± 0.00^{bc}	0.64 ± 0.13^{ab}
StFGT2	ND	4.63 ± 0.00^{defgh}	4.89 ± 0.51^{hij}	5.02 ± 0.13^{ijk}	2.96 ± 0.13^{def}	10.94 ± 0.13^{j}	6.82 ± 0.13^{efg}	ND
StFGT3	ND	4.50 ± 0.13^{bcdefgh}	4.89 ± 0.00^{hij}	4.89 ± 0.00^{ijk}	2.70 ± 0.13^{de}	10.81 ± 0.00^{j}	6.56 ± 0.13^{def}	ND
StFInT1	ND	3.73 ± 0.13^{abcdef}	5.02 ± 0.13^{ijk}	5.15 ± 0.00^{k}	3.09 ± 0.00^{efg}	8.62 ± 0.13^{i}	7.72 ± 0.26^{fg}	ND
StFInT2	ND	4.76 ± 0.13^{efgh}	4.38 ± 0.26^{efgh}	4.50 ± 0.64^{hij}	2.57 ± 0.26^{d}	7.98 ± 0.00^{h}	8.11 ± 0.13^{fg}	ND
StFInT3	ND	4.76 ± 0.13^{efgh}	5.15 ± 0.00^{ijk}	4.38 ± 0.51^{ghi}	0.51 ± 0.00^{b}	4.25 ± 0.13^{de}	4.38 ± 0.00^{bc}	ND
CCExt	1.02 ± 0.00^{a}	1.88 ± 0.04^{ab}	0.13 ± 0.00^{a}	0.73 ± 0.03^{a}	0.06 ± 0.00^{ab}	1.65 ± 0.01^{a}	ND	ND
ECExt	3.86 ± 0.23^{b}	6.99 ± 0.42^{hi}	0.51 ± 0.00^{a}	1.05 ± 0.03^{a}	0.22 ± 0.03^{ab}	1.79 ± 0.06^{a}	1.15 ± 0.00^{a}	ND

Note: CFG = Control cookie under fasting condition at gastric phase; CFIn = Control cookie under fasting condition at intestinal phase; CPsG = Control cookie under postprandial condition at gastric phase; CPsIn = Control cookie under postprandial condition at intestinal phase; EFG = Enriched cookie under fasting condition at gastric phase; EFIn = Enriched cookie under fasting condition at intestinal phase; EPsG = Enriched cookie under postprandial condition at gastric phase; EPsIn = Enriched cookie under postprandial condition at intestinal phase; T1 = Initial sampling time; T2 = Intermediate sampling time; T3 = Final sampling time; StF = Standard food; CCExt = Control cookie extract obtained by ultrasonic bath (20 min, 120 W, 40 KHz); ECExt = Enriched cookie extract obtained by ultrasonic bath (20 min, 120 W, 40 KHz); ND = Not detected; PtAc = Protocatechuic acid; ChAc = chlorogenic acid; CuAc = coumaric acid; CiAc = cinnamic acid; Rt = rutin; Q + L = quercetin + luteolin; Hpn = hesperidin

Table S4. Bioaccessibility of individual polyphenols from the control cookie and the cookie enriched with microencapsulated habanero pepper leaf extract during simulated digestion

Cookie type	Bioaccessibility (%)						
	PtAc	Ctc	ChAc	CuAc	CiAc	Rt	Q + L
CC-F	764.39 ± 2.53^{b}	296.23 ± 6.00^{b}	3,932.58 ± 449.79^{b}	913.16 ± 36.69^{c}	0.00 ± 0.00^{a}	335.99 ± 1.11^{d}	NQ
CC-P	0.00 ± 0.00^{a}	245.05 ± 6.16^{b}	3,699.05 ± 94.73^{b}	615.17 ± 10.37^{b}	0.00 ± 0.00^{a}	208.63 ± 5.62^{b}	NQ
EC-F	0.00 ± 0.00^{a}	860.52 ± 51.98^{c}	651.97 ± 0.70^{a}	633.00 ± 18.72^{b}	0.00 ± 0.00^{a}	248.68 ± 8.61^{c}	1,642.00 ± 0.00^{b}
EC-P	0.00 ± 0.00^{a}	92.63 ± 0.40^{a}	920.54 ± 19.70^{a}	258.11 ± 7.61^{a}	1,909.07 ± 271.18^{b}	175.56 ± 6.13^{a}	581.88 ± 0.48^{a}

Note: CC = Control cookie; EC = Enriched cookie; F = Fasting; P = Posprandial; PtAc = Protocatechuic acid; ChAc = chlorogenic acid; CuAc = coumaric acid; CiAc = cinnamic acid; Rt = rutin; Q + L = quercetin + luteolin; Hpn = hesperidin; NQ = Not quantifiable