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Modified wheat starch for the development of 3D-printed red propolis gels for dysphagic diets: phenolic content and bioactivities during *in vitro* gastrointestinal digestion

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ABSTRACT

This study developed functional foods for dysphagic diets using Brazilian Red Propolis, modified starch, and 3D printing technology. The obtained gels were evaluated in relation to their printability, textures suitable for people with dysphagia, propolis phenolic bioaccessibility and bioactive properties. The gels were formulated using native and Dry Heating Treatment (DHT)-modified wheat starches (2 and 4 h), at 4–10 % concentrations, with propolis (4 %). Lower starch concentrations (≤6 %) led to agglomerates hindering 3D printing, while higher starch concentrations (8–10 %) produced homogeneous, printable gels. Fork test confirmed that DHT_2h 8 %, DHT_2h 10 %, DHT_4h 10 % gels met dysphagic criteria, and these 3D-printed gels were submitted to the *in vitro* digestion. Considering 60 % infill density (which showed the best results), consuming three cubes would deliver 500 mg propolis. *In vitro* gastrointestinal digestion demonstrated that the starch matrix stabilized phenolic compounds, with 10 % starch-propolis gels enhancing bioaccessibility and antioxidant activity. Anti-inflammatory tests revealed that starch alone had no effect, but propolis gels reduced NF-κB activation and pro-inflammatory cytokine release post-digestion, demonstrating anti-inflammatory potential. These highlight the synergy between 3D printing and functional ingredients, offering a promising approach for developing functional foods tailored to meet the specific dietary and health needs of dysphagic populations.

1. Introduction

Propolis is a complex resinous substance produced by bees mainly from leaf buds, flowers, stems, and bark fissures of various tree species [1]. Several biological properties of different types of propolis have been reported, including antioxidant, anti-inflammatory, and anti-proliferative activities [2]. In this context, Brazilian Red Propolis stands out due to its unique chemical composition, particularly rich in phenolic compounds such as isoflavonoids, flavanones, and chalcones [3]. Those active compounds have attracted attention due to their potential functional applications, including the management of inflammatory conditions and increased antioxidant defenses [4]. These benefits can help people with health complications, such as people with dysphagia, considering they often face significant nutritional challenges due to limited food intake [5]. For instance, people undergoing treatment for head and neck cancer may suffer from swallowing problems, chronic inflammation, and oxidative stress [6]. In this context, the safe inclusion of Brazilian Red Propolis in the diet could offer significant benefits due to its biological properties [6].

However, the administration of propolis can be a challenge in some cases.

Dysphagia is characterized by difficulty in swallowing foods and/or beverages. This condition affects a significant portion of the population, particularly the elderly [7], people suffering from neurodegenerative

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diseases [8], or those undergoing radiotherapy as treatment for head and neck cancer [6]. People with dysphagia experience several challenges related to eating, including malnutrition, dehydration, and aspiration pneumonia [9], which can significantly reduce their quality of life, and even threaten their lives. Therefore, to ensure safe eating for people with dysphagia, it is necessary to modify the texture of foods and beverages, ensuring that they are safe and easy to swallow, thus minimizing the risk of choking or aspiration. In this context, the traditional way of propolis intake, as an ethanolic solution, can be a challenge for those people.

Liquid foods, in particular, present a high risk of aspiration if consumed by people with dysphagia, therefore requiring adjustment in texture so that they meet specific criteria, ensuring safe swallowing [10]. Ethanol, although in small quantities, is not desirable in this context as well.

Some strategies have been adopted to achieve a personalized texture for liquid foods. For instance, several hydrocolloids are widely used to enhance the consistency and cohesiveness of different types of foods [11]. In fact, previous studies have investigated the use of various polysaccharides and proteins in combination with 3D printing technology to tailor the texture of liquid foods, making them solids easier to administrate, becoming suitable for people with dysphagia [12–14]. Among these polysaccharides, starch stands out as an excellent option due to its abundance, low cost, and natural origin, making it a widely accessible and sustainable agent. In addition to its gelling and thickening properties, starch can be structurally modified to enhance its functionality, improve printability, stability, and the protection of bioactive compounds, such as phenolic compounds, in 3D-printed gels. These findings demonstrate that incorporating starch in combination with 3D printing technology is a promising approach for developing tailored foods for people with dysphagia.

In this context, this study aimed to develop 3D printed gels to administrate Brazilian Red Propolis, using native and modified wheat starch to tailor texture and enhance suitability for people with dysphagia. The objectives included evaluating the gel-forming ability, the performance of these gels during 3D printing, and their suitability for people with dysphagia. Additionally, the study investigated the bioactive properties of the gels during simulated gastrointestinal digestion, focusing on their phenolic profile, bioaccessibility, and antioxidant and anti-inflammatory activities.

2. Materials and methods

This study is divided into three steps. Firstly, wheat starch was modified by DHT at 130 °C for 2 h or for 4 h to modify their properties. Then, Brazilian Red Propolis gels were developed using native and modified starches as a gelling ingredient, at different concentrations (4, 6, 8, and 10 %), and added with a fixed concentration of propolis (4 %). The second step consisted in the evaluation of the 3D printing performance of the gels carried out with different infill densities (40, 60, 80 and 100 %). In this step, the printed gels were also evaluated for suitability for people with dysphagia. Finally, in the third step, the gels at the concentrations that showed the best performance in 3D printing and were suitable for people with dysphagia were submitted to simulated

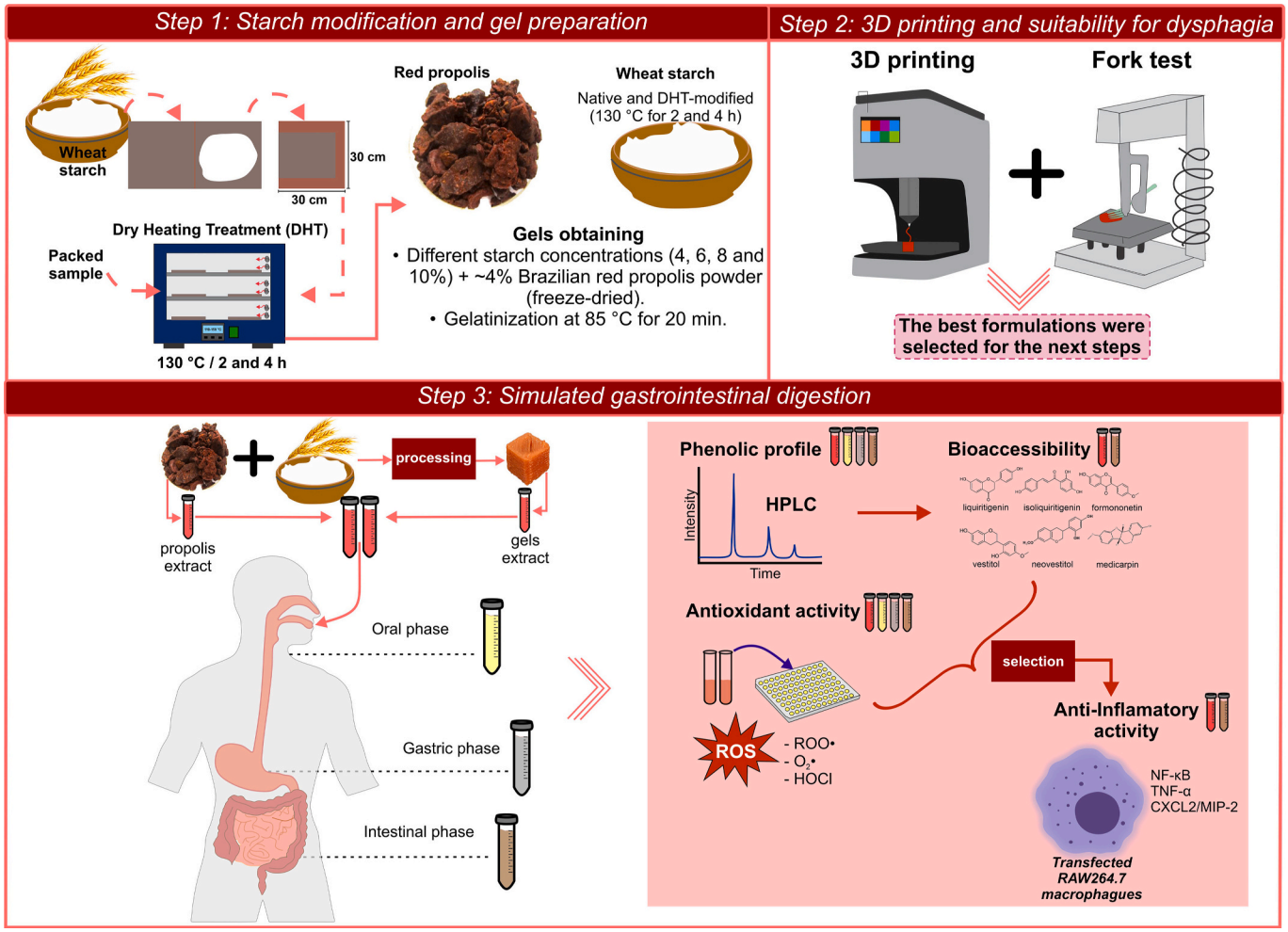

Fig. 1. Illustrated workflow diagram of the experiments carried out.

gastrointestinal digestion according to the INFOGEST 2.0 method. The phenolic profile was evaluated at each stage of the *in vitro* digestion (oral, gastric and intestinal), as well as the phenolics bioaccessibility and scavenging activity against reactive oxygen species (ROS). Based on the results, a selection of samples was made, and the anti-inflammatory activity was assessed on the selected samples. An illustrated workflow diagram is shown in Fig. 1.

2.1. Brazilian Red Propolis extraction

The crude Brazilian Red Propolis was collected in the city of Maceió - Alagoas, Brazil (S 9° 38′ 53.3004″ W 35° 43′ 2.0604″ - legal approval to evaluation from SisGen, Registration n° A305815). Extraction was carried out as described by Alencar et al. [3], with slight modifications. Initially, the material was ground in liquid nitrogen, followed by a mixture in ethanol in the proportion of 0.3:1 (p/v) (crude propolis: ethanol). The mixture was then maintained under gentle stirring at 25 °C for 24 h. Following, the solution was stored in a freezer at −20 °C to separate the beeswax and then filtered through filter paper.

The extract obtained after filtration was rotoevaporated at 50 °C to remove the solvent, then freeze-dried. The propolis powder obtained after freeze-dying was used for gel preparation and subsequent analyses.

To simplify this text, the Brazilian Red Propolis is also called "propolis" throughout the manuscript.

2.2. Starch modification by dry heating treatment (DHT) and gel elaboration

The gels were made with wheat starch (gelling agent) purchased from Merck KGaA (Germany) and the produced propolis powder.

Wheat starch was modified by DHT (the conditions were determined by Maniglia et al. [15] as optimal for 3D printing performance - 130 °C for 2 and 4 h). For this, 50 g of wheat starch was spread in aluminum foil sheets (30 × 30 cm) and sealed to avoid losses. The packages were placed in a convective hot air oven (MA 035, Marconi, 1 ± 0.1 $m{\cdot}s^{-1}$) at 130 °C for 2 and 4 h, resulting in the samples named as DHT_2h and DHT_4h, respectively. Once the heating process was complete, the envelopes with the modified wheat starch were removed from the oven and placed in a desiccator to cool down to ambient temperature. Posteriorly, the samples were then sieved (250 μm/60 mesh) and stored in sealed glass containers until the next procedures. A comprehensive characterization of the obtained starches, including XRD, FTIR, pH, molecular size distribution, DSC, rheology, and firmness, of the granules, paste, and hydrogel, is presented by Maniglia et al. [15].

The hydrogels were prepared in a thermal bath at 85 ± 2 °C for 20 min, under constant agitation (350 rpm) in different concentrations of wheat starch (4, 6, 8 and 10 %) plus 4 % of propolis powder (a discussion about this concentration is provided on Section 3.1). Subsequently, the gels were placed in syringes for 3D printing (60 mL) and stored at 5 °C for 24 h to be used later in 3D printing.

2.3. 3D printing

After storage (5 °C/24 h), the gels were printed using a 3D printer (Wiiboox Sweetin, Naajing Wiiking3D Technology Co. Ltd., China) featuring a 0.84 mm metal nozzle in a controlled ambient temperature at 20 °C. The 3D printing model was designed using Tinkercad software (online version, Autodesk) and slicing was done with Cura (version 15.02.1). A printing speed of 10 $mm{\cdot}s^{-1}$ was set for both nozzle travel and infill [12]. The infill density of the solid cubes (1.5 cm) varied at 100, 80, 60 and 40 %, and the width, length, and height of the final products were determined using a vernier calipers.

2.4. Suitability for people with dysphagia: the fork test

The fork test was performed using an accessory designed to attach the fork to a texturometer (TA.XT Plus, Stable Micro Systems Ltd., Surrey, UK) [16]. This accessory was developed to simulate the analysis of the fork test described by the International Dysphagia Diet Standardization Initiative - IDDSI [17,18], but with standardized analysis conditions in relation to the force applied by the fork, speed, and rest time of the fork in the gels, recording the curve force vs distance covered by the fork on the sample. According to IDDSI [18], a food is suitable for people with dysphagia when it is squashed by a fork to the point where the blanching of the thumbnail occurs and does not return to its original shape once the fork is removed. The force necessary for blanching the thumbnail occurs when the pressure exceeds the mean arterial pressure and is connected with the tongue force used during swallowing [19]. To measure the force needed to blanch the thumbnail (4.8 N) a scale was used, as described by Bitencourt et al. [12].

Briefly, the analysis consisted of penetrating the gels with a fork probe coupled to the texturometer up to 4.8 N of force, remaining unchanged in force for 5 s, followed by returning to the initial position, simulating the IDDSI test procedure. During and after the fork test, data from the texturometer and images of the gels were recorded.

2.5. Simulated gastrointestinal digestion

Simulated gastrointestinal digestion was performed on the gels suitable for people with dysphagia, according to the INFOGEST 2.0 protocol [20] with a schematic representation provided in Fig. 1. As controls and for comparison, the same gels without propolis, and their respective modification controls (native starch gels (8 and 10 % starch solids) with and without propolis) were evaluated. Additionally, ethanolic extracts of propolis (EEP) and propolis heated (EEP-H) at the same concentration and gelatinization conditions of the gels (85 °C for 20 min) were also digested as controls, as well as the enzyme control, using water instead of the sample.

Simulated saliva (SSF), gastric (SGF), and intestinal (SIF) fluids were previously prepared, while the enzymatic solutions were prepared immediately before the experiment. Process began with the oral phase, where the samples were mixed with SSF (1:1 *w*/w) and salivary amylase (75 U/mL), and the pH was adjusted to 7 with NaOH 10 M. Samples were then incubated at 37 °C for 2 min with continuous agitation. The gastric phase started after incubation, mixing the bolus of the oral phase with SGF (1:1 *w*/w) and pepsin (2000 U/mL), and the pH was adjusted to 3 with HCl 6 M. Samples were again incubated at 37 °C for 2 h under constant agitation. After this period, the intestinal phase began, where SIF (1:1 w/w) was mixed with gastric chyme, along with pancreatic lipase (2000 U/mL), bile salts (10 mM) and pancreatin (100 U/mL). The pH was adjusted to 7 using NaOH 10 M. Samples were incubated at 37 °C for 2 h under constant agitation.

From each stage of the simulated gastrointestinal digestion, aliquots were taken for further analysis, which were named as oral fraction (OF), gastric fraction (GF), and intestinal fraction (IF). They were all centrifuged at 10,000 *g* and 4 °C for 45 min and the supernatants collected and immediately frozen at −80 °C for further analysis. The supernatant from the intestinal phase was considered the bioaccessible fraction and used to access phenolics bioaccessibility (Section 2.6.2).

2.6. Phenolic profile by HPLC-PDA and phenolics' bioaccessibility

In the present work, we quantified phenolic compounds from Brazilian Red Propolis in the gels and in the different stages of simulated gastrointestinal digestion process to assess whether the process of texture modifying propolis through the formation of a starchy gel could affect its phenolic compounds and their bioaccessibility.

2.6.1. Extracts production

The EEP, EEP-H, and gels were diluted with ethanol at the same concentrations obtained from the digested ones. The solutions were vortexed for 1 min, sonicated for 20 min, vortexed for another minute,

centrifuged at 10,000 *g* and 4 °C for 15 min, filtered on filter paper, rotaevaporated at 50 °C and freeze-dried. After freeze-drying, samples were resuspended in ethanol and again filtered through PVDF (0.22 μm) and cellulose (0.20 μm) membranes. All samples were at the same concentration (62.5 mg/mL) before being injected in the high-performance liquid chromatography (HPLC).

2.6.2. HPLC-PDA analysis

A HPLC system from Shimadzu (Tokyo, Japan), consisting of a SCL-10AVp controller, LC-6 CE pumps, SPD-M10AVp photodiode array detector (PDA), CTO-ASVp oven, and SIL-10AF auto injector, was employed. The separation was carried out using a C18 Luna column (250 × 4.6 mm; 5 μm, Phenomenex, Torrance, CA, USA). The mobile phase was water:acetic acid (99.5:0.5 % *v*/v) (A) and methanol (99.8 % v/v) (B) at a flow rate of 1 mL/min. The mobile phase gradient started at 30 % (B) and gradually rose to 40 % (B) at 15 min, to 50 % (B) at 30 min, to 60 % (B) at 45 min, to 75 % (B) at 65 and 85 min, and reached 90 % (B) at 95 min, then decreased back to 30 % (B) at 105 min. The run concluded at 120 min. Injection volume was 20 μL. Chromatograms were processed using Class-VP software (Shimadzu, Tokyo, Japan). Some major compounds were identified by comparison with available authentic standards (Sigma–Aldrich, Buchs, Switzerland), which was the case of formononetin, liquiritigenin, and isoliquiritigenin. For them, calibration curves were constructed to calculate their concentrations, expressed as mg/g of crude propolis. Other three major compounds from Brazilian Red Propolis - vestitol, neovestitol and medicarpin – were tentatively identified based on retention time, elution order, and maxima absorption compared to data previously reported by our research group and obtained under the same chromatographic conditions by both HPLC-PDA (same equipment apparatus) and HPLC-ESI-QTOF-MS [3]. These three isoflavonoids were quantified as formononetin equivalents (mg/g crude propolis). Phenolics' bioaccessibility was accessed according to Eq. (1):

$$\textit{Phenolics bioaccessibility}\ (\%) = \frac{\textit{phenolics concentration after gastrointestinal digestion}}{\textit{phenolics concentration before gastrointestinal digestion}} * 100 \quad (1)$$

2.7. Scavenging activity towards reactive oxygen species (ROS)

Antioxidant activity was performed on samples before (extracts), during (OF and GF), and after (IF) the simulated gastrointestinal digestion. All analyses were performed in 96-well microplates and read in a microplate reader (SpectraMax M3, Molecular Devices, CA, USA), following the protocols described by Chisté et al. [21], with slight modifications.

The determination of peroxyl radical scavenging capacity (ROO•) was evaluated by the fluorescence degradation resulting from ROO• induced oxidation of fluorescein, which was expressed as the Oxygen Radical Absorbance Capacity (ORAC). Briefly, 30 μL of the sample was added to a 96-well plate and mixed with 60 μL of fluorescein disodium (508.25 mM) and 110 μL of AAPH (76 mM). The absorbance was measured every minute for 2 h at an excitation wavelength of 485 nm and an emission wavelength of 528 nm, at 37 °C. A calibration curve was constructed with different concentrations of Trolox (μM) and the results expressed in μmol Trolox equivalents per mg of sample (μmol TE/mg) [21].

The superoxide radical ($O_2^{\bullet-}$) scavenging capacity was performed at room temperature by reacting 100 μL of sample at different concentrations, 50 μL of NBT (645 μM), 50 μL of PMS (16.2 μM) and 100 μL of NADH (498 μM). After 5 min in the dark, the absorbance was recorded at 560 nm. A control was prepared by performing the same reaction with the sample being replaced by potassium phosphate buffer (19 mM, pH 7.4) and the blank consisted of buffer only. The results were presented as IC_{50}, which indicates the average amount (μg/mL) of sample required to eliminate 50 % of the superoxide radicals [21].

The evaluation of hypochlorous acid (HOCl) scavenging capacity was carried out by observing the impact of propolis on the HOCl-induced oxidation of DHR to rhodamine 123. Firstly, HOCl (30 μM) was prepared from NaOCl solution (1 %), adjusting the pH to 6.2 using H_2SO_4 (10 %). Afterward, 100 μL of sample at various concentrations was added to 100 μL of phosphate buffer (100 mM, pH 7.4), 50 μL of DHR (7.5 μM) and 50 μL of HOCl (30 μM) in a 96-well plate and fluorescence was recorded immediately after mixing at emission wavelength of 528 nm with excitation at 485 nm using a microplate reader at 37 °C. Control and blank were prepared as previously described for $O_2^{\bullet-}$ scavenging. The analysis was conducted in the dark and the results were then expressed as IC_{50} (μg/mL) [21].

2.8. Anti-inflammatory activity

2.8.1. Cell culture and viability test

The RAW 264.7 murine macrophages, transfected with the NF-κBpLUC gene (NF-κB), purchased from BPS Bioscience, were cultured in DMEM medium with 10 % (*v*/v) fetal bovine serum (FBS), 1 % (v/v) penicillin/streptomycin, and 700 μg/mL of geneticin. All reagents used were purchased from Sigma–Aldrich (Buchs, Switzerland). The cells were cultured in a humidified environment with 5 % CO2 at 37 °C, and the medium was replaced every 48 h until the cells reached exponential growth with 60–80 % confluence. At this point, they were scratched and resuspended in complete DMEM (5 × 10^5 cells/mL).

Cell viability was conducted in EEP, EEP-H and gels before (extracts) and after simulated gastrointestinal digestion (IF), to determine the concentration of the samples non-toxic to the cells by the MTT (3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium) assay. The procedure consisted of seeding cells in 96-well plates, at a density of 2 × 10^4 cells/well, and incubated at 37 °C for 24 h in complete DMEM. After the incubation period, the medium was carefully removed from the plate and the cells were treated with 100 μL of incomplete DMEM with the samples (at 5, 10, 25, 50, and 100 μg/mL). Controls were prepared following the same procedures, without adding samples to the medium. Then, the plate was incubated in an atmosphere with 5 % CO_2 at 37 °C for 4 h. After the incubation time, the samples were carefully removed from the wells and 100 μL of the MTT solution (0.03 mg/mL) were added and, again, the plate was incubated in an atmosphere with 5 % CO_2 at 37 °C for 3 h. After the incubation time, the MTT solution was removed and 100 μL of DMSO was added to each well, then the absorbance was measured in a microplate reader (SpectraMax M3, Molecular Devices, CA, USA) at 570 nm.

Once the non-toxic concentration to cells was defined, the anti-inflammatory assay was performed, as follows.

2.8.2. Activation of NF-κB and release of TNF-α and CXCL2/MIP2

Macrophages (3 × 10^5 cells/well, in a 24-well plate) pre-treated with samples (extracts and IF) in concentrations that were determined by the MTT assay were incubated for 30 min before being stimulated with 100 ng/mL (final concentration) of lipopolysaccharide (LPS) for 4 h. Afterwards, the supernatants were collected and sandwich ELISA (R&D

Systems, Minneapolis, MN, USA) were utilized to access tumor necrosis factor-alpha (TNF-α) and chemokine ligand 2/macrophage inflammatory protein 2 (CXCL2/MIP2). The remaining cells stimulated for 4 h were analyzed for NF-κB activation by luminescence quantification. In brief, the cells were lysed with Tris-NaCl-Tween buffer, and 25 μL of the lysate was added to 50 μL of luciferase (0.5 mg/mL), and luminescence was read in a microplate reader as described elsewhere [22].

2.9. Study design and statistical evaluation

The procedures and analysis were conducted in triplicate using a fully randomized design, with the results expressed as mean values ± standard deviation. The normality of the distributions was assessed by the Ryan-Joiner test and homoscedasticity by the Bartlett test. Analysis of variance (ANOVA) was carried out, with Tukey's test applied at 5 % significance to compare the means. All statistics were performed using Minitab® software (version 19.2, USA).

3. Results and discussion

3.1. 3D printing performance

Gels were produced with different concentrations of native and modified wheat starches (DHT 130 °C for 2 and 4 h) and propolis as active ingredient, to produce 3D printed structures suitable for people with dysphagia. However, the gels prepared with the smallest concentrations of starch (i.e. 4 and 6 %), as well as the one made with DHT_4h with 8 % starch, were not able to form homogeneous gels with propolis. Those gels showed agglomerates of propolis, obstructing the nozzle and made it impossible to perform the 3D printing. Meanwhile, for the other gel formulations (Native 8 and 10 %, DHT_2h 8 and 10 %, and DHT_4h 10 %), propolis was well dispersed in the medium, and the gels were able to be printed and evaluated for their printability and dysphagia diet feasibility (Section 3.2). The results are shown in Fig. 2.

Brazilian Red Propolis is a natural product composed mainly of a complex mixture of resins, waxes, fatty acid class, aromatic acids and flavonoids, among other components [23]. Due to its chemical composition, propolis is not soluble in water and its consumption is normally as an ethanolic solution. However, the consumption of ethanolic substances is not desirable for people with dysphagia, and alternative texturizing options are preferable. In this study, to enable the creation of alcohol-free propolis gels, propolis powder was mixed with water and starch, without a prior dilution in ethanol.

In the gels with lower starch concentrations (4 and 6 %), the amount of starch was not enough to "protect" propolis and disperse it into the hydrogel (Fig. 2). Furthermore, DHT_4h with 8 % starch also formed gels with agglomerated propolis particles, although less than the lower concentrations, while native and DHT_2h starches with 8 % starch formed homogeneous gels without propolis agglomerates. This result is probably related to the molecular and structural changes that starch undergoes during DHT, especially with the more intense duration of 4 h [15] that may affect its ability to encapsulate and disperse propolis particles homogeneously at lower concentrations. In fact, DHT modification can promote rearrangement of crystalline regions, and reorganization of starch chains, which may increase or decrease crystallinity, affect swelling, solubility, and molecular interactions [24]. Maniglia et al. [15] applied DHT to modify wheat starch under the same conditions described in the present study, producing hydrogels only using water. They reported that the DHT led to slight molecular depolymerization and reduced crystallinity, which contributed to improving the hydrogels printability.

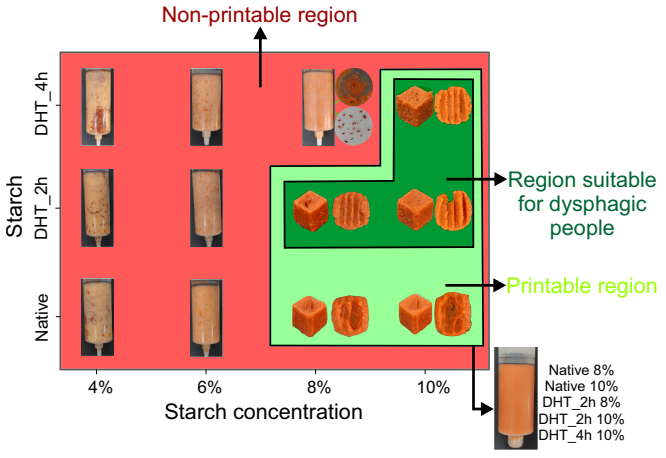

Fig. 2. Diagram of the gels prepared with red propolis and 4, 6, 8 and 10 % of native and modified (DHT 130 °C, 2 and 4 h) wheat starch, showing the non-printable, printable and/or suitable gels for people with dysphagia.

Other starch modification techniques have also been applied to tailor its properties, such as heat-moisture treatment (HMT), which usually reduces the swelling power and cause partial gelatinization [25], or chemical cross-linking, which reinforces internal hydrogen bonding and enhances resistance to enzymatic degradation [26]. However, compared to these techniques, DHT stands out as a simple and clean-label physical method, able to achieve good functionality of the final products [27]. In this study, DHT was selected because it allows starch modification without the use of chemical agents or excess moisture, offering a balance between structural integrity and improved performance in food 3D printing applications.

At higher concentrations of starch (Native 8 and 10 %, DHT_2h 8 and 10 %, and DHT_4h 10 %), the propolis particles were well dispersed in the medium, resulting in homogeneous gels. One possible explanation for this phenomenon lies in the encapsulating properties of starch. When starch is hydrated and gelatinized, it forms a matrix that encapsulates the propolis particles, improving the solubility of propolis in the aqueous phase [28]. Therefore, the starch molecules act as carriers, allowing the propolis to be better dispersed and integrated into the gel.

These results suggest that starch, in addition to acting as a thickening agent, can facilitate the integration of bioactive compounds such as propolis into food matrices that require an efficient and stable dispersion system. Although the complex composition of propolis makes its use in aqueous systems challenging, the use of starch in adequate concentrations enables the development of new products that offer the functionality and benefits of propolis in formats more suitable for individuals with difficulty in swallowing food or with restrictions on alcohol use.

Fig. 3 presents the 3D printing with several infill densities (40, 60, 80, and 100 %) of propolis hydrogels that were able to be 3D printed (Fig. 2, Table 1), using native or modified wheat starch in different concentrations. Additionally, the 3D printability was evaluated regarding the width (Fig. 3a), length (Fig. 3b), height (Fig. 3c), and weight (%) (Fig. 3d) of the 3D printed cubes. It is possible to observe that all gels printed with infill densities of 100, 80, and 60 % were able to form the solid cube, approaching the pre-designed model. However, in gels with an infill density of 40 %, the printed cubes show visible empty spaces inside.

Regarding width and length (Fig. 3a and b), the cubes printed with an infill density of 100 % differed mostly from the pre-designed model (1.5 cm), followed by 80 %, 60 % and 40 %, respectively (being 60 and 40 % the ones most similar to the model). The observed higher values for those dimensions can be a result of Barus effect (swelling of a viscoelastic material when squeezed through an orifice) [29], and therefore, when using a very high infill density, the 3D printed gel ends up having larger dimensions than those pre-designed.

However, even though the width and length of the gels with infill density of 40 % were very similar to the pre-designed solid cube, the 3D printing was visibly impaired by the low infill density, showing hollow cubes, for all starches. On the other hand, solid cubes printed with 60 % infill density were clearly similar to the pre-designed model, with no

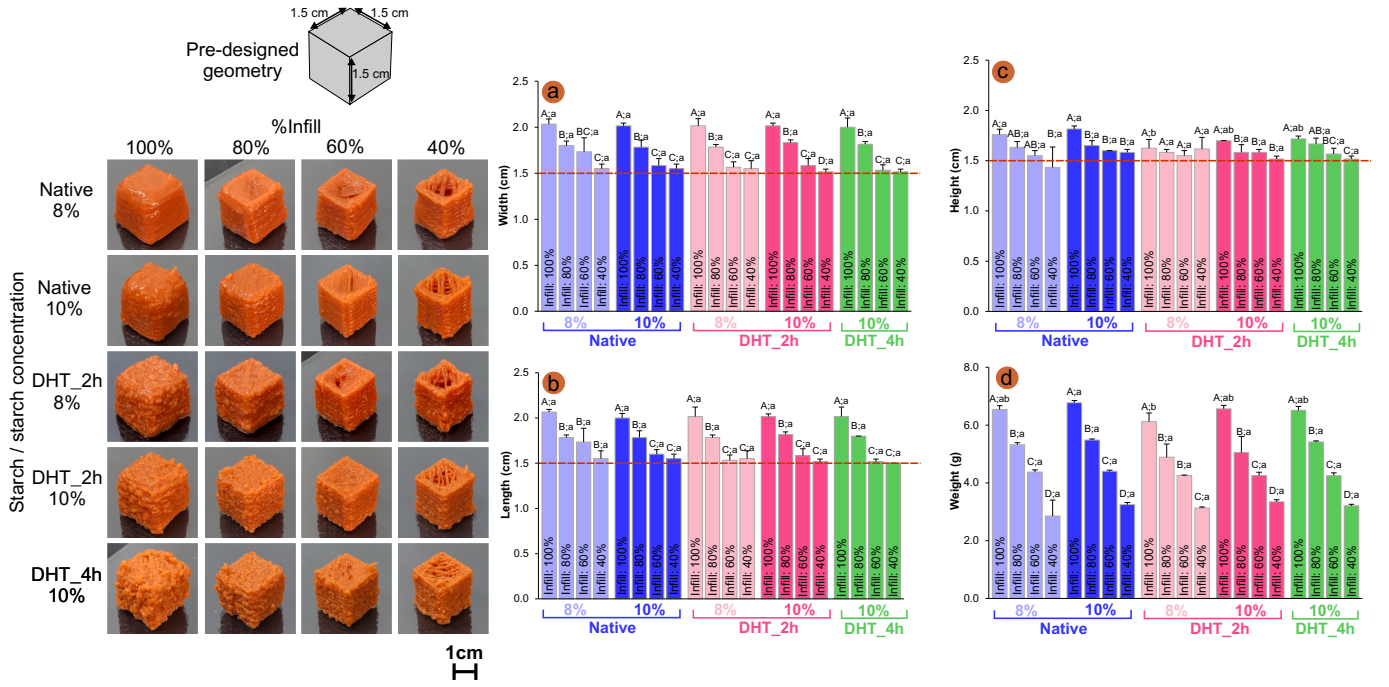

Fig. 3. 3D printed solid cubes with different infill densities (100, 80, 60, and 40 %) of gels prepared with red propolis and 4, 6, 8, and 10 % of native and modified (DHT 130 °C, 2 and 4 h) wheat starches, where (a) indicate width (cm), (b) indicate length (cm), (c) indicate height (cm), (d) indicate weight (g). The red line indicates the target value of the pre-designed geometry. Statistical analysis by Tukey's test ($p < 0.05$): Different uppercase letters indicate significant differences within the same sample but at different infill densities. Different lowercase letters indicate significant differences between native and modified starch at the same infill density.

appearance of hollow spaces for gels prepared, especially with 10 % of starch content. Furthermore, there was no significant difference regarding the length and width of the gels prepared with different starches (native or modified).

The same behavior described for width and length was observed for height, where when the infill density was 100 %, the height was larger and more significantly different from the pre-designed cube, while 80, 60 and 40 % approached more of the theoretical value (1.5 cm) showing similarity ($p > 0.05$) between them (Fig. 3c).

The weight of the solid cubes was proportional to the infill density, i.e. as the infill density increased, the weight also increased. On the other hand, there was no significant difference in the weight of gels prepared with different starches (native or modified) (Fig. 3d). Considering the amount of propolis added for gel preparation (4 %), to reach the recommended daily intake of 500 mg of propolis [30], consumption would need to be adjusted according to the infill density: for gels with 100 % infill density, two cubes per day would be required; with 80 % infill, two cubes per day; with 60 % infill, three cubes per day; and with 40 % density, four cubes per day.

Among the gels developed in this study, different 3D printing infill densities (100, 80, 60, and 40 %) were evaluated. Cubes printed with 60 and 40 % infill densities showed the closest dimensions to the pre-designed model, indicating good 3D printing fidelity. However, cubes printed with 40 % infill density exhibited structural weaknesses and visible internal voids, making them unsuitable for practical use. Based on these results, 60 % infill density was selected as the best parameter, balancing print fidelity and structural integrity.

Furthermore, it remains essential to determine whether these formulations are appropriate for people with dysphagia. Additionally, we also evaluated the performance of the propolis gels in fork tests for all the formulations that were printable (Fig. 2 and Table 1), and considering the different 3D printing parameters (infill densities of 100, 80, 60 and 40 %), as described in the following section.

3.2. Suitability for dysphagic diets: the fork test

The DHT modification changed the behavior of the gels on the fork test. It is possible to observe in Fig. 4a that the gels prepared with 8 and 10 % of native starch tend to return to the initial shape after removing the fork from it, not being classified as suitable for consumption by people with dysphagia, according to IDDSI (Table 1) - [17,18].

On the other hand, when the starch was modified by DHT 2 or 4 h, the gels did not tend to return to their original shape after being completely penetrated by the fork when applying a force equivalent to the blanching of the thumbnail (see description on Section 2.4), with the marks of the fork tines being clearly observed on the surface of the gels (Fig. 4a).

Fig. 4b illustrates the energy required to penetrate the gel - all samples were fully penetrated by the fork. In general, the higher the infill density, the greater the energy required to penetrate the gel. This could be due to the greater quantity of product deposited during printing (i.e. greater weight) or due to the formation of a denser structure while layer-by-layer are being deposited [31]. In addition, gels prepared with 10 % starch required greater force for penetration by the fork than those with 8 % starch, but there was no significant difference ($p > 0.05$) between native or modified starch at the same starch concentration.

From the obtained results of 3D printing and suitability for people with dysphagia, it is possible observe that only DHT-modified starches showed both good 3D printing performance and texture suitability for people with dysphagia (Table 1), i.e. DHT_2h 8 and 10 %, and DHT_4h 10 %. Therefore, the 3D printed treatments DHT_2h 8 and 10 %, and DHT_4h 10 % at 60 % infill density were digested through the protocol INFOGEST 2.0 and evaluated for phenolic content before, during, and after the simulated gastrointestinal digestion, as well as the phenolic bioaccessibility and antioxidant and anti-inflammatory activities. In addition, gels with Native 8 % and Native 10 % starches were also evaluated as controls.

Table 1
Parameters analyzed for 3D printing and fork test on gels in 3D printed solid cubes with different infill densities (100, 80, 60, and 40 %) of gels prepared with red propolis 8 and 10 % of native and modified (DHT 130 °C, 2 and 4 h) wheat starches. Symbols: ✓ = yes; ✗ = no.

			3D printing performance					Fork test		Is the treatment suitable for dysphagia and had good 3D printing performance? (selected for further analysis)
Starch	Gelling solids centration	Infill percentage	Is it printable, and similar to the designed?	Is propolis well dispersed?	Continuous lines and smoothly extruded?	Retains its shape after printing?	The printed dimensions agree with the projected?	Squashed by the fork	Tends to return to original form	
What is desirable?			✓	✓	✓	✓	✓	✓	✗	
Native	4%	-	✗	✗		Non-printable		Non-printable		✗
	6%	-	✗	✗		Non-printable		Non-printable		✗
	8%	100%	✓	✓	✓	✓	✗	✓	✓	✗
		80%	✓	✓	✓	✓	✗	✓	✓	✗
		60%	✓	✓	✓	✓	✓	✓	✓	✗ (Control)
		40%	✗	✓	✓	✓	✓	✓	✓	✗
	10%	100%	✓	✓	✓	✓	✗	✓	✓	✗
		80%	✓	✓	✓	✓	✗	✓	✓	✗
		60%	✓	✓	✓	✓	✓	✓	✓	✗ (Control)
		40%	✗	✓	✓	✓	✓	✓	✓	✗
DHT_2h	4%	-	✗	✗		Non-printable		Non-printable		✗
	6%	-	✗	✗		Non-printable		Non-printable		✗
	8%	100%	✓	✓	✓	✓	✗	✓	✗	✗
		80%	✓	✓	✓	✓	✗	✓	✗	✗
		60%	✓	✓	✓	✓	✓	✓	✗	✓
		40%	✗	✓	✓	✓	✓	✓	✗	✗
	10%	100%	✓	✓	✓	✓	✗	✓	✗	✗
		80%	✓	✓	✓	✓	✗	✓	✗	✗
		60%	✓	✓	✓	✓	✓	✓	✗	✓
		40%	✗	✓	✓	✓	✓	✓	✗	✗
DHT_4h	4%	-	✗	✗		Non-printable		Non-printable		✗
	6%	-	✗	✗		Non-printable		Non-printable		✗
	8%	-	✗	✗		Non-printable		Non-printable		✗
	10%	100%	✓	✓	✓	✓	✗	✓	✗	✗
		80%	✓	✓	✓	✓	✗	✓	✗	✗
		60%	✓	✓	✓	✓	✓	✓	✗	✓
		40%	✗	✓	✓	✓	✓	✓	✗	✗

3.3. Phenolic profile and bioaccessibility during the simulated gastrointestinal digestion

Red propolis has a complex chemical composition that can vary depending on factors such as the biodiversity and phytogeographic composition of the hives, with >300 compounds [4,23]. Brazilian Red Propolis, in particular, is rich mainly in isoflavonoids that give it a series of biological properties, such as antibacterial, antifungal, anti-caries, anti-inflammatory, antioxidant, and antiproliferative properties [4], and for this reason it has attracted attention and been used for different applications [32].

In fact, flavonoids and isoflavonoids are recognized for their active properties that may be beneficial for patients with dysphagia. A recent study evaluated the topical use of propolis in patients undergoing treatment for head and neck cancer who suffer from dysphagia often caused by acute oral toxicities, such as mucositis, oral candidiasis, and exacerbated inflammation [6]. Promising results were observed, where the group that used propolis presented lower rates of dysphagia, dysgeusia, and oral mucositis, in addition to reduced levels of interleukin-1β (IL-1β) and tumor necrosis factor alpha (TNF-α), indicators of inflammation. These results were associated with the active properties of propolis, which can be applied in functional formulations for the prevention and management of symptoms associated with dysphagia [6]. Fig. 5 presents the quantification by HPLC-PDA of the active compounds of EEP, EEP-H and 3D printed propolis gels in the extracts (initial quantity - before digestion) and in all phases of the gastrointestinal digestion (OF, GF and IF).

Six active compounds were identified, classified as isoflavonoids (formononetin, vestitol, neovestitol, and medicarpin), flavanones (liquiritigenin), and chalcones (isoliquiritigenin). Overall, all extracts before digestion presented similar amounts of the phenolic compounds evaluated (except for formononetin), demonstrating that the processing methods applied to produce the gels did not affect the initial amounts of most compounds.

On the other hand, temperature seemed to negatively affect formononetin level, in which all gels and heated propolis (EEP-H) presented lower initial levels compared to the propolis extract without any type of processing (EEP). This result suggests that formononetin is particularly sensitive to the heating conditions used to prepare the gels (i.e. 80 °C for 20 min). Indeed, thermal degradation or chemical transformation are common phenomena for certain phenolic compounds [33].

Furthermore, for the gels, the complexation of propolis with starch apparently partially prevented the release of phenolic compounds in the first phases of digestion (oral and gastric phases), including for formononetin and medicarpin, no amount of these compounds was detected in these two fractions (OF and GF). Although smaller amounts of phenolic compounds were found in OF and GF, this does not necessarily mean that the compounds were preserved or would be fully released in IF. In fact, there was a significant decrease in the content of phenolic compounds in the intestinal phase for all treatments tested (propolis or gel) compared with the initial amount (extracts – before digestion). This behavior is commonly reported in the literature and can be explained by the structural changes that the phenolic compounds may have undergo during digestion, mainly due to changes in pH [34], reflecting in the bioaccessibility of these compounds.

Fig. 6 presents the bioaccessibility of the bioactive compounds evaluated for EEP, EEP-H, and all 3D printed propolis gels. Bioaccessibility refers to the compounds present in the intestinal fraction after gastrointestinal digestion, which can be absorbed and utilized by

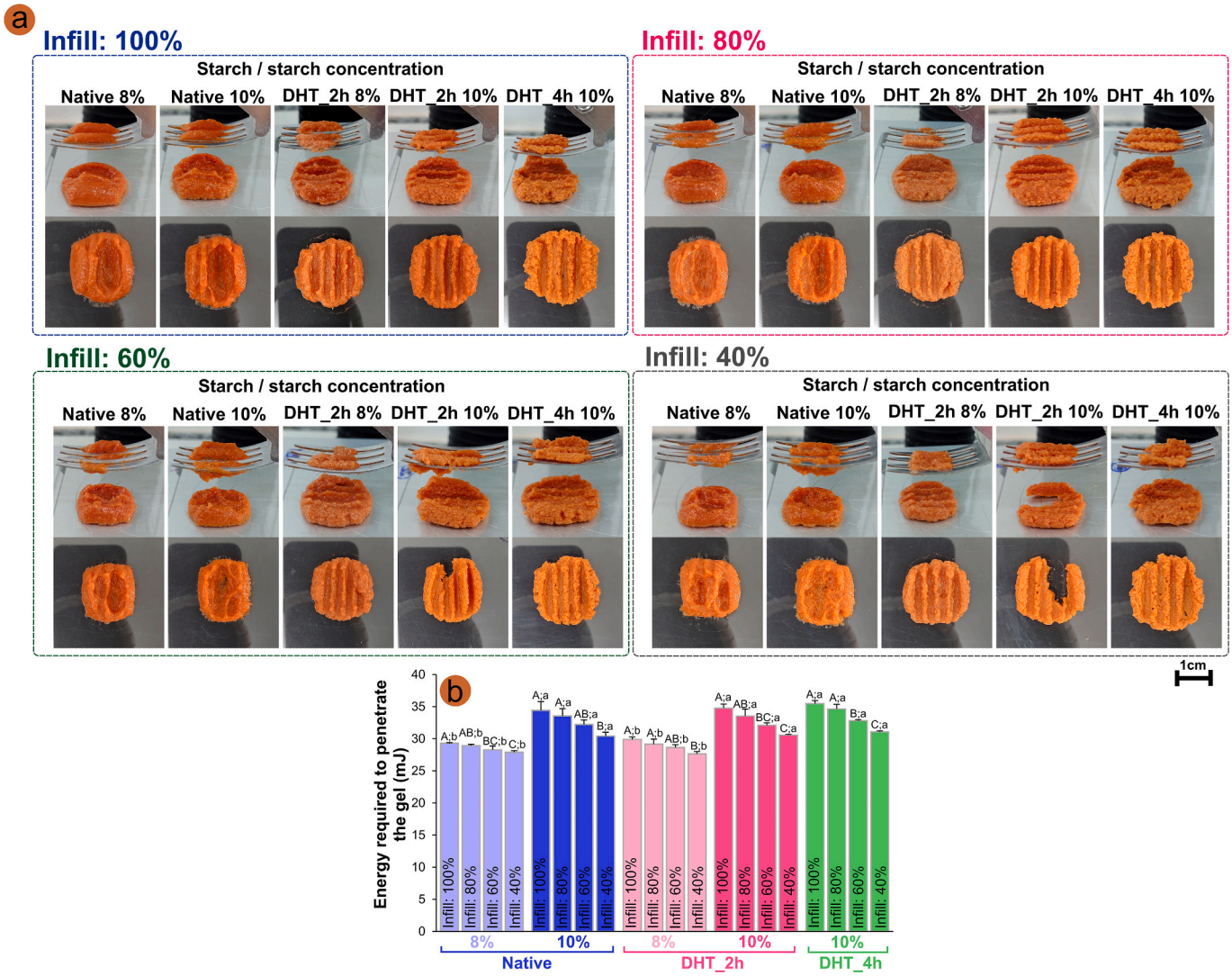

Fig. 4. (a) Fork test, and (b) Energy required to penetrate the sample (mJ) in 3D printed solid cubes with different infill densities (100, 80, 60, and 40 %) of gels prepared with red propolis 8 and 10 % of native and modified (DHT 130 °C, 2 and 4 h) wheat starches. Statistical analysis by Tukey's test ($p < 0.05$): Statistical analysis by Tukey's test ($p < 0.05$): Different uppercase letters indicate significant differences within the same sample but at different infill densities. Different lowercase letters indicate significant differences between native and modified starch at the same infill density.

the human body.

Interestingly, the results showed that for formononetin and medicarpin, the bioaccessibility in the gels was similar to that observed for EEP and EEP-H. This behavior can be attributed to a possible protection of propolis by the starch matrix during gastrointestinal digestion. On the other hand, for the other phenolic compounds evaluated, the bioaccessibility was lower in the gels compared to EEP and EEP-H, suggesting that encapsulation within the starch matrix may have negatively influenced their release or stability during the gastrointestinal digestion.

Another relevant point to highlight is that, for liquiritigenin, the 3D printed propolis gels prepared with a higher starch concentration (10 %) exhibited greater bioaccessibility compared to gels with a lower starch concentration (8 %). A possible explanation for this effect lies in starch–phenolic interactions, which occur mainly through non-covalent bonds such as hydrogen bonding and hydrophobic forces [35]. These interactions typically involve the hydroxyl groups of starch chains and the hydroxyl or aromatic groups of phenolic compounds [35]. At higher starch concentrations, the gel matrix becomes more compact, which may restrict the diffusion and enzymatic degradation of phenolic compounds during gastrointestinal digestion, thereby favoring their release in the intestinal phase. In fact, Karim, et al. [36] reported that increased polysaccharide-induced consistency can reduce compound exposure to enzymatic activity and pH changes throughout the digestive process, leading to enhanced protection against degradation. These findings support the hypothesis that the higher density of the starch matrix may offer more effective protection for the compound during gastrointestinal digestion, resulting in increased bioaccessibility in the intestinal fraction. Consequently, it is important to study gelling ingredients for 3D printing of foods for special needs, not only to ensure adequate printability and texture (original objectives of this research), but also protect some bioactive compounds and to increase the product functionality.

It is also important to describe the limitations of the *in vitro* gastrointestinal digestion model used in this study. Although this standardized method allows the evaluation of different phases of the gastrointestinal digestion (oral, gastric, and intestinal), it is a static model and cannot replicate the complex dynamics of digestion or the physiological interactions with the host [20]. Therefore, the bioaccessibility values reported here represent an estimate of the compounds potentially available for absorption, rather than actual bioavailability. Future studies using intestinal cell models or in vivo systems are encouraged to validate and expand upon these findings.

Still, it is essential to evaluate whether the differences in phenolic

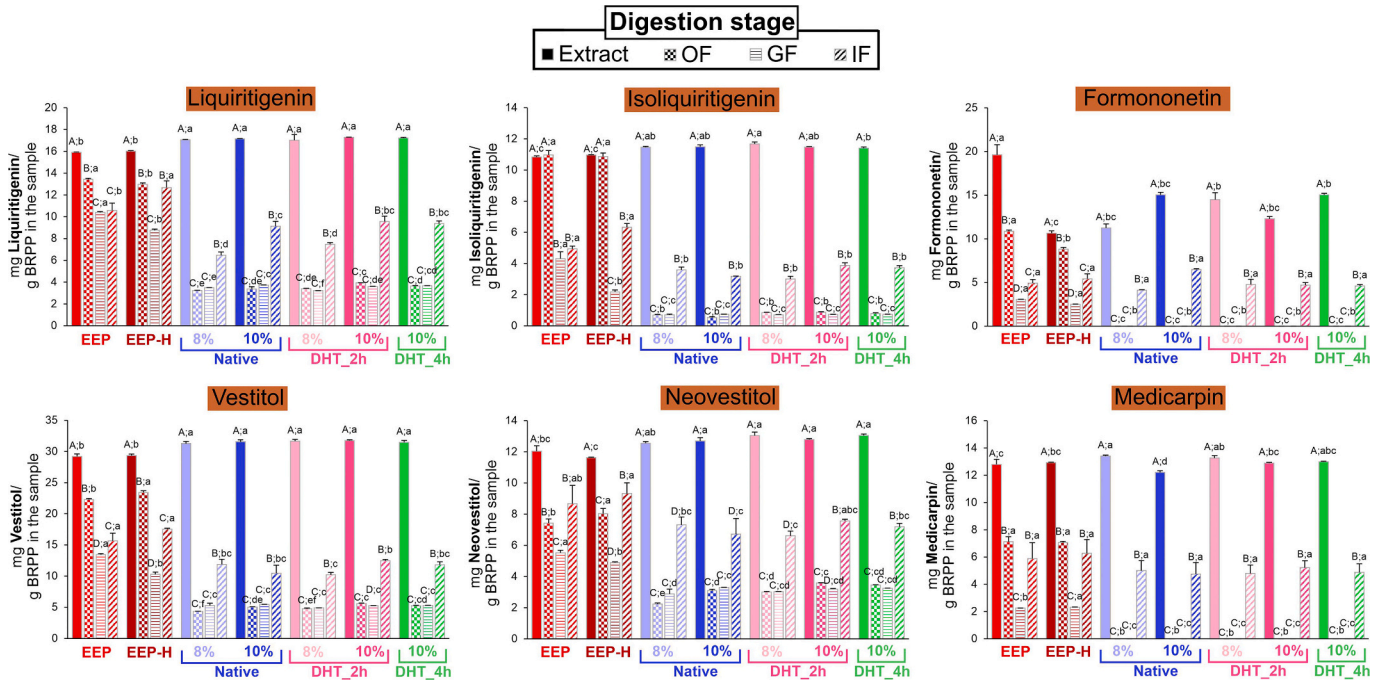

Fig. 5. HPLC-PDA quantification of phenolic compounds in the extracts (before the gastrointestinal digestion), and during the simulated gastrointestinal digestion: oral fraction (OF), gastric fraction (GF), and intestinal fraction (IF) of 3D printed propolis gels (infill density: 60 %) with 8 and 10 % of native and modified (DHT 130 °C, 2 and 4 h) wheat starches. Statistical analysis by Tukey's test ($p < 0.05$): Different uppercase letters indicate significant differences within the same sample but at different digestion stages (extract, OF, GF, and IF). Different lowercase letters indicate significant differences between samples (EEP, EEP-H, Native 8 % and 10 %, DHT_2h 8 % and 10 %, or DHT_4h 10 %) at the same digestion stage.

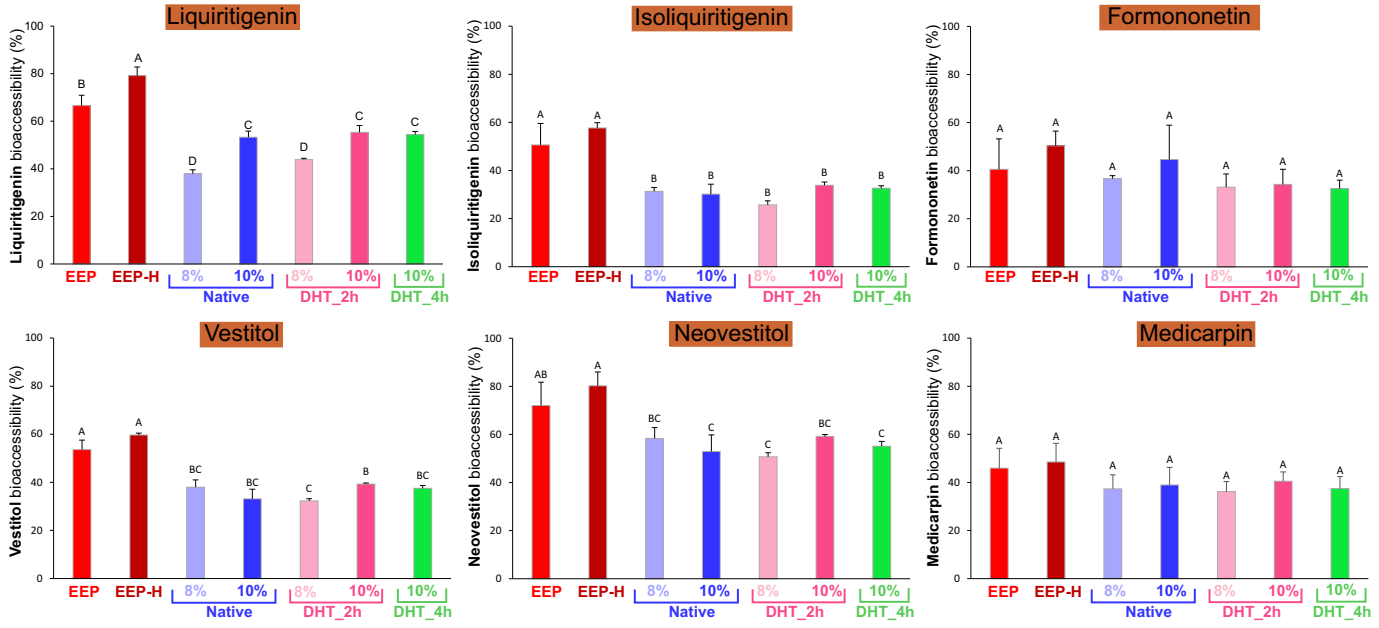

Fig. 6. Bioaccessibility of phenolic compounds in EEP, EEP-H, and 3D printed propolis gels (infill density: 60 %) with 8 and 10 % of native and modified (DHT 130 °C, 2 and 4 h) wheat starches. Statistical analysis by Tukey's test ($p < 0.05$): Different uppercase letters indicate a significant difference ($p < 0.05$) among the formulations.

compound content after gastrointestinal digestion influenced the bioactive properties of the gels, such as their antioxidant and anti-inflammatory activities.

3.4. Scavenging activity towards reactive oxygen species (ROS)

Dysphagia can lead to antioxidant nutrient deficiency due to difficulty in food consumption [37]. Furthermore, studies have indicated that lack of consumption of antioxidant foods may be related to the development of neurodegenerative diseases, such as Alzheimer's disease [38]. In addition, patients undergoing cancer treatment are often exposed to radiation (curative radiotherapy), which can induce excessive production of ROS [39]. Particularly in head and neck cancer treatment, patients may also develop dysphagia as a side effect of

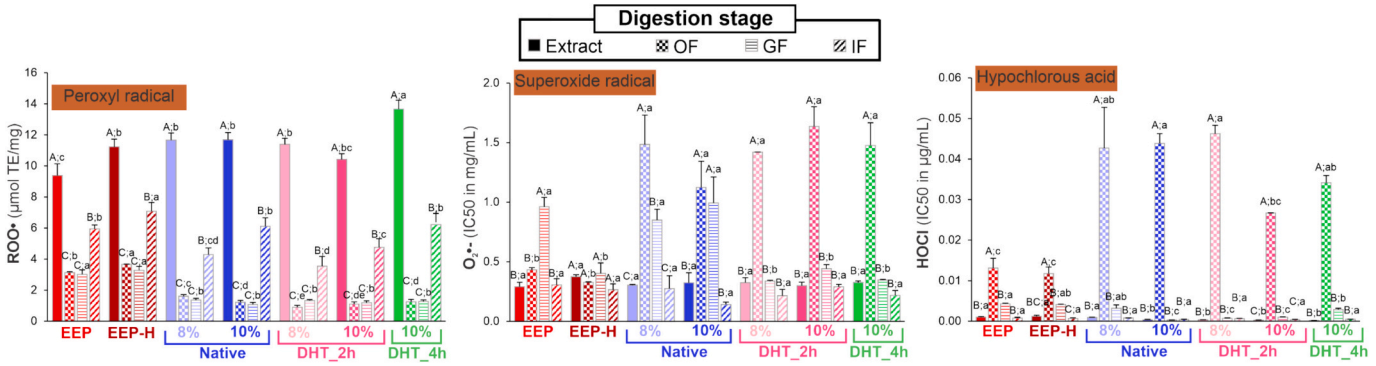

Fig. 7. Reactive oxygen species scavenging in the extracts (before the gastrointestinal digestion), and during the simulated gastrointestinal digestion: oral fraction (OF), gastric fraction (GF), and intestinal fraction (IF) of 3D printed propolis gels (infill density: 60 %) with 8 and 10 % of native and modified (DHT 130 °C, 2 and 4 h) wheat starches. Statistical analysis by Tukey's test ($p < 0.05$): Different uppercase letters indicate significant differences within the same sample but at different digestion stages (extract, OF, GF, and IF). Different lowercase letters indicate significant differences between samples (EEP, EEP-H, Native 8 % and 10 %, DHT_2h 8 % and 10 %, or DHT_4h 10 %) at the same digestion stage.

therapy [39], which makes the development of foods with adequate texture and active properties even more relevant.

In Brazilian Red Propolis, flavonoids are the main antioxidant compounds, which exhibit antioxidant activity against ROS by donating an electron from a hydroxyl group linked to their B ring [40]. In this study, we evaluated the power of the 3D printed propolis gels in the scavenging activity of three ROS: peroxyl ($ROO^{\bullet}$) and superoxide ($O_2^{\bullet-}$) radicals, and hypochlorous acid (HOCl) (Fig. 7).

All extracts (before digestion) of propolis (with or without heating) and 3D printed propolis gels showed higher $ROO^{\bullet}$ scavenging activity compared to the fractions analyzed during the different phases of gastrointestinal digestion. The activity was lower in the oral (OF) and gastric (GF) phases and increased again in the intestinal phase (IF), likely due to the greater release of phenolic compounds in this phase. The reduction in $ROO^{\bullet}$ scavenging activity observed after gastrointestinal digestion is probably related to the degradation of the phenolic compounds of propolis during the gastrointestinal digestion (Fig. 5), as flavonoids play a key role in $ROO^{\bullet}$ scavenging capacity. In fact, during the gastrointestinal digestion, the phenolic compounds can interact with enzymes and/or undergo structural changes, or even be degraded by prolonged exposure to low pH, therefore reducing their bioactivity [41].

Additionally, the 3D printed propolis gels with higher starch concentration (10 %) exhibited greater activity compared to their respective gels with only 8 % starch, demonstrating again the protective action of starch on the active compounds of propolis. This result suggests that a higher concentration of starch results in greater encapsulation and protection of propolis compounds, highlighting the importance of optimizing the proportions between starch and bioactive compounds to maximize bioactivity.

For $O_2^{\bullet-}$ and HOCl scavenging activities, the oral and gastric fractions (OF and GF) generally showed reduced activity, with significantly higher IC_{50} values ($p > 0.05$). Furthermore, there was no significant difference ($p < 0.05$) between the extracts and the intestinal fraction (IF) for all treatments. This indicates that, although some compounds were

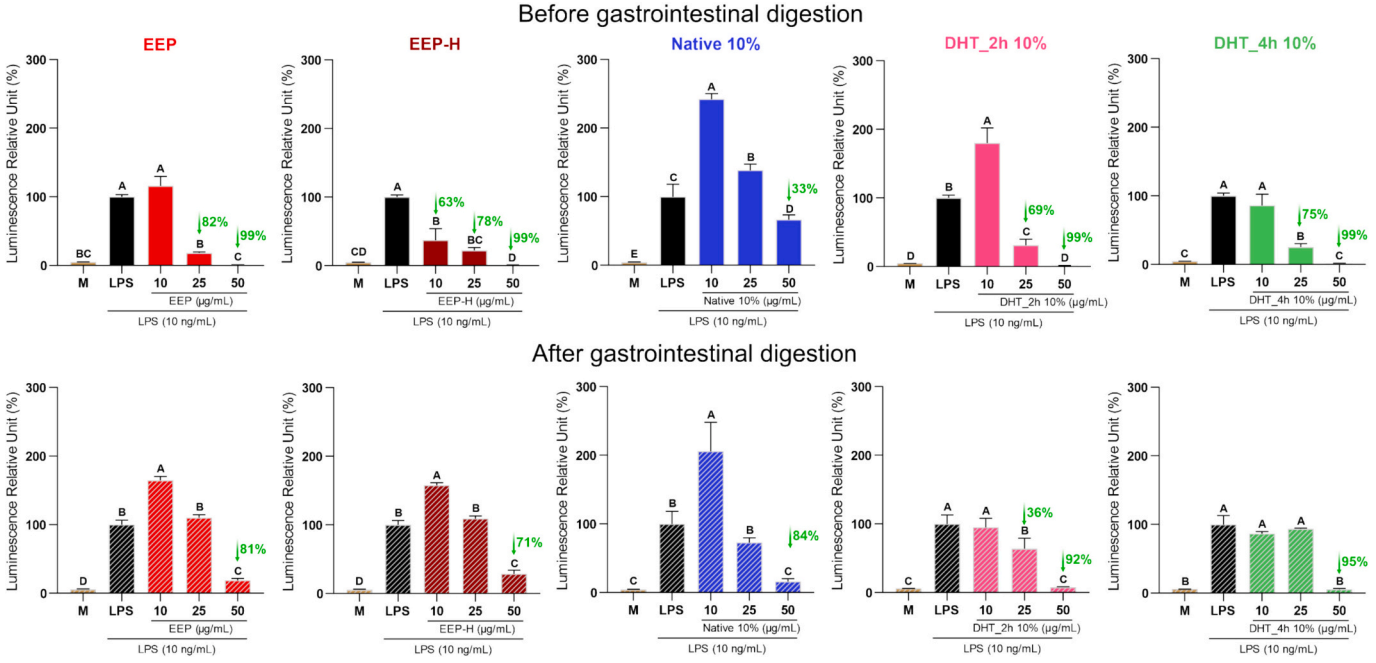

Fig. 8. Activation of NF-κB by LPS in RAW 264.7 macrophages incubated with extracts (before gastrointestinal digestion) and intestinal fraction (after gastrointestinal digestion) of 3D-printed propolis gels (infill density: 60 %) with 10 % native and modified (DHT 130 °C, 2 and 4 h) wheat starches. Statistical analysis by Tukey's test ($p < 0.05$): Different uppercase letters within each graphic indicate significant difference among the tested concentrations. M: negative control, representing only macrophages. LPS: positive control, representing only LPS-induced macrophages.

degraded during gastrointestinal digestion (Fig. 5), the scavenging activity of important ROS in the intestinal phase (after digestion) was not impaired – an interesting result.

Finally, the results of the phenolic profile, bioaccessibility, and ROS scavenging activity indicated that 3D printed propolis gels prepared with higher concentrations of starch tended to preserve more of the compounds of interest in propolis, as well as its active properties. For this reason, the analysis of anti-inflammatory activity (next section) was performed only on gels with 10 % starch (i.e. Native 10 %, DHT_2h 10 %, and DHT_4h 10 %). The propolis extracts without (EEP) and with (EEP-H) heating were also evaluated to separate the effect of composition and processing. Furthermore, the anti-inflammatory activity was assessed only in samples before digestion (extracts) and after digestion (IF), as these stages are the most representative and relevant in the process. It is important to note that it is in the intestine where macrophages play a crucial role in modulating local inflammation.

3.5. Anti-inflammatory activity

Inflammation is a protective body response to infections and foreign molecules, but it becomes detrimental when persistent and prolonged [42,43]. Inflammation is a key factor that increases the risk of disease and death in the elderly [42]. Particularly in people with dysphagia (mostly elderly), chronic inflammation can lead to a worsening of the general health status and impair the recovery of swallowing function [44], further aggravating their difficulties. Therefore, it is essential to develop foods that meet not only specific textural requirements, but also offer functional properties, such as anti-inflammatory activity.

Hence, we evaluated the anti-inflammatory properties against NF-kB transcription factor pathway, TNF-α cytokine, and MIP-2 chemokine before and after gastrointestinal digestion of red propolis gels produced with native and modified wheat starch, in addition to the control treatments – ethanolic extract of propolis without and with heating treatment under the same preparation conditions as the gels (i.e. 85 °C/20 min), and the gels made only with starch (without propolis). Initially, the viability of RAW 264.7 macrophages treated with different concentrations of the samples was evaluated, as shown in Fig. S1. The cells treated with the control samples and propolis gels, before and after gastrointestinal digestion, presented cell viability above 80 % up to the concentration of 50 μg/mL, which was considered the maximum concentration for the anti-inflammatory activity assay.

NF-κB transcription factor pathway activation plays a key role in regulating inflammation by controlling the expression of pro-inflammatory genes [45]. In this study, heating (EEP-H) appears to have increased the inhibition capacity of LPS-induced NF-κB pathway activation, compared to unheated propolis extract (EEP) for samples before gastrointestinal digestion (Fig. 8). Actually, all concentrations tested (10, 25, and 50 μg/mL) for EEP-H showed inhibition of NF-κB activation, while only 25 and 50 μg/mL showed the same effect for EEP. On the other hand, after gastrointestinal digestion, the concentrations 10 and 25 μg/mL of both control samples (EEP and EEP-H) did not show any effect in the activation of NF-κB pathway, only at the concentration of 50 μg/mL, with a decrease of ~81 and 71 % in the Luminescence Relative Unit (%) for EEP and EEP-H, respectively.

Besides, we studied the effects of gels produced with native and modified starches with or without propolis before and after gastrointestinal digestion on anti-inflammatory parameters. Gels containing only starch (without propolis) were evaluated as controls, to determine the isolated effects of starch on anti-inflammatory activity (Fig. S2, S3, and S4). For all parameters evaluated (i.e. NF-κB activation, TNF- α, and CXCL2/MIP-2 release), no significant anti-inflammatory activity was observed for any of these controls accessed, before or after gastrointestinal digestion (Fig. S2, S3, and S4). This indicates that the structural and molecular modifications of starch during the DHT-modification do not trigger an inflammatory response.

Regarding propolis gels before gastrointestinal digestion (Fig. 8), an increase in NF-κB activation was observed in relation to LPS-treated cells in some concentrations of gels prepared with native starch (10 and 25 μg/mL) and DHT_2h (10 μg/mL). In fact, Romier et al. [46] reported that polyphenols can modulate the NF-κB activation pathway by reducing or increasing the activation caused by inflammatory stimuli, such as LPS. Furthermore, it was reported that this effect may vary depending on the

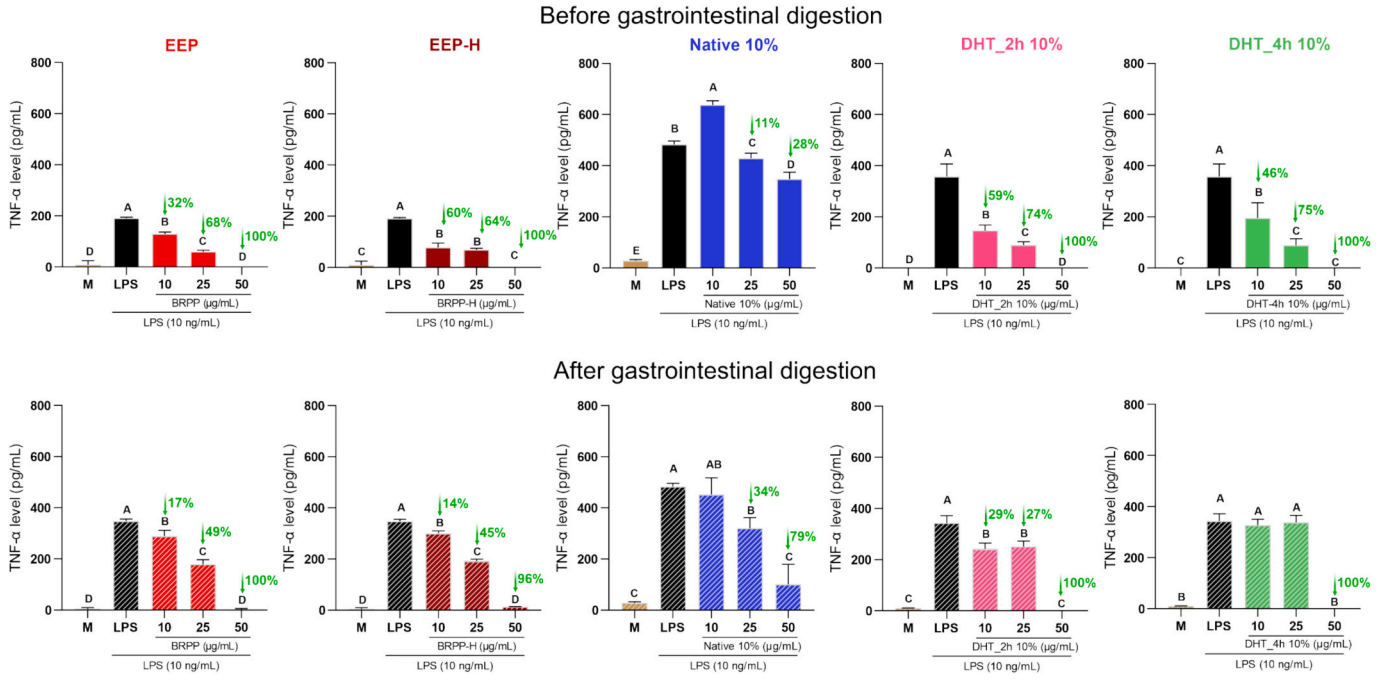

Fig. 9. TNF-α release induced by LPS in RAW 264.7 macrophages incubated with extracts (before gastrointestinal digestion) and intestinal fraction (after gastrointestinal digestion) of 3D-printed propolis gels (infill density: 60 %) with 10 % native and modified (DHT 130 °C, 2 and 4 h) wheat starches. Statistical analysis by Tukey's test ($p < 0.05$): Different uppercase letters within each graphic indicate significant difference among the tested concentrations. M: negative control, representing only macrophages. LPS: positive control, representing only LPS-induced macrophages.

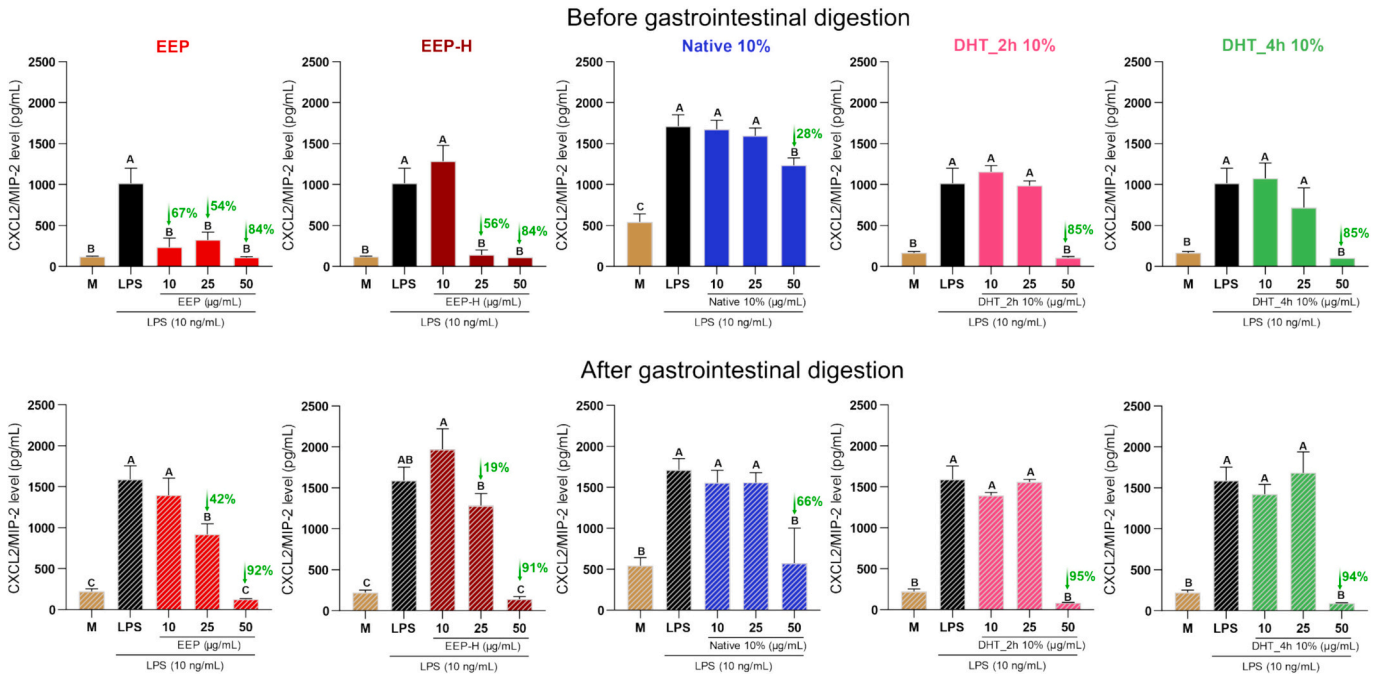

Fig. 10. CXCL2/MIP-2 release induced by LPS in RAW 264.7 macrophages incubated with extracts (before gastrointestinal digestion) and intestinal fraction (after gastrointestinal digestion) of 3D-printed propolis gels (infill density: 60 %) with 10 % native and modified (DHT 130 °C, 2 and 4 h) wheat starches. Statistical analysis by Tukey's test ($p < 0.05$): Different uppercase letters within each graphic indicate significant difference among the tested concentrations. M: negative control, representing only macrophages. LPS: positive control, representing only LPS-induced macrophages.

cell model [46]. On the other hand, at a higher concentration tested (50 μg/mL) for native starch propolis gels reduced NF-κB activation by ~33 %. When the starches were modified, the propolis gels prepared with DHT_2h and DHT_4h showed even greater power in reducing NF-κB activation at concentrations of 25 and 50 μg/mL, similar to the behavior observed for the propolis extracts – EEP and EEP-H.

After gastrointestinal digestion, propolis extracts (EEP and EEP-H) and propolis gels (native, DHT_2h, and DHT_4h) exhibited anti-inflammatory activity, as evidenced by the reduction in NF-κB activation at the highest concentration tested (50 μg/mL). Notably, only the propolis gels formulated with modified starch DHT_2h reduced the NF-κB activation (~33 %) at a concentration of 25 μg/mL, suggesting a possible synergistic effect between DHT_2h starch and propolis.

In fact, the modified starch matrix may have delayed or modulated the release of phenolic compounds during gastrointestinal digestion, resulting in a more pronounced presence of bioactive compounds in the simulated intestine phase, where the anti-inflammatory activity was evaluated. Moreover, interactions between phenolic compounds and the starch matrix (e.g., hydrogen bonds or hydrophobic interactions) may have contributed to the stabilization and protection of these compounds from enzymatic degradation, enhancing their bioactivity [35]. Furthermore, this hypothesis is further supported by the bioaccessibility results obtained for liquiritigenin (Fig. 6), as previously discussed. This synergistic mechanism opens new avenues for investigation in future studies.

NF-κB activation triggers a cascade of immune responses, including the modulation of cytokines such as TNF-α and chemokines such as CXCL2/MIP-2 [45], which in turn also induce NF-κB activation [47]. In fact, excessive production of TNF-α and CXCL2/MIP-2 has been associated with chronic inflammation and the progression of several inflammatory diseases [48]. Therefore, evaluating anti-inflammatory activity through the reduction of TNF-α and CXCL2/MIP-2 release is important to understand the potential of propolis gels to mitigate inflammation and its harmful effects. Based on this, in this work we also evaluated the release of TNF-α and CXCL2/MIP-2 in samples before and after gastrointestinal digestion.

Figs. 9 and 10 show the results of TNF-α, and CXCL2/MIP-2 release, respectively, induced by LPS in RAW 264.7 macrophages incubated with extracts (before gastrointestinal digestion), and intestinal fraction (after gastrointestinal digestion) of propolis extracts (EEP, and EEP-H) and propolis gels (native, DHT_2h, and DHT_4h). All concentrations tested (10, 25, and 50 μg/mL) for EEP and EEP-H showed a significant decrease in TNF-α release (Fig. 9) for both before and after gastrointestinal digestion. For native starch propolis gels, only concentrations of 25 and 50 μg/mL reduced TNF-α release, and gastrointestinal digestion had a positive effect reducing the TNF-α release. In propolis gels produced with DHT_2h and DHT_4h, all concentrations tested showed a significant reduction in TNF-α release before and after gastrointestinal digestion (except DHT_4h after digestion). However, gastrointestinal digestion reduced the effectiveness of TNF-α release inhibition at concentrations of 10 and 25 μg/mL, but even so, a 100 % reduction in TNF-α release was observed for the concentration of 50 μg/mL for both DHT_2h and DHT_4h after gastrointestinal digestion. These results suggest that gastrointestinal digestion can influence the bioactivity of phenolic compounds in different ways, depending on the formulation and concentration, with the higher concentration (50 μg/mL) of propolis extract and propolis gels generally retaining stronger anti-inflammatory potential.

Regarding the CXCL2/MIP-2 release (Fig. 10), unlike NF-κB, heating negatively affected the propolis extract, where for EEP all concentrations tested in samples before gastrointestinal digestion showed a reduction in CXCL2/MIP-2 release, while for EEP-H at a concentration of 10 μg/mL no effect was observed. Although cytokine release is closely linked to NF-κB activation, a decrease in NF-κB activation does not necessarily imply a corresponding decrease in CXCL2/MIP-2 release, as other signaling pathways might also regulate its expression [49,50].

After gastrointestinal digestion, however, EEP and EEP-H showed similar behavior in reducing the CXCL2/MIP-2 release. The propolis gels showed a reduction of CXCL2/MIP-2 release only at the highest concentration tested (50 μg/mL) before or after gastrointestinal digestion, and the modification of starch DHT_2h and DHT_4h showed a positive effect with a reduction of >90 % in the release of CXCL2/MIP-2 at a

concentration of 50 μg/mL comparing with the native one.

This pattern of response observed for TNF-α and CXCL2/MIP-2, particularly in the gels formulated with DHT_2h and DHT_4h, aligns with the potential protective and modulatory effects of the modified starch matrix previously discussed. Although these markers are regulated by distinct pathways, the enhanced anti-inflammatory outcomes may still be influenced by the enhanced stability and availability of bioactive compounds in the intestinal phase, as suggested by the NF-κB results.

In summary, although the propolis heating (EEP-H) improved the inhibition of NF-κB activation before gastrointestinal digestion, the effect was reduced after gastrointestinal digestion. Additionally, propolis gels prepared with native and modified starches showed varying effects of NF-κB modulation, with gels made from DHT-modified starches exhibiting the most promising anti-inflammatory potential, especially at higher concentrations. Furthermore, evaluation of TNF-α and CXCL2/MIP-2 release further supported the anti-inflammatory effects of the samples, particularly for modified starch propolis gels, emphasizing the importance of formulation strategies to improve the functional properties of foods for individuals with chronic inflammation, such as those with dysphagia. These findings suggest that combining bioactive compounds, such as those present in propolis, with functional ingredients, such as modified starches, may lead to developing a product with tailored texture for people with dysphagia, and with active properties.

4. Final considerations

This work demonstrates the possibility of developing a 3D-printed propolis hydrogel using native and DHT-modified starch with a texture suitable for people with dysphagia. This work evaluated the properties of the gels after preparation, such as 3D printing performance, suitability for people with dysphagia, and during and after gastrointestinal digestion simulated by the INFOGEST 2.0 protocol, such as the phenolic profile and bioaccessibility, antioxidant and anti-inflammatory activities. The findings highlight the potential of combining propolis with starch-based matrices to create innovative functional foods tailored for specific dietary needs.

Although this study achieved its objectives, it does not intend to be a definitive reference in the medical or nutritional field. Further clinical investigations are essential to validate the use of adapted diets for dysphagia and to evaluate the inclusion of different foods in the composition of a balanced diet. It is essential to consider the varying degrees of dysphagia, perform comprehensive clinical evaluations and ponder factors such as allergens, sensitivities and intolerances before prescribing specific foods. This work highlights the relevance of developing innovative and functional solutions, such as tailored foods through 3D printing, to meet special needs more effectively, expanding the available options and promoting an integrated vision of health and well-being.

5. Conclusion

This study demonstrated the feasibility of producing 3D printed gels from different concentrations of native and DHT-modified wheat starch, incorporated with Brazilian Red Propolis for people with dysphagia. The results showed that starch, in addition to being a gelling and texturizer ingredient, facilitates the incorporation and protection of propolis into stable food matrices, enabling functional products for people with dysphagia or alcohol restrictions. DHT modification resulted in gels with a texture suitable for people with dysphagia. Besides, starch alone, whether modified or not, did not affect the anti-inflammatory response. Additionally, the propolis gels showed a significant reduction in NF-κB activation and the release of pro-inflammatory cytokines, demonstrating anti-inflammatory potential. Additionally, higher starch concentrations tend to better preserve the phenolic compounds of propolis, as well as its active properties. Therefore, the integration of bioactive compounds and functional ingredients, such as modified starches, offers a promising strategy for developing foods with functional properties and tailored texture for people with dysphagia. Nevertheless, the mechanisms involved in these effects need to be elucidated, and further studies are required to gain a deeper understanding.

CRediT authorship contribution statement

Bruna Sousa Bitencourt: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jaqueline Souza Guedes:** Validation, Methodology, Investigation. **Ana Sofia Martelli Chaib Saliba:** Validation, Methodology, Investigation, Formal analysis. **Severino Matias de Alencar:** Writing – review & editing, Visualization, Supervision, Resources, Methodology, Funding acquisition. **Alan Giovanini de Oliveira Sartori:** Writing – review & editing, Investigation. **Bianca Chieregato Maniglia:** Writing – review & editing, Visualization, Supervision. **Pedro Esteves Duarte Augusto:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors state that they have no recognized financial conflicts of interest or personal affiliations that could have potentially impacted the research presented in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijbiomac.2025.145240.

Data availability

Data will be made available on request.

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