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3D printing of ascorbic acid-rich protein-starch gels for dysphagia diets: texture, nutritional and bioactive properties

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ABSTRACT

Dysphagia affects the ability to safely consume regular-texture foods, requiring the development of modifiedtexture products with adequate nutritional and structural properties. 3D printing can be used to tailor the texture, and shape of the food, allowing the development of customized foods that meet with the need of people with dysphagia. Therefore, this study evaluated the texture, nutritional and bioactive properties of ascorbic acidrich 3D-printed gels for dysphagic diets, using acerola pulp, native or Dry Heating Treatment (DHT) maize starch and concentrated pea protein as gelling ingredients. The gels were prepared using 5 %, 10 %, and 15 % of gelling ingredients, with starch:protein ratios of 100:0, 50:50, and 0:100. Gels were evaluated for 3D printability (under 40-100 % infill rates) and suitability for dysphagia (texture), based on IDDSI criteria. The selected gels were evaluated according to ascorbic acid content and antioxidant capacity (using both FRAP and ABTS methodologies). Formulations with 15 % ingredients starch-protein ratios (50:50) exhibited smooth extrusion, good shape fidelity, and structural stability after printing. Regarding nutritional content, the starch-containing gels preserved ascorbic acid content (up to 94.3 % compared to fresh pulp), while the protein-containing formulations showed slightly greater losses (up to 27 %). However, the protein-containing formulations showed higher antioxidant capacity, indicating the contribution of bioactive compounds from the protein. In conclusion, this work demonstrated desirability ranges in formulation and processing conditions to produce gels suitable for both 3D printing and dysphagic diets, obtaining gels with the potential to be used as ascorbic acid supplements. These results highlight the potential of combining bioactive acerola-based ingredient modified starch and vegetable protein to produce printable, functional foods/supplements for individuals with dysphagia.

1. Introduction

Dysphagia is a health condition characterized by difficulty swallowing liquids and foods. It affects almost 580 million people worldwide, especially the elderly and infants, and it can lead to nutritional deficiencies, dehydration, pneumonia, and health complications (Raheem et al., 2021). For instance, solid foods often result in choking, while liquids can lead to pulmonary aspiration, both resulting in other health problems. This condition, therefore, drastically limits not only food intake, but also any capsules, tablets or powder to be dissolved in

water for medicine or nutritional supplements.

Moreover, dysphagia can significantly impair quality of life, contributing to psychological distress, social isolation, and increased healthcare costs (Niezgoda et al., 2012). Given this context, developing foods and supplements with suitable textures is essential to facilitate safe swallowing while maintaining sensorial appeal (Pereira et al., 2021).

Despite the availability of commercial products aimed at this population, many of them lack sensory appeal, format variability, and adequate nutritional value (Zhu et al., 2025). As a result, there is a

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growing interest in technologies enabling more personalized and functional nutrition. Among these technologies, 3D food printing stands out, which allows the development of structures with specific shapes, controlled textures, and adjustable nutritional composition, simultaneously meeting clinical and sensory requirements (Herrada-Manchón et al., 2020; Liu et al., 2024; Wang et al., 2021; Zhu et al., 2023).

Ascorbic acid (vitamin C) plays multiple roles in human body, including acting as an antioxidant, an enzyme cofactor, and an immune system protector (Ali et al., 2024; NIH, 2021). However, dysphagic individuals may have difficulty consuming conventional vitamin supplements, highlighting the potential of fortified, texture-modified foods. This vitamin supplementation can be obtained using natural sources, such as acerola cherry (*Malpighia emarginata* DC.), a promising fruit rich in ascorbic acid, in addition to its pleasant flavor and intense color (da Silva et al., 2023; Takino et al., 2020).

For 3D printing, fruit pulps require gelling agents to form extrudable structures that maintain their shape. Polysaccharides (such as starch) and proteins have been used as gelling ingredients to produce foods with modified texture due to their technological and functional properties (Guo et al., 2024; et al., 2023; Shanthakumar et al., 2022; Zhang et al., 2024). Vegetable proteins add nutritional value and can influence the retention of antioxidants, in addition to providing elasticity and cohesion to the structure (Shanthakumar et al., 2022; Wang et al., 2022). Among them, pea protein has gained attention due to its nutritional and functional properties, such as gelation, emulsification, and low allergenicity (Shanthakumar et al., 2022). However, it tends to produce weaker gels compared to other proteins, which can limit its ability to provide structural stability when used alone in 3D printing (Mittal et al., 2023). Moreover, starch has been used in 3D printing to improve the rheological properties of food matrices (Azam et al., 2018), although native starches present limited performance. Consequently, starch modification emerges as a promising strategy to improve its functionality and expand its applicability in complex food systems. Among different starch modification techniques, dry heating treatment (DHT) stands out as a simple physical technique that alters the molecular structure and modifies the gel characteristics, making it more suitable for 3D printing (Maniglia et al., 2020).

This study advances the field by integrating three key components rarely combined in practice: a real bioactive-rich fruit matrix (acerola pulp) as a natural ascorbic acid source, maize starch selectively modified by DHT, and pea protein concentrate, to improve nutritional and texture properties. Their integration represents a novel strategy for the development of 3D-printed foods targeted to dysphagia-oriented applications.

2. Materials and methods

This work was developed in three steps, as described in Fig. 1. In the first step, maize starch was modified using the dry heating treatment (DHT) process, pea protein concentrate was obtained from dry peas, and gels were produced by combining maize starch (native and modified), pea protein, and acerola pulp at different concentrations and proportions (36 formulations in total). At this stage, formulations that failed to form gels with sufficient cohesion or that could not be extruded through the 3D printer were excluded.

In the second step, the printable gels were further evaluated for both 3D printing performance and suitability for dysphagia diets. Only formulations that simultaneously showed adequate printability (continuous extrusion and maintenance of printed shape) and compliance with dysphagia texture requirements (structural cohesion and absence of phase separation) were retained.

Finally, in the third step, the selected gels were analyzed for bioactive and nutritional properties, focusing on ascorbic acid retention and antioxidant capacity. The detailed methodology is described as follows.

2.1. Materials

Native maize (corn) starch was supplied by Ingredion Brasil Ingredients Ltd. (\sim 28 % of amylose, Agri Cs 3400). Frozen acerola pulp (Ice fruit, Ice Fruit Indústria e Comércio de Alimentos LTDA., Tatuí - SP) was obtained from the local market in Piracicaba (SP, Brazil), with 3.5 pH and 6.0° Brix.

2.1.1. Pea protein extraction

The extraction process and comprehensive characterization of this pea protein are described by Magalhães et al. (2025). In summary, pea protein concentrate was extracted from dry peas (*Pisum sativum*) obtained from a local supplier (Cerealista Pereira e Barbosa Ltda, MG, Brazil). Pea beans were used without roasting and kept at room temperature. The seeds were ground using a blender (Power NL-26, Mondial, Brazil, at 550 W for 5 min) and the resulting material was sieved through a 1.4 mm mesh to obtain a flour with homogeneous particle size. This flour was used in the control treatment for protein extraction as described by Magalhães (2025). The pea flour was dispersed in sterile distilled water (1:10, w/v), and the pH was adjusted to 10.0 using a 1 mol·L⁻¹ NaOH solution. The suspension was kept under constant magnetic stirring (Fisatom, model 752 A, Brazil) at 300 rpm and 25 °C for 120 min. After stirring, the mixture was centrifuged at 5000g for 15 min

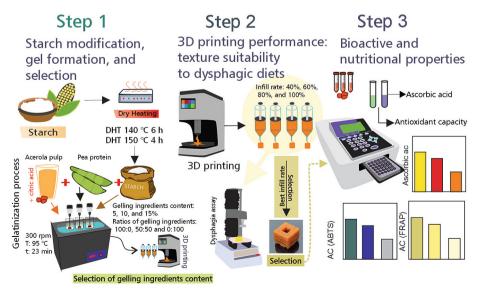


Fig. 1. Diagram of the experiments performed.

at 15 °C, and the supernatant was collected. The pH was then adjusted to 4.5 using a 1 mol·L solution to induce isoelectric precipitation. The resulting precipitate was separated by centrifugation under the same conditions and lyophilized (Liobras, model LP510, Brazil). The protein concentrate obtained was stored under vacuum at -18 °C until further analysis. After extraction, the protein content was 82.5 %, and the soluble protein content was 18.6 % at pH 6 (Magalhães et al., 2025). The protein solubility as a function of pH and water holding capacity is presented in the supporting material.

2.2. Step 1: starch modification, gelatinization process, and selection

2.2.1. Starch modification by dry heating treatment (DHT)

The DHT process was performed on maize starch using the best conditions established in our previous work (Guedes et al., 2023), as they resulted in improved 3D printability and structural self-support, even in formulations with a lower pH value. The maize starch (30 g) was spread on aluminum foil (30 \times 30 cm), sealed, and placed in a hot air oven at 140 °C for 6 h and 150 °C for 4 h. After this, the package was placed in a desiccator until room temperature. Then, they were sieved through 250 μm sieves and stored in closed glass flasks for further analysis. Each process was conducted in three replicates, and the reduction in sample's moisture was measured to ensure the correct solid content for gel preparation.

2.2.2. Acerola gel preparation

The acerola pulp was acidified to a pH of approximately 2.3 using 0.5 mol·L citric acid. Acidification was performed to promote solubilization of the concentrated pea protein, facilitating its interaction with the other components and ensuring adequate gel formation. The gradual addition of an aqueous solution of citric acid was accompanied by continuous monitoring of the pH using a pH meter (TEC-5, Tecnal, SP, Brasil).

Gels were formulated using acerola pulp as dispersion medium, to which gelling ingredients (pea protein, and either native or modified starch by DHT) were added at: 5, 10, and 15 % (w/w). For each gelling ingredient content, three different starch-to-protein ratios were evaluated: 0:100, 50:50, and 100:0 (starch:protein). The appropriate amounts of starch and pea protein were weighed and dissolved in acidified acerola pulp. The resulting mixtures were incubated in a water bath at 25 °C for 1 h to ensure complete hydration of the ingredients. Subsequently, the gelatinization process was initiated by transferring the solutions to a water bath at 95 °C for 23 min, with continuous stirring at 300 rpm. Following complete gelatinization, the samples were transferred into syringes for 3D printing and refrigerated at 5 °C for 24 h to facilitate gelation. After 24 h of gelation, the gels were evaluated for the ability to be printed using a 3D Food Printer (WiiboxSweetin, Wiibox, Nanjing, China), equipped with a 0.84 mm diameter nozzle at room temperature (20 °C). The printing speed was set to 10 mm·s, with parameters previously optimized in earlier studies (Bitencourt et al., 2023; Guedes et al., 2023). A cuboid with dimensions of 1.5 x 1.5 \times 0.75 cm was selected as the model, with various infill densities (40, 60, 80, and 100 %) being evaluated. The geometry of the model was created using the online version of Tinkercad software (Autodesk) for modeling, and Cura slices (version 15.02.1) were employed to prepare the printing files. Infill density was defined in the slicing software as the percentage of each cross-sectional plane ("slice") filled with the food ink, a parameter known to significantly affect the internal structure and texture of 3D printed constructs.

2.3. Step 2: texture suitability to dysphagic diets and 3D printing performance

2.3.1. Texture suitability to dysphagic diets: the instrumental fork assay The gels that were able to be printed were evaluated according to whether they were suitable for dysphagic diets.

An instrumental fork assay was conducted to obtain quantitative data based on the standard IDDSI test (IDDSI, 2019), using the accessory developed by Lancha et al. (2022), compatible with the texture analyzer (TA.XT Plus, Stable Micro Systems Ltd., Surrey, United Kingdom). This test was developed to replicate the standard manual fork test (IDDSI, 2019), but under controlled and reproducible conditions. Parameters such as the force applied, the speed of penetration, and the time the fork rested on the gel surface were standardized (Bitencourt et al., 2023; Guedes et al., 2023). Additionally, the force exerted during penetration was continuously recorded throughout the test. The accessory used was specifically designed for compatibility with the Texture Analyzer (TA.XT Plus, Stable Micro Systems Ltd., Surrey UK), allowing the fork test to be performed consistently and objectively. This configuration minimizes operator-related variability and provides a more comprehensive and accurate assessment of the mechanical properties relevant to texture classification in dysphagia foods.

A force of 4.8 N (490 gf), corresponding to the pressure required to blanch a thumbnail. During the analysis, the fork penetrated the 3D printed gel until reaching this force, which was maintained for 5 s, and then the fork was removed, following the same protocol used in the manual testing. The energy required to penetrate the printed gels was recorded. The qualitative aspects described by (IDDSI, 2019) were also evaluated, such as whether the gel was crushed by the fork or recovered its original shape after compression, providing further insight into the structural integrity and texture of the samples.

2.3.2. 3D printing performance evaluation

The gels selected in step 1 were analyzed for printability performance. Different infill rates were tested, and the printed geometries were compared with the digital model in terms of height, width, and length (cm) to evaluate dimensional fidelity. Based on these results, the most suitable infill rate was selected and used to print a larger structure, a cuboid with a central hole $(2.0 \times 2.0 \times 1.5 \text{ cm})$, in order to assess whether the gels could maintain structural integrity and stability at an increased scale. Additional qualitative aspects were also considered, such as the smoothness and continuity of the extruded lines, as well as the absence of breaks or irregularities.

2.4. Step 3: bioactive and nutritional properties

The selected gels, which showed the best printability and were classified as suitable for dysphagic diets, were analyzed for their ascorbic acid content and antioxidant capacity, along with two control treatments: homogenized fresh pulp and "Cooked" pulp (pulp heated under the same gelatinization conditions as the gels: 95 °C for 23 min).

2.4.1. Ascorbic acid determination

The determination of ascorbic acid was performed according to the methodology described by Egoville et al. (1988), with modifications. The extracting solution was prepared by dissolving 0.4 g of oxalic acid in 500 mL of distilled water, to which 200 mL of acetone was added. The solution was then diluted with 1 L of water. The pH of the solution (1.1) was adjusted using concentrated sulfuric acid. The stock solution of 2,6-dichloroindophenol (DCIP) was prepared by dissolving 100 mg of the dye in 100 mL of warm water, followed by the addition of 84 mg of NaHCO3. The solution was then diluted to 500 mL with water.

The ascorbic acid solution was prepared by dissolving 100 mg of ascorbic acid in 100 mL of the extracting solution. For the preparation of the extract, 2 g of the sample was mixed with 0.4 % oxalic acid containing 20 % acetone. The mixture was homogenized using a vortex, sonicated (ultrasonic bath Q13/25, Ultronique, Brazil; frequency of 25 kHz, 20 min at room temperature), and centrifuged at 5000 rpm for 10 min. The supernatant was collected and used for ascorbic acid determination.

For each analysis, 1 mL of the supernatant was mixed with 9 mL of the DCIP solution. The tubes were shaken for $46 \, \text{s}$, and the absorbance

was measured using a UV–Vis spectrophotometer (Agilent, Cary 60, USA) at 520 nm. A calibration curve was prepared by adding 1 mL of the ascorbic acid solution at concentrations of 10, 20, 30, 40, and 50 μ g, followed by the addition of 9 mL of the DCIP solution (1:10). A linear regression (R² = 0.9939) was used to calculate the samples ascorbic acid, and the results were expressed as the concentration of ascorbic acid content (mg \cdot 100 g).

2.4.2. Antioxidant capacity

Acerola-based gels, homogenized fresh pulp "Cooked" pulp (1 g, w. b.), were mixed with 15 mL ethanol:water, 80:20 (ν /v). The mixture was vortexed for 1 min, sonicated (ultrasonic bath Q13/25, Ultronique, Brazil; 25 kHz, 20 min at room temperature), shaken for 30 min, and then centrifuged at 10.000 xg at 25 °C for 15 min. The supernatant was stored in a refrigerated environment until further analysis.

The antioxidant activity (AC) was measured by both ABTS (2,2'azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) and FRAP (Ferric Reducing Antioxidant Power) assays. The antioxidant capacity was carried out using the ABTS method as described by Al-Duais et al. (2009) with modifications. First, the ABTS.⁺ radical was generated from the oxidation of ABTS (Roche, Germany) (7 mM) using potassium persulfate (Dinâmica LTDA, Brazil) (140 mM). The final solution concentration was 2.45 mM. The solution remained in the dark for 16 h to complete ABTS. + radical. Afterward, the ABTS. + radical was diluted in ethanol:water (80:20 v/v) until the absorbance 0.71 \pm 0.01 at 734 nm. 220 µL ABTS.⁺ radical and 20 µL extract were poured into the microplate for reaction. The absorbance was measured using a microplate reader (SpectraMax M3, Molecular Devices, CA, USA) at 734 nm. A calibration curve was obtained with different Trolox concentrations (0, 12.5, 50, 100, 150, 200, and 250 μ M). A linear regression (R² = 0.9655) was used to calculate the samples' antioxidant capacity, and the results were expressed in Trolox equivalents (μ M Trolox g^{-1} w.b.).

For the FRAP assay, the procedure was described by Müller et al. (2010), which was based on the reduction of a ferric-tripyridyltriazine complex to its ferrous, colored form in the presence of antioxidants. 20 μL of the sample was mixed with 30 μL of water in a 96-well microplate. Subsequently, 200 µL of freshly prepared FRAP reagent was added. The FRAP reagent was composed of 10 volumes of 300 mmol·L⁻¹ acetate buffer (pH 3.6), 10 volumes of 20 mmol·L⁻¹ FeCl₃, and 1 volume of 10 mmol·L⁻¹ TPTZ (2,4,6 -tripyridy-s-triazine, Sigma) in 40 mmol·L⁻¹ HCl. After 8 min, the absorbance was measured at 595 nm using a microplate reader (SpectraMax M3, Molecular Devices, CA, USA) at 37 °C. A calibration curve was obtained with different ferrous sulfate 2.5 mM concentrations (100, 250, 400, 550, and 700 μ M). A linear regression ($R^2 = 0.9871$) was used to calculate the samples' antioxidant capacity, and the results were expressed as the concentration of antioxidants having a ferric-reducing ability equivalent to that of 1 mmol/L FeSO₄.

2.5. Experimental design and statistical analysis

All analyses were performed in triplicate using a completely random design to carry out this work. A difference of p < 0.05 was obtained using Tukey's test using Minitab software (version 18). Graphs were plotted using Excel (version 365).

3. Results and discussion

3.1. Step 1: starch modification, gelatinization process, and screening of gelling ingredients

Fig. 2 shown 3D printed gels prepared with acerola pulp as the liquid phase and different gelling ingredients: pea protein concentrate, native maize starch, and maize starch modified by dry heating treatment (DHT), and Table 1 shows the codification of the formulations. Formulations were developed with three total concentrations of gelling

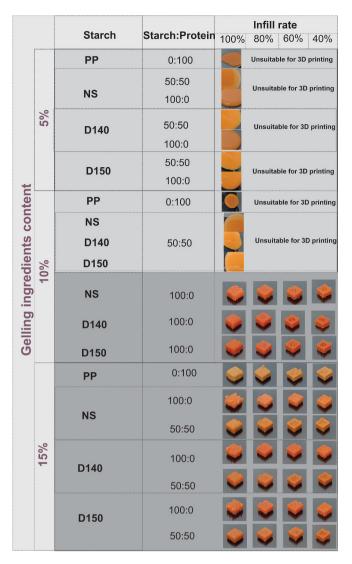


Fig. 2. 3D-printed gels formulated with acerola pulp and different gelling agents: native maize starch, maize starch modified by DHT (140 $^{\circ}$ C 6 h and 150 $^{\circ}$ C 4 h), and pea protein concentrate. Samples were prepared using three starch-to-protein ratios (0:100, 50:50, and 100:0), three gelling agent concentrations (5, 10, and 15 %), and four infill rates (40, 60, 80, and 100 %).

Table 1Codification and composition of gel samples according to gelling ingredients type, concentration, and starch-to-protein ratio.

Gels (treatment code)	Gelling ingredient concentration in the gel (%)	Type of maize starch	Starch: Pea protein ratio
NS-10	10	Native	100:0
D140-10	10	DHT 140 °C 6 h	100:0
D150-10	10	DHT 150 °C 4 h	100:0
PP-15	15	_	0:100
NS-15	15	Native	100:0
NSP-15	15	Native	50:50
D140-15	15	DHT 140 °C 6 h	100:0
D140P-15	15	DHT 140 °C 6 h	50:50
D150-15	15	DHT 150 °C 4 h	100:0
D150P-15	15	DHT 150 °C 4 h	50:50

ingredients (5 %, 10 %, and 15 %), combined in three starch-to-protein ratios (100:0, 50:50, and 0:100), and printed using infill rates of 40, 60, 80, and 100 %. All the gels made with 5 % gelling ingredient content were too weak to be extruded through the nozzle (i.e., they did not form self-sustaining gels but rather dropped from the nozzle instead of being extruded through it, indicating insufficient network formation and mechanical strength to withstand extrusion pressure) and, therefore, could not be successfully printed.

At 10 % gelling ingredients content of protein, and its mixtures with starch were also too weak to be printed, as shown in Fig. 2. This behavior is consistent with the characteristic of pea protein gelling, which forms relatively weak and less elastic gels compared to other plant proteins. As a result, even at higher concentrations, it may not provide sufficient structural strength (Zhang et al., 2021). This limited gelling capacity is mainly attributed to its lower content of sulfur-containing amino acids, such as cysteine, which are essential for forming strong intermolecular disulfide bonds during gelation (Husband et al., 2024; Shanthakumar et al., 2022). Additionally, the globulin-rich protein profile of pea (mainly legumin and vicilin) tends to aggregate through weaker hydrophobic and electrostatic interactions, forming less cohesive networks (Shanthakumar et al., 2022). On the other hand, the gels made with starch (native and modified by DHT for 140 °C 6 h and 150 °C 4 h) could be printed at 10 % content of gelling ingredients, as expected.

At 15 % gelling ingredients content (pure starch or protein, and their mixtures), all the gels could be printed. The gels flowed smoothly through the printer nozzle and maintained their structural integrity

across all infill percentages. At this concentration, the network formed by starch (native or modified) and/or pea protein was sufficiently dense and cohesive, allowing the material to withstand the mechanical stress of extrusion while maintaining its shape after deposition. Therefore, gels formulated with pure maize starch (Native and modified) containing 10 % gelling ingredients, as well as gels containing 15 % gelling ingredients: pure starch or protein, and their mixtures, as shown in Table 1, were selected to advance to step 2. In this second step, the suitability of gels for dysphagic diets was evaluated, along more detailed assessment of their printability.

3.2. Step 2: 3D printing performance and suitability to dysphagic diets

Fig. 3 a-b shows all the gels printed in different infill rates, that is, the internal filling percentage of the printed structure, which directly affects its density and mechanical behavior, before and after applying the fork test using a prototype attached to a texturometer. The purpose of the prototype was to perform the fork method of the International Dysphagia Diet Standardisation Initiative (IDDSI) and to obtain more information about the sample than using the IDDSI standard method (Lancha et al., 2022). Consequently, the necessary energy to penetrate the fork into the gels was obtained by recording the force using a texture analyzer. Moreover, following the IDDSI standard (IDDSI, 2019) two qualitative points were also evaluated: if the fork could squash the sample with the applied force (which is desirable), and if the sample could return to its original shape after the fork was removed (which is

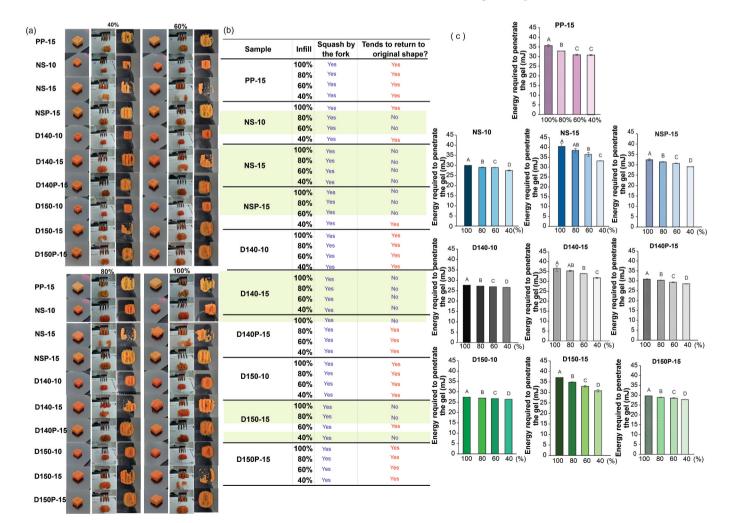


Fig. 3. (a-b) Fork test assay obtained from the fork test using a texture analyzer; (c) energy required to penetrate the gel (mJ) of the gels made with different concentrations of starch (native or modified) and protein, as shown in Table 1.

undesirable). This can be seen in Fig. 3 - a. The samples NS-10 with 80 and 60 % of infill rate, NS-15 in all infill rates, NSP-15100, 80, and 40 % of infill rates, D140–15 in all infill rates, D140P-15 and D150–15100, 80, and 40 % infill rates could be classified as suitable for dysphagic diets by IDSSI. All the other samples, after removing the fork, returned to their shape. This behavior is an indication that the sample could pose a swallowing risk for individuals with dysphagia. Therefore, they were not classified as suitable for dysphagia diets.

Fig. 3 c shows the energy required to penetrate the gels. In general, consistent behavior was observed among the samples: gels with a $100\,\%$ infill rate required a higher amount of energy to be penetrated, especially compared to gels with a $40\,\%$ infill rate. This result can be attributed to the greater volume of material present in the samples with total infill, which increases the density and contributes to a more compact and deformation-resistant structure.

Gels made by the mixtures of starch and protein had slightly lower penetration energy than those made with separate macromolecules. The mixture of starch and protein may interfere with the three-dimensional organization of the matrix, limiting the formation of a continuous network as observed in formulations with only one type of gelling ingredient. This possible competition for water or intermolecular bonds may contribute to a more fragile structure, making it less resistant to penetration.

The 3D printing performance of the gels was also evaluated in two steps. In the first step, a cuboid with a lower height was used to evaluate different infill rates and check how closely the printed material approximated the proposed model. In the second step, the optimal infill rate was selected and applied to the printing of a cuboid with a larger height and a void space in the middle, aiming to evaluate the self-supporting capacity of the printed gels.

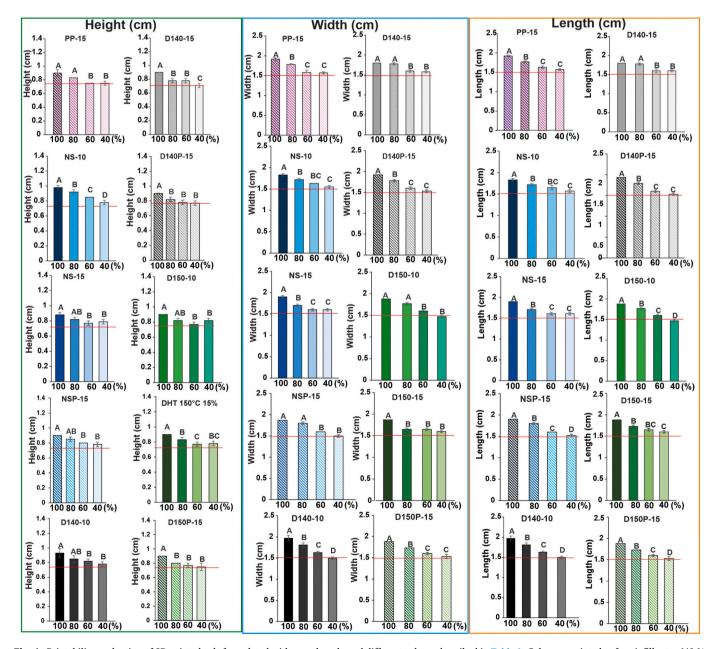


Fig. 4. Printability evaluation of 3D-printed gels formulated with acerola pulp and different gels, as described in Table 1. Gels were printed at four infill rates (40 %, 60 %, 80 %, and 100 %) to assess the effects of composition and internal structure on shape fidelity and print quality. The red line indicates the target value corresponding to the pre-designed geometry. Statistical analysis was performed using Tukey's test (p < 0.05): different letters indicate significant differences within the same sample. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

While the physicochemical properties of the gel matrices are fundamental to determining their printability and post-processing stability, printing parameters such as infill rate also play a crucial role in defining the final quality of the printed structure. In this work, cuboids were printed at four different infill rates: 40, 60, 80, and 100 %, and the printability was evaluated as shown in Fig. 4.

The target height of the model was $0.75~\rm cm$, and all the gels with $100~\rm \%$ infill rate were higher than normal. The same behavior was observed in width and length for all gels. It was observed that the starch ink tends to swell after being extruded by the very small nozzle. The swelling effect of the starch gels was also observed by Cui et al. (2022), who reported that the material with high viscoelasticity can easily swell when extruded due to the absence of a constraining force from the tube wall of the printer.

This swelling behavior when exiting a tube (in this case, when extruded through the printer nozzle), is called the Barus Effect, being characteristic of viscoelastic materials, and manifests as the expansion of the material jet as it exists in a capillary (Ibarz & Barbosa-Canovas, 2020; Kishi & Iizuka, 1964). During the extrusion, the orientation and deformation of the polymer chains inside the capillary generate normal stresses that, when released at the exit, promote elastic relaxation and result in an increase in the diameter of the extrudate.

The gels with 40 % infill rate were the closest to the model, followed by 60 % and 80 % of infill. Although the gels with low infill rates were suitable for the geometry measurements, the printed shape presented some defects, especially a hole formed in the geometry after printing, as can be seen in Fig. 4, in which 40 % and 60 % presented these characteristics. Despite the higher fidelity to the original design, the structure obtained with low infill was not sufficient to support the weight of the material and eventually collapsed. Liu et al. (2018) observed similar behavior with mashed potatoes, which when printed with infill below

40 %, presented partial internal collapse and visual defects. This behavior was attributed to the insufficient mechanical strength provided by low infill percentages, which rendered the printed structures unable to support themselves.

Rong et al. (2023) in a review on starch-based products highlighted that the mechanical structure of printed products is favored when the infill rate is higher than 40 %. However, they also reported that rates between 70 % and 100 % can compromise the accuracy of 3D printing food materials, mainly due to the Barus Effect described above.

The analysis shows that both dimensional accuracy and visual quality are critical in food printing. While 40, 60, and 100 % infill levels resulted in defects or oversizing. However, the 80 % infill rate demonstrated better printability for most gels and was selected for the next print.

In the second step, the geometry of a cuboid with a central cavity was selected for printing the gels, using the previously determined infill ratio, 80 %. The corresponding results are shown in Fig. 5.

At 10 % gelling ingredients, only the NS-10 was able to maintain its structure. The gel D150–10 collapsed as the number of layers increased and was unable to self-support. The gel D140–10 showed a tendency towards partial collapse, as evidenced by a slightly flattened base compared to the gel with native starch. The results differ from those reported by Guedes, Bitencourt, & Augusto (2023), in which gels prepared with 10 % of gelling ingredients, especially the DHT-modified maize starch (140 °C for 6 h), were able to maintain printed layers. This difference may be attributed to the low pH (2.3) used in the formulation of the present gels, which could have influenced their structural integrity at this concentration of gelling ingredients. Furthermore, the dispersion medium used in the preparation of the gels must be considered, since the fruit pH and solids are different in the present study and those developed by Guedes, Bitencourt, & Augusto

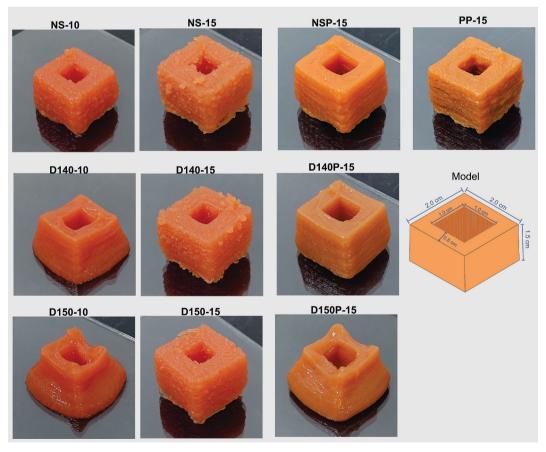


Fig. 5. 3D-printed gels formulated with acerola pulp and different gelling systems, as described in Table 1.

(2023).

Gels with 15 % gelling ingredients (pure protein, and its mixtures with native and DHT 140 °C 6 h starches) presented a smoother surface and the ability to be self-sustaining. Although the samples formulated with just starch as a gelling ingredient at 15 % concentration were able to maintain their structure, they exhibited an irregular surface compared to the protein and starch, and just protein gels at 15 %. 3D printing gels made with starch, especially in higher concentrations, may result in a more fragmented deposit, with less cohesive edges and a rougher surface texture (Oh et al., 2024; Wedamulla et al., 2023). The gels formulated with protein (pure or with starch), on the other hand, did not exhibit the irregular surface observed in the pure starch gels. The smoother surface observed in the protein gels may be attributed to the acid pH (2.3), which was selected to enhance protein solubility and dispersion. Under this condition, the protein formed a homogeneous and cohesive matrix that allowed for uniform extrusion and smoother deposition. However, the gel D150P-15, under the same conditions, did not maintain its structure despite having a similarly smooth surface - reflecting the interactions among the modified starch, protein, and acerola pulp resulted in a gel unable to self-sustaining after printing.

The main forces involved in starch-protein interactions include covalent bonds, electrostatic interactions, hydrogen bonds, ionic bonds, hydrophobic interactions, and size exclusion (Zhang et al., 2025). When maize starch is subjected to DHT at 140 °C for 6 h and 150 °C for 4 h, both carboxyl and carbonyl groups are formed as a consequence of partial oxidation of hydroxyl groups (Guedes, Bitencourt, & Augusto, 2023). These modifications can promote different and additional interactions with proteins through hydrogen bonding and electrostatic forces. In addition, a partial molecular depolymerization is observed in starch molecules (Guedes, Bitencourt, & Augusto, 2023), resulting in mobile chains with different packing factors that increase the surface area and facilitate binding with proteins.

In the present system, the low pH provided by acerola pulp plays a decisive role, resulting in the protonation of charged amino acid residues that may interact with starch (Dumetz et al., 2008; Scott & Awika, 2023), and possibly the carboxyl groups of DHT-modified starches. Proteins with a high proportion of charged amino acids are more likely to influence starch retrogradation, either through electrostatic repulsion or attraction (Scott & Awika, 2023). Consequently, the interactions and stability of the starch-protein system in the acidic environment may be significantly affected. Therefore, complex interactions among starch, proteins, and acerola pulp molecules are expected, and further studies are needed to understand them.

Subsequently, the gels that demonstrated the best performance in 3D printing were those made with 15 % gelling ingredients, coded as NSP-15, D140P-15, and PP15. These gels exhibited self-sustaining capacity, with a smoother surface, and did not collapse after printing. They were then evaluated in terms of texture.

To summarize, the selection process was performed in three sequential steps based on the following objective criteria:

- Initial 3D printing screening (step 1): All 36 formulations were tested for their ability to extrude through the printer nozzle. Only 10 formulations successfully extruded, indicating they were suitable for further evaluation. This step ensured that subsequent analyses focused on practically printable gels.
- Dysphagia suitability evaluation (step 2): The 10 printable formulations were assessed according to IDDSI criteria, including structural cohesion, absence of phase separation, and resistance to fork penetration (texture). Formulations meeting these criteria were NS-10, NS-15, D140-10, D140-15, D150-15, NSP-15 and D140P-15.
- Advanced 3D printing performance (step 2): The same 10 formulations were evaluated for shape fidelity, extrusion continuity, and stability. Only NSP-15, D140P-15, and PP-15 showed superior printability.

Therefore, only two formulations, NSP-15 and D140P-15, simultaneously present the desirable texture and suitability for 3D printing, as shown in Fig. 6. These results highlight the relevance of the synergy between the type of starch, its modification, and the presence of protein in the formulation for the development of gels that are balanced in terms of printability and swallowing safety. The next step explored the bioactive and nutritional properties of these selected gels (as well as the control treatments NS-10, NS-15, PP-15, Fresh pulp, and "Cooked" pulp). Even so, further studies are needed to deeply understand the interactions among modified starches, proteins (in special plant-based), and food matrices (containing different compounds and at different pHs). Starting from the molecular interactions and going until the bulk (gel) properties, different processing parameters must be evaluated, considering starch modification techniques and conditions, gel production, and evaluation.

3.3. Step 3: bioactive and nutritional properties

According to the literature, the ascorbic acid content in fresh acerola fruits can reach 4500 mg•100 g, being up to 100 times higher than that found in citrus fruits such as orange or lemon (Prakash & Baskaran, 2018). However, it is a highly sensitive compound, whose stability can be affected by several factors, including pH variations, high temperatures, exposure to light, presence of oxygen, and metal ions (Huang et al., 2022). Such conditions can accelerate the degradation of ascorbic acid during processing and storage, resulting in considerable losses in its final content in the formulated products. Consequently, the ascorbic acid content and antioxidant capacity of the selected gels were evaluated, as shown in Figs. 7 and 8.

Despite this sensitivity, it is important to highlight that the processing conditions at which the gels were prepared (95 $^{\circ}$ C for 23 min) did not promote drastic degradation of ascorbic acid. This is evidenced by the fact that the pulp heated under these same conditions did not differ significantly from the fresh pulp (p > 0.05), suggesting that thermal degradation during gel preparation was minimal.

Among the formulations, the gels NS-10 and NS-15, and D140–10 presented ascorbic acid contents statistically similar to those observed in both fresh and "cooked" pulp (p > 0.05). On the other hand, the gels formulated with combinations of starch and protein – NSP-15, D140P-15, as well as the gel PP-15, presented significantly lower contents compared to the fresh pulp (5.74, 23.7, and 24.20 % respectively), being similar to the 'cooked' pulp, suggesting that factors beyond dilution contributed to the losses observed.

Several mechanisms can explain the lower ascorbic acid content in protein-based gels. One possible factor is the presence of trace minerals (Fe, Cu, Zn, and Mg) naturally retained by pea protein, which may catalyze oxidation reactions under thermal conditions (López-Calabozo et al., 2025). In additions, it is also possible that amino acids or peptides containing free thiol groups (such as cysteine residues) can be released during protein unfolding, interact with ascorbic acid or its oxidative intermediates (Yu & Zhang, 2010). These sulfur compounds may participate in redox reactions or form conjugates with ascorbic acid degradation products, resulting in a decrease in their measurable content (Yu et al., 2012). Although some proteins can provide a shielding effect, reducing oxygen permeability and delaying oxidation (Choi et al., 2023; Khin et al., 2024), this protective behavior was not observed in our gels. Another factor that may contribute to the loss of ascorbic acid in protein-containing gels is the oxidation of ascorbic acid to dehydroascorbic acid (DHA) (Yin et al., 2022). DHA can be irreversibly degraded, producing highly reactive carbonyl intermediates, which may react covalently with amino groups of proteins, leading to glycosylation (Yin et al., 2022). This process can further reduce the measurable ascorbic acid content and alter protein structure within the gel matrix. Finally, the pH can also influence the degradation of ascorbic acid. It is well established that ascorbic acid is more stable under acidic conditions (Farah et al., 2020; Moura et al., 1994), where the protonated form is

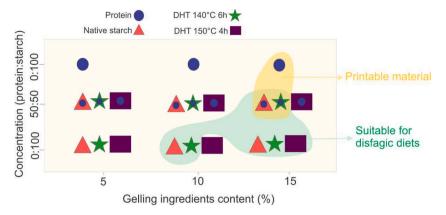


Fig. 6. Diagram showing the classification of samples as printable materials and/or suitable for dysphagic diets, according to the different gelling ingredient contents described in Table 1.

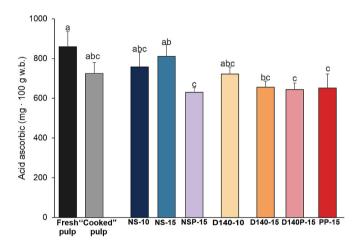


Fig. 7. Acid ascorbic content of the gels formulated with acerola pulp, maize starch (native and modified by DHT), and/or pea protein, as shown in Table 1.

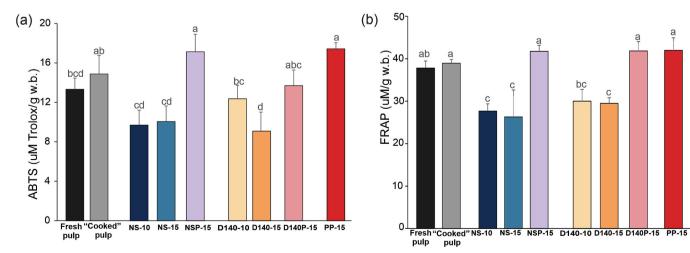
less prone to oxidation. According to Yin et al. (2022), under alkaline conditions, ascorbic acid is first reversibly oxidized to dehydroascorbic acid (DHA), which then irreversibly hydrolyzes to 2,3-diketogulonic acid. Therefore, the observed degradation is more likely related to other factors, such as interactions with protein components, the presence of trace metals, rather than to pH effects.

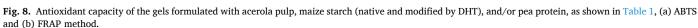
Therefore, although some loss of ascorbic acid was expected due to ingredient dilution and thermal exposure, the results suggest that specific chemical interactions involving pea protein components played a major role in the ascorbic acid content of those gels.

In contrast, starch-based (native and modified) gels showed an ascorbic acid content very similar of the fresh and "cooked" pulp. These results are interesting as recent findings indicate that ascorbic acid can also inhibit pancreatic α -amylase, slowing starch hydrolysis and increasing resistant starch content (J. Guo et al., 2021). In this sense, the preservation of ascorbic acid observed in the native and DHT-starch gels of our study may not only contribute to antioxidant activity but also play a secondary role in modulating starch digestibility, thus enhancing the functional value of these formulations – it is worth mentioning that further studies on this topic are suggested.

Although the protein gels showed a lower ascorbic acid content compared to fresh acerola pulp, this reduction is expected given the combined effects of freezing, thermal processing, interactions, and dilution with the gelling ingredients. Even so, the gels still retained a reasonable amount of ascorbic acid (629 to 859 mg/100 g) when compared to fruit-based products, such as orange juice (~33.4 to 66.67 mg/100 mL) (Maria et al., 2022) or cashew apple juice (293 mg/100 mL) (Sivaprakasam et al., 2025).

For reference, it is interesting to compare the obtained results with the "Fact Sheet for Health Professionals" from the US National Institute of Health (NIH, N. I. of H, 2021). According to this guideline, the recommended dietary allowance for ascorbic acid in adults ranges from 75 mg (for females) to 120 mg (for females who are lactating). To achieve





those values by ingesting the acerola gels here produced, quantities of only $8.7\,\mathrm{g}$ to $19\,\mathrm{g}$ of gels are needed. It is also important to highlight that the same document describes the tolerable upper intake levels of ascorbic acid in adults as 2000 mg (adults), which would represent 233–318 g of the gels here produced. Those values support the gel's potential as a functional food or vitamin supplement, although clinical investigations are essential to validate the use of adapted diets for dysphagia before any prescription.

The antioxidant capacity of the gels was evaluated using both the ABTS and FRAP methods, as shown in Fig. 8. The antioxidant capacity of fresh and "cooked" pulp evaluated in this study presented values of 13.33 and 14.87 µmol Trolox•g $^{-1}$ (ABTS), and 37.79 and 38.94 µmol Fe^{2+} •g $^{-1}$ (FRAP), respectively. These results are similar to those reported by Paiva et al. (2023), who found 11.14 µmol Trolox•g $^{-1}$ and 21.99 µmol Fe^{2+} • g $^{-1}$ in acerola pulp. However, they are considerably lower than those observed in other studies that used the whole fresh fruit (pulp and peel) or specific varieties, such as those by Ferreira et al. (2021), whose values ranged from 42 to 208 µmol Trolox•kg $^{-1}$ and from 293 to 535 µmol Fe^{2+} • kg $^{-1}$. Differences may be attributed to several factors, including the part of the fruit analyzed (pulp versus whole fruit), varietal differences, cultivation conditions, ripeness stage, and extraction methodologies employed.

The evaluation of the antioxidant capacity of the gels revealed that the thermal treatment used to prepare the gels did not compromise the antioxidant activity of the matrix, since the values obtained for the fresh pulp and the "cooked" pulp were statistically similar (p>0.05) in both ABTS and FRAP.

Despite this thermal stability, the gels prepared with only starch and pulp presented significantly lower values of antioxidant capacity when compared to the pure pulp samples, even with the dilution being considered. Specifically, the antioxidant activities were NS-10 - 27.2 % (ABST) and 26.80 % (FRAP); NS-15 - 24.68 % (ABTS) and 30.45 % (FRAP); D140–10 - 7.26 % (ABTS) and 20.6 % (FRAP); D140–15 - 31.90 % (ABTS) and 21.96 % (FRAP). These results can be attributed to the physical or chemical interactions between the antioxidant compounds and the gelatinized starch matrix, resulting in the encapsulation of the compounds in the starch matrix (Zuo et al., 2024), which can limit their action as reducing agents (in FRAP) or as radical scavengers (in ABTS). As reported by Lee and Hwang (2023) starch-based hydrogels are capable of effectively protecting molecules such as antibiotics, analgesics, and drugs, since their three-dimensional hydrogel matrix creates a protective environment that minimizes degradation and enhances their stability during storage and release.

On the other hand, in the gels containing pea protein in the formulation, an antioxidant content similar to that of "cooked" and fresh pulp samples was observed in FRAP assay, and comparable to that of the "cooked" pulp in the ABST assay. This behavior may be related to the composition of pea protein itself, which contains amino acids with potential antioxidant properties (Shanthakumar et al., 2022). The presence of residues such as tyrosine and cysteine, for example, has already been associated with some antioxidant properties in food systems, especially after heating, which may favor structural modifications and the exposure of reactive groups (Ebrahimi et al., 2025; Irankunda et al., 2025).

In general, the results obtained by both methods show that the composition of the gel matrix plays a central role in the final antioxidant activity of the system, with protein being a more significant contributor than starch. Furthermore, the combined use of the ABTS and FRAP assays allowed a more comprehensive evaluation, considering different mechanisms of antioxidant action: while ABTS is sensitive to the free radical scavenging capacity, FRAP detects only compounds with reducing potential (Jones et al., 2017; Mariutti et al., 2008). The consistency between the two techniques reinforces the reliability of the data and the functional importance of pea protein in the developed formulations.

The antioxidant activity of the gels, evaluated by both ABTS and FRAP assays (range from 9.07 to 17.43 μ mol Trolox \bullet g $^{-1}$, and from 26.28

to 41.95 µmol Fe²⁺•g⁻¹, respectively), demonstrated values comparable to those found in several foods recognized as sources of antioxidants. ABTS and FRAP results were similar to those of açaí fruit (15.1 µmol Trolox•g⁻¹, 32.1 µmol Fe²⁺•g⁻¹), higher than Yellow mombin (7.8 µmol Trolox•g⁻¹, 11.8 µmol Fe²⁺•g⁻¹), and Caju apple (11.2 µmol Trolox•g⁻¹, 22.9 µmol Fe²⁺•g⁻¹), and lower than the pure accrola fruit (96.6 µmol Trolox•g⁻¹, 148 µmol Fe²⁺•g⁻¹) (do Rufino et al., 2010). Consequently, the results demonstrate that the composition of the gelled matrix significantly influences the final antioxidant activity, with pea protein being a key component to preserve this functionality. The similarity of the values obtained with fruits known to be antioxidants reinforces the potential of the developed formulations not only as suitable textural alternatives for dysphagia diets, but also as functional products or supplements.

However, although the objective of our study was achieved, we highlight the relevance of applying the developed gels as functional foods or supplements, in regular or dysphagic diets, which should be evaluated in future studies, by dedicated groups and maybe clinical trials. Moreover, different levels of dysphagia, associated with an overall clinical evaluation, demand specific approaches in relation to food and nutrition. Consequently, this study does not aim to be a reference from the medical nor nutritional points of view, even so we highlight its importance by demonstrating alternatives to improve the well-being of people with special needs. Nonetheless, this work reinforces the relevance of developing tailored food products and supplements for individuals with special dietary needs, and the key role of gelling ingredients and 3D printing technology.

4. Final considerations

This work demonstrates the feasibility of developing 3D-printed, ascorbic acid-rich gels for dysphagic diets, obtained using protein and starch. The gels presented adequate texture and bioactive properties, highlighting the potential of combining acerola-based ingredients, modified starch, and vegetable protein to produce functional, tailormade, and printable foods.

However, although this work describes an interesting approach to tailor food composition and texture for people with special needs, achieving its objectives, it does not intend to be a definitive reference in the medical or nutritional field. Laboratory indicators, such as texture and Ascorbic acid retention, provide preliminary guidance that is essential for advancing research towards real-world application (namely, this study is a necessary step before approving further studies involving humans). For the next steps, clinical and sensory evaluations are crucial to ensure palatability, ease of swallowing, and patient compliance. Further investigations are needed to evaluate these formulations in dysphagic diets and their integration into balanced nutritional plans, considering dysphagia severity, allergens, and food intolerance.

Even so, we highlight the importance of this study by demonstrating alternatives to improve the well-being of people with special needs concerning food ingestion. It is important to highlight that the number of available foods for dysphagic diets is limited, affecting not only the dysphagic people's nourishment, but also their mood, which indirectly impacts their medical condition. Therefore, providing new alternatives for dysphagic people is a topic of high interest.

5. Conclusions

This study demonstrated that acerola pulp-based gels, formulated with native or DHT-modified maize starch and pea protein, can be successfully applied in 3D printing, also achieving textures compliant with IDDSI standards for dysphagia diets.

Formulations with 15 % gelling ingredients, particularly those combining protein with either native or DHT-modified starch (140 $^{\circ}$ C 6 h), exhibited better printability, structural stability, and texture

performance. Importantly, these formulations preserved ascorbic acid content and maintained high antioxidant capacity, thus ensuring nutritional and functional value.

By integrating a bioactive-rich fruit matrix with starch modification and plant-based protein, this work advances the design of functional foods tailored to dysphagic populations. Those findings highlight the potential of DHT-modified starch as a strategy to improve printability without compromising bioactive retention.

CRediT authorship contribution statement

Jaqueline Souza Guedes: Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. Bruna Sousa Bitencourt: Methodology, Investigation, Data curation. Isabela Soares Magalhães: Validation, Resources, Methodology, Investigation, Data curation. Bruna de Oliveira Gomes: Methodology, Investigation. Bruno Ricardo de Castro Leite Junior: Writing – review & editing, Validation, Resources, Methodology, Data curation. Wanessa R. Melchert: Writing – review & editing, Resources, Methodology, Conceptualization. Pedro Esteves Duarte Augusto: Writing – review & editing, Visualization, Supervision, Project administration, Methodology, Formal analysis.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ifset.2025.104283.

Data availability

Data will be made available on request.

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