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Mineral bioaccessibility in 3D printed gels based on milk/starch/κ-carrageenan for dysphagic people

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ABSTRACT

Dysphagia is a condition that affects the ability to chew and swallow food and beverages, having a major impact on people's health and wellbeing. This work developed gel systems with a customized texture suitable for intake by dysphagic people using 3D printing and milk. Gels were developed using skim powdered milk, cassava starch (native and modified by the Dry Heating Treatment (DHT)), and different concentrations of kappa-carrageenan (κC). The gels were evaluated in relation to the starch modification process and concentration of gelling agents, 3D printing performance, and suitability for dysphagic people (following both the standard fork test described by the International Dysphagia Diet Standardization Initiative (IDDSI), and also using a new device coupled to a texture analyzer). Moreover, the best formulations were evaluated for mineral bioaccessibility through simulated gastrointestinal digestion based on INFOGEST 2.0 standardized method. The results showed that κC had a dominant effect compared to the DHT-modified starch on gel texture, 3D printing performance, and fork tests. The gels obtained by molding or 3D printing resulted in different behaviors during the fork test, which was associated with the gel extrusion process that breaks down their initial structure. The strategies applied to tailor the texture of the milk did not affect the mineral bioaccessibility, which was kept high (>80%).

1. Introduction

Dysphagia is a condition that affects the ability to chew and swallow foods, impacting the psychological and physical health of people with this condition (Rodd et al., 2021; Sukkar et al., 2018), and also affecting their well-being. In fact, dysphagic people can choke and aspirate foods or drinks, which can be fatal (Sungsinchai et al., 2019). Dysphagia mainly affects elderly people due to the weakening of the muscles involved in swallowing (Sura et al., 2012), but it can also be a consequence of multiple neurological, muscular factors (Thiyagalingam et al., 2021) and diseases. For instance, it has recently been reported that patients severely affected by Covid-19 are prone to dysphagia (Dziewas et al., 2020), as well as head and neck cancer patients even after oncologic treatment (Fernandes et al., 2022). Therefore, the development of foods with modified texture is essential to improve the life quality of dysphagic people, although it is a challenge for the food industry.

Particularly, low-viscosity liquids pose a risk of being consumed by dysphagic people, as they can be aspirated (Newman et al., 2016). It limits the consumption of some food products with relevance as nutritional intake - such as milk.

Therefore, there is a need for developing new ingredients to modify liquid foods towards consumption in dysphagic diets, as well as new strategies of personalization and serving. Given this scenario, using the 3D printing technology, combined with tailored ingredients, is an interesting approach to producing foods with customized textures for dysphagic people.

In fact, 3D printing is a technology that offers numerous advantages in the development of foods for special needs. For instance, people with chewing and swallowing difficulties often lose their desire to eat, mainly due to the unpleasant appearance of foods with modified texture. 3D printing is a useful tool to produce tailor-made foods, with suitable texture, more attractive, and with high nutritional value. However, the

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foods used in 3D printing must have physical and chemical properties that allow extrusion through the printing needle, while being able to withstand the various layers deposited during the 3D printing process. To achieve this goal, biopolymers such as starch and kappa-carrageenan (κC) are interesting options to act as gelling agents, being accessible and widely found in nature (Bemiller, 2011; Maniglia et al., 2020).

However, most studies focus on the evaluation of model foods, using only the gelling agents and water, being the evaluation of real foods essential. Considering the possible interactions and structuring, the impact of food formulation (using the gelling ingredients) and 3D printing on the proposed food nutritional aspect is an important factor to be evaluated, even more considering the consumers with special needs. For instance, the mineral bioaccessibility is an important factor to be evaluated, as it indicates the proportion of minerals released from the food matrix and made available for absorption by the human body. Therefore, once different ingredients (gelling agents) are mixed with milk, a new food matrix is formed by the interactions that occur among the components of this matrix, which can directly influence the kinetics of digestion and release of minerals in the human body, affecting its bioaccessibility. Since milk is a food source of essential minerals such as calcium, but hardly consumed by dysphagic people, it is important to assess the extent to which that mineral bioaccessibility is affected by treatments and interactions with gelling agents applied to obtain a product with good performance for 3D printing.

Accordingly, the first objective of this study was to develop gels based on starch (native or modified cassava starch by DHT), κC and milk, evaluating the starch modification, 3D printing performance and texture suitability for dysphagic people. Furthermore, the second objective was to evaluate the mineral bioaccessibility in the developed gels, through simulated gastrointestinal digestion based on application of the INFOGEST 2.0 method.

2. Materials and methods

This study was developed in three steps. In the first step, the cassava starch was modified by the Dry Heating Treatment (DHT) process (130 °C for 2 and 4 h) with the intention of improving the gel properties to allow 3D printing. Then, the gels based on starch (native and modified), κC (in different concentrations) and skim powdered milk were evaluated in relation to the effects of gelling agents on texture and syneresis. Second, the performance of the gels for 3D printing and their suitability for dysphagic people were evaluated. The gels that presented the best performance for 3D printing and dysphagia were selected for the last step. In the third step, we evaluated the mineral bioaccessibility through simulated gastrointestinal digestion based on INFOGEST 2.0 method. The experimental flowchart, involving all stages of this study, is

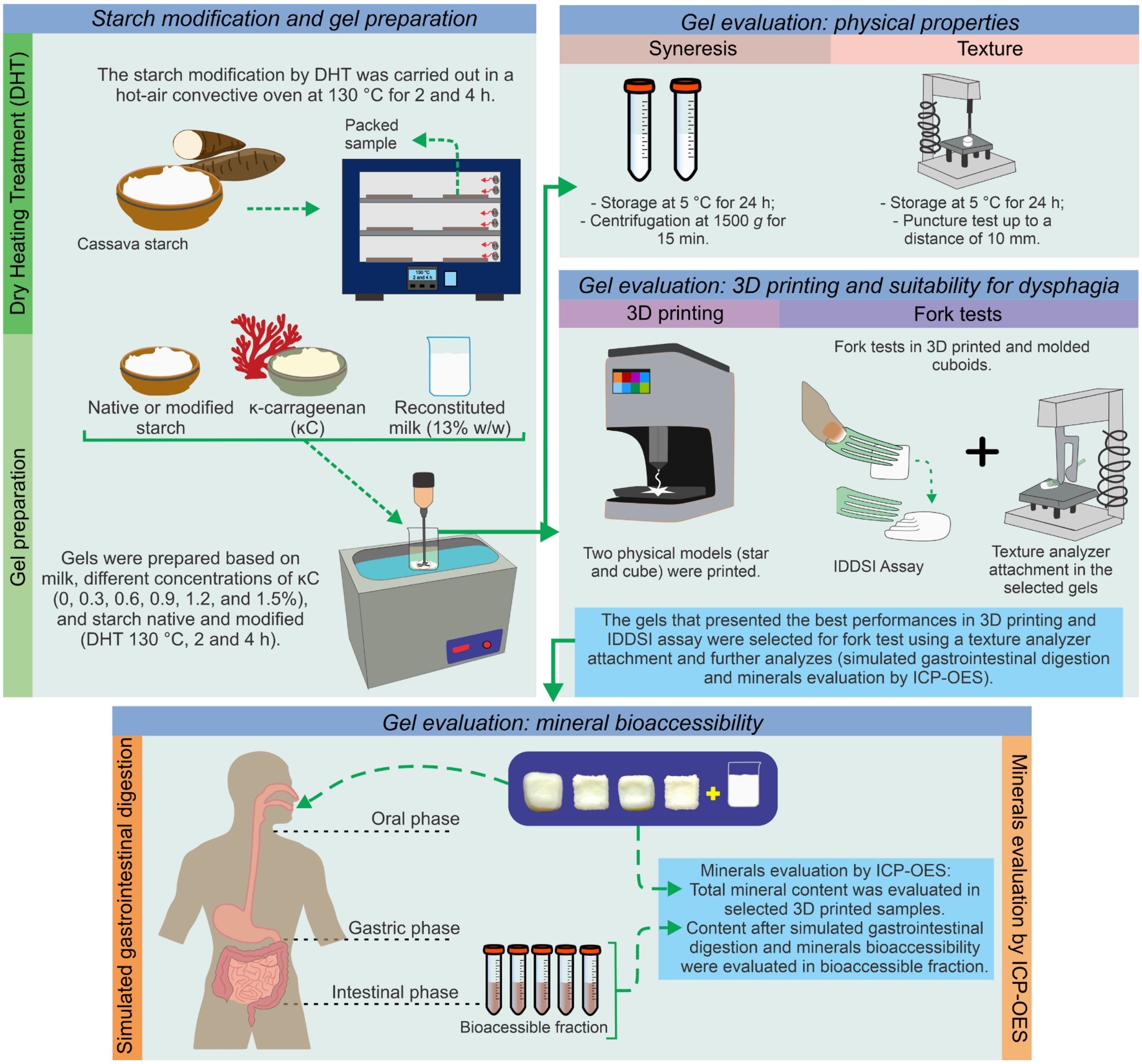

Fig. 1. Illustrative scheme of the study.

shown in Fig. 1.

2.1. Starch modification and gel preparation

The gels were produced using milk and gelling ingredients (native or modified starches and/or κC (AgarGel, Brazil)). The native cassava starch was kindly supplied by Podium Alimentos (Tamboara, Brazil).

The modification of cassava starch was performed by DHT as described by Lim et al. (2002), under the same conditions that resulted in better 3D printing by Maniglia et al. (2020) - (130 °C for 2 and 4 h). The process consisted in a thin layer of cassava starch (50 g) distributed and packed in aluminum foil (30 × 30 cm) which was closed with adhesive tape to avoid losses during the process. The samples were taken to a hot-air convective oven (MA 035, Marconi, 1 ± 0.1 $m \cdot s^{-1}$) at 130 °C for 2 and 4 h (named DHT-2 h and DHT-4 h, respectively). After DHT processing and cooling to room temperature, the samples were sieved through a 250 μm (60 mesh) sieve and stored in glass containers.

For the preparation of the gels, the skim powdered milk was reconstituted with distilled water following the procedure recommended by the manufacturer (Piracanjuba, Brazil), which resulted in a reconstituted milk with 13% w/w solids. It was stored for 12 h under refrigeration before preparing the gels, to assure complete hydration. The gels were prepared using 18% w/w total solids, from which 13% were the dairy solids and 5% were the developed gelling ingredients (starch and/or κC). Among the 5% of gelling ingredients, the proportions of starch (100, 99.7, 99.4, 99.1, 98.8 and 98.5%) and κC (0, 0.3, 0.6, 0.9, 1.2 and 1.5%) were varied.

The samples were gelatinized in a glass beaker covered with aluminum foil and placed in a water bath for 20 min at 90 ± 3 °C, under constant agitation (250 rpm) using a mechanical stirrer. Then, the pastes were cooled down and stored at 5 °C for 24 h in appropriate containers for each analysis performed, which were placed in a desiccator with water at the bottom to maintain uniform moisture.

2.2. Characterization of the obtained gels: Firmness and syneresis of the gels

Gel firmness was measured by the puncture method using a Texture Analyzer (TA.XT Plus, Stable Micro Systems Ltd., Surrey, UK) with a load cell of 50 kgf (490.3 N). The test consisted of penetrating the samples with a cylindrical probe (P/0.5R, 12.7 mm of diameter) to a distance of 10 mm at 1 $mm \cdot s^{-1}$, being recorded the force over penetration distance.

Syneresis analysis followed the method described by Yeh and Yeh (1993). Briefly, the starch pastes, prepared as described previously, were deposited in 15 mL Falcon tubes, and stored at 5 °C for 24 h. Then, the gels were centrifuged at 1500 *g* for 15 min and syneresis was determined as the amount of water (g water/100 g gel) released.

2.3. 3D printing of the obtained gels

The 3D printing foods (i.e., the gels) were prepared as described in section 2.1. The pastes were transferred to printing syringes (60 mL) and stored at 5 °C for 24 h before printing. Then, the gels were immediately processed in a 3D printer (Wiiboox Sweetin, Naajing Wiiking3D Technology Co. Ltd., China), using a 0.84 mm diameter metal nozzle at 20 °C. Two physical models (star and cuboid) were designed using Tinkercad (online running, Autodesk) software and Cura software (version 15.02.1) was used as slicing software.

Each model had different dimensions and printing properties. The star was designed with dimensions of 5 × 5 cm (width × length) and 5 layers were deposited, with nozzle travel and infill speed of 5 $mm \cdot s^{-1}$; this model was chosen to evaluate the ability of the gels to form well-defined angles and lines. The cuboid had dimensions of 1.5 × 1.5 × 0.75 cm (width × length × height) and printing parameters of nozzle travel and infill speed of 10 $mm \cdot s^{-1}$; this model was chosen to be tested by the fork tests as proposed by IDDSI, and additionally, the texture analyzer attachment, as described in the next section.

2.4. Fork test of the obtained gels: IDDSI assay and texture analyzer attachment

It was evaluated if the gel properties suit the needs of people who have swallowing and chewing difficulties (dysphagia), considering the procedure established by the International Dysphagia Diet Standardization Initiative - IDDSI (2019a, 2019b). The fork test was conducted to define whether the gels could be classified according to the IDDSI standard. The analysis consisted of pressing down the tines of the fork on the gel, with the thumb on the base of the fork, until observing the blanching of the thumbnail, and then the classification was made based on the IDDSI descriptions for each level food. The force required to blanch the thumbnail is consistent with the tongue force used during swallowing, justifying the procedure (IDDSI, 2019a, 2019b).

The test was applied to the printed and molded cuboids with the same dimensions (1.5 × 1.5 × 0.75 cm; width × length × height), both after storage at 5 °C for 24 h. The molded cuboids were cut from the cylindrical mold (36 × 21 mm, diameter × height), while the printed cuboids were produced as described in section 2.3. The gels that presented the best performances in 3D printing and dysphagia suitability tests were selected for instrumental analysis of the fork test, using an attachment developed to couple the fork with a texture analyzer (Lancha et al., 2022). This test was conducted to simulate the manual fork test, but with controlled force, speed, and resting time of the fork on the gels, as well as registering the force across sample penetration. This attachment is compatible with the Texture Analyzer (TA.XT Plus, Stable Micro Systems Ltd., Surrey, UK) and allows fork test analysis to be carried out on foods under standardized conditions, avoiding errors and being able to assess food characteristics more deeply. More details are provided by (Lancha et al., 2022). An analyzes similar to that described by Pematilleke et al. (2022) was conducted.

A scale was used to define the force needed to blanch the thumbnail. For this, different tests were conducted to ensure accuracy: (1) the thumb was positioned in the center of the scale and pressed until the nail was blanched; (2) the thumb was placed on the base of the fork, placed on the scale and pressed until the nail was blanched; (3) the sample, with the same dimensions mentioned before, was positioned in the center of the scale and the fork test was performed until the nail was blanched; (4) the same trained person conducted all the tests. The exercised “weight” needed to blanch the nail was 490 g (corresponding to a force of 4.8 N).

The analysis consisted of penetrating the selected gel (molded or 3D printed) with the fork attached to the Texture Analyzer until reaching a force of 4.8 N. Upon reaching 4.8 N, the fork remained exerting that force for 5 s, then returned to the initial state, simulating the analysis described by the IDDSI.

Then, an additional assessment of mineral bioaccessibility was performed on the selected gels, as described below.

2.5. Simulated gastrointestinal digestion and mineral bioaccessibility

In vitro digestion was performed for the gels selected in the previous steps (section 2.4). Furthermore, as control treatments, it was also performed for the reconstituted milk cooked under the same gelatinization conditions as the gels (90 °C and 20 min; named as “Cooked milk”), in addition to a blank correspondent to the enzymes, using water instead of the sample.

The INFOGEST 2.0 *in vitro* static gastrointestinal simulation was performed according to the protocol described by Brodkorb et al. (2019), with some modifications. The schematic representation can be seen in Fig. 1. In vitro gastrointestinal digestion consisted of a 3-phase process: oral, gastric, and intestinal. All reagents used were purchased from Sigma–Aldrich, Buchs, Switzerland. For the oral phase, the samples were mixed with water (1:1, w/w) and salivary amylase (75 U/mL). The samples were incubated at 37 °C for 2 min under agitation. After incubation, the gastric phase was started, adding water (1:1, w/w) and pepsin (2000 U/mL) in the bolus from the oral phase and the pH was adjusted to 3 using HCl 5 M. The samples were then incubated under agitation at 37 °C for 2 h. Finally, for the intestinal phase, water (1:1, w/w), pancreatic lipase (2000 U/mL), bile salts (10 mM) and pancreatin (100 U/mL) were added to the gastric chyme, being the pH adjusted to 7 using NaOH 5 M. The samples were again incubated at 37 °C for 2 h under agitation. Then, the samples were centrifuged at 10,000 *g* for 10 min at 4 °C, and the supernatants (bioaccessible fraction) were collected, filtered through filter paper, and stored at −80 °C until the time of subsequent analyses. The supernatant was further evaluated to measure the mineral bioaccessibility (as described in section 2.6).

2.6. Determination of minerals by Inductively coupled plasma Optical emission Spectrometer (ICP-OES)

Mineral quantification was performed to determine the total content, content after simulated gastrointestinal digestion and mineral bioaccessibility. Descriptions of the evaluated samples are specified below:

1) Total mineral content: was performed in cooked milk and in selected gel samples (section 2.4), after gelatinization, storage at 5 °C for 24 h, and 3D printing (sections 2.1 and 2.3). The samples were freeze-dried, ground, and mineralized before being evaluated in ICP-OES. The mineralization of the samples was performed to release all the mineral content present in the gels and followed the subsequent procedure: 250 mg of sample was mixed with 6 mL of HNO_3 (20% v/v) and 2 mL of H_2O_2 (30% v/v) (Merck KGaA, Germany), and heated (160–230 °C) in a microwave oven with a high-pressure reactor (UltraWAVE MCLA 1000–60, Milestone, Italy). After the heating procedure, the samples were cooled to room temperature and the volume was made up to 25 mL with demineralized water. Blank samples using water instead of the samples were included. After mineralization, the samples were evaluated in ICP-OES.

2) Content after simulated gastrointestinal digestion and mineral bioaccessibility: were evaluated after simulated gastrointestinal digestion, and in enzymatic blank samples (section 2.5), through ICP-OES. Bioaccessibility was calculated by dividing the content after simulated gastrointestinal digestion and the total mineral content.

The total mineral content, content after simulated gastrointestinal digestion and mineral bioaccessibility was determined through an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES, iCAP 7000, Thermo Scientific, USA). The following operational parameters were used in the analysis by the ICP-OES: power radio frequency = 1.2 kW, plasma gas flow = 12 $L \cdot min^{-1}$, auxiliary gas flow = 0.5 $L \cdot min^{-1}$, nebulizer gas flow = 0.6 $L \cdot min^{-1}$, sample aspiration rate = 1.5 $mL \cdot min^{-1}$, emission lines: Ca II (λ = 184.006 nm), Mg II (λ = 285.213 nm), K I (λ = 769.896 nm), and P I (λ = 213.618 nm). To test the accuracy of the method, a SRM 1549 - Non-Fat Milk Powder - NIST (National Institute of Standards & Technology) certified reference material was used.

2.7. Experimental design and statistical analysis

All processes and analyzes were performed in triplicate in a completely randomized design and the results were expressed as means ± standard deviation. To determine statistical differences, the results were evaluated by Analysis of variance (ANOVA) and Tukey test at 5% significance using Minitab® software (version 19.2, USA).

3. Results and discussion

3.1. Characterization of the obtained gels: Effect of gelling ingredients (native and DHT modified starches + κC) on gel firmness and syneresis

In this study, we evaluated the firmness of gels prepared with skim milk gelled with native or modified cassava starches (by the DHT process at 130 °C for 2 and 4 h) and added with different concentrations of κC. The gel firmness was evaluated by the puncture/penetration test, being the results shown in Fig. 2.

The process of starch modification by DHT did not result in significant differences for the gel firmness, differently of some previous studies (La Fuente et al., 2022; Lima et al., 2021; Maniglia et al., 2020), due to the high variability of this particular raw material (Maniglia et al., 2021). On the other hand, the increase in the concentration of κC leads to a significant increase in the gel firmness (Fig. 2), given that κC has a dominant effect on the textural properties of the gels (Verbeken et al., 2004).

Wang et al. (2021) reported the same behavior for κC. In fact, as the concentration of κC or κC + konjac gum increased in milk puddings, the structure of milk pudding was more compact, reflecting in greater energy required for gel deformation. The authors suggested the network structure formed by κC and konjac gum and the specific interaction between the positively charged amino residues of casein and the negatively charged sulfate groups of κC are responsible for the observed compacting gel. In fact, there is an interaction between κC and milk casein micelles (Spagnuolo et al., 2005), and between κC and calcium (Agoda-Tandjawa et al., 2017), which increase gel firmness (Verbeken et al., 2006). The increase in the gel firmness due to addition of κC in cassava starch gels is an interesting result, since they become more suitable for industrial applications than the pure cassava starch gels, especially considering application in 3D printing.

The syneresis analysis was also conducted on the gels based on milk, native or modified cassava starches (native, DHT-2 h, and DHT-4 h), and added with different concentrations of κC. According to Pant et al. (2021), syneresis refers to the unwanted release of water from the gels, impairing the visual and technological aspect of the product. In addition, a product that exhibits syneresis is not interesting for application in this study, due to two reasons: (i) considering 3D printing, it results in non-stable prints, that easily collapse; (ii) and, most importantly, syneresis can result in aspiration of dysphagic foods (Funami, 2011), potentially resulting in health complications.

In this study, syneresis was not observed in any condition evaluated after storage at 5 °C for 24 h up to 7 days (Figure S1). Therefore, this result is interesting from the point of view of application in 3D printing and to be consumed by dysphagic people – whose potential were evaluated, as follows.

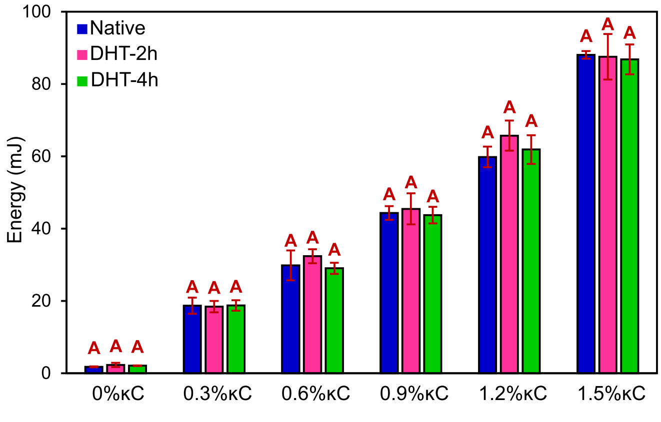

Fig. 2. Firmness (evaluated as the energy necessary for a 10 mm punctual assay) of gels based on milk, different concentrations of κC (0, 0.3, 0.6, 0.9, 1.2, and 1.5%), and cassava starch, native and modified by (DHT 130 °C, 2 and 4 h). Gelling ingredients (κC + starch) was fixed in 5%. Vertical bars indicate the standard deviation. Equal letters indicate that there were no significant differences ($p > 0.05$) among the different formulations with native or modified starches at the same κC concentration.

3.2. 3D printing performance

Fig. 3 shows the 3D printed star-shaped structure using the milk gels. Prior to 3D printing, the gels were stored at 5 °C for 24 h for gelling. 3D printing performances were evaluated in terms of comparing the printed sample with the pre-designed star geometry (3D design project), formation of well-defined angles, continuous line formation, smooth extrusion, surface spread, and shape retention after printing, as described in Table 1.

In fact, many factors are related to the quality of extrusion-based 3D food printing, such as whether the food can be extruded and support the layers that are deposited above it, without collapsing. In this context, literature reports that native cassava starch hydrogels are unsuitable for application in 3D printing, as they form printed "lines" that spreads out on the surface and are not strong enough to maintain their shape after printing (Maniglia et al., 2020). In fact, cassava starch is known to form a weak hydrogel in comparison with other sources (Castanha et al., 2021). However, cassava is an important starch source due to its versatile properties, applications and high worldwide production. For this reason, it is important to study alternatives to improve the gel properties of this starch.

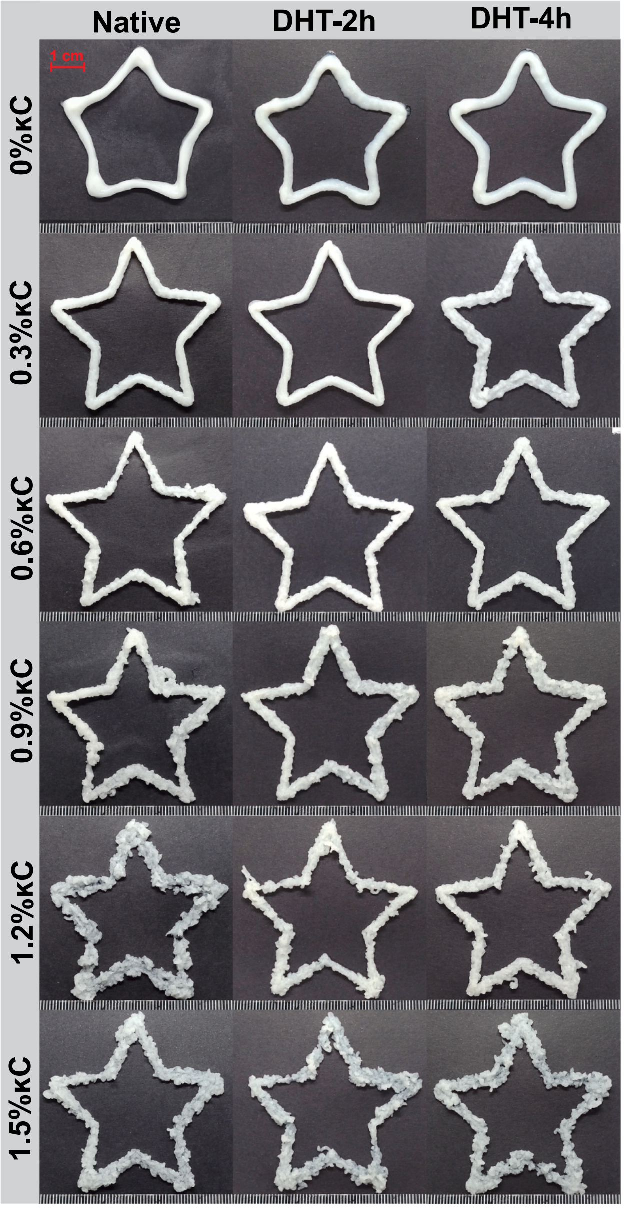

Fig. 3. Images of the stars printed by 3D printing for gels based on milk, different concentrations of κC (0, 0.3, 0.6, 0.9, 1.2, and 1.5%), and native and modified starch (DHT 130 °C, 2 and 4 h). Gelling ingredients (κC + starch) was fixed in 5%.

The 3D printed star-shaped structure using gels based on milk and native starch, without addiction of κC (0% κC) did not present good printability, without definition of the star angles (Fig. 3).

As the concentration of κC was increased, the firmness of the gels also increased, which was reflected in the printing quality. At a concentration of 0.3% κC, the gels prepared with native starch and DHT-2 h presented accurate impressions, very similar to the pre-designed star geometry, with well-defined star angles, smooth extrusion, and continuous lines, with no surface spreading and no deformation of the structure as the layers were deposited.

The same behavior was not observed for the printing of the gels with modified starch DHT-4 h and 0.3% κC, where the extrusion was hampered, resulting in lower resolution and extrusion of non-continuous lines. During the treatment with DHT in cassava starch, partial depolymerization of the starch molecules occurs, which results in better reassociation and packaging and, consequently, a change in behavior during 3D printing - as observed by Maniglia et al. (2020).

From 0.6% κC, an increasing loss in printing quality is observed for all starches (native, DHT-2 h, and DHT-4 h), with a high surface spreading index during printing, low printing resolution, and difficulty of extrusion. We can correlate this behavior with the texture previously evaluated (Fig. 2), where a significant and gradual increase in gel firmness was observed with increasing κC concentration. Cai et al. (2022) also observed similar behavior in the 3D printing of custard cream. The authors observed that a concentration of 0.5% κC was essential for maintaining the shape of the custard cream after 3D printing; however, the continuous increase in the concentration of κC impaired the printing performance.

In fact, both very weak and very strong gels result in poor-quality 3D printing, due to their difficulty in maintaining the shape after impression (formulations containing 0% κC) or difficulty in extrusion (formulations containing from 0.6% κC).

Considering the gels prepared in this study, only two formulations met all the desired requirements for good-quality 3D printing: native starch with 0.3% κC, and DHT-2 h starch with 0.3% κC (Table 1). However, it is still necessary to assess whether these formulations are suitable for consumption by dysphagic people. For this reason, we also evaluated the performance of the gels based on skim milk and gelling agents - native or modified cassava starches (DHT-2 h and DHT-4 h) and different concentrations of κC in the fork tests, described in the next section.

3.3. Suitability for dysphagic diets: The fork test

3.3.1. IDDSI standard assay

The IDDSI (2019a, 2019b) provides standardized terminology as well as testing procedures using simple utensils such as a fork to determine at what level a food fits and its suitability for dysphagic people. As described, the fork test was used in this study to assess whether gels (printed in the cuboid shape or molded in the same dimensions) are suitable for the dysphagic people's diet (Fig. 4).

The fork test can be used to assess whether foods fall into the categories "4 - Puree", "5 - Minced and moist", "6 - Soft and bite-sized", and "7 - Easy to chew", according to IDDSI (2019a). In this study, due to the characteristics of the gels and their behavior during the test proposed by the IDDSI, the samples were classified between levels 5 and 6, as shown in Table 1. According to IDDSI, for the food to be classified as Level 5 - Minced and moist, it must be easily mashed with little force exerted on the fork, without blanching the thumbnail, the particles must be easily separated and pass through the tines/prongs of the fork. In order to be classified as Level 6 - Soft and bite-sized, during the fork test the sample must squash, break apart, change shape, and not return to its original

Table 1

Parameters analyzed for 3D printing of star-shaped design (Fig. 3), and IDDSI fork test on gels (printed in the cuboid shape or molded in the same dimensions, Fig. 4) based on milk, different concentrations of κC (0, 0.3, 0.6, 0.9, 1.2, and 1.5%), and starch native and modified (DHT 130 °C, 2 and 4 h). Gelling ingredients (κC + starch) was fixed in 5%. IDDSI fork test was performed on 3D printed or molded gels. Symbols: ✔ = yes; X = no. The colors to define the classified level 5 (orange) and level 6 (blue) were based on IDDSI framework graphic (Cichero et al., 2017).

			IDDSI fork test				3D printing performance				
κC concentration	Treatment		Squashed by the fork	Tends to return to original form	Thumbnail blanching is observed	Level	Similar shape to the pre-designed star geometry with well-defined angles	Continuous lines	Smoothly extruded	Does not spread on the surface and retains its shape after printing	Was the treatment selected for further analyses?
What is desirable for Level 5?			✓	✗	✗	-	✓	✓	✓	✓	-
What is desirable for Level 6?			✓	✗	✓	-					
0%	Native	3D printed gel	✓	✓	✗	-	✗	✓	✓	✗	✓ (Control)
		Molded gel	✓	✓	✗	-	-	-	-	-	
	DHT-2h	3D printed gel	✓	✓	✗	-	✗	✓	✓	✗	✓ (Treatment control)
		Molded gel	✓	✓	✗	-	-	-	-	-	
	DHT-4h	3D printed gel	✓	✓	✗	-	✗	✓	✓	✗	✗
		Molded gel	✓	✓	✗	-	-	-	-	-	
0.3%	Native	3D printed gel	✓	✗	✗	Level 5	✓	✓	✓	✓	✓
		Molded gel	✓	✗	✓	Level 6	-	-	-	-	
	DHT-2h	3D printed gel	✓	✗	✗	Level 5	✓	✓	✓	✓	✓
		Molded gel	✓	✗	✓	Level 6	-	-	-	-	
	DHT-4h	3D printed gel	✓	✗	✗	Level 5	✓	✗	✗	✓	✗
		Molded gel	✓	✗	✓	Level 6	-	-	-	-	
0.6%	Native	3D printed gel	✓	✗	✗	Level 5	✓	✗	✗	✓	✗
		Molded gel	✓	✗	✓	Level 6	-	-	-	-	
	DHT-2h	3D printed gel	✓	✗	✗	Level 5	✓	✗	✗	✓	✗
		Molded gel	✓	✗	✓	Level 6	-	-	-	-	
	DHT-4h	3D printed gel	✓	✗	✗	Level 5	✓	✗	✗	✓	✗
		Molded gel	✓	✗	✓	Level 6	-	-	-	-	
0.9%	Native	3D printed gel	✓	✗	✗	Level 5	✗	✗	✗	✗	✗
		Molded gel	✓	✗	✓	Level 6	-	-	-	-	
	DHT-2h	3D printed gel	✓	✗	✗	Level 5	✗	✗	✗	✗	✗
		Molded gel	✓	✗	✓	Level 6	-	-	-	-	
	DHT-4h	3D printed gel	✓	✗	✗	Level 5	✗	✗	✗	✗	✗
		Molded gel	✓	✗	✓	Level 6	-	-	-	-	
1.2%	Native	3D printed gel	✓	✗	✗	Level 5	✗	✗	✗	✗	✗
		Molded gel	✗	-	✓	-	-	-	-	-	
	DHT-2h	3D printed gel	✓	✗	✗	Level 5	✗	✗	✗	✗	✗
		Molded gel	✗	-	✓	-	-	-	-	-	
	DHT-4h	3D printed gel	✓	✗	✗	Level 5	✗	✗	✗	✗	✗
		Molded gel	✗	-	✓	-	-	-	-	-	
1.5%	Native	3D printed gel	✓	✗	✗	Level 5	✗	✗	✗	✗	✗
		Molded gel	✗	-	✓	-	-	-	-	-	
	DHT-2h	3D printed gel	✓	✗	✗	Level 5	✗	✗	✗	✗	✗
		Molded gel	✗	-	✓	-	-	-	-	-	
	DHT-4h	3D printed gel	✓	✗	✗	Level 5	✗	✗	✗	✗	✗
		Molded gel	✗	-	✓	-	-	-	-	-	

shape when the fork is removed; moreover, thumbnail blanched is observed during the test. In addition, the behavior of the gels during the fork test (IDDSI assay) was evaluated in relation to some requirements described by the IDDSI: (1) Whether the gel is squashed by the fork; (2) Whether it is necessary to apply sufficient force to blanch the thumbnail for gel to be squashed; (3) Whether the gel tends to return to its original shape (Table 1).

The gels prepared with native starch and without κC (0% κC) were not suitable for consumption by dysphagic people, as they tend to return to their original shape after the force exerted by the fork is removed. In addition, this gel visually presented adhesive and sticky characteristic, that requires greater muscular effort during chewing (Steele et al., 2015). Foods with these characteristics can accumulate in the individual's oropharynx and lead to aspiration after swallowing (Schmidt et al., 2021; Park et al., 2017; Sungsinchai et al., 2019). The modification process (DHT-2 h or DHT-4 h) showed a similar result to Native starch gels, in that the gels tend to return to their original shape right after the fork is removed. Therefore, gels prepared with 0% κC were not classified within the levels established by the IDDSI and were not suitable for diets for dysphagic people.

When 0.3% κC was added to native or modified starches (DHT-2 h and DHT-4 h), the firmness of the obtained gels was increased (Fig. 2). This was reflected in the behavior of the gels during the IDDSI fork test: as seen in Fig. 4 and Table 1, the gels were easily squashed by the fork and their shape did not return to the original shape after the force exerted by the fork was removed, both for 3D printed and molded gels. However, we observed a difference in thumbnail blanching for the printed and molded gels during the test.

In the 3D printed gels, the force required to squash the gel with the fork was not enough to blanch the thumbnail, which characterizes the gels 3D printed prepared with native or modified starch (DHT-2 h and DHT-4 h) and 0.3% κC at Level 5 (Table 1). In fact, foods classified as Level 5 indicate they can be chewed with minimal effort and do not require biting, with tongue pressure alone being sufficient to disintegrate the food. Foods classified as Level 5 can be ingested by people who experience fatigue during chewing due to very weak masticatory muscles, poor tongue mobility, difficulty to eat Level 6 or 7 foods, missing teeth or ill-fitting dentures (Cichero et al., 2017; IDDSI, 2019a).

On the other hand, blanching of the thumbnail was observed to squash the molded gels, which characterizes molded gels prepared with native or modified starch (DHT-2 h and DHT-4 h) and 0.3% κC at Level 6 (Table 1). Foods classified as Level 6 are described as foods that mimic a "bite of food". Although the ability to bite is not required, the individual must have chewing skills. These foods can be consumed by people who

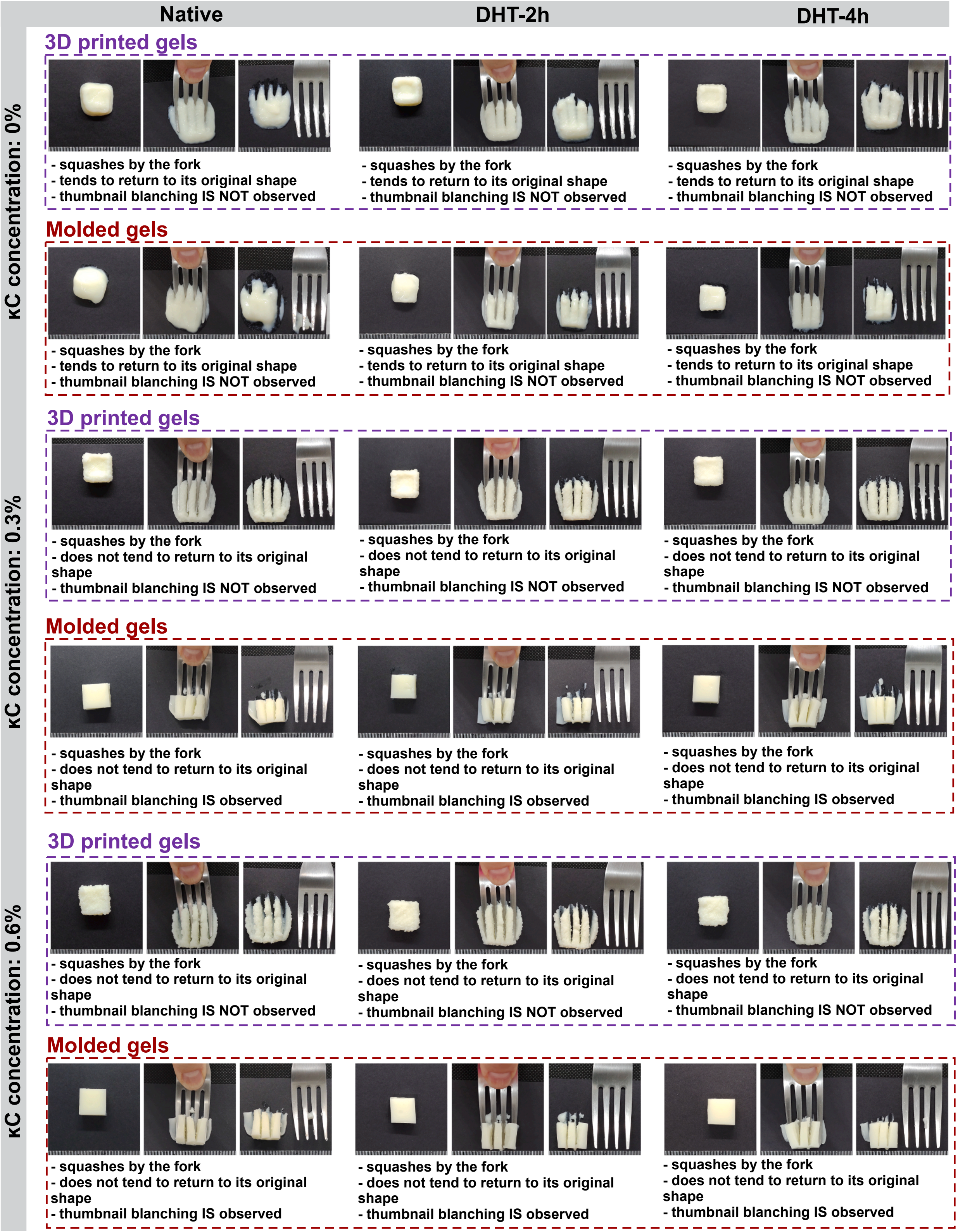

Fig. 4. IDDSI fork test on 3D printed and molded cuboids for gels based on milk, different concentrations of κC (0, 0.3, 0.6, 0.9, 1.2, and 1.5%), and native and modified (DHT 130 °C, 2 and 4 h) starches. Gelling ingredients (κC + starch) was fixed in 5%.

have weak masticatory muscles that lead to fatigue when chewing over time, asymmetrical chewing ability and limited rotational chewing, poor ability to reduce the size of Level 7 food particles, missing teeth or ill-fitting dentures (Cichero et al., 2017; IDDSI, 2019a).

For the other evaluated κC concentrations (0.6, 0.9, 1.2 and 1.5%), the behavior during the IDDSI fork test of the 3D printed cuboids for all starches (native, DHT-2H and DHT-4H) was similar to formulations containing 0.3% κC: the sample was easily squashed by the fork, does

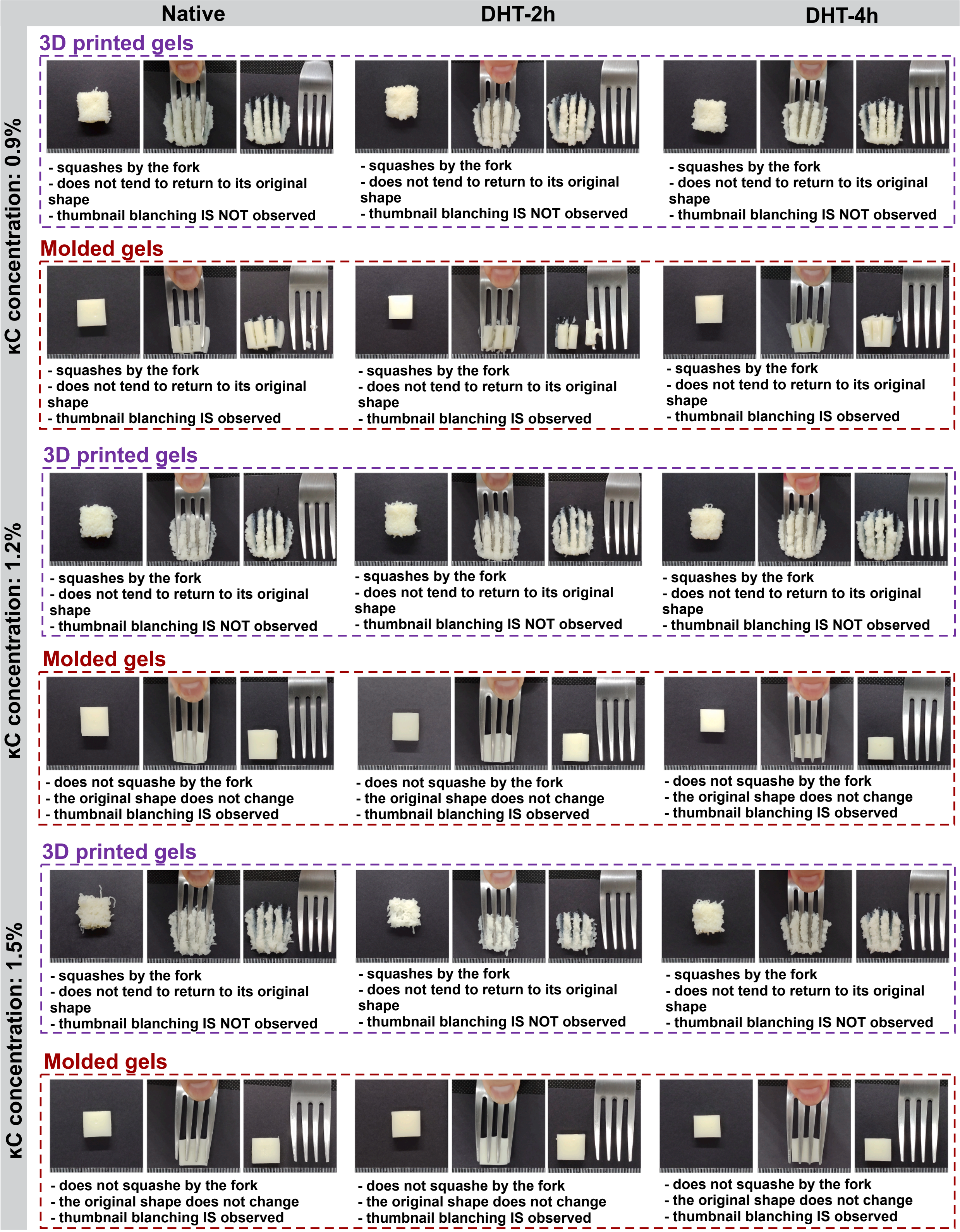

Fig. 4. (*continued*).

not tend to return to the initial shape and thumbnail blanching was not observed, being classified as Level 5.

Furthermore, molded gels prepared with 0.6 and 0.9% κC also exhibited similar behavior to gels molded with 0.3% κC: the gels were crushed by the fork while the thumbnail was observed to blanching and did not tend to return to the original shape, classified at Level 6. However, the molded gels with 1.2 and 1.5% κC were not suitable for dysphagic diets, as they were not squashed by the fork with the force necessary to blanch the thumbnail due to the large increase in firmness (Fig. 2). Therefore, they were not classified among the levels established by the IDDSI – and so they were considered not appropriate for dysphagic diets.

Besides, we observed that 3D printing allowed gels prepared with 1.2 and 1.5% κC, to be consumed by dysphagic people, independently of starch (native or DHT-2 h or DHT-4 h). On the other hand, this same formulation, when molded, was not suitable for this purpose. In fact, during 3D printing, the gels were extruded by a nozzle, which causes the initial structure of the gel to break. In study carried out by Le Tohic et al. (2018), changes in the texture of 3D printed cheeses were also observed, which showed lower hardness compared to non-printed (molded) materials. On the other hand, for cereal-based snacks, a behavior contrary to that reported in this study was observed. The 3D printed cereal-based snacks showed greater hardness compared to the product prepared in a traditional way, this is because the layer-by-layer deposition produced by 3D printing resulted in a cuboid with a denser structure, with larger pores and fewer pores inside when compared to the non-printed model (Derossi et al., 2020). Moreover, Strother et al. (2020) observed the same texture behavior and sensory properties in 3D printed or molded carrot puree mixed with different gums. Then, we can see that 3D printing can affect the texture in different ways for different products, and it may be possible to adapt them for different objectives.

In summary, 3D printing resulted in a product with broken structure due to the extrusion process and for this reason, 3D printed gels were classified at Level 5 while the same gels, when molded (non-printed), were classified at Level 6. This can be interesting and intended for individuals with different needs. Moreover, the gels based on milk, native starch and DHT-2 h with 0.3% κC were able to be consumed by dysphagic people and presented the best performances in 3D printing (discussed in section 3.2). Consequently, they were selected for the next evaluations. In addition, native starch and DHT-2 h gels without added κC (0% κC) were selected as control formulations.

3.3.2. *IDDSI assay using a texture analyzer*

The gels selected by the fork test of IDDSI (Native-0%κC, Native-0.3%κC, DHT-2 h-0%κC and DHT-2 h-0.3%κC) were then evaluated using an attachment developed to apply the fork test using a texture analyzer (Lancha et al., 2022). The results are shown in Fig. 5, as the energy required to penetrate the gel with the fork. Both 3D printed and molded gels were evaluated.

The fork test using an attachment coupled to the texture analyzer allows obtaining more accurate information about the behavior of the gels when penetrated by the fork. For instance, when we compared the different gel formulations (native or DHT-2 h starch, with 0 or 0.3% κC), we observed DHT for 2 h did not affect the energy needed to penetrate the gel (Fig. 5), where no differences were obtained by varying the starch treatment. We can also correlate the results of Fig. 5 with the gel firmness (Fig. 2), in which the gels prepared with 0.3% κC were firmer (Fig. 2) and required more energy for their penetration (Fig. 5), when compared to gels with 0% κC. In fact, firmer gels require more energy to be deformed. When comparing 3D printed and molded gels, prepared with the same formulation, we observed that 3D printing reduced the firmness of the gels in relation to the molded ones. It confirms the result presented for the IDDSI assay (section 3.3.1), where the 3D-printed gels were penetrated with the fork with a small force, not enough to blanch the thumbnail (Table 1).

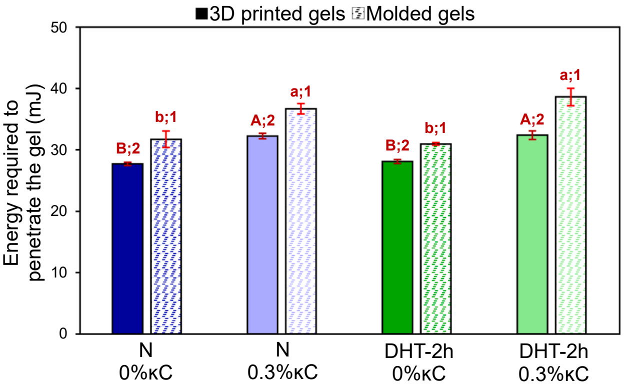

Fig. 5. Energy required to penetrate the gel (mJ) obtained from the fork test using a texture analyzer attachment on 3D printed and molded cuboids for previously selected gels based on starch/κC/milk: native (N) + 0 or 0.3% κC; and DHT-2 h + 0 or 0.3% κC. Gelling ingredients (κC + starch) was fixed in 5%. Statistical analysis by Tukey's test ($p < 0.05$): different big caps indicate a significant difference between the 3D printed gels for the different treatments; different small caps indicate a significant difference between the molded gels for the different treatments; different numbers indicate significant difference between 3D printed and molded gels for the same treatment.

Therefore, we observed that the selected gels (native and DHT-2 h with 0.3% κC) were suitable for dysphagic people and the addition of 0.3% κC was efficient to increase the firmness of the gels and improve their behavior during the fork test (evaluated both by the IDDSI assay and also by the texture analyzer). Therefore, analyze of mineral bioaccessibility was conducted to assess whether modifying milk structure and texture, by adding gelling agents, affect the release of food matrix of calcium, magnesium, potassium, and phosphorus.

3.4. *Mineral bioaccessibility*

Minerals are essential inorganic micronutrients that perform fundamental functions in the human body, from building strong bones to transmitting nerve impulses (Gharibzahedi & Jafari, 2017; Thakur et al., 2020). Being indispensable to the functioning of the body, minerals must be regularly present in the diet (Quintaes & Diez-Garcia, 2015). The essential minerals such as calcium, magnesium, potassium, and phosphorus are present in milk (Górska-Warsewicz et al., 2019); however, their presence does not guarantee bioaccessibility and/or bioavailability in the human body.

Bioaccessibility is defined as the portion of the compound (the mineral in this case) released in the gastrointestinal tract after the digestion process and which is available for intestinal absorption. To evaluate the mineral bioaccessibility in gels prepared with native or DHT-2 h starch, κC and milk (selected formulations in section 3.3.1), simulated gastrointestinal digestion using the INFOGEST 2.0 method was used.

Fig. 6 presents the results of bioaccessibility (ratio between calcium content after simulated gastrointestinal digestion and total calcium content) of calcium, magnesium, potassium, and phosphorus, for samples of cooked milk and previously selected gels based on starch/κC/milk (native + 0 or 0.3% κC; and DHT-2 h + 0 or 0.3% κC). All the gels were prepared considering the moisture content of each added product (starch, κC, and milk), to have the same final moisture and solids content: all gels had moisture of 82.5% (w.b.), and the reconstituted skim milk had 86.7% (w.b.).

It has been reported that calcium bioaccessibility is associated with changes in the balance of this mineral in the soluble and colloidal phases, as well as in the milk matrix, which is affected by processing (Teixeira et al., 2022a). By adding new ingredients to milk (gelling agents), we obtained a new food matrix/structure (gel). The food matrix is important in the kinetics of digestion and release of nutrients (Teixeira et al., 2022a), which could affect the bioaccessibility of the evaluated minerals. Even so, all samples presented the same calcium bioaccessibility ($p > 0.05$), showing high values (>80%). This is a very interesting result, reinforcing the texture modification strategy that does not reduce the calcium bioaccessibility in the proposed product. Moreover, the obtained results were similar to those reported for fortified, whole, semi-skim, and skim milks (Fioravanti et al., 2020; Lacerda Sanches et al., 2020; Perales et al., 2006) and higher than those reported for different types of cheese (Teixeira et al., 2022b) and yogurt (Teixeira et al., 2022a). It worth mentions those results are of high interest, since minerals, such as calcium, play essential roles in the functioning and development of the human body.

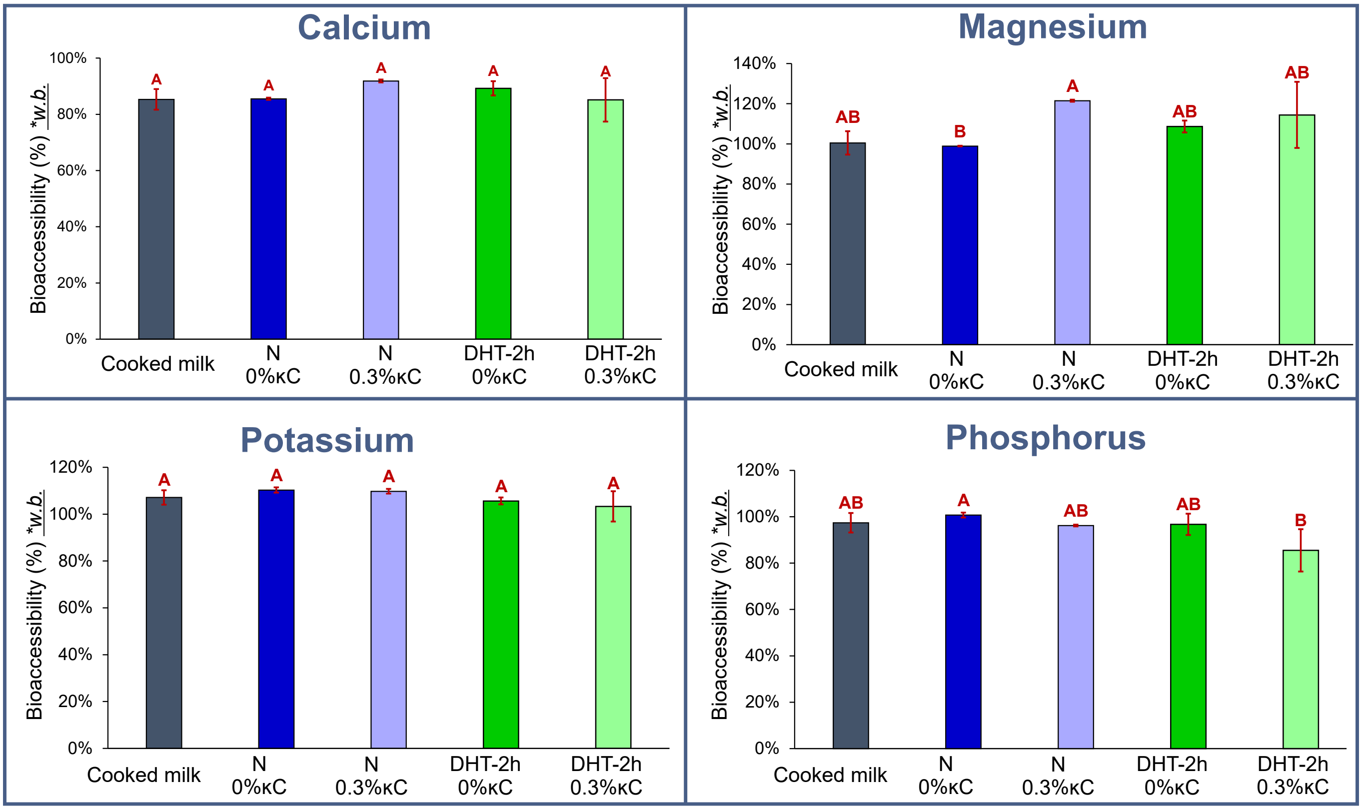

Fig. 6. Bioaccessibility of calcium, magnesium, potassium, and phosphorus for samples of cooked milk and previously selected gels based on starch/κC/milk: native + 0 or 0.3% κC; and DHT-2 h + 0 or 0.3% κC. Gelling ingredients (κC + starch) were fixed at 5%. Different letters indicate a significant difference ($p < 0.05$) among the formulations. The results are presented on wet basis (w.b.). Further information such as total mineral content and content after simulated gastrointestinal digestion is provided as supplementary material (Figure S2).

The obtained results were similar for magnesium, potassium, and phosphorus. It is possible to observe that the mineral bioaccessibility in the samples was always high than 90% (magnesium, potassium, phosphorus) or 80% (calcium), showing the potential for absorption of these minerals.

The strategy here presented, therefore, can be interesting considering milk is an important source of minerals, with difficult consumption by the dysphagic population. Calcium, for instance, plays crucial roles in human health, from children to senior people. Being a main component of bones and teeth, calcium is also important in muscle and nerve impulse reactions, cell division, among others (Zoroddu et al., 2019). Similarly, potassium, acts in the transmission of nerve impulses to the brain (Zoroddu et al., 2019), and phosphorus is an element present in key molecules, such as DHA and RNA (Elser, 2012). Magnesium participates in>300 enzymatic reactions, being essential for physiological functions such as heart rate, muscle contraction and relaxation, in addition to being necessary for bone formation (Jahnen-Dechent & Ketteler, 2012; Schwalfenberg & Genuis, 2017). Furthermore, it has been reported that dysphagia is one of the important symptoms of magnesium deficiency in the human body (Flink, 1978; Hamed & Lindeman, 1978). Indeed, one of the consequences of magnesium deficiency in the human body is neuromuscular decline (Holmes et al., 2016; Swaminathan, 2003), which may result in the manifestation of dysphagia symptoms (Flink, 1981).

Overall, the high bioaccessibility reported for these minerals is a positive result in terms of human consumption, highlighting 3D printing can be an interesting approach to tailor diets.

4. Final considerations

This study focused on developing ingredients and using 3D printing as strategies to modify the texture of milk, an important nutrient-rich food for humans, aiming its consumption by dysphagic people. Therefore, different technologies were studied, such as the addition of gelling agents - native or modified starch by the DHT technique in combination with κC. In addition, the gels formed by milk/starch/κC were 3D printed, evaluating the printing performance and the ability to develop a product that can be tailored to dysphagic diets. The suitability for dysphagic people was evaluated by the standard fork test, as suggested by the IDDSI (2019b), and also using a new probe that allows a fork to be attached to be a texture analyzer. Finally, the mineral bioaccessibility was evaluated through simulated gastrointestinal digestion following the INFOGEST 2.0 method.

Our study describes an interesting approach to tailor food texture for people with special needs. However, it is important to emphasize that, although the objective of our study was achieved, we have no intention of recommending the ingestion of these products indiscriminately, whose relevance should be evaluated in future studies, by dedicated groups and maybe clinical trials.

Moreover, this manuscript does not suggest the 3D printing of gelled milk as the only possibility of dairy products consumption. There are different dairy products already available, and their suitability for dysphagic diets must be evaluated (not only in relation to their texture, but also composition and relevance).

Different levels of dysphagia, associated with an overall clinical evaluation, demand specific approaches in relation to food and nutrition. Aspects such as possible allergens, sensitivity, and intolerance must also be considered before any prescription. Consequently, this study does not aim to be a reference from the medical nor nutritional points of view, nor does it proposes that starch and/or κ-carrageenan are the best gelling agents for milk in any case.

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 the number of available foods for dysphagic diets is limited, affecting not only the dysphagic people nourishment, but also their mood, which indirectly impacts their medical condition. Therefore, providing new alternatives for dysphagic people is a topic of high interest.

3D printing technology can be a protagonist in tailoring foods for special needs, but the ingredients to allow this must be developed. Our group, among others, is dedicated to provide technical alternatives to allow this development.

5. Conclusion

This study evaluated the effect of different formulations of gels based on milk and gelling agents (native and modified cassava starch by DHT and κC) to explore the emerging technology of 3D printing, as an alternative to create food for special needs – in this case, to hep dysphagic people. DHT slightly changes the starch performance for 3D printing, while κC was the agent with the greatest influence on the gel texture. The addition of 0.3% κC increased the gel firmness and achieved better 3D printing performance. The addition of gelling agents made the milk-based gels suitable for dysphagic people, considering the desired texture. The combination of ingredients and 3D printing was able to modify the texture of the gels, making them classified into Level 5 (3D printed gels) and Level 6 (molded gels), according to the evaluation proposed by IDDSI. In addition, all gels showed calcium bioaccessibility>80% and magnesium, potassium, and phosphorus>90%, demonstrating that, despite the techniques used to modify the texture, minerals can still have a high absorption potential. In conclusion, this study demonstrated an approach to modify the texture of milk, tailoring it for dysphagic diets, without affecting the mineral bioaccessibility.

CRediT authorship contribution statement

B.S. Bitencourt: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization. **J.S. Guedes:** Conceptualization, Methodology, Validation, Investigation. **A.S.M.C. Saliba:** Methodology, Validation, Investigation. **A.G.O. Sartori:** Methodology, Validation, Investigation. **L.C.R. Torres:** Methodology, Validation, Investigation. **J.E.P.G. Amaral:** Methodology, Validation, Investigation. **S.M. Alencar:** Methodology, Resources, Visualization, Supervision, Writing – review & editing. **B.C. Maniglia:** Methodology, Visualization, Supervision, Writing – review & editing. **P.E.D. Augusto:** Conceptualization, Methodology, Formal analysis, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: [One of the authors is an associate editor of the Food Research International - P.E.D. Augusto.].

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2023.113010.

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