DEVELOPMENT OF FORMULATIONS BASED ON VEGETABLE PROTEINS AND POLYSACCHARIDES FOR 3D FOOD PRINTING

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Abstract *Formulations with soy protein, corn starch, water, and hydrocolloids (guar gum and xanthan gum), at 1.25% and 2.50%, were used in a piston-driven extrusion 3D printer. The formulations were evaluated according to mechanical strength, stability, printing accuracy, syneresis, and partial centesimal composition. The formulation with 1.25% guar gum was the most efficient, standing out for its greater height of the layers, good printability, and lower syneresis, revealing great potential for 3D-printed food applications. Based on this result, this formulation was adapted, incorporating new ingredients to bring it closer to a meat burger. The 3D printing tests were repeated, along with comparative analyses of color, texture, and cooking yield, using a burger made from animal origin to evaluate appearance and flavor. The results showed promising potential; however, further studies are needed to optimize formulations and explore new ingredients, aiming to advance 3D printing technology in the food sector.*

Keywords. *3D food printing, Hydrocolloids, Printability, Analog meat burger.*

Introduction

The development of food processing technologies and the growing demand for customized and sustainable solutions are prompting the food industry to explore new methodologies to meet consumer needs (Isodeinovação, 2024). In this context, the 3D extrusion printing technique has been applied, offering benefits such as customization for the individual needs of elderly consumers, athletes, and patients with dysphagia (Lorenz *et al.*, 2022). In addition, there is the automation of the food production process, and the enhance sustainability by reducing food waste, minimizing packaging, and using alternative sources of nutrients (Yang *et al.*, 2015).

Extrusion 3D printing, also known as additive manufacturing, is a process in which a syringe, driven by heads, extrudes food materials onto a platform. The object is built layer by layer, based on a pre-established digital model (Ma & Zhang, 2022). The food materials used in this process are often called 'inks' because they can be extruded in a manner like ink used in traditional printing methods (Godoi *et al.*, 2016). According to Waseem *et al.* (2024), a successful 3D printing requires an understanding of the physical and chemical properties of the materials. Additionally, it is important to comprehend the printing process itself. This includes controlling the flow properties of the materials, testing different sizes and shapes of syringe nozzles, and analysing layer interactions to ensure precise deposition. Finally, the study of post-processing processes, including heat treatments, may be necessary to obtain the desired texture and flavor.

Some applications of extrusion 3D food printing include edible hydrogel inks, such as combining two hydrocolloids with flavor concentrates (Cohen *et al.*, 2009); and inks created from plants or meat, such as plant protein-based salmon fillets (Tay *et al.*, 2023).

Food inks formulated with vegetable proteins and hydrocolloids were developed to create meat analogues, reproducing the sensorial and nutritional characteristics of animal meat. The use of these raw materials not only allows the production of high-quality meat substitutes, but also contributes to reduce the environmental impact associated with the production of foods of animal origin (Kevany, 2019). This innovation follows the growing demand for sustainable food alternatives and reflects a trend towards more conscious and responsible consumption in the food industry.

This project was a collaboration with the PhD research project of Fernanda Sviech, supervised by professors PhD Ana Silvia Prata and PhD Kaciane Andreola, from the State University of Campinas (UNICAMP). The focus was on the isolation of a hydrocolloid from the ora-pro-nóbis (*Pereskia aculeata* Miller) mucilage, for the purpose of producing meat analogues using 3D food printing technology.

Objectives

This research aims to gather data on formulations that utilize widely commercialized hydrocolloids, such as xanthan and guar gums, in 3D food printing. This information will be crucial in determining whether the ora-pro-nobis mucilage demonstrates equivalent or superior performance in 3D food printing. Furthermore, the formulation that performed best was adapted for creating a meat-like product using plant-based ingredients.

Development

The food inks were prepared with xanthan gum (Ticaxan® Xanthan EC, Ingredion, Brazil) or guar gum (Pre-Hydrated® Guar Gum 8/24 Powder, Ingredion, Brazil), modified cornstarch (S) (National® 465, SP, Ingredion, Brazil), soy protein isolate (P) (Supro® 500e, IFF, SP, Brazil), 100% pure beetroot powder (Armazém Bezerra, Brazil), and distilled water according to the formulations described in Table 1.

To prepare 100 g of food inks, the hydrocolloids were first weighed and dispersed in distilled water for approximately 24 h. For the samples containing 1.25% of hydrocolloid, the mixture was stirred continuously without a lid on a magnetic stirrer, while the samples with 2.50% of hydrocolloid were left to stand, with the lid on, in the refrigerator. The more concentrated samples were not stirred due to the high consistency of the mixture, which required equipment with greater rotation capacity. After full dispersion, the remaining ingredients of each formulation were weighed and added to the mixture. Homogenization was performed manually using a glass stick to ensure that all components were thoroughly mixed. The samples were transferred using a spatula into 10 ml syringes, which were then attached to the 3D printer.

Table 1 – Composition of food ink formulations containing soy protein, cornstarch, guar gum, xanthan gum, beetroot powder and distilled water.

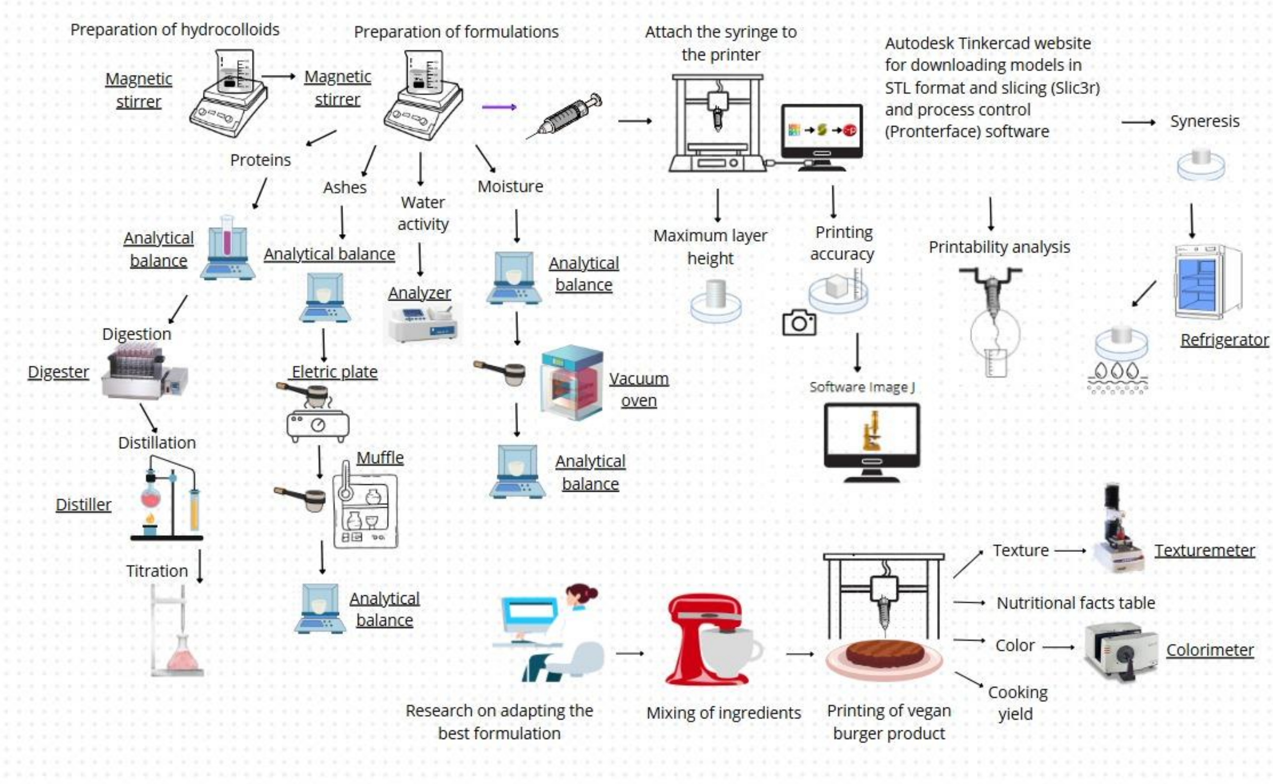
Formulation	%Soy protein	%Corn starch	%Guar gum	%Xanthan gum	%Beetroot powder	%Distilled water
1.25G	17.40	4.95	1.25	-	1.00	75.40
1.25X	17.40	4.95	-	1.25	1.00	75.40
2.50G	17.40	4.95	2.50	-	1.00	74.15
2.50X	17.40	4.95	-	2.50	1.00	74.15

The 3D printed models were made on an extrusion-based piston-driven 3D printer (Genesis II -3DBS, 3D Biotechnology Solutions, Brazil), as shown in Figure 1.

Figure 2 presents the research graphical abstract, illustrating the steps and tests carried out throughout the development of the project.

Figure 1 – Genesis II -3DBS 3D Printer

Figure 2 – Research graphical abstract



Maximum layer height provides the mechanical resistance of food inks, helping to understand the stability of stacked layers. The methodology described by Arii and Nishikawa (2023) was used in triplicate, in which a circular perimeter with a diameter of 28 mm was printed. The maximum layer height was recorded when the printed object fell over.

Printing accuracy was determined based on Wang *et al.* (2024). The printed model was in cuboid shape (15 mm x 15 mm x 15 mm, with 13 layers), with a filling density of 70%. The parameters, including the syringe nozzle diameter (1.2 mm), retraction speed (1.5 mm/s), and printing speed (1.5 mm/s), were determined based on preliminary tests (Sviech, 2021). After the obtention of three 3D print models, photographs were recorded and analyzed in the Image J program (National Institute of Mental Health, USA), in which the necessary measurements could be made. Thus, the printing accuracy was determined by Equation 1.

$$Printing\ accuracy\ (\%) = \frac{\left(\frac{(1-(h1-h2))}{h2}+\frac{(1-(h3-h2))}{h2}+\frac{(1-(l1-l2))}{h2}\right)*100}{3} \quad (1)$$

In which:
h_1: height of the edge of the printed samples (mm);
h_2: the defined height of the model (mm);
h_3: central height of the printed samples (mm);
l_1: the bottom length of the printed samples (mm);
l_2: the bottom length of the model (mm).

Printability is the characteristic that ensures both printing quality and efficiency, enabling the production of objects with precision and without interruptions. In this analysis, the methodology described by Shi *et al.* (2023) was followed in triplicate. Three 10 ml syringes, with a 1.2 mm diameter nozzle, were filled and attached to the 3D printer, which operated at a speed of 15 mm/s. To analyze only the filament, stars models were printed using previously established parameters, to evaluate the printability of figures. The classification used in this test was: Obstructed: formulations that obstructed the nozzle; Excessive fluidity: formulations that demonstrated spontaneous flow; Printable: formulations that produced a regular and continuous filament.

Syneresis is the process by which a gel releases liquid. It is often observed in food gels (Damodaran *et al.*, 2007) and is an undesirable characteristic in starch gels that are refrigerated. This experiment was based on Alvarez *et al.* (2024) in triplicate, in which a cylinder with 2 cm diameter and 1 cm height was printed. After printing, the samples were stored for 12 h at 5 °C. The degree of syneresis can be determined by measuring the mass lost before and during refrigeration.

The moisture was determined by desiccation using a vacuum oven drying (Instituto Adolfo Lutz, 2008), in triplicate. 3 g of sample were heated for 24 h in a vacuum oven (TE-395, Tecnal, Brazil) at 70°C, under a reduced pressure of 100 mmHg. The moisture content was calculated by Equation 2, in which *n* represents the mass loss during the process (g), and *p* represents the mass of the sample (g).

$$Moisture\ (\%) = \frac{100*n}{p} \quad (2)$$

Water activity (aw) indicates the availability of the water for biological and chemical processes (Food Safety Brasil, 2016). It was determined in triplicate using a water activity analyzer (Aqualab® 4TE, Meter, Brasil), which expresses a ratio between the vapor pressure of water in the food and the vapor pressure of pure water at the same temperature, in this case 22 °C. The scale ranges from 0 (no water available) to 1 (fully available water) and is essential for food safety, preservation and product quality over time.

Ash is the total mineral content present in a sample after the complete combustion of organic material. It includes essential elements such as calcium, phosphorus, iron, sodium, potassium, and magnesium. It was determined according to the Adolfo Lutz Institute (2008), in triplicate. 4 g of sample were carbonized on an electric plate and incinerated in a muffle furnace (Q.318.24, Quimis, Brazil) at 550°C, until the complete elimination of carbon. They were calculated by Equation 3, in which *c* represents the mass of ash (g), and *p* represents the mass of the sample (g).

$$Ash\ (\%) = \frac{100*c}{p} \quad (3)$$

Protein determination is essential to assess its nutritional value, since they are essential biological macromolecules composed of amino acids, playing crucial roles in cellular and tissue structures and in the functioning of the human body. The Kjeldahl method (Instituto Adolfo Lutz, 2008) was adapted for this test. 0.5 g of the sample was weighed into test tubes, and 1.5 ml of copper sulfate was added as a catalyst to accelerate the oxidation of organic material. Thus, the decomposition of organic material was carried out by digestion of the sample at 400 °C with concentrated sulfuric acid. Finally, the nitrogen present in the resulting acid solution was determined by steam distillation, followed by titration with dilute hydrochloric acid. Equation 4 presents the calculation required for this analysis.

$$Proteins\ (\%) = \frac{fca*(V1-V2)*14*0{,}02*fcp}{10*p} \tag{4}$$

In which:
V_1: volume of hydrochloric acid solution used in the sample titration (ml);
V_2: volume of hydrochloric acid solution used in the blank titration (ml);
fca: hydrochloric acid correction factor;
fcp: soy protein correction factor (5.71);
p: sample mass (g).

After determining the best-performing ink food formulation, NotCo's Vegan Smash Burger was selected as the benchmark for vegan burger. To achieve the texture and flavor, new ingredients were incorporated into the previously studied food ink. The new 250 g formulation was composed of 1.0% guar gum, 0.20% methylcellulose stabilizer (2304A, Denver®, Brazil), 12.96% pea protein (Pisane® C9 3120240, Blumos, Brazil), 6.96% soy protein, 1.98% modified cornstarch (S), 9.60% extra virgin coconut oil (Copra®, Brazil), 9.20% sunflower oil (Liza®, Brazil), 1.0% cocoa powder (Genuine®, Brazil), 0.40% chlorophyll powder (Chlorella®, Ocean Drop, Brazil), 0.40% 100% pure beetroot powder (Armazém Bezerra, Brazil), 0.24% monosodium glutamate (Ajinomoto®, Brazil), 0.96% salt (Cisne®, Brazil), 0.80% nutritional yeast, 1.60% chia flour, 1.74% onion powder, 0.40% garlic powder purchased from Casa do Norte Sabor do Brasil and 0.40% meat aroma 20533784 SD (Vogler®, Kerry, Brazil), and distilled water until 100%. It was established that practically 50% of the formulation would be composed of the food ink previously identified with the best performance, and the remaining ingredients based on the ingredients present in NotCo's Vegan Smash Burger.

Printability, print performance, maximum layer height, and syneresis tests were performed with new food ink formulation. These trials ensured that the new formulation was printable and met the desired quality standards.

The new food ink was charactered after post-processing by grill in a 22 cm diameter frying pan for 3 min at approximately 170°C. The texture, color, and yield were measured on the most similar and least burnt sides. These parameters were compared to those of an animal-based burger (Sadia), seeking to identify sensory similarities and differences.

The texture analysis evaluated the mechanical and sensory properties of the product, essential factors for consumer acceptance. Texture profile analysis (TPA) was conducted at 20 °C with a TA-XT2i texture analyzer (Stable Micro Systems, England), according to Libório *et al.* (2019). Three samples with dimensions of 3.5 cm wide, 4.5 cm long, and 7.5 cm high were used, maintaining a thickness equivalent to that of a hamburger of animal origin. The width was limited by the capacity of the 3D printer while the length followed the texture analyzer probe, resulting in an oval shape. The samples were subjected to two compression cycles, reaching 40% of their height, with a 3.5 cm probe (P36/R) and a speed of 5 mm/s. The parameters evaluated were hardness, elasticity, cohesiveness, gumminess, chewiness, and resilience. The color was evaluated in triplicate as described by Libório *et al.* (2019), using a HunterLab spectrophotometer (ColorQuest XE, USA).

The yield of the burgers after grilled was obtained by measuring the weight in triplicate of the samples before and after cooking on a frying pan, (Equation 5), as described in Piñero *et al.* (2008).

$$RC\ (\%) = \left(\frac{PACo}{PACr}\right) * 100 \tag{5}$$

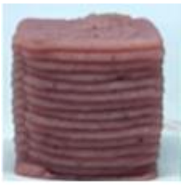
In which:
RC: cooking yield;
PACo: weight of the cooked sample;
PACr: weight of the raw sample.

The obtained results were subjected to analysis of variance (ANOVA) and Tukey's test for more than two formulations. For comparisons between two formulations, Student's t-test was used. All statistical analyses were performed in Minitab® software version 16.2.3.

Results and Discussion

The results in Table 2 showed that a higher concentration of hydrocolloid did not guarantee a greater number of printed layers. The formulations with guar gum showed the best performance. All formulations accurately reproduced the dimensions and details of the pre-established shape. The 1.25G formulation showed the lower mass lost over a 24 h period, a superior performance compared to the other formulations. This indicates greater stability, maintaining the desired consistency for a longer period, which is important to guarantee the quality and shelf life of the formulated product.

Table 2 – Maximum layer height, accuracy and syneresis test for 1.25G, 1.25X, 2.50G and 2.50X food inks

Formulation	1.25G	1.25X	2.50G	2.50X	HSD Tukey
Maximum number of layers (mm)	68 ± 4^{a} 	54 ± 6^{b} 	64 ± 3^{a} 	52 ± 2^{b} 	10
Precision (%)	$98.0 \pm 0.4^{a\,b}$ 	99.2 ± 0.6^{a} 	$98 \pm 1^{a\,b}$ 	96 ± 1^{b} 	1
Mass lost in 24h (g)	0.138 ± 0.009^{b} 	0.17 ± 0.01^{a} 	0.19 ± 0.01^{a} 	$0,192 \pm 0.005^{a}$ 	0.04

* Averages with the same letter, in the same line, do not differ significantly (p>0.05)

All formulations were considered suitable for 3D printing. This indicates that they presented a constant and controlled flow of material during printing. Furthermore, the results regarding star-shaped printing (Figure 3) were better in the formulations containing guar gum, which showed fuller and more stable designs.

Figure 3 – Star-shaped prints of the 1.25G, 1.25X, 2.50G, and 2.50X food inks

The moisture results (Table 3) indicate that the formulations with 2.50% gum were close to the expected value of 74.15%. On the other hand, the formulations with 1.25% gum presented results further from the expected value, which would be 74.40%. The difference can be attributed to the different process of the samples. There was no significant difference in water activity between the formulations. The ash contents were higher in formulations with xanthan gum, which may be related to its chemical composition and its purification process (Phillips & Williams, 2009). The protein contents in the formulations were slightly lower than 17.40%. This can be attributed to the inhomogeneous distribution of ingredients and the protein purity, which is around 90%.

Table 3 – Moisture, ash and proteins contents and water activity (*aw*) of the 1.25G,1.25X, 2.50G, 2.50X food inks

Formulation	Moisture (%)	*aw*	Ash (%)	Proteins (%)
1.25G	70.9±0.2 [d]	0.995±0.001 [a]	0.86±0.01 [b]	15.04±0.08 [a]
1.25X	72.65±0.09 [c]	0.991±0.003 [a]	0.91±0.00 [a]	14.53±0.04 [a]
2.50G	74.66±0.06 [a]	0.994±0.002 [a]	0.74±0.01 [c]	12.4±0.2 [c]
2.50X	74.3±0.2 [b]	0.992±0.001 [a]	0.93±0.02 [a]	13.2±0.3 [b]
HSD Tukey	0.41	-	0.05	0.9

* Averages with the same letter, in the same column, do not differ significantly ($p>0.05$)

Due to the unavailability of a rheometer suitable for characterizing the rheological properties of the samples, the tests initially proposed in the project could not be conducted.

While the maximum height did not differ between 1.25G and vegan burger, the 1.25G presented lower syneresis, indicating greater stability over time, while the vegan burger was more precise, suggesting greater consistency. To optimize the formulations, it is important to reduce the syneresis of this formulation, thus improving its stability and quality (Table 4). Figure 4 shows the results of maximum layer height, printing performance, printability, syneresis tests for vegan burger ink, and vegan burger formulation after printing and grill.

Table 4 – Comparison of maximum layer height, printability, printing performance, and syneresis tests of the vegan burger and 1.25G food ink formulations.

Formulation	Maximum layer height (mm)	Precision (%)	Syneresis (g)
1.25G	68 ± 4 [a]	98.0 ± 0.4 [b]	0.138 ± 0.009 [b]
Vegan burger	67 ± 1 [a]	99.5 ± 0.3 [a]	0.17 ± 0.02 [a]

* Averages with the same letter, in the same column, do not differ significantly ($p>0.05$)

Figure 4 –Maximum layer height (a), printing performance (b), printability (c), syneresis tests (d), and (e) vegan burger formulation after grill.

(a) (b) (c) (d) (e)

Table 5 presents the vegan burger nutritional fact table, considering the data of Vitat website (2024) and information from suppliers. According to RDC 429/2020 and IN 75/2020 (Brasil, 2020a, 2020b), the burger (80 g) does not contain added sugars and has sodium within the regulatory limits for front-of-pack lable (FOP) (184 mg/100 g). However, saturated fats total 9.1/ 100 g, exceeding the permitted limit for FOP of 6 g saturated fat/100 g. Therefore, it is necessary to include FOP labelling indicating "rich in saturated fats". It is necessary to perform the calculations for the product after grilling, considering water mass loss.

Table 5 – Nutritional facts table of the vegan burger food ink formulation before cooking/grill, according to Brazilian laws

INFORMAÇÕES NUTRICIONAIS

Porções por embalagem: 1

Porção: 80 g (1 unidade)

	100g	80 g	%VD*
Valor energético (kcal)	256	205	10
Carboidratos (g)	4.9	3.9	1
Açúcares totais (g)	0.18	0.15	
Açúcares adicionados (g)	0	0	0
Proteínas (g)	19	15	31
Gorduras totais (g)	18	14	22
Gorduras saturadas (g)	9.1	7.3	37
Gorduras trans (g)	0	0	0
Fibras Alimentares (g)	2.8	2.3	9
Sódio (mg)	184	147	7

*Percentual de valores diários fornecidos pela porção.

The texture parameters in the vegan and animal-based burgers were significantly different, except for hardness and elasticity. This information is essential for the development and optimization of food products, allowing targeted adjustments in the formulation to achieve the desired characteristics. The standard deviation was high for hardness and springiness, which indicates the need for a greater number of essays to ensure the reliability of the results, considering the possibility of experimental errors that could compromise the precision of the data obtained.

In addition, the results show that the meat-based burger has higher values of a^*, b^*, L^* indicating a more reddish, yellowish and light hue, while the vegan burger is darker and less saturated. In terms of cooking yield, the vegan burger retains more water (85%) compared to the animal-based burger (77.4%), which may favor its texture and juiciness after preparation (Table 6).

Table 6 - Texture, color and cooking yield tests for vegan burger and animal-based burger food ink formulations

	Vegan burger	Animal-based burger
Hardness	36 ± 17 [a]	71 ± 11 [a]
Springiness	0.7 ± 0.2 [a]	1.03 ± 0.05 [a]
Cohesiveness	0.36 ± 0.08 [a]	0.801 ± 0.007 [b]
Gumminess	14 ± 8 [a]	57 ± 9 [b]
Chewiness	9 ± 6 [a]	69 ± 15 [b]
Resilience	0.18 ± 0.06 [a]	0.35 ± 0.03 [b]
Color		
a^*	2.20 ± 0.02 [a]	5.5 ± 0.4 [b]
b^*	2.1 ± 0.3 [a]	9.1 ± 0.2 [b]
L^*	37 ± 2 [a]	46 ± 1 [b]
Cooking yield (%)	85 ± 2 [a]	77.4 ± 0.3 [b]

* Averages with the same letter, in the same line, do not differ significantly (p>0.05)

Conclusion

This project demonstrated the potential of food ink formulations based on soy protein, hydrocolloids, cornstarch and water for 3D printing of food, highlighting the feasibility and applicability of this emerging technology. The results highlighted the 1.25G food ink formulation as the most effective, as it presented superior results in maximum layer height, printability and syneresis, indicating fuller and more stable figures. This research provided essential information to explore the potential of ora-pro-nobis mucilage that is being developed by PhD student Fernanda Sviech. The adaptation of this formulation to produce a vegan burger resulted in a product with satisfactory textural characteristics, although there are still differences in relation to the animal-based burger, especially in texture and color. However, there is a need for additional studies to optimize the formulations and explore new ingredients that can further improve the stability, texture and flavor of 3D printed products.

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