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# 3D printing of microalgae-enriched cookie dough: determining feasible regions of process parameters for continuous extrusion

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## Abstract

Microalgae can be part of the solution to the global food crisis, as they have high nutritional values. Recently, 3D printing of microalgae-enriched snacks has been reported with the capability to customize nutritional profiles, shapes, and textures of the snacks. Because the process parameters of extrusion-based 3D printing affect the printability of cookie dough, it is important to know the levels of process parameters leading to continuous extrusion. This study investigated feasible regions of printing process parameters for continuous extrusion of microalgae (*Arthrospira Platensis*) enriched cookie dough. The process parameters studied were nozzle diameter, printing speed, and air pressure. The feasible regions were determined by visual inspections of printed strands. The results show that, for smaller nozzle diameters and higher printing speeds, higher air pressures are required to ensure continuous extrusion. The identified feasible regions from this study would be helpful when deciding the appropriate nozzle diameter, printing speed, and air pressure to print microalgae-enriched cookie dough and other materials with similar rheological properties in extrusion-based 3D printing.

**Keywords:** Microalgae, extrusion-based 3D printing, continuous extrusion, feasible region



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## INTRODUCTION

It is expected that the world population will reach 9.7 billion in 2050 and 11.2 billion in 2100<sup>[1]</sup>. The number of people in the world affected by hunger in 2020 increased from 8.4 to 9.9 percent due to the COVID-19 pandemic. In addition, healthy diets are out of reach for more than 3 billion people<sup>[2]</sup>. Microalgae can help to mitigate this global food crisis.

Microalgae are microscopic photosynthetic unicellular organisms and can be found in both seawater and freshwater. Compared to the cultivation of conventional crops, microalgae cultivation has a lower carbon footprint because it requires fewer resources such as land, water, and fertilizers. Additionally, microalgae will consume carbon dioxide during photosynthesis, which can help reduce greenhouse gas in the atmosphere<sup>[3]</sup>. Microalgae contain health-beneficial components, including proteins, minerals, and antioxidants<sup>[4]</sup>. In some cases, they contain a higher protein amount than soybean, corn, and wheat<sup>[5]</sup>. Among thousands of microalgae species, some are listed as “Generally Recognized as Safe” for human consumption, such as *Chlorella*, *Dunaliella*, *Haematococcus*, *Schizochytrium*, and *Spirulina*<sup>[6]</sup>. *Spirulina* (*Arthrospira platensis*) is one of the most well-known superfoods, according to the World Health Organization<sup>[7]</sup>. *Spirulina* is a rich source of protein, with 60%-70% of its dry weight being protein. This protein is considered of high quality and contains all the essential amino acids needed for human health. It is a good source of omega-3 fatty acid alpha-linolenic acid (ALA), which is important for brain and heart health. In addition, it is a good source of vitamins (vitamin A, vitamin K, and vitamin B), minerals (iron, calcium, magnesium, and potassium), and antioxidants (beta-carotene, zeaxanthin, and phycocyanin) which can help to protect the body against oxidative damage<sup>[8-10]</sup>. It has been reported that *Spirulina* crackers show higher antioxidant activity and better sensory analysis scores compared with other microalgae crackers<sup>[11]</sup>. Microalgae, in the form of dried powder, capsules, pressed pills, and fresh liquid, are all considered healthy food products<sup>[12]</sup>. Commercially available food items that contain microalgae include candies, noodles, ice cream, burgers, smoothies, waffles, soft drinks, protein bars, and crispy biscuits<sup>[13]</sup>.

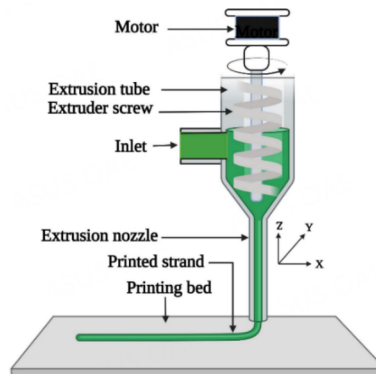
Recently, 3D printing has been used to fabricate both solid (cookie, pizza) and semi-solid food (fish surimi gel)<sup>[14-16]</sup>. It utilizes digital data to fabricate a 3D food structure. Available 3D food printing technologies include selective laser sintering, binder jetting, inkjet printing, and extrusion printing<sup>[17,18]</sup>. **Table 1** shows some examples of food products fabricated by different printing technologies. 3D printing can automatically do repetitive work on a large scale, create complex geometries, and minimize waste. Most importantly, the nutritional profiles, shapes, and textures of the food can be customized easily with 3D food printing<sup>[33]</sup>. Researchers have investigated 3D food printing for both earth and space missions<sup>[34,35]</sup>.

Extrusion-based 3D food printing has numerous advantages, such as ease of use, compact size, low maintenance cost, and ability to print malleable or slurry material. Compared to other 3D printing methods, extrusion-based 3D printing often has inferior resolution<sup>[36]</sup>. Also, printed samples by extrusion-based 3D printing often have visible layer lines, making the surfaces rougher. Despite these disadvantages, extrusion-based printers are easily accessible, cheaper, and highly customizable. In general, extrusion-based 3D printers have higher Technology Readiness Levels (TRL). This technology has been around for decades, and extrusion-based printers are usually more reliable and accessible<sup>[37]</sup>.

In extrusion-based 3D printing, the printing material (usually liquid or semi-solid material) is pushed through a nozzle to fabricate a 3D object. Three mechanisms (screw, syringe, and air pressure) have been used to extrude the printing material<sup>[38]</sup>. **Figure 1** illustrates extrusion-based printing using the screw mechanism. The illustration was created with BioRender.com. The printing material is fed to the extrusion tube through the inlet. The extruder screw driven by the motor pushes the dough through the nozzle<sup>[39]</sup>. The

**Table 1. Examples of reported studies on 3D printing of food products by different 3D printing technologies**

3D printing technology	Food product	Reference
Extrusion printing	Customized chocolate	[19]
	Customized pizzas	[20]
	Cookies	[21-26]
	Microalgae-enriched cookies and snacks	[27-29]
Binder jetting	Sugar cube in full color	[30]
Inkjet printing	Chocolate-decorated donuts, cookies, and ice creams	[31]
Selective Laser Sintering	Toroidal coil structure	[32]

**Figure 1.** Illustration of extrusion-based 3D printing using the screw mechanism.

extrusion nozzle can move along the X, Y, and Z-axis. In some cases, the printer has a material storage container with a piston. Air pressure is required to push this piston to supply the printing material into the extrusion tube. Major printing parameters include nozzle diameter, printing speed, and air pressure. Nozzle diameter is the diameter of the opening at the tip of the extrusion nozzle. Printing speed is the speed at which the nozzle moves along the X and Y-axis while printing. Air pressure is the pressure used to supply the printing material from the material storage container to the extruder tube.

In reported studies on extrusion-based 3D food printing, cookies were commonly used food items. It has been reported that cookie dough composition and printing process parameters (nozzle diameter, printing speed, extrusion pressure, and layer height) affected the geometric accuracy of 3D printed cookies<sup>[24-26,32,40]</sup>. One study showed that adding microalgae to dough improved shape retention capacity<sup>[30]</sup>. However, the feasible regions of extrusion-based 3D printing process parameters for continuous extrusion of microalgae-enriched cookie dough have not been reported yet.

In extrusion-based 3D printing, the extruded strand is the fundamental unit for printed constructs. The strand needs to be continuous for high-quality printing. Process parameters affect the continuous extrusion and the quality of the printed strand<sup>[41,42]</sup>. Each process parameter has its feasible region. If the value of any parameter is outside its feasible region, a continuous strand of acceptable quality cannot be printed. Thakare et al. studied the feasible regions of extrusion pressure and nozzle diameter on continuous extrusion of bioink containing algae cells<sup>[42]</sup>. They also studied the effects of these parameters on the algae cell quantity in printed samples<sup>[43]</sup>. They used a pneumatic extrusion printer to print green bioink. Therefore, a comprehensive study on feasible regions of process parameters for extruding continuous strands of microalgae-enriched cookie dough should be conducted before other comprehensive studies on extrusion-

based 3D printing of microalgae-enriched cookie dough.

The objective of this study is to determine the feasible regions of process parameters for continuous extrusion. It is a necessary first step for further studies on extrusion-based 3D printing of microalgae-enriched cookie dough. For further studies, the values of process parameters should be in their feasible regions. Otherwise, extruded strands might be discontinuous, making it very difficult to print samples that have the designed dimensions. The selected process parameters are nozzle diameter, printing speed, and air pressure. The rest of the paper has three sections: materials and methods, results and discussions, and conclusions.

## MATERIALS AND METHODS

### Preparation of microalgae-enriched cookie dough

**Table 2** lists the ingredients and their percentages in the cookie dough. This composition was obtained by modifying the composition for printable and thermally stable traditional cookies<sup>[44]</sup>. The microalgae species used in this study is *Spirulina* (*Arthrospira platensis*). All-purpose flour (Brand: Great Value), unsalted butter (Brand: Great Value), powdered sugar (Brand: Great Value), and *Spirulina* (Organic *Spirulina* Powder, Manufacturer: Nutricost) were purchased from a local Walmart store (Bryan, Texas). A digital analytical balance scale (USS-DBS2-50, US Solid, Cleveland, Ohio, USA) was used to measure the required amount of ingredients according to the recipe.

**Figure 2** illustrates the preparation steps of microalgae-enriched cookie dough. The illustration was created with [BioRender.com](https://www.biorender.com).

Step 1, butter (111 g) was sited out for 15 min from the refrigerator. Then it was put in a mixing bowl of the electric stand mixer (KitchenAid Classic Plus, Whirlpool Corporation). The butter was softened at speed 4 of the mixer for 1 min.

Step 2, powdered sugar was added to the softened butter in the mixing bowl in two batches (25 g/batch). The mixing was performed by the mixer at speed 4 for 1 min after adding the first batch of powdered sugar, and for 4 min after adding the second batch. At the end of Step 2, the mixture had a lump-free texture.

Step 3, dry spirulina powder was added in a single batch (2.85g/batch). Mixing was performed by the mixer at speed 4 for 1 min.

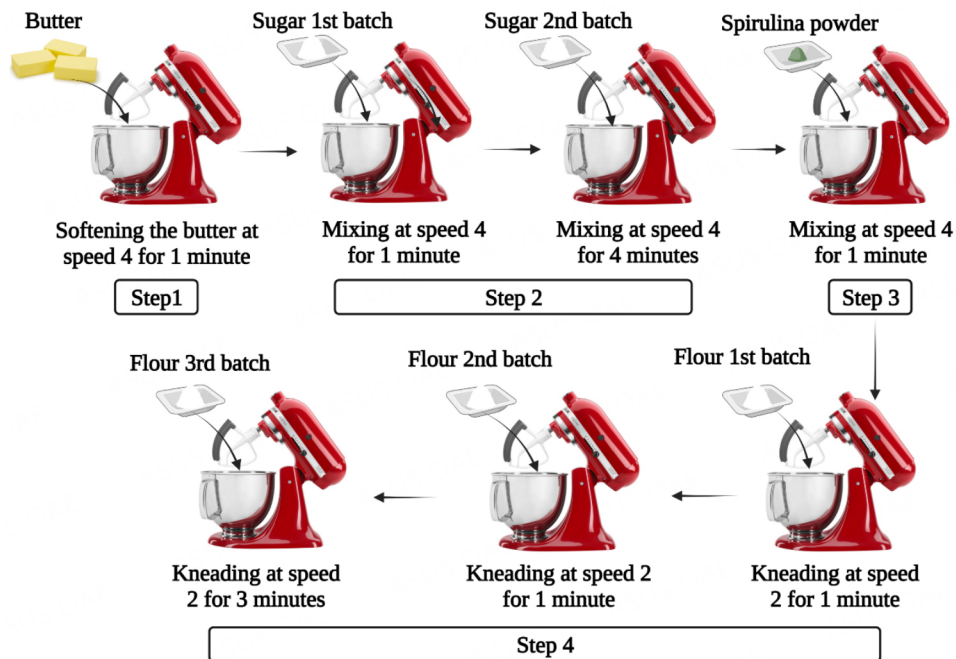
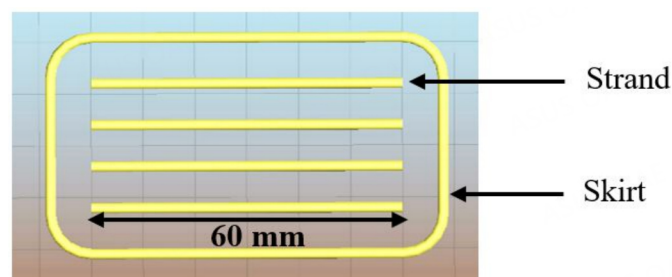
Step 4, flour was added in three batches (40.4g/batch). The kneading was performed by the mixer at speed 2 for 1 min after adding the first batch, 1 min after adding the second batch, and 3 min after adding the third batch. At the end of Step 4, uniform cookie dough was obtained and ready to be transferred by a spoon from the mixing bowl to the material storage container of the 3D printer.

### Design of printed samples

CAD software (Autodesk Fusion 360) was used to design the sample to be printed, and the design file was in the STL format. The design is shown in **Figure 3**. The design involved four straight line segments of strands, with each line segment being 60 mm in length. The Slic3r (version 1.3.0) application was used to prepare the printer instructions as G-code. In the Slic3r application, there is an option to include a skirt which is an outline surrounding the design and is extruded before printing the sample. In this study, the skirt option was enabled. The G-code file was then imported, via an SSD (Solid-State Drive) card, into the Delta WASP 2040 3D printer.

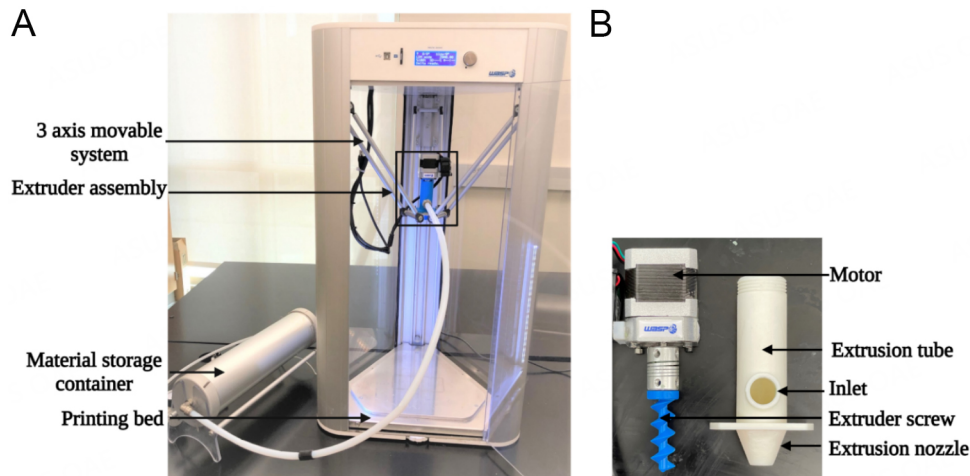
**Table 2. Ingredients and their percentages in the microalgae-enriched cookie dough**

Ingredient	Weight percentage
Flour	42.5%
Unsalted butter	39%
Powdered sugar	17.5%
Spirulina powder	1%

**Figure 2.** Preparation steps of the microalgae-enriched cookie dough.**Figure 3.** Design of the sample to be printed, including four strands, each 60 mm in length.

### Experimental set-up for printing

The printing experiments were performed using the screw-driven extrusion-based 3D printer (Delta 2040, WASP, Italy), as shown in [Figure 4](#). The printer was purchased from Spectrum Scientific (Philadelphia, PA, USA)<sup>[45]</sup>. The printer has a material storage container with a plastic piston. Air pressure is provided from the air compressor (Kobalt 4.3-gallon Electric Twin Stack Quiet Air Compressor, Mooresville, North Carolina, USA) to push this piston to supply printing material to the extrusion tube<sup>[46,47]</sup>. Finally, with the help of a motor-driven extruder screw, the material passes through the nozzle. The sample is printed on parchment paper fixed onto the printing bed.



**Figure 4.** Extrusion-based 3D printer: (A) Delta Wasp 2040 printer used in this study; (B) extruder assembly including motor, extrusion tube, inlet, extruder screw, and extrusion nozzle.

### Design of experiments

Table 3 shows the process parameters studied and their levels. Nozzle diameter had three levels, and printing speed and air pressure both had seven levels. A larger nozzle can extrude more material more quickly, enabling faster printing. It can also handle larger particles in the cookie dough, making it less prone to clog than a smaller one. Also, a larger nozzle can extrude the same material with less force. However, if the nozzle diameter is too large, the printing resolution will be poorer. The values of nozzle diameters used in this study produce acceptable resolution while possessing the benefits of large nozzle diameters.

An experimental condition refers to a unique combination of levels of these process parameters. For example, one experimental condition could comprise a nozzle diameter of 1 mm, a printing speed of 5 mm/s, and an air pressure of 0.3 MPa. Hence, this study had a total of  $3 \times 7 \times 7 = 147$  experimental conditions. Under each condition, one sample was printed, and each sample had four strands, as illustrated in Figure 3. Three batches of the cookie dough (284.3 g/batch) were prepared, each for all experimental conditions with the same level of nozzle diameter. In this study, the distance from the nozzle tip to the print bed was 2 mm. It was kept fixed for all experimental conditions.

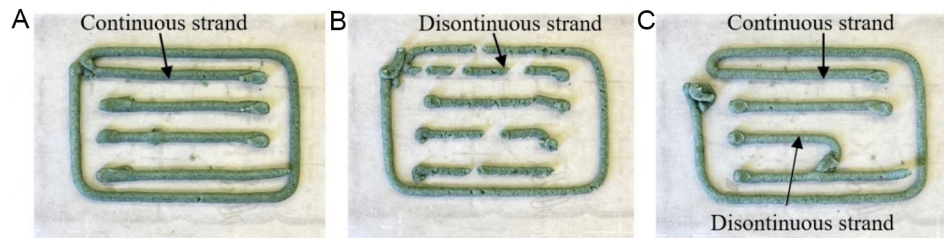
### Definition and determination of feasible regions

Four straight lines are used to evaluate the feasible regions. The outline (called a skirt) was generated automatically by the Slic3r software to ensure that the material could flow smoothly before printing any sample. Because the skirt was not part of the design sample, it was not included in the scoring. Each extruded strand was assigned a quality score based on visual inspection. When a strand was printed continuously, without any surface breaks, it was continuous and assigned “1”; for example, all four strands in Figure 5A. When a strand was not completely continuous, with at least one surface break, it was discontinuous and assigned “0”; for example, each of the four strands in Figure 5B had at least one surface break, so the assigned quality score of each strand was “0”.

The quality score of a sample was the average of the quality scores of its four strands. For example, for the sample shown in Figure 5A, all four strands were printed continuously without any surface break. So, the quality score of the sample was 1. For the sample shown in Figure 5B, all four strands were not continuous. So, the quality score of the sample was 0. For the sample shown in Figure 5C, three strands had a quality score of 1 and one strand had a quality score of 0. So, the quality score of the sample was “0.75”.

**Table 3. Selected process parameters of the extrusion-based 3D printer and their levels**

Process parameter	Level	Unit
Nozzle diameter	1, 2, 3	mm
Printing speed	5, 10, 15, 20, 25, 30, 35	mm/s
Air pressure	0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4	MPa



**Figure 5.** Examples of quality scores of the samples: (A) sample with a quality score of 1, where all four strands were continuous; (B) sample with a quality score of 0, where all four strands were discontinuous; and (C) sample with a quality score of 0.75, where three strands were continuous, and one strand was discontinuous.

For each experimental condition, whether it was inside or outside the feasible regions was decided based on the quality score of the sample printed under the condition. In this study, the value of 0.75 was selected as the cutoff value for feasible regions because it was the average score for four strands where three strands were continuous (with a quality score of 1) and one strand was discontinuous (with a quality score of 0).

## RESULTS AND DISCUSSIONS

Tables 4, 5, and 6 show the quality scores of the printed samples for each of the 147 conditions (unique combinations of the three process parameters: nozzle diameter, printing speed, and air pressure). For example, Table 4 shows that when the nozzle diameter was 3 mm and the printing speed was 5 mm/s, a continuous extrusion was feasible only if air pressure was 0.15 MPa or higher. It also shows that, for printing speeds of 10, 15, 20, 25, 30, and 35 mm/s, continuous extrusion was not feasible until air pressure was 0.2, 0.25, 0.25, 0.3, 0.3, and 0.3 MPa, respectively. Table 5 and Table 6 show that, for nozzle diameters of 2 mm and 1 mm, when the printing speed was 5 mm/s, continuous extrusion was feasible only if air pressure was 0.15 MPa or higher. They also show that, for printing speeds of 10, 15, 20, 25, 30, and 35 mm/s, continuous extrusion was not feasible until air pressure was 0.2, 0.25, 0.3, 0.3, 0.35, and 0.35 MPa, respectively. In addition, these tables show that, for smaller nozzle diameters and higher printing speeds, higher air pressure is required for continuous extrusion. For example, when the printing speed was 30 mm/s, for the 3 mm nozzle diameter, the minimum required air pressure was 0.3 MPa, whereas for the 1 mm nozzle diameter, the minimum required air pressure was 0.35 MPa.

Figure 6 shows some printed samples for a nozzle diameter of 3 mm at different combinations of printing speed and air pressure. For air pressure of 0.25 MPa, and a printing speed of 5 mm/s, the printed strands were continuous, whereas for a printing speed of 35 mm/s, the printed strands were discontinuous. These results are consistent with reported results on 3D printing of rice starch that too high a printing speed can result in discontinuous strands and poor product finishing<sup>[28]</sup>.

Figure 6 also shows that for printing speeds of 5 mm/s and 35 mm/s, the required minimum air pressure for continuous extrusion was 0.15 MPa and 0.3 MPa, respectively. This result concludes that a higher printing speed requires a higher air pressure to ensure continuous extrusion of cookie dough. Another study<sup>[40]</sup> on

**Table 4. Feasible regions of printing speed and air pressure for continuous extrusion in 3D printing of microalgae-enriched cookie dough when nozzle diameter is 3 mm**

	Air pressure (MPa)						
	0.1	0.15	0.2	0.25	0.3	0.35	0.4
5	0	1	1	1	1	1	1
10	0	0	1	1	1	1	1
15	0	0	0	1	1	1	1
20	0	0	0	0.75	1	1	1
25	0	0	0	0	1	1	1
30	0	0	0	0	1	1	1
35	0	0	0	0	1	1	1

**Table 5. Feasible regions of printing speed and air pressure for continuous extrusion in 3D printing of microalgae-enriched cookie dough when nozzle diameter is 2 mm**

	Air pressure (MPa)						
	0.1	0.15	0.2	0.25	0.3	0.35	0.4
5	0	0.75	1	1	1	1	1
10	0	0	1	1	1	1	1
15	0	0	0.25	0.75	1	1	1
20	0	0	0	0.5	1	1	1
25	0	0	0	0	1	1	1
30	0	0	0	0	0.25	1	1
35	0	0	0	0	0	0.75	1

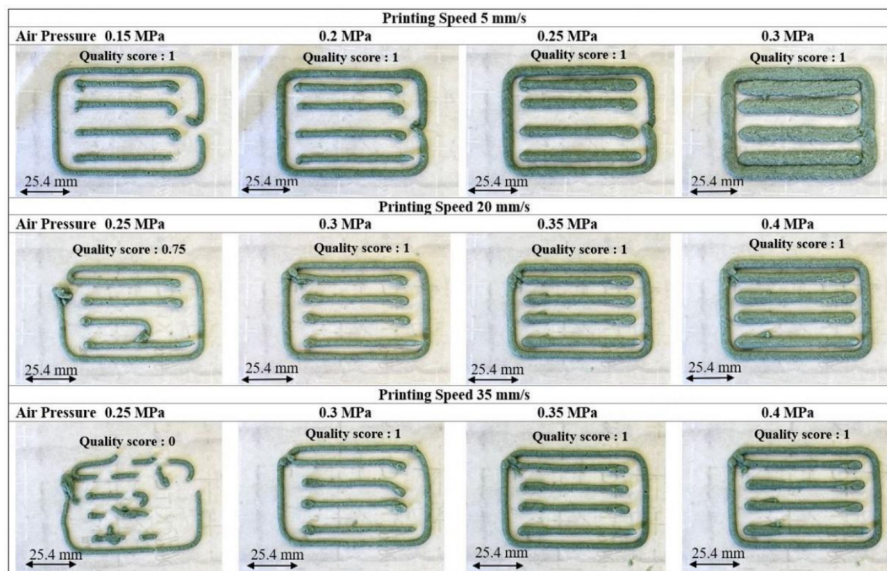
**Table 6. Feasible regions of printing speed and air pressure for continuous extrusion in 3D printing of microalgae-enriched cookie dough when nozzle diameter is 1 mm**

	Air pressure (MPa)						
	0.1	0.15	0.2	0.25	0.3	0.35	0.4
5	0	0.75	1	1	1	1	1
10	0	0	0.75	1	1	1	1
15	0	0	0	0.75	1	1	1
20	0	0	0	0	1	1	1
25	0	0	0	0	0.75	1	1
30	0	0	0	0	0.25	1	1
35	0	0	0	0	0.25	1	1

the 3D printing behavior of the *Nostoc Sphaeroides* system showed that there was a significant relationship between printing speed and extrusion rate for the continuous extrusion of the material. Syringe-based extrusion printing was used in that study<sup>[41]</sup>.

Finally, Figure 6 shows that, for a printing speed of 5mm/s, when air pressure was increased from 0.15 MPa to 0.3 MPa, though the printed strands were continuous, the width of the strand increased. This result is consistent with the reported results from a study on 3D food printing (using wheat flour, freeze-dried mango powder, olive oil, and water). It was shown that using too high an air pressure resulted in significant deformation of the printed samples<sup>[25]</sup>. Thus, the tradeoff between printing time and dimensional accuracy needs to be considered when selecting nozzle diameter, printing speed, and air pressure.





**Figure 6.** Printed samples of microalgae-enriched cookie dough for a nozzle diameter of 3 mm at different combinations of printing speed and air pressure.

## CONCLUSIONS

This paper presents an experimental study to determine feasible regions of extrusion-based 3D printing process parameters that result in continuous extrusion of the microalgae-enriched cookie dough. The experimental results show that, for smaller nozzle diameters and higher printing speeds, higher air pressure is required to ensure continuous extrusion. In addition, to obtain continuous extrusion, air pressure and printing speed should be at least 0.15 MPa and 5 mm/s, respectively, for all three levels of nozzle diameter (1, 2, and 3 mm).

In future studies, 3D structures will be printed with different infill patterns and infill densities. The simple design was used in this study because it was appropriate to achieve the objective of this initial study: to determine the feasible regions of process parameters for continuous extrusion. It is a necessary first step for future studies on multi-layered samples where the values of process parameters should be in their feasible regions. Otherwise, extruded strands might be discontinuous, making it very difficult to print samples that have the designed dimensions.

The information on feasible regions can be useful in deciding the appropriate nozzle diameter, printing speed, and air pressure while printing with microalgae-enriched cookie dough in extrusion-based printing. Though all the parameter combinations inside the feasible regions will produce continuous strands, some of the combinations may cause other issues, such as dimensional inaccuracy. For example, when nozzle diameter and printing speed were fixed, the width of the printed strands increased as air pressure increased. One future research topic is to quantify the effects of printing parameters and their interactions on the shape fidelity (geometrical accuracy) of printed samples.

## DECLARATIONS

### Authors' contributions

Methodology: Rahman TT, Pei Z, Rahman AM

Investigation: Rahman TT, Rahman AM, Thakare K

Writing - original draft preparation: Rahman TT

Writing - review and editing: Rahman TT, Pei Z, Qin H, Rahman AM, Thakare K, Khan A

### Availability of data and materials

The authors confirm that the data to support the findings of this study are available within the article or upon request to the corresponding author.

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### Conflicts of interest

All authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Copyright

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