



*Research article*

## TAILORED 3D FOOD PRINTING INK RECIPE WITH ISOLATED SOY PROTEIN, TEMPEH FLOUR AND PROBIOTIC : COMPARISON OF DURIAN SEED FLOUR, SODIUM ALGINAT AND XANTHAN GUM AS A HYDROCOLLOID

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### Abstract

This study is a part of the 3DFP product development which is formulated with isolated soybean protein (ISP), defatted tempeh flour (DTF), durian seed flour (DSF), and probiotic. The potential of durian seed flour as an alternative hydrocolloid ingredient for 3D food printing ink will be investigated. The food-inks were printed at different temperatures of 30°C, 40°C and 50°C, then baked at 145°C for 6 min. The printed results were analyzed for printability and microstructure. The rheology of the food ink formulated with DSF measured at different temperatures showed comparable behavior to those formulated with SA and XG. Moreover, the higher the shear rate the higher the shear stress and the lower viscosity of those food-inks. The results also showed that food-ink had lower yield stress at higher temperatures. These results indicate that DSF is a suitable ingredient for food-ink formulation with acceptable printability, comparable to those formulated with SA and XG. Based on the printability and microstructure analysis, food inks printed at 40°C were superior to those printed at 30°C and 50°C. Therefore, DSF is a qualified ingredient that can be used in the formulation of 3DFP food-ink.

## 1. Introduction

With the constant increase of the global population reaching 8 billion in 2024, sustainable food production to supply nutritious food for each individual is a major Sustainable Development Goal (SDG) and is becoming a major food security challenge to many governments to provide a sustainable food supply. Therefore, various efforts to overcome these problems continue to be explored, one of which is the development of 3D food printing (3DFP) technology which opens up opportunities for the use of various materials in designing food-ink to produce palatable nutraceutical food products with new characteristics that are in accordance with consumer needs from the physical, nutritional, sensory and functional health value aspects. The working principle of 3DFP is to make 3-dimensional food products from edible ink (food-ink) layer by layer according to the design and size made with specific. Computer software and controlled electronically. The widely used 3D food printer is a printer that involves an extrusion process, where food-ink made from food ingredients is inserted into a cylinder and extruded out through a hole or mold with a certain shape (nozzle) with the push of a hydraulic piston (Altiparmak *et al.*, 2022). The desired characteristics of food-ink are that it can flow through the nozzle and can solidify after printing (Lee, 2021). Food-ink that is suitable for printing (printable) is a food-ink that has a viscosity and rheology with certain viscoelasticity that has good extrudability and self-supporting stability.

Food materials that have been widely used commercially as food-ink are chocolate, but various studies have shown that protein solutions and materials that contain a lot of hydrogels such as starch and hydrocolloids can also be formulated into 3D printable food-ink (Liu *et al.*, 2018; Kewuyemi *et al.*, 2022; Ma and Zhang, 2018). One of the potential materials that can be used as food-ink materials is the flour of durian seed. Based on its chemical composition (dry matter), durian seeds contain 40.29% starch and 55.44% gum (Permatasari *et al.*, 2021), so it has the potential

to produce printable food-ink. One of the largest durian producers in the world is Indonesia. Based on data from the Central Bureau of Statistics Indonesia, durian production in Indonesia reached 1,582,172 tons in 2022 (CBSI, 2023). Durian seeds make up 5-15% of the total weight of durian and are generally discarded as waste (Rozikhin *et al.*, 2020), so on an annual basis there are around 79,109 to 237,326 tons of durian seed waste in Indonesia. The use of durian seeds as food ingredients is expected to support a green economy in terms of sustainability while supporting the achievement of the 2030 SDGs goals, namely zero hunger [SDG-2]. The use of durian seed flour (DSF) as a food-ink material has never been studied, even though it has the potential to develop sustainable superfoods using 3DFP technology. In developing sustainable superfoods with 3DFP, DSF needs to be combined with other complementing nutritious ingredients such as functional protein. An economical source of protein that is widely consumed by the community is tempeh. Tempeh is a fermented soybean product that is coined as a superfood owing to its high nutrient density (especially protein and vitamin B12); bioactive compounds that have functional health value such as isoflavones and  $\gamma$ -amino butyric acid (GABA); and its production process that is classified as traditional so it is environmentally friendly, sustainable food product (Romulo and Surya, 2021; Maskar *et al.*, 2022). Isolated Soybean Protein (ISP) is also an economical protein source material that has various functional values and can improve the texture and flavor of the final product (Lee, 2015). Other ingredients that can be combined with DSF are probiotics. Probiotic bacteria from local isolates that have been widely used for food product development are *Lactobacillus plantarum* Dad-13 (*L. plantarum* Dad-13). This probiotic bacteria has various health benefits, namely improving digestive tract health, anti-diarrhea, boosting the immune system, facilitating the production of folic acid, and enhancing growth among malnourished children (Kamil *et al.*, 2023).

The combination of DSF, tempeh flour, ISP, and *L. plantarum* Dad-13 is expected to produce nutrient-dense tailored printable food-ink in order to develop sustainable food using 3DFP. In this study, potential of durian seed flour as a hydrocolloid alternative ingredient of 3D food printing ink will be investigated.

## 2. Materials and methods

### 2.1. Materials

Materials for food-ink formulation were durian seed flour, isolated soy protein, defatted tempeh flour, gelatin, water and probiotic *L. plantarum* Dad-13. Durian seed flour was prepared from durian seed, obtained from a durian fruit processing unit in Surabaya, Indonesia. Durian seeds were washed, sorted, boiled, peeled, cut, sterilized, dried, and crushed into flour and sieved with a Tyler sieve shaker at 70 mesh. Isolated soy protein and gelatin edible grade 200 bloom were purchased from the local market. The defatted tempeh flour was prepared from hygienic tempeh purchased from the local market, then sliced, steam blanched at 90°C for 2 min, dried at 60°C for 8 h, then crushed and sieved at 70 mesh with a Tyler sieve shaker. The tempeh flour was defatted with hexane at room temperature for 2 h, then dried at 60°C for 8 h.

### 2.2. Methods

#### 2.2.1. Preparation of food-ink and 3D printing

Food-ink was made according to the procedure outlined by Chen et al. (2022). In this research, 0.2% of DSF was mixed with water, 4% DTF, 10% gelatin, 17% ISP, and 0.1% probiotic *L. plantarum* Dad-13, stirred (100 rpm, 5 minutes) and settled (4°C, 24 hours), then heated to 35°C before characterization. For comparison, food-ink was also made in the same way, but the DSF was substituted with 0.5% xanthan gum (XG) and 0.5% sodium alginate (SA). The viscosity and rheology properties of the food-inks were determined before being printed. The 3DFP used in this study is an extrusion-based Foodbot 3D Food Printer, with the following conditions: 2 mm nozzle, 7 mm/s printing speed, and dimensions of 150x150x70 mm.

Different printing temperatures were applied (30°C, 40°C and 50°C), then baked at 145°C for 6 min. The printed results were analyzed for printability and microstructure.

#### 2.2.2. Determination of the viscosity and rheology properties

The viscosity and rheology properties of the food-ink were determined by using a rheometer (RM200 CP 4000, Lamy Rheology, France). Flow tests were carried out at different temperatures i.e. 25°C; 35°C and 45°C from the view of the experimental environment, edible condition and sample preparation. The 25°C is around the room temperature, the 35°C is around the body temperature (~37°C), and the 45°C is the temperature to solve the gelatin during the protein paste preparation. Casson model analysis was used for determining yield stress and viscosity.

#### 2.2.3. Printability determination

The printability was determined by observation of the shape and surface of the printed results, and photographed. The baked products were also observed for their shape and surface, and photographed.

#### 2.2.4. Microstructure analysis

Microstructural analysis was performed using SEM (Scanning Electron Microscope, EVO MA 10, Zeiss). The samples were freeze-dried and then coated by using Pb-Au. In the observation process with SEM, the samples were observed at a certain magnification. The results of the particle diameter magnification were analyzed using Image-J software.

#### 2.2.5. Statistical analysis

The obtained data were calculated for the mean and standard deviation.

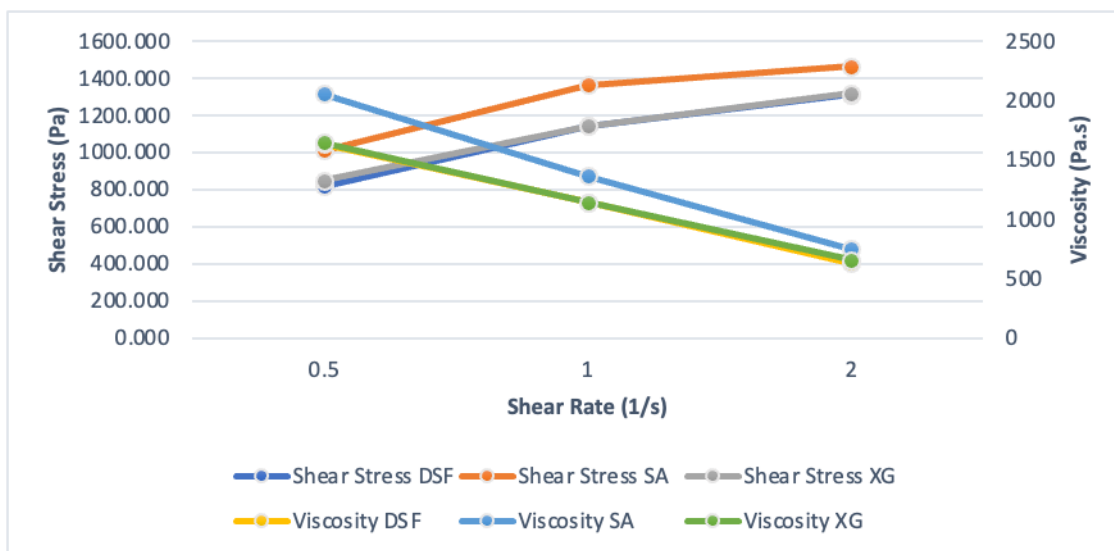
## 3. Results and discussions

### 3.1. Viscosity and rheology properties of food-ink

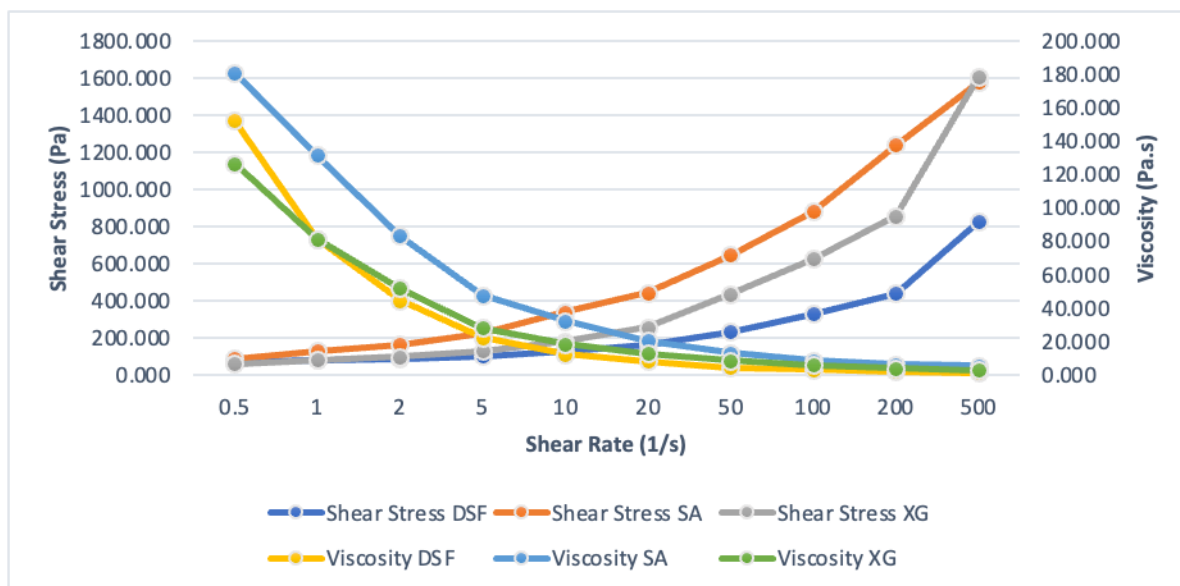
Food-ink prepared from isolated soy protein, defatted tempeh flour, gelatin and probiotic with 3 (three) different hydrocolloids (DSF, SA, and XG) were determined for the viscosity and rheology properties. According to Lee (2021), viscosity and rheology properties of food-ink affect its capability to flow through the nozzle and can solidify after 3D printing,

thus important to its printability. The viscosity of the food-inks should be low enough to ensure that the material can be smoothly extruded from the smaller nozzles, and high

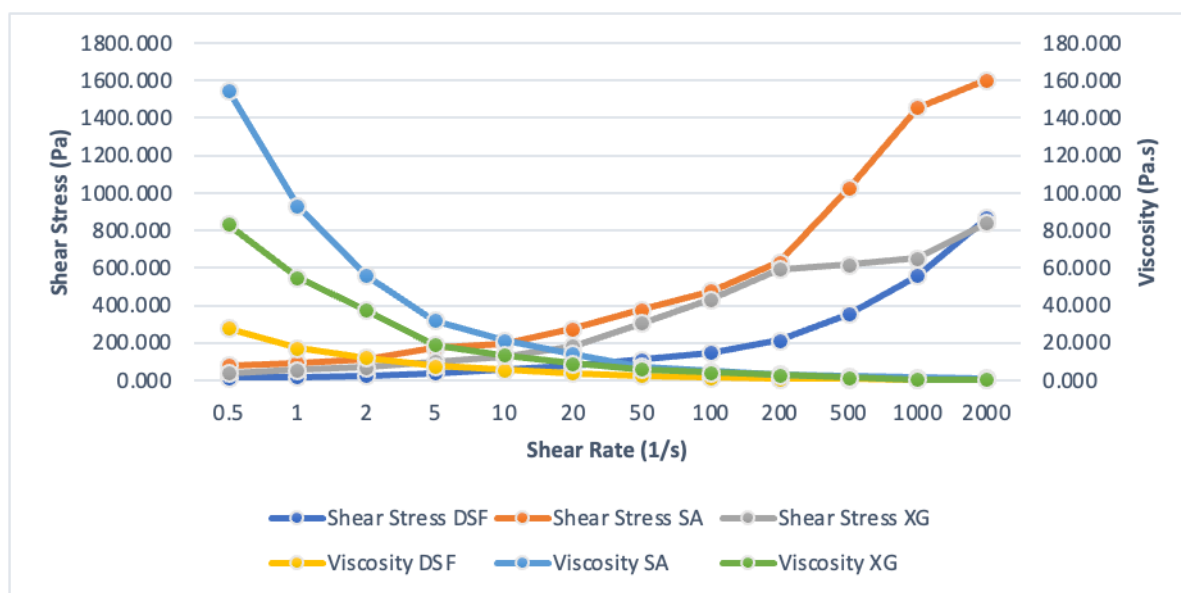
enough to ensure that the food-inks have sufficient mechanical strength to maintain the stability of the printing products (Guo et al., 2022).



**Figure 1A.** Viscosity and rheology properties of food-inks determined at 25°C



**Figure 1B.** Viscosity and rheology properties of food-inks determined at 35°C



**Figure 1c.** Viscosity and rheology properties of food-inks determined at 45°C

**Table 1.** Yield stress of food-ink with different hydrocolloids.

Hydrocolloid	Yield Stress (Pa)		
	25°C	35°C	45°C
DSF	453.45 ± 46.60	78.70 ± 8.34	47.4 ± 0.28
SA	825.45 ± 44.48	124.40 ± 2.83	78.7 ± 0.57
XG	644.3 ± 24.18	74.6 ± 4.53	59.95 ± 4.45

Figure 1 shows the viscosity and shear stress at different shear rates of the three food-inks which were determined at different temperatures of 25°C, 35°C, and 45°C. At 25°C measurement of food-ink with DSF, increasing shear rate, shear stress increased from 816.69 Pa at a shear rate of 0.5/s to become 1,311.18 Pa at a shear rate of 2.0/s and, viscosity decreased from 1,633.37 Pa.s at shear rate 0.5/s to become 630.59 Pa.s at shear rate 2/s. At higher temperatures (35°C and 45°C), a similar phenomenon has been found, increasing the shear rate resulted in increasing shear stress and decreasing viscosity. At 35°C, the shear stress was 73.61 Pa at shear rate of 0.5/s increased to 89.99 Pa at 2/s. The similar trend is also can be observed at 45°C, the shear stress was 13.54 Pa at shear rate of 0.5/s become 24.61 Pa at 2/s, while the viscosity decreased from 27.72 Pa.s become 12.01 Pa.s. During preliminary research, food-ink with 0.5% DSF concentration could not be printed because the food-ink had too high viscosity. The durian seed contains hydrocolloids in the form of

starch and gum, which contribute to the rheology behavior. Amin et al. (2017) reported that durian seed gum had been purified and characterized. The viscosity of 1% durian seed gum solution was 65 mPa.s at a shear rate of 1000/s at 29.8°C. The viscosity of fully hydrated durian seed gum solution decreased as temperature increased.

It was noted that the rheology behavior of food-ink formulated with DSF was similar to those formulated with SA, and XG at the same temperatures. When the shear rate increases, the shear stress decreases and viscosity increases. However, the values of shear stress and viscosity among those three food-inks are different. The shear stress and viscosity values of food-ink formulated with DSF are between the values of those food-inks formulated with SA and XG. For example, shear stress of food-inks formulated with DSF, SA and XG measured at 35°C and 0.5/s were 73.61, 90.46, and 63.37 Pa, respectively. The viscosity values at those conditions were 152.22, 180.93, and 126.75 Pa.s, respectively.

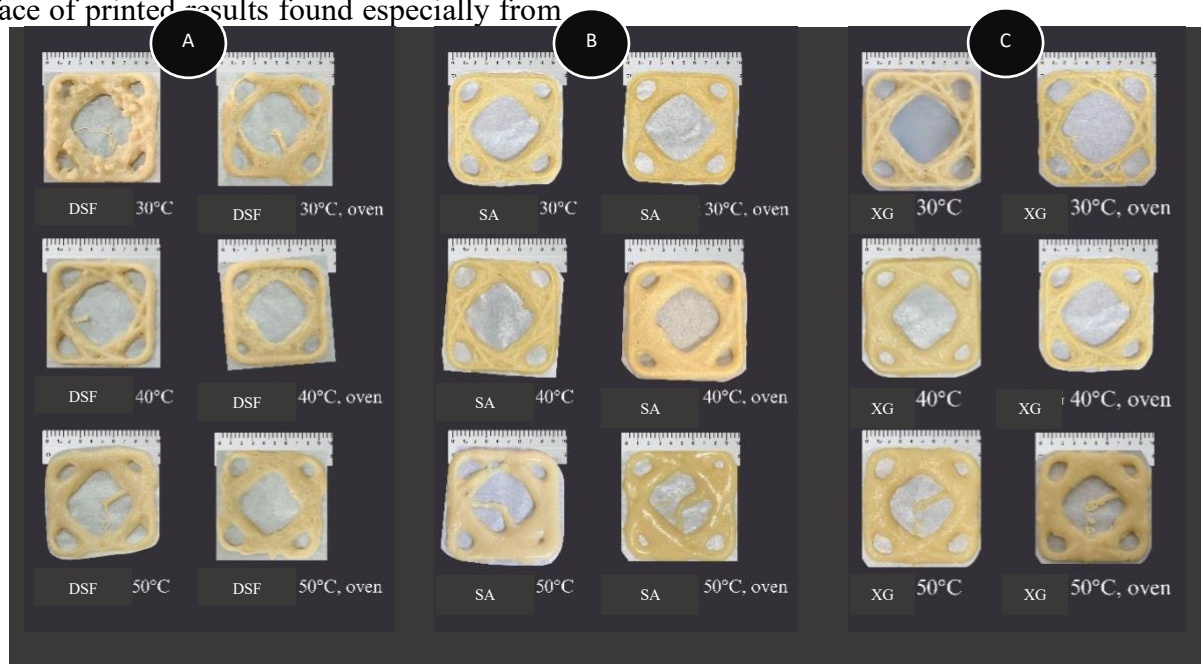
Table 1 shows the yield stress of food-ink with different hydrocolloids. The yield stress values decrease when the temperature increases. The yield stress values of the food-ink formulated with DFS at 25°C, 35°C, and 45°C were 453.45 Pa, 78.70 Pa, and 47.4 Pa, respectively. Those values were comparable to yield stress values of food-ink formulated with pregelatinized corn starch, in a range of 87.5 to 883.2 Pa (Maldonado-Rosas et al., 2022). Based on the dry matter, durian seed contains hydrocolloids in the form of starch (40.29%) and gum (55.44%) (Permatasari et al., 2021), which contribute to the rheology behavior.

### 3.2. Printability of food-ink

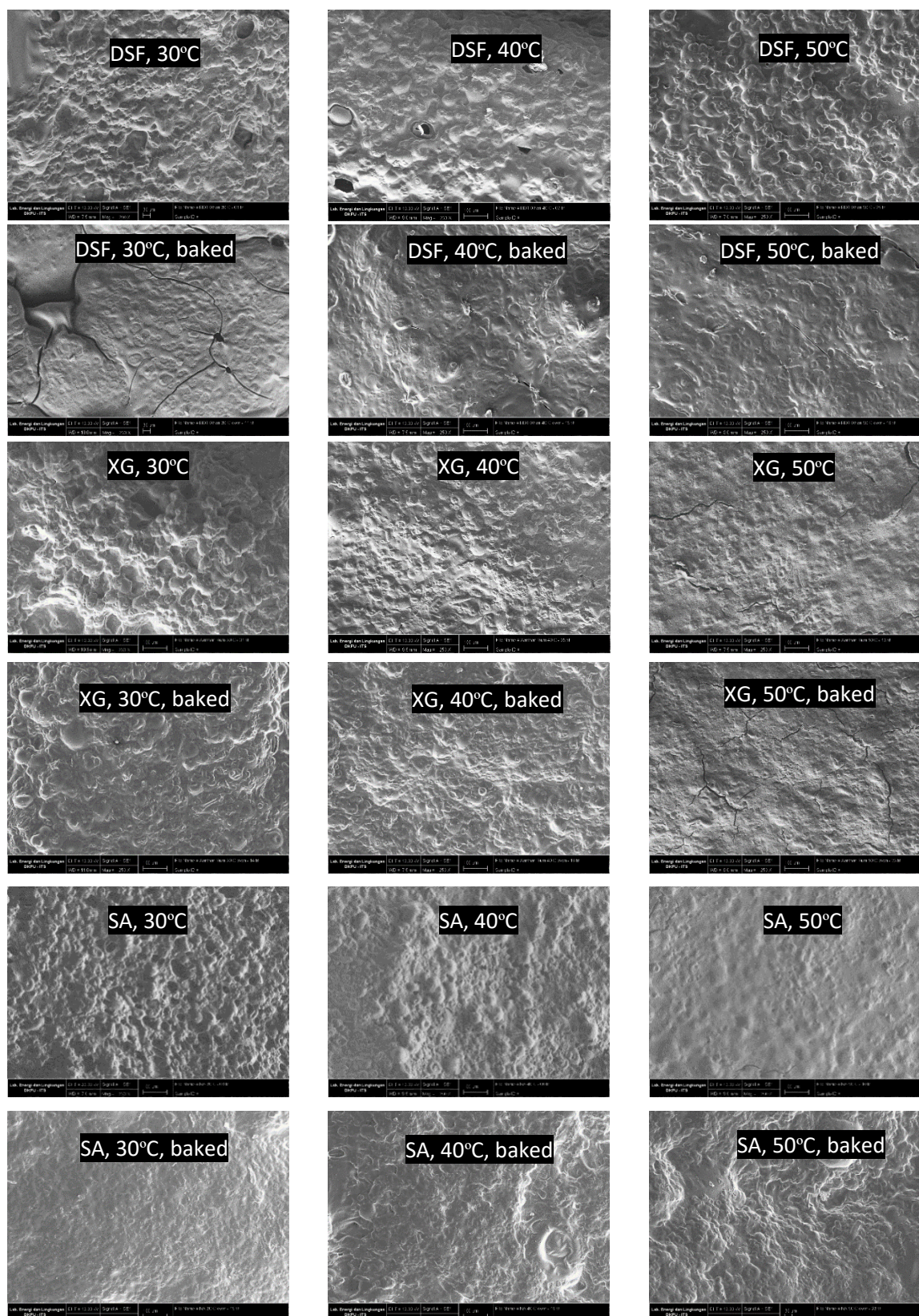
The highly desirable materials for extrusion-based printing are pseudoplastic fluids with shear thinning behavior. A low viscosity indicates strong shear-thinning behavior and materials with this behavior could be easily extruded out due to low viscosity with the application of shear stress (Liu et al., 2018). Figure 2 shows the printing results of the different printing temperatures. Temperature is known to affect the rheological properties of food material and printability of the material. The figures show that at 30°C, less smooth surface of printed results found especially from

food-inks formulated with DSF and XG. All three food-inks resulted smooth surface when printed at 40°C and 50°C. Chen et al. (2022) reported the similar finding that the printing temperature (25, 35 and 45°C) affect the printability, higher temperature enhanced texture properties of 3D printed objects of protein-based food-ink formulated with isolated soy protein. However, higher temperature printing can cause the surface of the printed food to widen. We found that all three food-inks resulted widen surface when printed at 50°C. In case of food-ink with DSF that contains starch, temperature of 30°C is not sufficient for interaction of the starch.

According to Liu et al. (2020), with the increase of the 3D printing temperature, the sufficient chain interactions between starch particles led to the formation of a uniform gel structure. Figure 2A shows that food-ink with 0.2% DSF produced good printability at higher temperatures, and was comparable to food-ink formulated with SA and XG (Figure 2B and C). XG and SA are hydrocolloids that are frequently used in the food-ink formulation thus the printability results confirm that DSF can be used as hydrocolloids in 3DFP food-ink formulation.



**Figure 2.** Printed food-ink formulated with DSF(A), SA(B), and XG(C) at different temperatures and its baked products



**Figure 3.** Microstructure of printed food-ink formulated with different hydrocolloids at different temperatures and its baked products

### 3.3. Microstructure of printed food-ink and baked 3DFP product

SEM was used to observe the microstructure of the printed food-inks formulated with 0.2% DSF with various printing temperatures and compared to food-inks formulated with SA and XG (Figure 3). Temperature is also known to affect the microstructure of food material. Higher printing temperature tends to produce better structure, indicated by fine particle distribution status. This is possibly caused by the gelatinization of hydrocolloids in the food-inks. As the printing temperature increases, the gelatinization process increases. This was further confirmed by the microstructure of baked food-ink that showed a better particle distribution status. The DSF seemed like a cracked structure, which might be caused by partial starch gelatinization. Higher temperature printing showed no cracking in the structure. Other research also showed the microstructure of printed food-ink based on starch-rich materials. Compared to food-inks formulated with SA and XG, the microstructure of food-inks formulated with 0.2% DSF exhibits a coarser particle distribution status. This is possibly caused by different gelatinization profiles of those hydrocolloids and different concentrations of hydrocolloids used. SA and XG used for the food-ink was 0.5% while DSF used was only 0.2%. However, baked food-ink formulated with 0.2% DSF showed a finer particle distribution status than those formulated with SA and XG.

### 4. Conclusions

The characterization of tailored food-ink formulated with ISP, DTF, DSF, and probiotic *L. plantarum* Dad-13 has been accomplished. DSF produced 3DFP food-ink with viscosity, shear stress values, and SEM results comparable to those formulated with SA and XG. Therefore, DSF is a qualified ingredient that can be used in the formulation of 3DFP food-ink. Further research is needed to confirm the nutritional values and functional properties of this newly tailored food-ink formula.

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