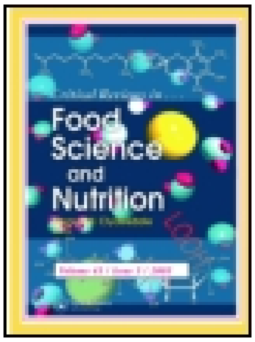

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REVIEW

Advancements in 3D food printing: a comprehensive overview of properties and opportunities

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ABSTRACT
3D printing has numerous applications in the food industry that may enhance diversity, quality, healthiness, and sustainability. This innovative additive manufacturing technology has the ability to specifically tailor food properties for individuals. Nevertheless, several challenges still need to be overcome before 3D printing can be utilized more widely in the food industry. This article focuses on the development and characterization of "food inks" suitable for 3D printing of foods. Specifically, the main factors impacting successfully printed foods are highlighted, including material properties and printing parameters. The creation of a 3D printed food with the appropriate quality and functional attributes requires understanding and control of these factors. Food ink printability is an especially important factor that depends on their composition, structure, and physicochemical properties. Previous studies do not sufficiently describe the precise design and operation of 3D printers in sufficient detail, which makes comparing results challenging. Additionally, important physicochemical characteristics utilized in traditional food are not consistently reported in 3D inks, such as moisture content, water activity, and microbial contamination, which limits the practical application of the results. For this reason, we highlight important factors impacting 3D ink formulation and performance, then provide suggestions for standardizing and optimizing 3D printed foods.

KEYWORDS
3D printing; food; printability; food safety; ink; macronutrients

Introduction

Three-dimensional (3D) printing, also known as additive manufacturing, utilizes digital data to create a physical three-dimensional object; typically, by laying down thin layers of material in succession. 3D printing is a unique technique that enables users to create highly complex materials that are difficult to create otherwise by using traditional mechanical manufacturing techniques. Additionally, 3D printed objects have immense customizability through the design parameters as well as the use of different types of printing materials. These advantages have led to rapid advances in the application of 3D printing in recent years, as highlighted by the steep rise in publications in this area since 2010 (Figure 1).

In general, 3D printing has a diverse range of applications in many fields, including military, space, medical, and manufacturing sectors (Yuan et al. 2019; Flowers et al. 2017; Bose, Vahabzadeh, and Bandyopadhyay 2013; Yan et al. 2018; Godoi, Prakash, and Bhandari 2016; Sun et al. 2015a; Dankar, Haddarah, et al. 2018). 3D printers have been utilized to print the parts for automobiles, aircrafts, and spaceships, thereby reducing the need to stockpile parts that are not needed (Yuan et al. 2019). Depending on their design, 3D printers can be used to print various kinds of materials, metals and plastics (Yuan et al. 2019). This technology has also been utilized for printing individual electronics components (Flowers et al. 2017). In the biomedical field, 3D printers have been used for several purposes, including designing bone tissue scaffolds to mimic the extracellular matrix, printing organs, and even printing whole tissues (Bose, Vahabzadeh, and Bandyopadhyay 2013; Yan et al. 2018).

More recently, 3D food printing has been rapidly developing. Within the last five years alone, there has been a surge of publications regarding 3D printed food applications (Figure 1). Researchers have used this technology to print cell cultured meat and meat analogues (Godoi, Prakash, and Bhandari 2016), which uses many of the same principles used to print organs and tissues in the biomedical field. 3D printing has also been used to create various kinds of solid or semi-solid foods, including cookies, cakes, burgers, apples, and chocolates (Lipton et al. 2015; Yang, Zhang, Prakash, et al. 2018; Godoi, Prakash, and Bhandari 2016; Wang et al. 2018; Pérez et al. 2019; Feng, Zhang, and Bhandari 2019). A major advantage of 3D printing for this purpose is that the shapes, textures, and nutritional profiles of the foods can be customized (Derossi et al. 2018). In general, several different designs of 3D printers are available, including selective laser sintering, powder bed printing, inkjet printing, and extrusion printing, however, the latter is currently the most commonly used for foods (Sun et al. 2015a; Wegrzyn, Golding, and Archer 2012). Typically, 3D

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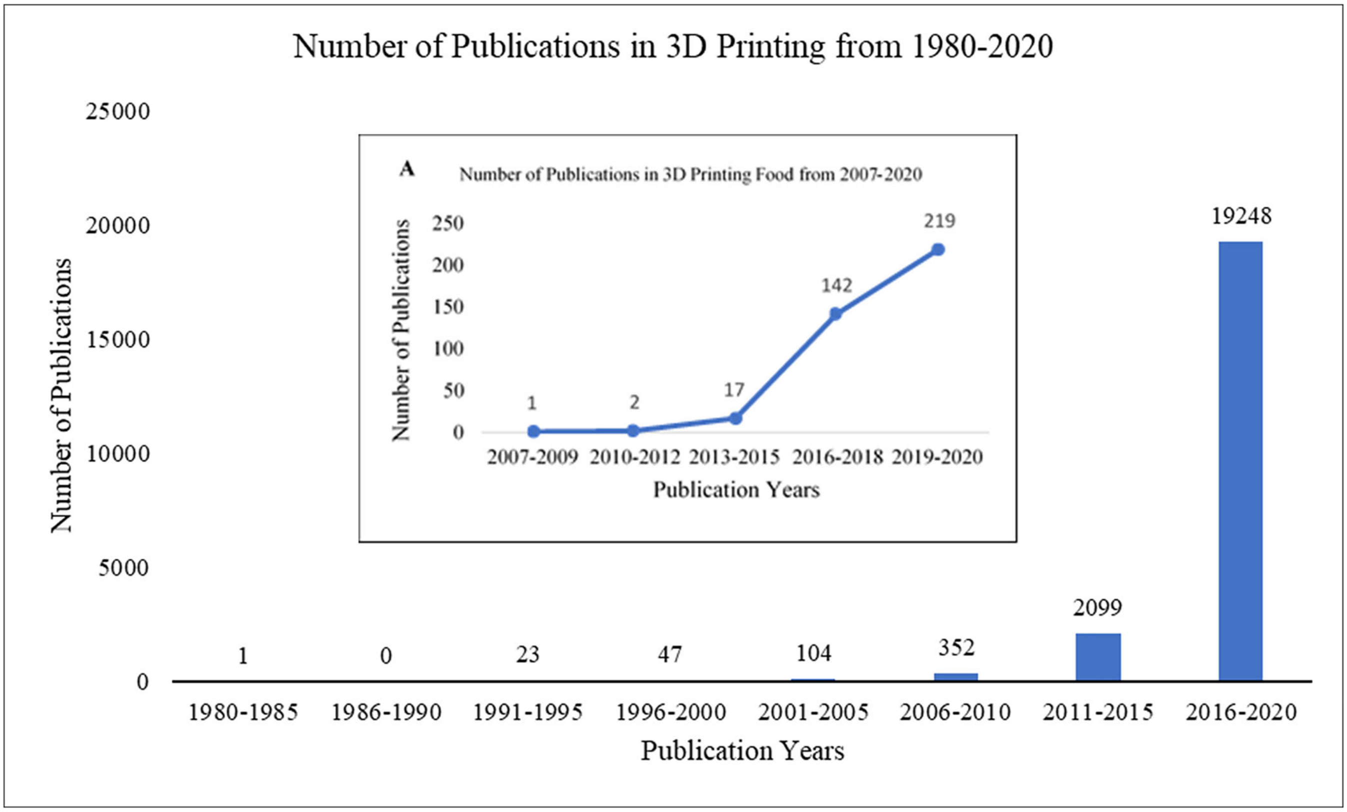


Figure 1. Number of Scientific Publications reported by Web of Science Clarivate Analytics containing the term 3D Printing in the title, keywords, and abstracts from the years 1980-2020. The insert A shows the number of 3D Printing Food Publications with the terms "3D printing, food" in the title, keywords, and abstracts. Both searches applied filters for articles and reviews.

food printers have one or more extruders, that create food products by drawing shapes layer-by-layer from point-to-point. Food inks, which may contain one or more ingredient, are typically added to an extruder, pushed out by applying an external force, and then printed into pre-designed shapes using spatial data stored in the instrument software (Feng, Zhang, and Bhandari 2019). Usually, the force required to extrude a food ink through a nozzle is provided by connecting the printer to an air pressure unit. The formulation of food inks with the required physicochemical and functional properties is one of the most critical factors in any successful 3D food printing application (Pérez et al. 2019).

To successfully print food, important material properties such as rheology, surface tension, and phase behavior of the food ink need to be considered. In addition, the operating parameters of the 3D printer used, such as nozzle height, nozzle diameter, flow rate, and printing speed, are also crucial as they impact the structure and stability of the foods printed. Researchers have identified several important parameters that impact the successful 3D printing of foods, including hardness, springiness, plasticity, and viscoelasticity (Feng, Zhang, and Bhandari 2019; Dankar, Haddarah, et al. 2018; Sun et al. 2015a; Sun et al. 2015b; Sun et al. 2015c; Wegzryn et al. 2012). Nevertheless, many other important parameters that are critical for the development of high quality and safe food products are often ignored, such as moisture content, water activity, microbial contamination, and mouthfeel. For this reason, this review will focus on the importance of physicochemical and microbiological attributes that are crucial to assessing consumer acceptability and safety of 3D foods, but often ignored in other studies.

Food materials for 3D printing

One of the most important factors impacting the success of 3D printing in the food industry is the availability of suitable "food inks". Typically, these materials should be able to flow through a nozzle, but then set after being deposited on a surface. In this section, we highlight some of the most important food components that can be used to form food inks.

Suitability of food materials for *3D* printing

When 3D printing food, there are multiple criteria that should be considered when selecting food ingredients to achieve printability. Herein, we define printability as successful printing of a material by the selected printer while maintaining the structural integrity and shape of the printed object. The printability of a food ink mainly depends on its rheological and surface properties (Pérez et al. 2019; Dankar, Haddarah, et al. 2018). Typically, a food ink should be able to flow through a nozzle but then set after printing (Jiang et al. 2019). This can be achieved in a number of ways. First, a material with "plastic" rheological properties can be used. A plastic material is one that acts like an elastic solid below an applied stress, known as the yield stress, but flows like a liquid above this value (Jiang et al. 2019; Wang et al. 2018). Consequently, this type of material can be placed in a cylinder and then extruded by applying a force above the yield stress, after which it will set when the force is removed. Food materials that contain networks of colloidal particles or polymers often exhibit this kind of behavior, such as crystalline fats or concentrated emulsions (Gregersen et al. 2015). Second, a material can be made to undergo a liquid-solid transition in response to some change in environment or composition (Li et al. 2020; Liu, Zhang, et al. 2019). Food materials can be made to undergo this kind of transition due to various phenomenon, such as crystallization of fats and gelling of hydrocolloids (Li et al. 2020). These transitions may be induced by heat, cold, or cross-linking agents. Selecting the most appropriate food ingredients to obtain an appropriate transition temperature, initial viscosity, final gel strength, and other physicochemical properties are all important.

For food inks, the type, concentration, structural organization, and interactions all influence printability. In particular, how the rheological properties of the food ink change during the printing process when exposed to external stimuli such as shear, temperature changes, or addition of gelling agents are critical. Therefore, when formulating food inks, relationships between property, process, and functionality need to be established to optimize printability.

Major food ink constituents

The main structural components in foods are macronutrients, such as carbohydrates, proteins, and fats. These macronutrients have different chemical structures and perform different functions in the human body when metabolized (Pérez et al. 2019; Lipton et al. 2015). Carbohydrates are built from monosaccharides and mainly consist of sugars, starches, and dietary fibers, which are commonly found in plant-based foods such as cereals, fruits, and vegetables. Proteins, which consist of chains of amino acids linked together by peptide bonds, are essential for many metabolic functions. Proteins are found in a diverse range of animal and plant sources including dairy, eggs, meat, soy, peas, beans, and lentils. Edible fats are mainly comprised of triglycerides, which consist of three fatty acids attached to a glycerol. The length, saturation, and position of the fatty acids in a triglyceride molecule determine its physical, chemical, and nutritional properties (Gregersen et al. 2015; Godoi, Prakash, and Bhandari 2016). Common sources of edible lipids are animal fats and vegetable oils, such as lard, olive oil, sunflower oil, coconut oil, and palm oil. The ability of macronutrients to provide the required physicochemical attributes to food inks depends on their structure-forming properties, which varies greatly from ingredient to ingredient. For instance, the thickening or gelling of food inks may be due to processes such as biopolymer overlap, biopolymer cross-linking, fat crystallization, or particle packing. Moreover, thickening or gelling may be induced by different factors, including heating, cooling, drying, or cross-linking agents (Li et al. 2020; Liu, Zhang, et al. 2019; Schmidt et al. 2019; Zheng et al. 2019). Therefore, careful consideration must be made when selecting appropriate ingredients for food inks. In the remainder of this section, we review some of the most important properties of different kinds of structure forming ingredients that can be used to formulate food inks. These ingredients can be used in isolation or in combination depending on the required attributes of the final food product.

Carbohydrates

A wide range of carbohydrate-rich foods have been tested for their potential application in the formulation of 3D inks, including mashed potatoes, doughs, fruits, vegetables, and isolated polysaccharides (Liu et al. 2018; Dankar, Pujolà, et al. 2018; Severini, Derossi, et al. 2018; Derossi et al. 2018; Yang et al. 2018; Kim, Bae, and Park 2017). Studies conducted with mashed potatoes showed that they were too soft to extrude without additives but could be printed when agar or alginate was added (Dankar, Pujolà, et al. 2018, Liu et al. 2018). On the other hand, the addition of lecithin or glycerol was not suitable for creating 3D inks. Another study used a combination of potato starch and lemon juice to create a food ink that could be successfully extruded (Yang, Zhang, Bhandari, et al. 2018). Puréed fruits and vegetables (bananas and carrots) have also been tested, but they were unsuitable for this purpose (even after pectin addition) because the final materials did not have the appropriate structural integrity (Derossi et al. 2018; Severini, Derossi, et al. 2018).

Proteins

Various types of proteins have been utilized to formulate food inks for 3D printing due to their gelling properties. Proteins can be made to gel by various mechanisms depending on their molecular structure, including cold-set (gelatin), heat-set (globular proteins), ionic-set (charged proteins), and enzymatic-set (most proteins). Their ability to form gels under controlled conditions means that they can be printed and then hold their shape (Andersson et al. 2020). Researchers have shown that insect proteins can be 3D printed into structures with the required solidity for some food applications (Severini, Azzollini, et al. 2018). Other researchers have shown that fish (silver carp) protein (Wang et al. 2018) and plant (soy) protein (Chen et al. 2019) can be used as food inks). Prior to NaCl addition, the carp filets were unsuitable for extrusion and resulted in structures with poor precision and texture. Therefore, the carp filets were mixed with sodium chloride (1.5% NaCl) to form a fish surimi gel that could then be successfully printed with higher precision and a firmer texture (Wang et al. 2018). Soy protein isolate was mixed with alginate and gelatin to obtain a firmer texture to improve printability. Egg proteins that have undergone controlled denaturation have also been used for this purpose (Liu, Meng, et al. 2019a).

Lipids

Past research regarding lipids is limited but findings show that lipids can be used in a bulk or emulsified form to create food inks. Bulk lipids can form semi-solid materials with plastic rheological properties suitable for printing when they are partially crystalline (Godoi, Prakash, and Bhandari 2016). In these applications, it is critical to identify a lipid source that has an appropriate melting/crystallization profile, *e.g.,* solid fat content (SFC) *versus* temperature. At the application temperature, the SFC should be such that the material is able to flow when a force is applied but set when the force is removed. Initially, ink viscosity would be influenced by the starting temperature prior to the print. It is important to consider experimental and printing conditions beforehand as well as the temperature of the end product. If the final product has a temperature that is inappropriate, the structural stability and sensory characteristics could be compromised.

Most previous studies have explored lipid-based materials such as cheese (25% fat) and semi-skimmed milk powder (SSMP, 9% fat) as sources of fat (Le Tohic et al. 2018; Lille et al. 2018). In successful printing applications, commercially available cheese was used for 3D printing without additives whereas the SSMP was combined with several other ingredients (Le Tohic et al. 2018; Lille et al. 2018), including, cellulose nanofiber and corn starch in a 60:40 ratio (Lille et al. 2018). However, when researchers used skim milk powder containing no fat, it failed to print due to lack of hardness and lubrication. These experiments revealed that fat could be a potential lubricant for food inks improving 3D printing capability. As mentioned earlier, the crystallization and melting behavior of lipids can be used to create 3D inks (Godoi, Prakash, and Bhandari 2016). Currently, the most extensively researched fat material used for this purpose is chocolate (Pérez et al. 2019; Godoi, Prakash, and Bhandari 2016; Yang, Zhang, and Bhandari 2017; Jiang et al. 2019). Chocolate contains a network of interlinked fat crystals that provide the required semi-solid behavior (Godoi, Prakash, and Bhandari 2016; Jiang et al. 2019). Moreover, the temperature of chocolate and other crystalline fat sources can be varied to convert them from a flowable liquid to a firm solid. More research needs to be established on these other food ingredients and experiments should be conducted with foods of different lipid content and structure in order to collect more data and draw stronger comparisons to help advance 3D food printing.

Factors influencing 3D food printing

There are multiple components that influence successful 3D food printing such as food properties and external experimental design and parameters designed around food constituents. Critical food properties include rheology, texture, ingredient concentration and food ink composition. External influences include 3D printing equipment, printing parameters, processing techniques and print design. For the most effective printing, both food properties and external components need thorough consideration and analysis.

Food properties and characterizations

Food properties are an inclusive category which include both food inks and their constituents. Crucial components such as rheology and texture are impacted by properties such as food concentration, composition, and structure. Altogether, printability is composed of rheology, texture and their respective properties. Initially, rheology can be used as one tool that indicates printability and characterizes the food ink input (Figure 2). Subsequently, texture serves as a tool to assess the 3D printing output and structural stability of the final product (Figure 2).

Rheology

Rheology can study the food system's flow under controlled conditions in where we can consider on multiple components including viscosity, shear stress, shear rate, loss modulus, storage modulus and yield stress. Viscosity is the measurement of the ability of a material to resist force, which is a component of printability. Optimal viscosity can be achieved by optimizing the material concentration or by use of additives. When 3D printing, the food ink resists the force provided from the 3D printer and optimal ink flow is required to successfully print an object. Moreover, shear stress, shear rate, and yield stress also need to be considered when 3D printing food. Shear stress is the force that causes deformation and shear rate is the rate at which deformation is applied. Both principles directly affect viscosity which is key to 3D printing. As shear rate and shear stress increase, viscosity decreases due to the increased deformation applied to the matrix which tears down the resistance. The decreased resistance leads to easier ink flow through the extruder. Yield stress is the maximum amount of force that can be applied before the deformation is irreversible. The combination of shear rate, shear stress and yield stress are all important in determining the resilience and stability of a potential food ink. Another rheological component, shear modulus is the ratio of shear stress to shear strain. Shear modulus higher than 2000 Pa is usually a predictor of the material's printability (Kim, Bae, and Park 2017).

In terms of rheology, food can be categorized into viscoelastic and pseudoplastic (Jiang et al. 2019). Both types of food can behave in Newtonian and non-Newtonian manners. Newtonian foods have viscosity independent of shear rate whereas non-Newtonian foods possess viscosity that is dependent on shear rate. Viscoelastic foods can behave in both Newtonian and non-Newtonian manners and are characterized by the storage modulus (G') which is the sample's ability to store energy elastically; and the loss modulus (G") which is a ratio of viscosity to stress. When G' is higher than G", food can form an elastic gel-like structure which has better structural stability and resistance for printing (Jiang et al. 2019). Table 1 summarizes rheometers and experimental parameters used by past 3D food studies. Researchers have studied viscoelastic foods such as hydrocolloids, soy-alginate-gelatin mixtures, silver carp filet, SSMP, and cheese in 3D food applications and reported G' and G" values (Kim, Bae, and Park 2017; Chen et al. 2019; Wang

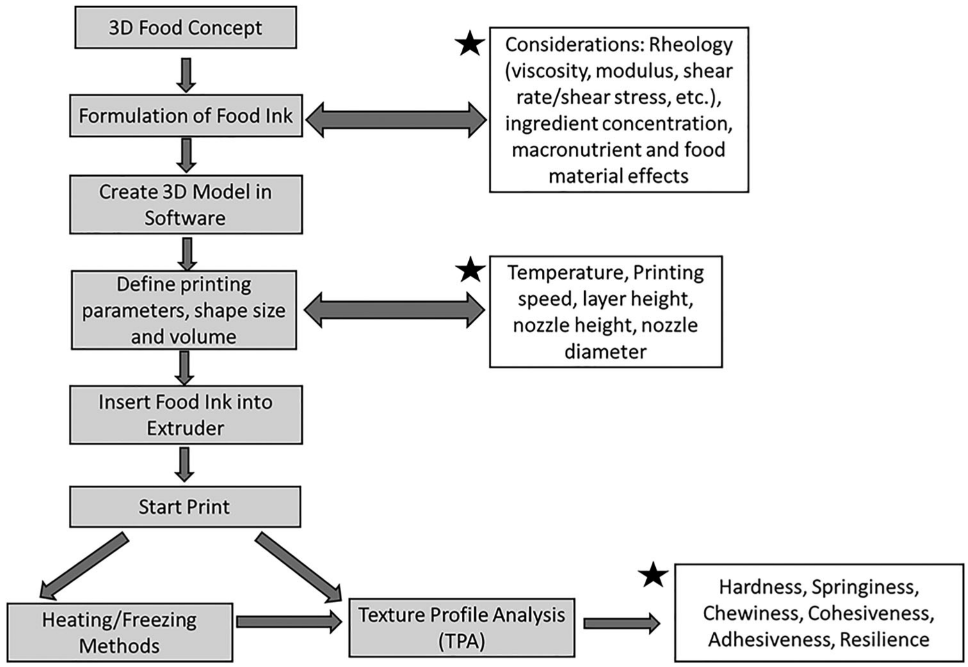


Figure 2. Process flow diagram detailing the 3D food printing process from start to finish and the considerations necessary for creating optimized and successful 3D printed structures. The stars represent key highlights discussed throughout the review and crucial checkpoints in the 3D printing process.

et al. 2018; Lille et al. 2018). These studies reported successful prints with G' and G" values in the range of 100 Pa to 10,000Pa for both properties (Wang et al. 2018; Yang et al. 2018; Lille et al. 2018).

On the other hand, pseudoplastic materials usually behave in a non-Newtonian manner. Pseudoplastic foods possess shear thinning behavior which means that as shear rate increases the viscosity decreases (Feng, Zhang, and Bhandari 2019). Shear thinning foods are ideal for 3D printing since decreased viscosity allows for easier extrusion from the nozzle. After the print is completed, there is no applied shear force and the food is able to recover to a viscous state that will maintain structural integrity (Feng, Zhang, and Bhandari 2019; Jiang et al. 2019). Pseudoplastic foods that have been successfully printed include baking dough, mashed potatoes, agar, vegemite, and marmite (Yang, Zhang, Prakash, et al. 2018; Liu et al. 2018; Hamilton, Alici, and In Het Panhuis 2018; Feng, Zhang, and Bhandari 2019). Researchers reported successful prints with viscosities ranging from 1000 to 10 Pa corresponding with shear rates of 0.1 to 500 s^{-1} (Hamilton, Alici, and In Het Panhuis 2018; Liu et al. 2018; Yang, Zhang, Prakash, et al. 2018). The wide range of values demonstrated that shear thinning principles applied directly to 3D food results in a capacity to provide a variety of successful prints.

Texture

Texture is a physical property relating to sensorial and structural elements of food. Textural analysis is essential for comprehending the structural stability of 3D food as well as indicating suitable mouthfeel and sensory characteristics. To analyze and quantify texture, most published 3D print researchers used the TA-xt2i instrument to conduct a texture profile analysis (TPA) (Table 1). Reported TPA parameters include hardness, chewiness, adhesiveness, cohesiveness, resilience, and springiness (Nishinari, Fang, and Rosenthal 2019). Parameters such as hardness, resilience, cohesiveness, and springiness are additionally good indicators of structural integrity as they represent the object's strength and recoverability to external forces. Hardness can be defined as the maximum force applied prior to the food fracturing, whereas springiness and resilience represent the rate and amount of recovery after deformation, respectively. Other parameters such as chewiness and adhesiveness can provide insight on the quality and mouthfeel of 3D printed food.

In past literature, the most frequently reported parameters were hardness and springiness (Table 2). Successful prints reported values from 4.48 N to 59.8 N for hardness and a springiness ratio under 1 (Table 2). Foods with a springiness values over 1 had poor structural stability because they fractured easily (Kim, Bae, and Park 2017). The data from these parameters demonstrate successful food outputs have a specific range of hardness and springiness. However, other TPA values such as chewiness, adhesiveness, cohesiveness, and resilience were not reported consistently across past published works (Table 2). These features are important textural descriptors and convey how well the food would recover from deformations. Structural stability of food is often assessed qualitatively but there needs to be definitive measures that quantify the changes in structural attributes. Researchers usually visually observe structural changes post print and then record their findings without quantitative measures (Table 2). Further discussion is in section "Printability." Thus, detailed investigation of these parameters would foster a deeper understanding of 3D food texture and thus overall print stability. Additionally, several researchers missed reporting the load cell weight and the

Table 1. Equipment used for Rheological and Texture Analysis.

	Rheometer		Texture Analyzer	
Reference	Make and Model	Parameters	Make and Model	Parameters
Kim, Bae, and Park 2017	Anton Paar. Paar Physica MCR 302	parallel plates with a diameter of 25 mm (PP25/S) and a gap of 1.5 mm.	Stable Microsystems, TAXT2i	30 mL sample height 20.0 mm in a beaker (inner diameter 44.0 mm) with 50 mL volume, compressed twice to 50% original height (cylindrical acryl probe 30.0 mm) Conditions: pretest 10.0 mm/s, test speed 1.0 mm/s, post-test speed of 10.0 mm/s. Load cell weight not mentioned
Chen et al. 2019	Anton Paar. Paar Physica MCR 302	parallel plate (diameter 50 mm) with the gap of 1 mm	Stable Microsystems, TAXT2i	4C. 50 kg load cell, 1 mm/s, 25% original height. compression-decompression cycle repeated after a time interval of 5 s. Triplicate experiment.
Yang et al. 2018	TA instruments, AG-G2 Rheometer	parallel plate (cone diameter 20 mm and gap 2 mm)	Stable Microsystems, TAXT2i	25C. Spherical probe. Initial distance of plate and probe is 3 cm. Down speed= 3 mm/s, compression= 2 mm/s, return speed = 10 mm/s. Tested in triplicates. Load cell weight not mentioned
Le Tohic et al. 2018	TA Instruments, Discovery Hybrid Rheometer	parallel plate (d 20 mm) samples cut as to thickness of 3 mm and diameter of 20 mm.	Stable Microsystems, TAXT2i	Two compression-decompression cycles. Time interval of 5 s. Rate of 1 mm/s, using a cylindrical aluminum geometry (diameter of 35 mm). 25% of initial height (5 mm/20 mm). Triplicate measurements
Dankar et al. 2018	N/A		Stable Microsystems, TAXT2i	50 kg load cell. Acrylic cylinders to a height of 35 mm. Extrusion process: locking distance of compression disk along cylinder to 20 mm. Two extruder holes (3 mm + 5 mm diameter). Speeds of 1 mm/s, 2 mm/s, 4 mm/s. Tests done in triplicates
Lille et al. 2018	TA instruments, AR-G2 Rheometer	parallel plates diameter of 20 mm gap of 1 mm Temp: 22 C	Stable Microsystems, TAXT2i	(no parameters mentioned. Only measured hardness)
Keerthana et al. 2020	Anton Paar, MCR 52 series	gap of 2000 μm between 25 mm diameter parallel plates, shear rate from 0.1 to 100 s^{-1}	Stable Microsystems, TA HD-Plus	Load cell not mentioned. 5 mm diameter cylindrical probe and knife cutting edge Pretest speed: 3 mm/s post-test speed: 5 mm/s
Liu L. et al. 2018	TA instruments, AR-G2 Rheometer	gap of 1000 μm	Stable Microsystems, TAXT2i	Load cell and probe type not mentioned. Room temperature. pretest speed: 1 mm/s; test speed, 2 mm/s; post-test speed, 2 mm/s; distance, 5 mm; time, 5 s;
Liu et al. 2018	TA instruments, Discovery HR-3 Hybrid Rheometer	20 mm parallel plate with the gap of 2000 mm	N/A	N/A
Wang et al. 2017	TA instruments, AR-G2 Rheometer	parallel plate (diameter 20 mm), gap of 2.0 mm.	N/A	N/A
Hamilton et al. 2018	Anton-Paar, Anton-Paar Physica MCR 301	conical plate measuring system (49.972 mm diameter, 0.992 angle, 97 mm truncation	N/A	N/A
Holland et al. 2018	Anton-Paar, Anton-Paar Physica MCR 301	Triplicate, used 100cSt silicone oil	N/A	N/A
Derossi et al. 2017	Brookfield Engineering, Brookfield DV2-HBT	10 rotational speeds ranging from 0.5 to 100 rpm, using the RV/HB/HB-4 spindle.	N/A	N/A

Table 2. Textural and structural analysis of 3D printed foods.

Reference	Materials	Concentra-tion (%)	Hardness (N)	Chewiness (N)	Adhesiveness	Cohesive-ness	Resilience (%)	Springiness (ratio)	Structural Integrity
Chen et al. 2019	Soy	20	1.16 ± 0.1	103.08 ± 5.72	−30.73 ± 7.45	0.91 ± 0.01	69.81 ± 0.54	0.9527 ± 0.0432	Soy alone was extruded easily but the layers deformed
	Soy + alginate	20 + 2 respectively	1.67 ± 0.01	145.90 ± 0.66	−21.30 ± 2.19	0.88 ± 0.01	62.49 ± 0.10	0.9715 ± 0.0090	Had the same issue but performed better than Soy alone
	Soy + gelatin	20 + 10 respectively	16.16 ± 0.4	1548.10 ± 42.82	0.00 ± 0.00	0.94 ± 0.00	80.57 ± 0.94	0.9967 ± 0.0025	Better structural integrity was reported
	Soy + alginate + gelatin	20 + 2 + 2 respectively	4.48 ± 0.28	422.34 ± 28.98	−0.91 ± 1.01	0.94 ± 0.00	74.42 ± 1.44	0.9837 ± 0.004	
		20 + 2 + 6 respectively	10.65 ± 0.3	1007.60 ± 26.0	0.00 ± 0.00	0.94 ± 0.00	78.13 ± 0.86	0.9826 ± 0.0037	
		20 + 2 + 10 respectively	15.66 ± 0.22	1455.01 ± 38.77	0.00 ± 0.00	0.94 ± 0.00	79.06 ± 0.30	0.9723 ± 0.0132	
Kim, Bae, and Park 2017	Methyl cellulose	8	7.30 ± 0.40	N/A	N/A	N/A	N/A	0.9575 ± 0.01	Wide strength ranges and lower springiness values reported, making them more suitable for printing
		10	13.62 ± 1.14					0.9785 ± 0.00	
		12	16.04 ± 0.71					0.9894 ± 0.02	
	Guar gum	8	5.76 ± 0.24					0.952 ± 0.00	
		10	7.85 ± 0.46					0.9762 ± 0.02	
		12	11.47 ± 1.20					0.9881 ± 0.04	
	Xanthan gum	8	1.96 ± 0.29					0.956 ± 0.00	
		10	2.21 ± 0.17					0.9783 ± 0.00	
		12	2.72 ± 0.13					0.9891 ± 0.00	
	Gellan gum	8	68.64 ± 7.86					0.95 ± 0.08	Fractured easily and were too springy which did not allow the structures to hold together
		10	84.99 ± 7.37					0.9703 ± 0.15	
		12	76.79 ± 3.60					0.9856 ± 0.28,	
	Gelatin	8	28.10 ± 6.11					0.947 ± 0.18	
		10	31.59 ± 3.13					0.9723 ± 0.13	
		12	48.25 ± 4.32					0.9873 ± 0.10	
	Agar	8	5.62 ± 0.22					0.9605 ± 0.01	
		10	6.43 ± 0.17					0.9758 ± 0.03	
		12	10.55 ± 0.84					0.9876 ± 0.02	
	Locus bean gum	8	3.39 ± 0.56					0.9535 ± 0.00	
		10	6.02 ± 0.93					0.977 ± 0.00	
		12	8.73 ± 0.31					0.9888 ± 0.03	
	Hydroxypropyl methyl cellulose	8	2.34 ± 0.49					0.953 ± 0.00	
		10	5.59 ± 0.61					0.977 ± 0.01	
		12	7.40 ± 0.52					0.9889 ± 0.00	
Yang, Zhang, Prakash, et al. 2018	Optimized baking dough	NA	N/A	N/A	8.895	N/A	N/A	N/A	If the structure held for 20 min, the print was successful. Butter content of 3 g and lower were failed prints.
Le Tohic et al. 2018	Low-Speed printed cheese	NA	18.4 ± 1.52	N/A	−0.8 ± 0.23	0.60 ± 0.03	0.25 ± 0.03	0.80 ± 0.04	Printing generated weaker structures when compared to untreated cheese.
	High-speed printed cheese		19.6 ± 1.49		−0.75 ± 0.24	0.58 ± 0.03	0.23 ± 0.02	0.78 ± 0.03	
	Untreated cheese		36 ± 7.54		−1.69 ± 0.32	0.53 ± 0.10	0.22 ± 0.06	0.80 ± 0.06	
	Melted cheese		32.3 ± 3.64		−0.96 ± 0.52	0.59 ± 0.04	0.25 ± 0.02	0.84 ± 0.02	
Dankar et al. 2018	Potato puree with alginate	0.5	3.3 ± 0.54	N/A	N/A	− 3.4 ± 0.5	N/A	N/A	Best structural integrity was obtained using alginate and agar.
		1	11.79 ± 1.56			− 4.3 ± 0.5			
		1.5	57.51 ± 1.9			− 2.7 ± 0.4			

(*continued*)

Table 2. Continued.

Reference	Materials	Concentra-tion (%)	Hardness (N)	Chewiness (N)	Adhesiveness	Cohesive-ness	Resilience (%)	Springiness (ratio)	Structural Integrity
	Potato puree with agar	0.5	0.13 ± 0.01			− 74.0 ± 36.5			Glycerol and lecithin led to deformation of structures
		1	0.16 ± 0.01			− 245.3 ± 31.7			
		1.5	0.15 ± 0.006,			− 687.7 ± 86.5			
	Potato puree with lecithin	0.5	0.136 ± 0.009			− 3.1 ± 0.5			
		1	0.144 ± 0.014			− 2.4 ± 0.4			
		1.5	0.139 ± 0.009			− 2.3 ± 0.6			
	Potato puree with glycerin	0.5	0.151 ± 0.009			− 3.6 ± 3.0			
		1	0.147 ± 0.014			− 2.4 ± 0.4			
		1.5	0.132 ± 0.015			− 2.5 ± 0.9			
Lille et al. 2018	Semi-skimmed milk powder	60	OD: 42.6 ± 3.1, FD: 36.7 ± 5.7,	N/A	N/A	N/A	N/A	N/A	Freeze dried structures generally showed better stability than oven drying since there was less moisture loss. For samples with higher dry matter content, comparable structures were obtained with both methods.
	Semi-skimmed milk powder + cellulose nanofiber	50 + 0.8 respectively	OD: 16.3 ± 4.2, FD: 9.4 ± 1.4,						
	Oat protein concentrate	35	OD: 13.0 ± 2.0, FD: 2.2 ± 0.0						
	Faba bean protein concentrate	45	OD: 2.9 ± 0.9, FD: 59.8 ± 16.3						
Wang et al. 2018	Fresh silver carp filet-minced + NaCl solution	82 + 0, 0.5, 1, 1.5 and 2 respectively	N/A	N/A	N/A	N/A	N/A	N/A	Gel strength was enhanced with 1.5% NaCl solution and was able to support its own weight.
Liu et al. 2018	Egg White Protein + Gelatin + Corn Starch + Sucrose	0 + 5 + 7 + 3 respectively	0.723 ± 0.110	0.489 ± 0.0990	− 0.093 ± 0.025	0.728 ± 0.003	0.394 ± 0.013	0.934 ± 0.006	Unable to retain structural stability due to low hardness and viscosity
		1 + 5 + 7 + 3 respectively	1.008 ± 0.144	0.595 ± 0.121	− 0.086 ± 0.013	0.736 ± 0.002	0.438 ± 0.018	0.960 ± 0.004	
		3 + 5 + 7 + 3 respectively	1.644 ± 0.174	1.195 ± 0.159	− 0.074 ± 0.036	0.750 ± 0.005	0.547 ± 0.009	0.956 ± 0.006	
		5 + 5 + 7 + 3 respectively	2.019 ± 0.211	1.222 ± 0.172	− 0.072 ± 0.038	0.764 ± 0.004	0.547 ± 0.010	0.962 ± 0.008	The best structural integrity was maintained.
		7 + 5 + 7 + 3 respectively	1.763 ± 0.192	1.468 ± 0.185	− 0.068 ± 0.039	0.777 ± 0.003	0.588 ± 0.013	0.948 ± 0.007	Difficulty printing due to high viscosity
Keerthana et al. 2020	Mushroom dough	100	100.21 ± 5.36	5.19 ± 0.49	−11.13 ± 2.90	0.10 ± 0.005	0.02 ± 0.004	0.43 ± 0.09	Texture was taken before printing and structures were not printable due to lack of particle aggregation
	Wheat Dough	100	87.39 ± 11.77	30.93 ± 2.93	−55.52 ± 4.29	0.46 ± 0.04	0.05 ± 0.016	0.77 ± 0.022	N/A
	20% mushroom dough + Wheat Flour	20 + 50 respectively	53.17 ± 11.86	49.33 ± 16.37	−197.80 ± 12.91	0.93 ± 0.09	0.02 ± 0.005	0.99 ± 0.003	Achieved the best structural stability and precision

- All hardness values were converted to newtons (N) for consistency.
- Optimized baking dough contained icing sugar, butter, low gluten flour, egg and water at ratio of 6.6:6:48:10.4:29
- Cheese used for the cheese data is commercially available processed cheese, containing 25% fat, 3% carbohydrate (2% lactose),18% protein and 3% salt.
- OD means oven dried and FD means freeze dried
- The mushroom and wheat doughs were mixed with water equal to flour in a 1:1 w/v ratio.

type of the compression probe (Table 1) which is a limitation as these conditions are necessary for calibration and replicability in future experiments.

Composition

The overall composition of the food matrix has a significant impact on its printability. This includes the macronutrient composition as well as the incorporation of additives. The food materials can be printed with different levels of effectiveness when printed alone compared to heterogeneous system containing multiple ingredients. A prime example is that a soy protein isolate (SPI) mixture with water was printed unsuccessfully and lacked structural integrity because SPI as a sole ingredient was unable to achieve optimal rheological properties suitable for 3D printing (Chen et al. 2019). When alginate was incorporated in the SPI solution, the printability improved slightly. However, the best synergistic effect of materials was reported when SPI was mixed with alginate and gelatin (Chen et al. 2019). The alginate and gelatin synergistically served as thickening agents which provided a better rheological profile lacked by soy proteins (Chen et al. 2019). For instance, the SPI and SPI-alginate solutions could not achieve hardness values over 2 N which was too low for extrusion but once gelatin was added to the mixture, the hardness ranged from 4.48 to 15.66 N which significantly improved the overall structural integrity (Table 2).

Apart from SPI, mashed potatoes are another ingredient that has been used by several researchers (Liu et al. 2018; Dankar et al. 2018). As stated before, mashed potatoes required additional ingredients such as potato starch, alginate, agar, lecithin, and glycerol to be printed successfully (Dankar, Pujolà, et al. 2018). The final results showed that alginate, agar, and potato starch were found to be the most suitable thickeners as they improved the hardness and extrudability (Dankar, Pujolà, et al. 2018). Contradictorily, lecithin and glycerol destabilized the internal microstructure and led to the softening of the mashed potato mixture preventing a stabilized print (Dankar, Pujolà, et al. 2018). As previously mentioned, silver carp filet printing required cross-linking with the presence of sodium chloride (concentrations between 0.5% and 2%; optimal: 1.5%) for structural integrity (Wang et al. 2018). However, texture profile analysis was not reported to better understand the performance indicators such as hardness, chewiness, and springiness.

Macronutrient composition could also factor in to how well the food matrix prints as past research has demonstrated. One such example is the SSMP and SMP study described in section "Lipids" where the researchers found that the presence of fat in SSMP (9% fat) served as a lubricant, optimizing extrudability when compared with SMP containing no fat. Another study involving 3D printed baking dough demonstrated the effects of both carbohydrates and lipids in optimizing printability. Here, the researchers examined the effects of sucrose and butter at approximate concentrations of 3–9% (Yang, Zhang, Prakash, et al. 2018). The results indicated that with the increase of sucrose content, the free water in the system decreases, allowing stiffer particle network formation which then increases the gel strength (Yang, Zhang, Prakash, et al. 2018). Likewise, sugar crystallization also helps to trap fat and control viscosity of the dough (Yang, Zhang, Prakash, et al. 2018; Godoi, Prakash, and Bhandari 2016). At 3.3% sucrose the printed structure lacked firmness and at 8.2% the high sucrose content led to breakage in the dough's structure due to lower free water content and lack of lubrication (Yang, Zhang, Prakash, et al. 2018). Meanwhile with the presence of butter, 3% concentration lacked adhesiveness and 9% decreased the dough resilience and increased viscosity which resulted in poor print resolution (Yang, Zhang, Prakash, et al. 2018). However, the best performing print reported at intermediate concentration of sucrose (6.6%) and butter (6%) at which the dough achieved the optimal rheological parameters.

Formula optimization

Ingredient ink concentrations directly affect factors such as viscosity and texture that needs to be optimized to obtain flowable ink that sets after printing. Higher ingredient concentrations usually increase the viscosity and could either assist or impede the finished 3D printing process. Past research explores the effects of concentration in materials such as gelatin, soy, alginate, and egg whites. (Chen et al. 2019; Kalkandelen et al. 2017; Lille et al. 2018; Liu, Meng, et al. 2019). Chen et al. used a mixture of SPI (92.1% protein), alginate and gelatin to print 28 mm (diameter) × 20 mm (height) cylinders. The SPI and alginate content were kept constant at 20% and 2% w/v respectively whereas gelatin concentration was varied in increments from 2% to 10% w/v (Chen et al. 2019). At different gelatin concentrations, the mixture obtained different functionality and printability (Chen et al. 2019). All the variations obtained good structural integrity however, lower concentrations such as 2% and 6% led to less firm and stable structures, due to the lower hardness value of soy-alginate-gelatin system (Table 2).

Kalkandelen et al. also conducted a similar experiment using only gelatin and water as ingredients. The researchers used numerous gelatin concentrations ranging from 1% to 20% and found a threshold value of 5% to obtain any structural integrity (Kalkandelen et al. 2017). In addition, the study found that the highest concentration (20%) was too viscous for extrusion (Kalkandelen et al. 2017). The optimal concentration for printing gelatin was found to be in the range of 10–15%. Contradictorily, in another study conducted by Kim et al., 10% and 12% gelatin concentrations were found unsuitable for printing. Between the two studies there were several inconsistencies. A critical point is that both papers did not state the type of gelatin used (bloom strength, type A/B) which may suggest discrepancies in results. Additionally, limited information was reported on the 3D printers as both studies used custom made or modified printers. Also, important printing parameters such as printing speed were not comparable as they were reported using different units. Such inconsistencies lead to difficulties in reproducibility and could produce contradictory results. Separately, Kim's study also examined the effect of concentration on methylcellulose, guar gum, and xanthan gum and reported that they were the most suitable for 3D printing due to having wide strength ranges and lower springiness (Table 2).

Research has also been conducted on 3D printing egg white proteins mixed with ingredients such as sucrose, gelatin, and corn starch (Liu L. et al. 2018). The concentration of sucrose, gelatin, corn starch were kept constant throughout the experiment at 3%, 5%, and 7%, respectively, while the egg white protein concentration varied from 0 to 7% (Liu, Meng, et al. 2019). Results showed that at low egg white protein concentrations (1, 3%) the print was unable to retain structural stability after printing due to low viscosity. In addition, a high concentration (7%) was unsuitable because the solution had difficulties being extruded (Liu et al. 2018). However, an intermediate concentration (5%) was optimal as it provided enough viscosity for extrusion and maintained structural integrity after the print (Liu et al. 2018). These results demonstrate that there is a need for careful consideration of the rheology of the food ink and the textural stability of the print used in order to successfully print self-supporting structures.

Food ink structure

In addition to composition and formulation, food ink structure can impact printability. Food ink structure can be modified by several methods such as using gel-like emulsions and high internal phase emulsions (Liu, Meng, et al. 2019; Li et al. 2020). Emulsions can be used to create semi-solid materials that can be printed. Specifically, gel-like emulsions were prepared with microfluidization and pickering emulsions. Additionally, oil phase properties were studied with regards to whey protein in 3D food printing (Liu, Meng, et al. 2019). The researchers used oil fractions ranging from 0.3 to 0.6 v/v and found that increasing the oil fraction transformed the ink into a more rigid structure suitable for printing (Liu, Meng, et al. 2019). Furthermore, high internal phase emulsions (HIPEs) have also been utilized for 3D food printing purposes (Li et al. 2020). HIPEs have an internal phase ratio exceeding 74% and result in high stability with a self-supporting structure (Li et al. 2020). Particularly, in 3D food applications, HIPEs were used with varying ratios of cod protein (10-50mg/mL) and results indicated an oil-phase ratio of 76%–88% coupled with 50 mg/mL cod protein led to prints with long term structural stability and plastic rheological behavior (Li et al. 2020). These findings demonstrate that there are alternative processes that can affect food properties and improve food ink printability.

Printability

As discussed previously, printability is the ability of the material to be successfully printed in a specific application. Printability of the material can be predicted via rheological and textural factors such as shear modulus, viscosity, hardness, and springiness (Kim, Bae, and Park 2017; Liu et al. 2018; Yang et al. 2018). Throughout past published work, printability has primarily been examined qualitatively using different methods. These assessments did not follow a consistent method correlated with time or texture, making them difficult to replicate for future researchers. Furthermore, inconsistent methodology leads to challenges in comparing different experiments and interpreting the data. For example, Lille's work, uses a five-point ranking scale to report the quality of prints (1 – lowest quality; 5 – highest quality) and noted factors such as nozzle clogging or stability of the final structure (Lille et al. 2018). Another study by Keerthana et al. assessed printability by considering flowability and structural stability of the printed product reported by flowability measured by printing precision percentages. However, the overall structural stability was visually assessed and reported with photo evidence. The photos demonstrate different levels of precision and stability, but they are not quantified. Contrary to Lille's work, there was no qualitative numerical scale incorporated and different parameters were evaluated for printability. Neither study had quantifiable data available for the measures of time, structural stability, or texture with regards to printability. Without numerical data for time, structural stability, or texture, it is difficult to determine quality, safety, and shelf life for potential consumers. While past literature provides framework, further work can be done to establish consistent interpretation of printability and structural stability. Future research should include TPA measurements post print over several different time intervals. These measurements should be combined with visual assessments, images and reporting to create a comprehensive scale and method for determining printability.

While there are many contributing factors that influence printability, standardizing methods and overall approach would help to improve efficiency and establish better advancements in 3D printing of foods. A well-considered standardized approach assesses all of the critical parameters for printability and ensures they also account for time and texture. In general, past published work does not take multiple textural measurements over time. By not tracking these changes, it is difficult to determine the overall integrity of the printed product. Taking measurements over established timeframes could give quantifiable data on structural integrity and printability. These times should be based on the food ink used; individual researchers need to define their rationale and timing based on experimental conditions.

Experimental and processing components

In conjunction with internal food components, experimental and processing components require optimal design and execution, of which entails fine tuning printing process parameters and processing techniques. Furthermore, experimental design and equipment need a higher degree of clarification for future reproducibility and success. In this section, we aim to discuss how external influences and parameters are critical to optimizing the 3D food printing process.

Process and mechanical parameters

Optimization of process parameters such as temperature and mechanical parameters like printing speed, layer height, nozzle height and diameter are necessary for successful 3D printing of food. Temperature is a critical parameter because it can affect rheological and textural properties. For example, temperature often influences viscosity with an inverse relationship. The majority of past research involving 3D printing food was conducted at room temperature (20–25 °C). However, some food inks were printed at higher temperatures due to inherently high viscosity at room temperature. The higher temperatures were used as a process parameter to decrease the viscosity to allow for improved flowability. This principle was applied to foods such as gelatin and cocoa (Feng, Zhang, and Bhandari 2019). Other examples include soy mixtures and cheese that were printed at 35 °C and 75 °C respectively (Table 3).

3D printing parameters such as printing speed, layer height, nozzle height and nozzle diameter also need to be optimized for successful results. Printing speed is a crucial mechanical parameter that directly affects the structural stability. While printing speed can improve efficiency, historically faster printing speeds lead to lower resolution prints and unstable structures due to the inaccurate extrusion, uneven layer formation and inability of the nozzle to maintain the inputted speed (Dankar, Haddarah, et al. 2018; Hertfaeld et al. 2019; Severini, Derossi, et al. 2018; Derossi et al. 2018). Lower printing speed slows down the printing process leading to structural instability since it is difficult for the layers to support itself for an extended time (Dankar, Haddarah, et al. 2018; Hertfaeld et al. 2019; Severini, Derossi, et al. 2018; Derossi et al. 2018). There are some consistencies in previously reported printing speed data and most research has found successful printing speeds around 10-30mm/s (Table 3).

Apart from printing speed, layer and nozzle height can significantly affect the final structure of 3D printed food. When layer height is too high, the structure has too few layers to support its own weight and therefore holds low structural integrity (Pérez et al. 2019; Dankar et al. 2018). Like low printing speed conditions, a layer height that is too low extends print time and again leads to structural instability. Even though layer height is not always a reported parameter throughout past literature, the available data reports successful prints with a height of 0.3–1.1 mm (Table 3). Furthermore, nozzle height and diameter affect the height and volume of the object printed (Yang, Zhang, and Bhandari 2017). Nozzle height determines the distance of the nozzle from the printing surface and needs to be optimized to obtain high resolution print. Prints at lower nozzle heights could clump together and at higher nozzle heights the prints could spread too far apart resulting in lower precision. Similarly, as nozzle diameter increases, the print becomes larger which leads decreased printing precision as a result leads to improper weight distribution due to imbalance of the shape's foundation (Yang, Zhang, and Bhandari 2017; Pérez et al. 2019; Dankar, Haddarah, et al. 2018). Throughout literature, nozzle diameter is consistently mentioned and successfully printed objects used diameters in the range of 0.41–2.00 mm (Table 3).

Processing techniques

Pre and post processing techniques such as cooling or heating by various means can affect the success of 3D printing of foods. These methods include cooking, baking, and freezing (Hertfaeld et al. 2019; Liu et al. 2017; Lille et al. 2018). Uncooked foods with lower rigidity sag which can increase the distance between the nozzle and the extruded layer, resulting in uneven printing with low structural integrity (Hertfaeld et al. 2019). Pre-processing methods such as microwaving and freezing have been utilized (Dankar et al. 2019; Keerthana et al. 2020). Dankar's study found that microwaving potatoes decreased moisture content which led to stronger inter-cohesive starch networks which improved rheological properties for 3D printing. Furthermore, Keerthana investigated the effect of blanching and freezing mushrooms on 3D printing for improved print quality and reported no loss of sensorial attributes from pre-processing.

IR heating has been used as a post processing step to improve rigidity and structural stability for several food inks consisting of ingredients such as sesame paste, chicken paste, shrimp paste and jujube jam (Hertfaeld et al. 2019). Likewise, microwaving has also been used as a post-processing cooking method and was reported to increase the hardness values of printed mushroom structures (Keerthana et al. 2020). Oven and freeze drying of ingredients have also been investigated as a post processing step on ingredients such as SSMP, oat protein concentrate, and faba bean protein concentrate (Lille et al. 2018). For all the ingredients except the faba bean protein concentrate, the oven drying lead to a higher hardness value when compared to the freeze-dried method (Table 2). However, oven drying led to shrinkage of the structure and reduced the structural stability compared to freeze drying which induced minimal structural changes in the 3D printed product (Lille et al. 2018).

Additionally, there are other techniques with potential for implementation in 3D food printing. These methods include utilizing the application of dual extrusion as a pre-processing step or the addition of other ingredients post-print. Primarily, single extrusion feeds are used in 3D food experiments. However, in other types of 3D printing experiments, dual extrusion has been utilized to bio-print hyaluronic acid (HA) hydrogels with materials such as alginate, gelatin, and agarose for photo-induced crosslinking (Ouyang et al. 2016). Ouyang found that crosslinking these materials led to shear-thinning behaviors which resulted in printed structures with high structural stability. These principles can be further applied to 3D food with ingredients such as calcium, pectin, and alginate. Another potential post-processing technique entails using a calcium chloride spray to improve textural properties and shelf life of the final product. Calcium chloride spray has been used in non 3D food applications with soy protein and fruits to improved nutritional content, extended shelf-life and improved important textural properties such as firmness, hardness and springiness (Zheng et al. 2019).

Table 3. Printing parameters and shapes of 3D printed foods.

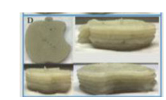
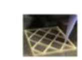
	Macronutrient Used for Printing			Printer Parameters						
Reference	Type	Material	Shape Printed	Nozzle Speed (mm/s)	Nozzle Diameter (mm)	Extrusion Speed (cm○3/s)	Layer Height (mm)	Temperature (°C)	Flow Rate	Image
Kim, Bae, and Park 2017	Primarily Carbohydrate	GeG, Gelatin, MC, GuG, MC, Agar, LBG, HPMC, XG	Spider web (2mm height), Maple Leaf (20mm height), Pentagon (40mm height), Spiral (40mm height) Cylinder (28*20mm, 40mm,80mm height)	20	1.1	N/A	0.9	20	N/A	
Yang et al. 2018		Baking Dough	Mickey Mouse (5.41cm length * 4.95 cm weidgth * 2.28 cm height)	25	2	0.118	N/A	24.8	N/A	
Yang et al. 2018			Square frame (same as mickey)	25	2	0.118	N/A	24.8		
Severini et al. 2018		Carrots, pears, kiwi, broccoli raab, avocado	Pyramid	10.97 15.87 20.77	1.2	N/A	1.1	Room temp	70%, 100%, 130%	
Keerthana et al. 2020		Mushrooms, Wheat Flour	Butterflies (44.39 mm±*30.20 mm±* 5 mm)	13.33	1.28	N/A	N/A	N/A	0.383g/min	
LeTohic et al. 2018	Primarily fat	Cheese		N/A	1.5	0.0666667, 0.2	N/A	75	N/A	N/A
Chen et al. 2019	Primarily Protein	Soy, Gelatin, Alginate	28x20mm cylinder	10	1.55	N/A	0.6	35	80%	SAG-10 
Wang et al. 2018		Fish surimi gel	Apples	28	2	0.0028	N/A	N/A	N/A	
Lille et al. 2018		SSMP, OPC, FBPC	25mmx25mm squares	2	0.41	N/A	0.3	Room temp	N/A	 
Liu L et al. 2019		Egg white protein, gelatin	Snowflakes, squares	70	2.5	0.003, 0.004, 0.006, 0.007	N/A	40	N/A	

Table 4. 3D Printers used in Past Research.

Reference	3D Printer: Model	Type	Description	Software
Kim, Bae, and Park 2017	Custom 3D Printer	Extrusion	Diagram of printer without explanation of components	Cura 2.4
Chen et al. 2019	Modified Felix 3.0	Extrusion	Felix 3.0 was modified with the motor-driven system for food matrix extrusion. Used a 60 mL syringe to print	Cura 15.04.6 (Ultimaker)
Yang, Zhang, Prakash, et al. 2018	Porimy CO	Extrusion	Four-part printer: conical hopper, electric heater and transport system, nozzle, stepper motor	N/A
Liu et al. 2018	Porimy CO	Extrusion	Four-part printer: conical hopper, electric heater and transport system, nozzle, stepper motor	N/A
Keerthana et al. 2020	Custom 3D Printer	Extrusion	Extrusion motor of 300 rpm. Externally equipped compressor (0.75 hp, 230 V AC). Pneumatically assisted screw driven mechanism	Simplify 3D (ver.±4.1.0)
Dankar et al. 2018	RepRap BCN3D + printer	Extrusion	Coupled with extrusion tool. (100 mL volume and 4 cm diameter)	N/A
Le Tohic et al. 2018	Modified Reprap Ormerod 1	Extrusion	Retrofitted with custom designed syringe-based extrusion configuration	CAD
Lille et al. 2018	Custom 3D Printer	Extrusion	No printer description, used VTT micron dispensing environment	N/A
Liu et al. 2018	FSE2 Bolimai Co	Extrusion	Four-part printer: conical hopper, electric heater and transport system, nozzle, stepper motor	N/A
Wang et al. 2017	Custom 3D Printer	Extrusion	3-part system: feed hopper, extrusion, XYZ positioning system	
Hamilton, Alici, and In Het Panhuis 2018	BioBot 1 Printer with Repetier Host software	Extrusion	none (commercially available)	N/A
Derossi et al. 2018	Modified Delta 2040 with Clay extruder kit 2.00	Extrusion	none	N/A
Holland et al. 2018	FujiFilm Dimatix ink jet printer	Ink Jet Printing	None	Cura 14.7 (Ultimaker), Tinkercad (AutodeAsk Inc).

Shape

In 3D printing, a shape is three-dimensional which means it has a length, width and height that creates a volume. Sustaining the final 3D printed shape is important for its identity and originality. Adjusting the shape of food can impact the 3D printing process by changing the final structural stability and the printing time. Shapes with infill and larger dimensions take longer to print since printing speed is capped to maintain precision. The shape can also affect the structural stability. Solid cylinders are a common test shape that is used throughout past published work (Chen et al. 2019; Kim, Bae, and Park 2017). While there is no direct justification for the use of cylinders, they are simple to set up geometrically.

While irregular and non-uniform shapes provide novelty, they are also more likely to have poor structural stability due to an asymmetrical distribution when compared to uniform shapes such as cubes or cylinders. Shapes can be used to form unique objects for printing, however, these introduce new challenges due to uneven weight distribution. Severini et al, reported unique shapes such as pyramids caused deformation and produced a curvature instead of triangular shape (Severini, Derossi, et al. 2018). In past research, there have been numerous attempts to print objects such as apples, butterflies, square frames, and Mickey Mouses with mixed results because of the shape complexity (Pérez et al. 2019; Keerthana et al. 2020; Severini, Derossi, et al. 2018; Yang, Zhang, Prakash, et al. 2018; Kim, Bae, and Park 2017; Wang et al. 2018; Lille et al. 2018). A summary of this work is reported in Table 3.

The printing parameters reported vary greatly across different shapes and even for the same shape (Table 3). For example, both Kim and Chen use cylinders as their testing shapes but used different printing parameters (Table 3) resulting in different outcomes in textural analysis and structural integrity. The varying conditions are likely because of the use of different food inks and 3D printers and the details have been captured between Tables 1–3. Based on past research, opportunities exist in identifying the effect of shape on textural output and structural integrity. For example, in past work, textural data was not always reported for all shapes printed in an experiment. For instance, Chen et al. (2019) used a soy-alginate-gelatin system to print several shapes such as cylinders, pears, and "CAAS" but textural data was only available for cylinders limiting comparisons of textural stability among shape. To further investigate the effect of shape, a diversity of shapes should be printed with the same food ink and undergo texture analysis.

Printers and customization

3D printing for food applications is a more recent development that is distinct from typical 3D printing. The majority of 3D printers use thermoplastics such as polycarbonate and are unsuitable for 3D food printing as the composition of food differs from thermoplastics (Dankar, Haddarah, et al. 2018; Yuan et al. 2019). Thermoplastics possess high hardness and viscosity values as well as little variation in the physicochemical properties which makes printing conditions more defined (Dankar, Haddarah, et al. 2018). In 3D food printing, the food inks possess a broad spectrum of characteristics which require specific parameters depending on ink composition for successful prints. The majority of reported food printing is extrusion based because it is the most affordable and suitable for the wide spectrum of unique physicochemical properties in food.

In past published works, many of the 3D printers used were modified or custom-built to effectively print food. Some examples include modifying the Reprap Pro Ormerod 1, Felix 3.0 and Delta 2040 printers (Table 4). The Reprap Pro Ormerod 1 was originally designed for plastic materials but was modified and reconfigured with a custom designed syringe that was appropriate for food extrusion (Table 4). Similarly, the Felix 3.0 was originally designed for plastic materials and lacked a food input system, however, it was then modified with a motor-driven system for food extrusion and a syringe for the input of food (Table 4). Another printer, the Delta2040 was customized with a clay extruder kit for softer food material extrusion (Table 4). Furthermore, some researchers made custom built printers for food applications and specifications were unavailable (Table 4). 3D printers have varying capabilities, specifications and design differences which makes standardization and reproducibility difficult. For future work, the setup, modifications, and customizations of 3D printers need to be clarified. On a larger scale, these specifications could provide further insight to 3D food printer advancement; thus, leading to the development of more universal printers for 3D food applications.

Future opportunities for characterizing 3D food

Current 3D food analysis accentuates the effects of rheology and texture on printability; however, future opportunities exist for using traditional food analysis methods to characterize 3D printed foods. 3D food analysis should also focus on physicochemical characterization such as moisture content, water activity, color and microbial safety and sanitation. These properties are essential as they are important predictors of the quality, shelf life and safety. As mentioned in section "Factors influencing 3D food printing," another limitation is the dearth of food materials that have been researched and suggestions will also be made as to what foods should have further exploration in 3D printing.

Physicochemical characterization

Understanding physicochemical properties of 3D printed foods is essential for the assessment of the safety and quality. For 3D food printing to increase its scope in markets, more research needs to be conducted to assess the safety, quality, and viability of edible prototypes for commercial applications. Below we discuss additional traditional food science-based techniques used to better characterize food that can be applied in 3D food systems to better understand their respective quality (and safety).

Moisture content

Moisture content can be defined as the amount of water in a product and is usually represented as a percentage or ratio. Water influences several critical properties of food such as weight, viscosity, shelf life, texture, and microbial safety (Isengard 2001). Due to moisture content's impact on critical aspects of printability such as rheology and texture, its influence on printability should be further investigated. Limited studies involving 3D food printing have reported moisture content data for food ink ingredients and printed foods. In 3D food, moisture content is usually measured by the dry oven method. Both Liu and Severini utilized dry oven methods to measure moisture content; Liu used the Chinese national standard GB/T8858-88 and Severini used gravimetric weighing. However, in a study conducted with lemon juice, the vacuum drying method was utilized (Yang, Zhang, Bhandari, et al. 2018). With those researchers, reports for food ink ingredients ranged from 78 to 81% for potatoes, 73.5–88.9% for a blend of fruits and vegetables that included carrots, pears, kiwis, broccoli, and avocado, and 59.82% for lemon juice (Liu et al. 2018; Severini, Derossi, et al. 2018; Yang, Zhang, Bhandari, et al. 2018). In addition, moisture content was examined after the 3D printing process (Keerthana et al. 2020). Prior to printing, the mushroom-based food ink had a moisture content of 7.45% and increased to 9.29% post processing consequent of adding water during ink preparation (Keerthana et al. 2020). Past work demonstrates moisture content has an inverse relationship with viscosity and can thus be used as a tool to adjust food viscosity without using additives (Severini, Derossi, et al. 2018). Future research on 3D foods should include moisture content as an experimental parameter to improve rheological properties and printability.

Water activity

Water activity (A_w) can be defined as the amount of water that is available for use for chemical reactions and microbial activity (Sandulachi and Tatarov 2012). There are multiple methods for measurement of water activity such as measuring vapor pressure, osmotic pressure, freezing point depression, boiling point, dew point and several other characteristics (Sandulachi and Tatarov 2012, Syamaladevi et al. 2016). Water activity is also an indicator of shelf stability; typically, a higher water activity (greater than 0.85) means that microbial growth is more likely (Sandulachi and Tatarov 2012).

In past 3D food research, there is one study with data on A_w. Water activity was reported for a mushroom-based food ink prior to printing and post-print and values were reported at 0.60 and 0.66 respectively (Keerthana et al. 2020). The slight increase in water activity is due to the entanglement of moisture in a fibrous network from water in preparation steps (Keerthana et al. 2019). Keerthana reports that bound water retention aided flowability and printability of the food ink. However, there are no additional studies reporting water activity in 3D food, providing an opportunity for further exploration.

If 3D printing food production were scaled up, water activity assessment is crucial for long term shelf-life stability and microbial safety. 3D food will need to meet the same established critical water activity standards as traditional food. By selecting and designing food inks and/or prints that have lower water activity, the microbial safety risks can be reduced significantly. However, decreasing water activity comes with its own potential challenges. While water activity reduction can impede microbial growth, at a low A_w ($\sim$0.5) food becomes more prone to oxidation and rancidity which leads to a decrease in quality and shelf life (Barden 2014). Decreasing water activity can also lead to increased thermal resistance in several strains of microbes (Syamaladevi et al. 2016). A possible remedy would be further heat treatment, which can cause issues with quality and appearance (Syamaladevi et al. 2016). Thus, careful considerations need to be made with water activity in 3D food to have a product that is safe, appealing, and high quality. Quality can be indicated by moisture sorption isotherms. Moisture sorption isotherms are the relationship between moisture content and water activity at constant temperature and pressure (Syamaladevi et al. 2016). Currently in food applications, isotherms can be used to determine food dehydration and storage conditions which impacts shelf life and stability (Syamaladevi et al. 2016). Applying these principles would help 3D food continue to transition into a larger scale.

Color

In food, consumers' initial evaluation of appearance and quality is based on color, therefore color measurement of 3D printed food is very important. Current literature has limited reports on the effect of the 3D printing process on color. For example, Dankar's study, the color parameters of hue angle, luminosity and chroma were taken prior to printing and post processing in a 3D printed potato puree application. The findings showed that there was little change in color caused by the 3D printing process, but additives such as agar and alginate caused decreases in the luminosity values (Dankar, Pujolà, et al. 2018). Additionally, studies with 3D cheese and mushroom ink reported color values of L, a, and b to show brightness and the color ranges (Le Tohic et al. 2018; Keerthana et al. 2020). In Le Tohic's study, the printing process caused a decrease in luminosity on the surface of the printed cheeses. Moreover, Keerthana's study investigated the effect of both printing and microwaving and found a significant decrease in lightness due to non-enzymatic browning from heat. These findings show that the 3D printing process could impact the overall color of food and therefore should be more widely assessed as a tool to determine overall food quality, appeal and as a preliminary indicator for comparison with traditional food products.

Microbiological analysis of *3D* food

With recent technological advancements in 3D food printing innovation and the potential of commercialization in the future, one key challenge that needs to be addressed is developing a validated sanitation procedure for microbial decontamination. Due to the unique design of 3D printers, standard cleaning procedures might not be effective. For traditional food manufacturing, microbial safety is one of the most crucial aspects that is studied and executed. Improper sanitation and storage practices can cause foodborne illnesses caused by microorganisms such as bacteria, yeast, and mold.

While past research regarding microbial load in 3D food is limited, Severini conducted a total plate count on the 3D printed fruits and vegetable mixture. The plates were tested under both aerobic and modified atmospheric packaging (MAP) environment with constant experimental conditions (Severini, Derossi, et al. 2018). Although the raw materials were thoroughly washed to decrease the microbial load, the study reported log CFU of 3-5 for the final 3D printed product for both conditions (Severini, Derossi, et al. 2018) suggesting that the preparation and the 3D printing process may have contributed considerable amount of microbial contamination. During the printing process, the ink came into contact with multiple parts of the printer such as the extruder, piston, and tube which have the potential to serve has harborage points for microbial growth (Severini, Derossi, et al. 2018). In this experiment and other past studies, the finished product was the focus of the microbial analysis and none of the printer components were swabbed and tested for microbial load to evaluate risk points and/or cleaning and sanitation efficacy. Additionally, no specific treatments for the sanitation of the 3D printer or 3D printed food have been discussed in past literature. The unavailability of data in both microbial load of 3D printers and potential sanitation treatments provides future opportunities for improving the microbiological safety and analysis of 3D food.

Food contact surfaces need to be cleaned to remove extraneous materials such as soil and then sanitized to eliminate microbes. On food contact surfaces, surfactants and alkali products can be used as cleaning agents to dissolve food debris by denaturing proteins and decreasing surface tension (Srey, Jahid, and Ha 2013). Sodium hypochlorite (bleach) is the most frequently used sanitizer for food contact surfaces due to the ease of use, effectiveness, and lower cost (Meireles, Giaouris, and Simões 2016). Moreover, other sanitation treatments such as ultrasound, UV light, and chemicals such as calcium lactate, electrolyzing oxidized water and hydrogen peroxide could be potentially used for

3D printing applications (Meireles, Giaouris, and Simões 2016; Srey, Jahid, and Ha 2013). However, the effectiveness of these treatments for 3D food printing applications needs to be evaluated and validated as not all of these approaches may be suitable for 3D equipment. This can be done by swabbing and plating food contact surfaces within the 3D printers targeting specific areas including the syringe, piston, extruder, tube, and printing platform prior to and post treatment.

Future foods for exploration

3D Food printing is an upcoming field that currently has limited success in food applications. As mentioned in Section 2, research on fats and protein is limited. Further exploration can be done on traditional protein sources such as chicken and beef, as well as plant-based proteins such as black beans, fava beans and chickpeas. Printing these proteins adds essential nutrients as well as increased customizability of food inks due to the unique properties gelling and denaturation. Fruits and vegetables are challenging ingredients for 3D applications due to their high moisture content and limited structural stability (Severini, Derossi, et al. 2018), however, more research can explore the applications of additives or adjusting moisture content in pre-processing steps. Moreover, lipids should have more extensive research so the effect of their structures on 3D printing can be studied. This should entail optimizing flow and crystallization for the print with different lipid blends. The effects of cocoa and butter in 3D food have been investigated (Lipton et al. 2015, Pérez et al. 2019, Yang, Zhang, Prakash, et al. 2018); however, other fat sources such as vegetable (palm, sunflower, etc.) and animal fats deserve consideration. Further examination of these foods can lead to advancements in creating new 3D foods and concepts.

Conclusion

3D food printing is a new and innovative field that has the potential to customize the design, nutrition, and composition of foods. Many unique and complex objects such as butterflies, Mickey mouses, apples, and pyramids have been printed (Keerthana et al. 2020; Yang, Zhang, Prakash, et al. 2018; Chen et al. 2019; Wang et al. 2018; Severini, Derossi, et al. 2018). However, there is a disconnect between traditionally manufactured food and 3D printed foods in their safety, formulation and large-scale production and replicability. There are multiple properties that require more data such as moisture content, water activity, color, and various textural parameters. Thus, this review examines the past scope of research and also identifies opportunities for improvement in 3D food printing such as moisture content, water activity, appearance, and microbial safety to close the gap between traditional and 3D foods.

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