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REVIEW

Recent advances in functional 3D printing of foods: a review of functions of ingredients and internal structures

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ABSTRACT
Three-dimensional (3D) food printing technology combines 3D printing and food manufacturing. Rapidly increasing number of publications on various aspects of 3D food printing indicate the importance of this technology to food industry. The potential of delivering personalized products tailored to meet the taste preferences and specific dietary needs is one of the reasons for increasing researches in this technology. Currently there is an absence of a systematic review on the functional 3D printing. Also, there is no review on four-dimensional (4D) food printing concept that has emerged recently. This paper systematically reviews the functional ingredients used for creating printable food formula and their functions, including physiological functions, beneficial for health and physico-chemical functions, affecting the quality of 3D printing. In addition, it analyzes the functions of internal structures used or developed during 3D printing (infill structure and infill density) and their effects on texture properties of 3D printed food. Finally, it also introduces the concept of 4D food printing and summarizes the current advances in this novel technology.

KEYWORDS
3D printing; 4D printing; personalized foods; functional ingredients; infill structures

Introduction

Three-dimensional printing (3D printing) is a additive manufacturing process that involves layer-by-layer construction of complex solid geometries and binds the layers by inducing chemical reactions or phase transitions (Severini, Derossi, and Azzollini 2016). The 3D printing system first emerged in 1980s and since then it has shown new promises and also encountered challenges in many research fields such as engineering, aerospace, gastronomy, medicine, food, and arts etc. (Rayna and Striukova 2016). The 3D printing technology is developing rapidly and its application fields are still broadening. A Shinnove 3D printer photo and its schematic diagram are shown in Figure 1 (Model No. Shinnove-D1, Shiyin Co. Ltd, Hangzhou, China). This 3D printer has two independent material tubes. These two tubes shared the same shaft and bearings. Each tube can be individually controlled to deposit food slurry.

The 3D food printing combines 3D printing technology and food manufacturing and uses edible materials such as fruit and vegetable juice and powder, starch, meat, chocolate, and algae etc. as printing materials (Jiang et al. 2019). Rapidly increasing number of publications on various aspects of 3D food printing indicate the importance of this technology to food industry (Perez et al. 2019).

As we know, the most important feature/advantage of 3D printing is the creation of complex 3D structures. But in food field, the potential of delivering personalized nutrition and personalized food choice may be the main reasons that the 3D food printing technology is advancing so rapidly. 3D food printing technology can enable formulation of food to meet the need of people having different preferences for taste, dietary needs and physical condition such as dysphagia (Feng, Zhang, and Bhandari 2019). Specifically, tailored foods by adding specific nutrients and functional compounds or eliminating/replacing certain ingredients in the formulation can help promote health and prevent diseases (Severini and Derossi 2016).

The 3D printing technology can seamlessly integrate nutrition, enable manufacturing of personalized foods that satisfy the requirements of consumers according to their occupation, gender, age, and lifestyle (Rodgers 2016). For instance, soldiers need customized foods to meet their nutritional needs: such foods should have long shelf life and also can be prepared and consumed in short time. Dehydrated raw materials are preferentially used for 3D printing to avoid the instability or deterioration of fresh ingredients (Nachal et al. 2019). The ability of 3D printers to use dehydrated foods or dehydrate the foods after printing makes 3D printing technology suitable for producing Space Food while it is a challenge for conventional processing methods as they require longer nutritional stability (Terfansky and Thangavelu 2013). 3D printing technology is also suitable for producing customized foods for athletes and pregnant women. This is achieved with relative ease by eliminating or reducing or altering the protein, fat, fiber, cellulose, and

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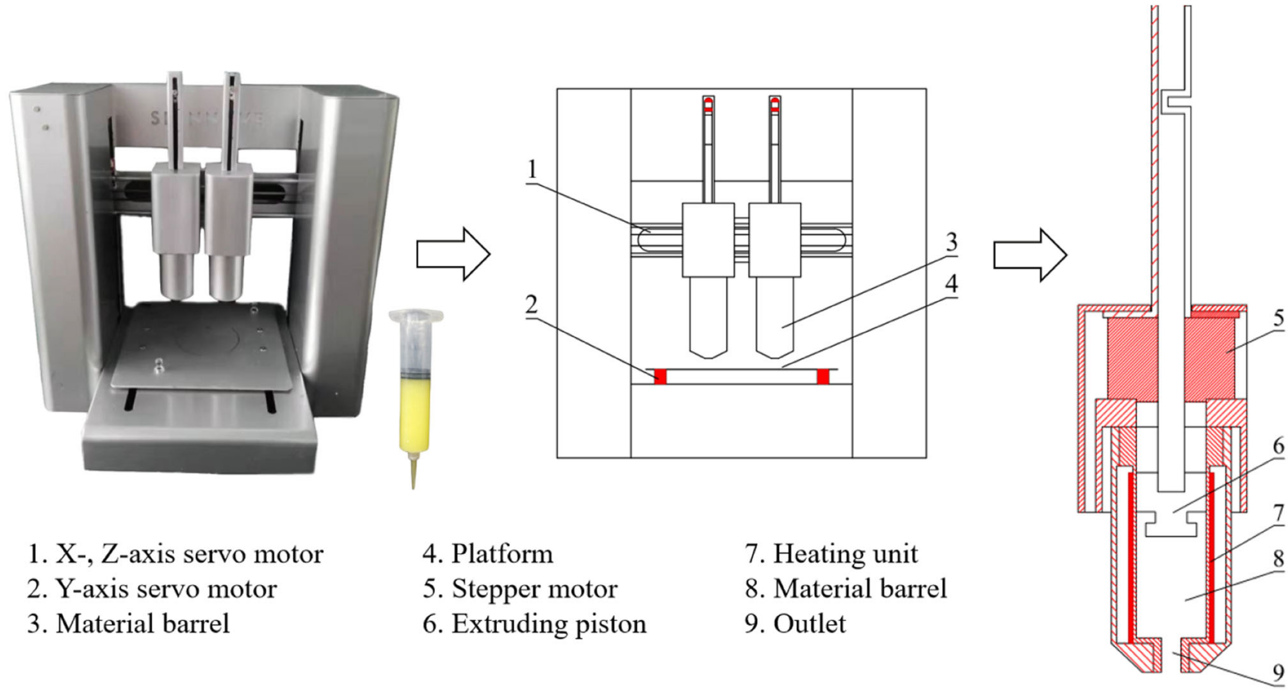


Figure 1. Schematic diagram of a 3D printer system (Liu, Zhang, and Yang 2018).

vitamin contents etc. and by introducing functional compounds such as anthocyanins, and carotenoids to impart desired functions (Dankar et al. 2018b). For example, patients with diabetes can upload their blood glucose data to cloud service platform, computer then calculates the nutrition-balanced recipe for next meal using internal program, and then 3D food printer produces the customized meal. In this way they can avoid consuming unhealthful and un-customized diet.

The 3D printed food with improved taste and visual appeal can be more suitable for elderly people with dysphagia and patients with stroke, paralysis, and Parkinson's disease (Portanguen et al. 2019). The 3D printed foods are more attractive alternatives of unappealing, unappetizing, mashed, thick, and pulpy foods. Thus, the 3D printed foods that can be specifically enriched with animal protein, can also be specifically designed to be soft, nutritious, suitably textured and appealing (Miguel Aguilera and Park 2016). Food with desirable texture have been produced using 3D printing by developing different nozzles and filling modes (Dick, Bhandari, and Prakash 2019a).

The 3D printed foods can be popular and more appealingly designed to cater for specific needs of teenagers and children. These two population groups are deficient in nutrients such as vitamins, minerals, especially iron and calcium (Hamilton, Alici, and Panhuis 2018). Commercially available fruit-based products also commonly lack these nutrients (Dankar et al. 2018b). 3D printing technology helps to create healthy snacks with novel shapes and rich in vitamins and minerals, attracting teenagers and children and becoming a model of personalized food. In fact, 3D printing enables manufacturing of personalized foods that have both health promoting and enjoyment elements (Severini and Derossi 2016). When it comes to joy of eating, development of 4D food printing technology may be more attractive. Now, food printing technology has evolved from 3D to 4D, which can enable self-transformation in color and/or shape of a printed food as a function of time, thereby increasing the visual enjoyment during the meal (Ghazal, Zhang, and Liu 2019; Wang et al. 2017).

Although the number of scientific publications covering various aspects of 3D printing technology and its application in food have increased in the last decade, there is no systematic review on the functional 3D printing. To fill this gap, this paper systematically reviewed the functional ingredients used for creating printable food formula and their functions, including physiological functions, beneficial for health and physico-chemical functions, affecting the quality of 3D printing. In addition, we have analyzed the functions of internal structures used or developed during 3D printing (infill structure and infill density) and their effects on texture properties of 3D printed food. We have also reviewed and summarized recent advances in 4D food printing. This review is expected to provide insights on ingredient-structure-function aspects of 3D printing and it is also an overview of 4D food printing and its importance.

Functional ingredients of 3D printing and its effects on the printing quality

Functional carbohydrate

Maltitol and xylitol

As sugar substitute, maltitol or xylitol were used to produce a 3D printed chocolate containing complex polysaccharides. Chocolate is loved by consumers from all over the world because of its delicate taste and unique flavor. Owing to the unique melting and solidifying characteristics of cocoa butter in chocolate, it is the most convenient and highly popular material to be used in 3D food printing (Li et al. 2014). However, due to high concentration of sucrose with high calorie density, eating too much chocolate is not conducive to human health. When sucrose in chocolates is replaced with maltitol or xylitol, the probability of chocolates causing obesity can be greatly minimized, owing to the low energy density of the latter sugars (Xiao et al. 2019). They produced a sugar-free chocolate material by replacing sucrose with maltitol and xylitol and by adding health-promoting complex polysaccharides. The complex polysaccharides obtained from ganoderma, wolfberry, and liriope have special physiological functions, such as to regulate immunity, assist in inhibiting tumor, resist oxidation, and slow down senescence (Li et al. 2019). The crystal nucleus provided by these complex polysaccharides can induce the crystallization of cocoa butter, shorten the crystallization time, accelerate the solidification of chocolate, and thus increase the stacking height of printed product. In addition, complex polysaccharides can reduce the migration rate of oils, and help to maintain the stability of the chocolate emulsification system. This work provides the guidelines for developing a modified chocolate, considered as a healthy 3D printing material.

Isomaltose

Isomaltose with a sweetness value of 40%–50% of sucrose is also an ideal substitute for the latter. It can also help to maintain the balance of normal bacterial flora including intestinal bifidobacteria, prevent dental caries, and boost immune system (Shan et al. 2006). In this context, Teng, Zhang, and Bhandri (2019) used isomaltose instead of sucrose together with *Cordyceps* powder, agar, and vegetable oil to prepare an unique 3D printing ink. Since isomaltose molecules can readily bind water, its inclusion in ink formulation maintains moistness, which acts as a lubricant. This property prevented contact between the *Cordyceps* powder particles, thus decreasing the formation of rigid network structure in the printed shape. The authors reported that isomaltose content of 12.4% (dry weight basis) had the best printing effect. It is worth noting that this ink was rich in cordycepsin, a functional compound from *Cordyceps* flower, in addition to isomaltose, and thus, had some unique functionality. *Cordyceps* flower is known to enhance the function of macrophages, positively regulate homeostasis and improve the immune function in the human body (Tuli, Sandhu, and Sharma 2014).

Xanthan gum

Xanthan gum is one of the most widely used hydrocolloids in 3D food printing, which functions as a gelling or a thickening agent and improves the mechanical stability of food materials after processing (Saha and Bhattacharya 2010). Actually, xanthan gum is found to have some benefits for health, such as functions of antioxidant and immune boosting (Liu et al. 2017). Most of the 3D printing studies related to xanthan gum are focus on its thickening property, rather than its physiological function in human body. Kim et al. (2019) showed that xanthan gum solved the deformation problem of 3D printed cookie products in post-treatment process such as baking. Adding 0.5 g/100 g xanthan gum into the 3D printing raw material improved the heat resistance of the 3D printed cookie sample, and its texture was similar to those of the control cookie in crushability and hardness. For 3D printed products which are possible to encounter high temperature environment during post-processing, the structuring and thickening properties of xanthan gum can be harnessed to improve their mechanical strength. Xanthan gum can inhibit the expansion of particles, control rheological properties of the ink due to its high hydration capacity. Kim et al. (2018) reported that xanthan gum improved the printing uniformity when different powders such as carrot, spinach and broccoli are used as printing materials. Addition of xanthan gum enhances binding and structural integrity even in heterogeneous systems such as cellulose composite powders (Holland, Tuck, and Foster 2018). Xanthan gum also has been used in starch-based formulations to modify the rheological properties of materials due to its strong water-holding capability (Liu, Zhang, and Bhandari 2018; Liu, Bhandari, et al. 2019).

Pectin

Pectin, a plant-based natural hydrocolloid, is also typically used in 3D printed objects which demand a gelling or a thickening agent. Inside the gastrointestinal tract, pectin preserves the ability to form gel or thicken solution and therefore, has many beneficial effects on human body, including diabetes prevention and control, improving lipid and cholesterol metabolism, and dumping syndrome (Lawaetz et al. 1983). It also has some unique abilities to treat or prevent other diseases such as obesity, cancer, atherosclerosis and intestinal infections (Lattimer and Haub 2010). Vancauwenberghe et al. (2018) have conducted a number of studies on pectin-based 3D printing including application of low methoxylated pectin gel as 3D-ink to print pectin-based food simulants. They also demonstrated that air bubbles and plant cells can be successfully encapsulated into pectin-based inks (Vancauwenberghe et al. 2019). In these studies, Ca^{2+} was used to produce pectin gels through the formation of calcium cross links between free carboxyl groups (Fraeye et al. 2010).

Dietary fiber

Vegetables, fruits and whole grains (including wheat bran, cereal, whole wheat flour, brown rice, oat etc.) are main source of dietary fibers. Dietary fibers, known as the seventh nutrient in the nutrition field, play an important role in promoting gastrointestinal peristalsis, speeding up the passage of food through the gastrointestinal tract, modulating absorption of nutrients, and preventing and treating constipation (Stroehle, Wolters, and Hahn 2018). The Food and Drug Administration's claim supporting the benefits of dietary fiber states that a diet high in vegetables, fruits, and whole grains and low in cholesterol and saturated fat can reduce the risk of causing coronary heart disease (Lattimer and Haub 2010). Therefore, not only from the perspective of product development, but also from the perspective of health-promoting functions as mentioned above, it is necessary to use dietary fibers for 3D printing.

Cellulose, the building block of plant cell walls, is the most abundant polymer on the earth and also one of the most important insoluble fiber. Human body does not have the necessary enzymes to digest it (Wuestenberg 2014). Holland, Tuck, and Foster (2018) successfully used finely milled (e.g., ball milled) cellulose fiber to produce 3D printed structures. Xanthan gum and glucomannan were used as printing-aid to facilitate printing. The cellulose-based formulations were used to produce low-calorie 3D-printed foods.

Vegetables contain a large proportion of dietary fiber and bio-active substances that are important in promoting health and preventing chronic diseases (Liu 2003). Kim et al. (2018) prepared 3D printing formulations containing 10% to 30% (wt/wt) vegetable powders (carrot, spinach and broccoli powders used individually), with the aid of different hydrocolloids, to investigate the effect of these vegetable powders on the rheological properties and printability. Their results indicated that, increase of powder from 10% to 30% substantially affected the rheological and printing properties. Similarly, the effect of the printing aid was also prominent at higher powder concentration. The addition of xanthan gum in the formulation was found to reduce the differences in printability between different vegetable sources. Lee et al. (2019) used spinach powders with different particle size as the cellular materials to study its printing performance and printability. The results showed that when the particle size of spinach powder was 307 μm, the mechanical strength of the printed object was improved, with its shape, structure and surface smoothness being maintained. Severini, Derossi, et al. (2018) used pears, carrots, broccoli leaves, kiwi and avocado as printing materials and produced 3D printed smoothie. These 3D printed smoothies were pyramid-shaped and were more appealing appearance than regular ones. The phenolic content, antioxidant capacity and sensory characteristics (color, taste, and odor) of the printed smoothies were not affected by the 3D printing process.

Huang, Zhang, and Bhandari (2019) produced 3D printed product using brown rice together with guar and xanthan gums in the formulation. Brown rice, that retains the cortex and embryo after husking, contains higher content of protein, fat, vitamins, minerals and other nutrients compared to white rice. It also retains dietary fiber, glutamate, glutathione, rice bran polysaccharide, inositol and other compounds with health promoting functions. The dietary fiber content of brown rice is six times more than that in white rice (Qi, Yu, and Yu 2015). This study shows that brown rice-based 3D printed foods can be produced, which will be suitable for diabetic people, requiring slow digestion of starch. Lille et al. (2018) developed 3D printed structures using formulations that were rich in protein, and fiber and low in sugar, and fat. Best printing structures were obtained in formulations containing 10% cold swelling starch + 15% skimmed milk powder, 60% semi-skimmed milk powder, 30% rye bran, 35% oat protein concentrate or 45% faba bean protein concentrate.

Other water-soluble dietary fibers such as guar gum, agar, maltodextrin have also been commonly used as 3D printing materials to study various printing parameters (Feng, Zhang, and Bhandari 2019). These hydrocolloids possess desirable printability and self-supporting functions They also have health-promoting functions to the human, such as improving gastrointestinal motility, promoting nutrient absorption, and preventing constipation (Darwiche et al. 2003; Rideout et al. 2008).

Functional protein

Pea protein

Pea protein is rich in essential amino acids except methionine (Palander et al. 2006). The anti-nutritional factor in pea protein is much less than that in soybean protein, thus, it gets digested more easily (Zhang, Han, and Li 2012). Feng et al. (2018) explored the effect of pea protein on the printability of potato starch-based 3D printing ink. They showed that the textural and thermal properties and structural properties (granular, crystalline, and chemical structure) of starch-based ink improved substantially when pea protein content was increased. Adding of 1% pea protein to the ink produced a high-quality 3D printed product. It was concluded, from this work, that incorporation of appropriate amount of pea protein in the ink mix improved the stability of structure, enhanced the texture and balanced the nutritive value of printed food product. Besides, pea protein also helps with gelation, contributes toward making the surface more hydrophobic and provides fat like taste sensation (Zhang, Han, and Li 2012). Therefore, it can be used to substitute the fat in low-calorie food products and to reduce dietary fat intake. All of them reduce the incidence of obesity, atherosclerosis and malignant tumors.

Whey protein isolate

Whey protein -isolates (WPI) and -concentrates (WPC) promote health and resist disease. WPI has some degree of biological activities that provide resistance to oxidation and virus, boost body immunity, and improve intestinal digestion (Zhang et al. 2020). Because of their nutritional value and functional characteristics, WPI and WPC are commonly incorporated in value-added foods, such as infant formula powder, high-protein foods for fitness enthusiasts and athletes etc. (Ji et al. 2017). Liu, Liu, et al (2018) designed and produced WPI- and WPC-based 3D printed foods and studied the effect of their concentration. The authors found that the incorporation of WPI and WPC decreased the apparent viscosity and soften resulting paste, which is beneficial to the printing process. The printing paste prepared using a WPI-to-WPC ratio of 2/5 was reported to have the best printability. In another study conducted by Liu, Yu, et al. (2019), a milk protein composite material was made by mixing WPC into sodium caseinate solution and the results indicated that the 3D ink containing appropriate total protein (400–450 g/L) showed the best printing performance and the best matching degree with the designed model.

Egg albumin

Egg albumin is the major protein in egg, comprising 54% of egg white protein (EWP) (Chen, Huang, and Ma 2015). It can form highly antioxidant covalent conjugates with sugars (glucose, maltose, and lactose) and soluble starch through Maillard reaction (Nakamura et al. 1992). Readily available methods such as microwave heating can hasten the Maillard reaction and also increase the antioxidant capacity of the adducts (Sun et al. 2006). Egg albumin readily forms edible gel by simple heating. Because of these benefits, egg albumin is now commonly used to develop 3D printed foods. Liu, Meng, et al. (2019) developed a 3D printing formulation that contained egg white protein, sucrose, cornstarch, and gelatin. The results of rheological and tribology studies showed that when the EWP content was 5.0% (wt/wt), the mixed gel system had the best printing effect. Incorporation of EWP at this level enhanced the springiness and hardness of gel and helped to maintain the shape of the printed food. This work suggested that EWP was a valuable ingredient to produce protein-based printing ink. Moreover, Liu, Yang, et al. (2020) optimized a formulation of 3D-printed complex containing EWP viz., 12.98 g of EWP, 19.72 g of cornstarch, 4.27 g of gelatin, and 8.02 g of sucrose in 250 mL of total water. However, egg albumins are most commonly known food allergens (Chen et al. 2007); Therefore, people who are allergic to egg albumin can consider adding other non-allergic proteins such as soy protein to customize 3D food to have protein balance.

Insect protein

Researchers have investigated the suitability of edible insect powders as an ingredient of 3D printed food. This is because insect powders are rich in protein, which varies 40%–70% (wt/wt), depending on the species and life cycles (Verkerk et al. 2007). As a sustainable source of high-value protein and minerals (e.g., calcium), edible insects are increasingly harvested to meet the growing demand for protein (Van Dyck et al. 2014). Use of insect protein as ingredient for 3D printing is a novel way of promoting this sustainable food source and an innovative way to soften people's reluctance in consuming insects. Soares and Forkes (2014) mixed larvae of yellow mealworms (*T. molitor*) with fondant and produced cakes, top part of which was decorated using 3D printing. Severini, Azzollini, et al. (2018) produced a 3D-printed snack containing different amount of wheat flour with yellow mealworm powder and then baked (200 °C). The total essential amino acid content of these 3D-printed snacks was 41.3 g/100 g protein.

Functional lipids

Lecithin molecules are composed of fatty acids, phosphates and choline and its importance is that it is the main component of biofilms. As an antioxidant, lecithin can delay the aging of the body. Choline (metabolite of lecithin) is a precursor for the biosynthesis of phosphatidylcholine (PC) which plays an important role in the intestinal absorption of lipids. Choline reacts with acetyl-CoA and the resulting acetylcholine can promote transmission of brain signals (Colares et al. 2016). Lecithin is used as emulsifier in many food emulsion, confectionery, and chocolate products (Loncarevic et al. 2013). When used in 3D inks, it can reduce the wear and tear of nozzle as a lubricant. Dankar et al. (2018a) investigated the effect of lecithin on the rheological properties and changes in starch's microstructural properties in commercial potato purees. Higher number of swollen starch aggregates were observed in the presence of lecithin, which affected the rheological properties of the purees. The effects of lecithin, agar, sodium alginate and glycerin in varying concentrations (0.5% to 1.5%) on color, microstructure, strength, and mechanical properties of potato puree were also studied (Dankar, Pujola, et al. 2018). The specific mechanical energy value of the potato puree decreased significantly when 1% lecithin was added. When the concentration of lecithin was increased, a smooth surface with tiny pores was produced in potato puree. But, under the formulations described in this article, lecithin was not as effective as agar and sodium alginate in improving the printability and stability of the product.

Cotabarren, Cruces, and Palla (2019) designed a nutraceutical oral dosage forms using mixtures of phytosterols (PS), and monoglycerides (MG) oleogels. At present, monoglycerides combined with phytosterols have been used to obtain food-grade oleogels as a delivery agent for functional ingredients or as a substitute for saturated fats (Matheson et al. 2018). As a lipid-soluble bio-active substance, phytosterols have special significance in the prevention of cardiovascular diseases and the treatment of hypercholesterolemia. This natural compound has a function and structure similar to cholesterol, can interfere with intestinal absorption and allow its excretion (Moss, Williams, and Ramji 2018). The incorporation of a certain amount PS promotes the gelation process of MG oleogels and produces higher levels of stabilization. On a unit dose basis, the PS content in the final successfully printed oral forms ranges from 230 mg to 285 mg (Cotabarren, Cruces, and Palla 2019).

The feasibility of introducing an oil phase in casein matrix was studied by Schutyser et al. (2018). They used olive oil as the oil phase and sodium caseinate as the water phase and created a premix with well dispersed oil droplets. Passing this premix from side-inlet of 3D printer enabled to create various structures that caused spatially well distribution of oil droplets. Olive oil, especially extra-virgin olive oil, contains high percentage of monounsaturated fatty acids, which, in addition to providing calorie, also helps to balance the high- and low-density lipoproteins and prevents the formation of excessive cholesterol. Moreover, olive oil also contains oil soluble vitamins and antioxidants. Currently, the most healthy and popular diet pattern is the "Mediterranean diet", which uses olive oil as the main cooking fat (Derossi et al. 2020). The ability of 3D printing to incorporate olive oil provides innovative ways to design healthy and personalized foods.

Vitamins and minerals

Sufficient daily intake of fruits and vegetables is necessary in preventing many diseases because they are rich sources of vitamins and minerals. Although Dietary Guidelines Advisory Committee (2010) has advised that people should intake five portions of fruits and vegetables on daily basis, many do not follow this guide lines (Sofi, Dinu, and Procedia 2016). Inadequate consumption of fruits and vegetables means that people are deficient in several vitamins and/or minerals. Deficiency of calcium, iron and Vitamin D negatively affects the healthy growth in adolescents and children (Derossi et al. 2018).

Vitamin D is essential for the promotion of calcium absorption and maintaining the bone health, modulation of immune function, neuromuscular and cell growth, prevention of cancer and cardiovascular disease, and reduction of inflammation (Povoroznyuk et al. 2015). Azam, Zhang, Bhandari, et al. (2018) added liquid Vitamin D to a blend of orange concentrate, wheat starch and gum to produce 3D printed food enriched with Vitamin D. A mixed formulation of wheat starch, k-carrageenan and Vitamin D enriched orange concentrate was found to be the best match for 3D printing.

Scerra et al. (2018) designed a 3D printing material containing Vitamin E, which is a fat-soluble vitamin with proven antioxidant property. Vitamin E acetate was added to extend the shelf life of peanut butter as well as to compare the influences of printing and post-printing heat treatment on the stability of vitamin E. The concentration of Vitamin E decreased by 24% (0.59–0.45 IU/kg) in the 3D printing process, which was much lower than a 42% loss (0.59–0.34 IU/kg) due to thermal treatment indicating that the former is a much benign process for this vitamin.

Calcium caseinate contains ionic calcium and it is an effective calcium supplement. It can be easily absorbed and utilized by human body without the need of the presence of Vitamin D. Zhang, Lou, and Schutyser (2018) added calcium caseinate into dough, which not only improved the printability but also improved stability after printing. The printing performance of dough was greatly improved when calcium caseinate content was 3%.

Derossi et al. (2018) developed 3D printed snacks using banana as the main ingredient and skim milk, mushroom, white beans, ascorbic acid (an antioxidant) etc as other ingredients. Viewing from micro-nutrient requirements of children, this snack formula provides sufficient calcium, iron and Vitamin D to children. The authors also noted that cowpeas, parsley or broccoli can also be added in the formulation to provide zinc and folic acid as well.

So far, a number of studies used fruit juice as the source of micronutrients. Fruit juices are rich in vitamins and minerals that human body needs (Feng, Zhang, and Bhandari 2019). Also, incorporation of fruit juice in printing mix can provide desired flavor in the printed food (Yang et al. 2018; Yang, Guo, et al. 2019; Yang, Zhang, and Liu 2019; Azam, Zhang, Mujumdar, et al. 2018). Thus, fruit juice acts as part of raw materials for 3D printing increases the intake of vitamins, and provides more options while developing personalized foods.

Probiotics and algae

Probiotic bacteria provide healthy balance of intestinal microorganisms. When provided in appropriate dose, they bring health benefits to the host (Hill et al. 2014). Probiotics are also able to produce some antimicrobial substances and play an immunomodulatory role (Onbas, Osmanagaoglu, and Kiran 2019). Besides, they are beneficial to people who suffer from some diseases such as inflammatory bowel diseases, metabolic diseases and periodontal diseases (de Almada et al. 2015).

The feasibility of producing probiotics (*Lactobacillus plantarum* WCFS1) incorporated and cereal (wheat)-based 3D printed foods and their survival in the baking process were studied by Zhang, Lou, and Schutyser (2018). There was no significant difference in the survival of incorporated probiotics in these products when baked in 145 °C to 205 °C range. However, the survival of probiotics in 3D printed "honeycomb" structure was $>10^6$ CFU/g, when the product was baked at 145 °C for 6 min and it met the requirement for a probiotic product. Liu, Bhandari, and Zhang (2020) studied the feasibility of incorporating probiotics (*Bifidobacterium animalis subsp. Lactis* BB-12) into 3D printed mashed potatoes. The authors chose this probiotic strain as it is known for its beneficial immune function and gastrointestinal health including protection against diarrhea, promoting bowel function and reducing side effects of antibiotics (Jungersen et al. 2014). The authors also investigated the effect of nozzle diameter (0.6, 1.0 and 1.4 mm) and printing temperature (25, 35, 45 and 55 °C) on the survival of the bacteria upon printing and during storage. They reported that viable bacterial cells reduced only slightly (9.93 log CFU/g to 9.74 log CFU/g) when the smallest diameter (0.6 mm) was used during printing. However, a significant reduction in survival of bacteria was observed (10.07 log CFU/g to 7.99 log CFU/g), when the mashed potatoes was kept in a heating nozzle tube at 55 °C for 45 minutes.

Nostoc sphaeroides is an algal food which is highly valued in traditional medicine. It has been historically suggested that *Nostoc sphaeroides* can cure a variety of medical conditions, including digestion, night blindness, inflammation, chronic fatigue, indigestion, anxiety, and burns. Some studies have also demonstrated various health benefits from *Nostoc sphaeroides*, including antiinflammatory, anticancer, antiviral, and cholesterol lowering activities (Park et al. 2008). *Nostoc sphaeroides* biomass, being a natural gelling material with rheological properties, has also been used for 3D printing. An et al. (2019) studied three forms of *N. sphaeroides*-based 3D formulation, including fresh biomass, powder, and a mixture of powder and potato starch. It was observed that although fresh biomass was able to form a gel and used for printing, the shape of the 3D printed product

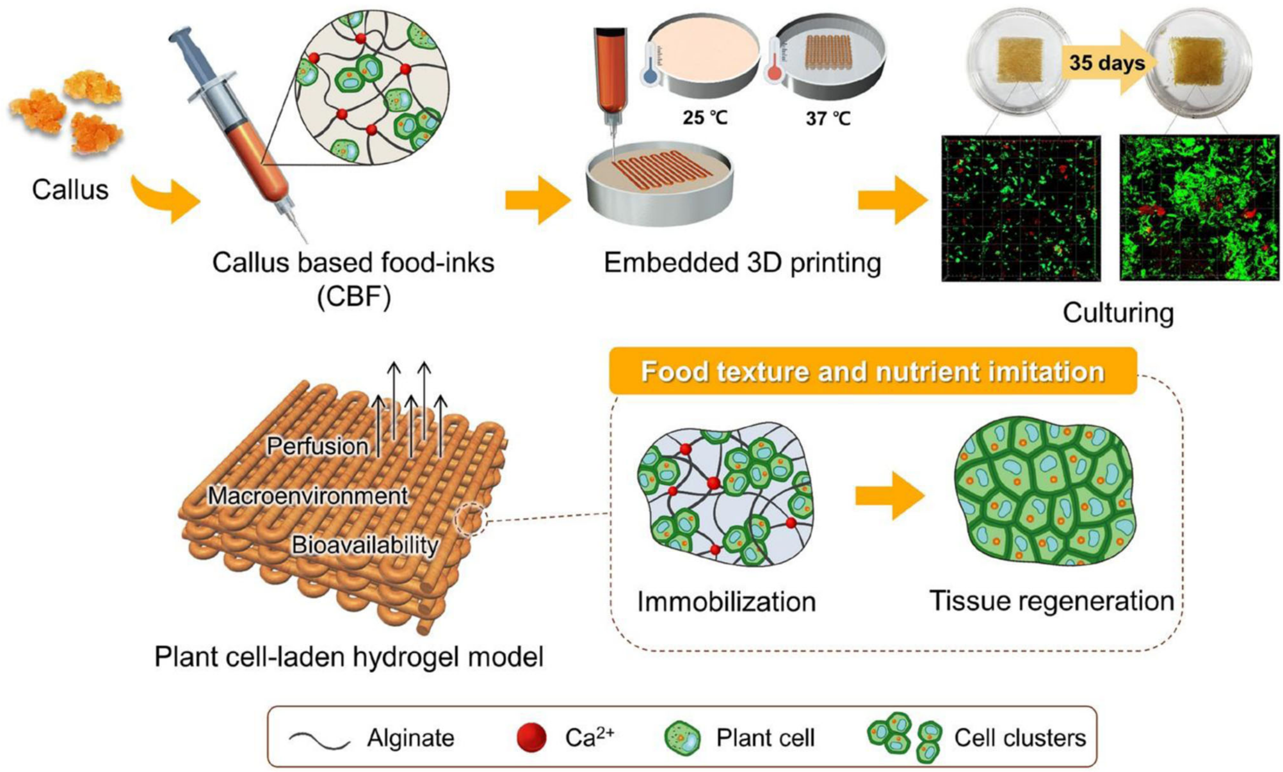


Figure 2. Integration of 3D printing and plant cell culture technologies (Park, Kim, and Park 2020).

was uneven and unstable. Blanching of the cells before preparing gel for printing also did not improve printing performance. The rehydrated gel, however, was found to be more suitable to 3D printing than the fresh one. Incorporation of starch, heating and rehydration for relatively longer time significantly improved 3D fabrication of *N. sphaeroides* powder.

Alive plant cells

There are many raw materials that can be used for 3D food printing, but the current level of 3D printing technology is difficult to imitate the texture of real foods, which limits the development of 3D food printing. Incorporation of alive cells as printing materials may be a viable solution to this limitation. By proliferating, cells can replicate nutritional compositions and functional factors exist in real foods, as well as inherent textures.

Vancauwenberghe et al. (2019) tried a new method to produce a 3D-printed food similar to a plant tissue. They encapsulated alive lettuce cells (*Valerianella locusta*) and bubbles into a pectin-based ink and demonstrated that the ink containing the plant cells was successfully 3D printed with good accuracy and reproducibility. However, the mechanical properties of the printed products in this study were highly reliant on the porosity changes and the pectin concentration. Furthermore, the expression of cellular tissue by alive cell growth was not studied. Park, Kim, and Park (2020) produced a plant cell laden hydrogel model with cellular structure using plant cells isolated from carrot (*Daucus carota* L.) (Figure 2). In order to study the effect of initial carrot cell content on tissue development efficiency of 3D printing objects, carrot cell dispersion was mixed with alginate solution at ratios of 1:1, 1:2 and 2:1 (wt/wt) to prepare callus based food-inks. According to the results of 3D printing, the 1:1 and 1:2 samples showed proper shape fidelity. Plant cell-laden scaffolds with different callus concentration observed high viability and extended cell growth over a 35 days culture cycle. The cells blended in the hydrogel developed cell clusters during culturing and showed unique texture of artificial plant tissues. These results verified the possibility of using alive cells to obtain 3D printed foods with textural properties similar to natural foods.

Functional ingredients used in 3D printed foods and the purpose of their incorporation are provided in Table 1.

Functions of internal structures and its effects on texture properties of 3D printed food

Customized food can satisfy the nutritional requirement of specific consumer groups, including infants, the elderly people, pregnant women, and bodybuilders. It also can meet the requirement of special eating environment, including in the harsh environment of the battlefield, in the state of exercise, anoxia and fatigue during high altitude activities, weightlessness during space travel, space restriction, and low pressure in the air craft. Both the physical conditions of specific consumer groups and specific eating environment will reduce the pleasure of eating and even increase the risk, such as cough, choke, sputter etc. Therefore, in addition to nutritional ingredients, texture design is also necessary for customized 3D printing food. Altering structural properties like infill structure and infill density to modify the textural properties of 3D printed foods is relatively new concept in 3D

Table 1. Functional ingredients used in 3D printed foods and the purpose of their incorporation.

	Ingredients	Purpose of incorporation	References
Functional carbohydrates	Xanthan gum	Modify the matrix properties Play a role of thickening or gelation Enhance the mechanical stability of food materials in the post-treatment process	Feng, Zhang, and Bhandari (2019)
	Maltitol and xylitol	Sucrose replacement Reduce the risk of obesity caused by high calorie chocolate	Xiao et al. (2019)
	Isomaltose	Prevent the contact between the *Cordyceps* flower powder molecules Decrease formation of rigid network structure	Teng et al. (2019)
	Pectin	Produce pectin-based food simulants	Vancauwenberghe et al. (2019)
Functional Protein	Pea protein	Explore the effect of pea protein on the printability of potato starch-based 3D printing ink	Feng et al. (2018)
	Whey protein isolate	Study the influence of different whey protein isolates content on the printing performance of milk protein concentrate	Liu, Liu, et al. (2018)
	Egg white protein	Investigate the effects of egg white protein addition on the rheological, texture properties of the mixture system	Liu, Meng, et al. (2019)
	Insects powders	Explore the potential of insects as a sustainable food source	Severini, Azzollini, et al. (2018)
Dietary Fiber	Amorphous cellulose	Use finely milled (e.g., ball milled) cellulose fiber to produce 3D printed structures	Holland, Tuck, and Foster (2018)
	Carrot, spinach and broccoli powders	Investigate their influence on the rheological properties and printability	Kim et al. (2019)
	Spinach powder	Investigate the particle size on printing performance and printability of printing medium	Lee et al. (2019)
	Pears, carrots, broccoli leaves, kiwi and avocado	Produce a new healthy smoothie	Severini, Derossi, et al. (2018)
	Brown rice	Evaluate the printability of brown rice	Huang et al. (2019)
	Rye bran, cellulose nanofiber	Design a healthy new structures with higher protein and fiber content, lower sugar or fat content	Lille et al. (2018)
Functional lipids	Lecithin	Study the effects of lecithin on the rheological properties of commercial potato puree Study the effects lecithin on the strength, mechanical properties, microstructure and color of potato puree	Dankar, Pujola, et al. (2018)
	Olive oil	Study the feasibility of adding olive oil to casein matrix	Schutyser et al. (2018)
	phytosterols and monoglycerides oleogels	Design a nutraceutical oral dosage forms	Cotabarren, Cruces, and Palla (2019)
Vitamins and minerals	Ionic calcium of calcium caseinate	Provide good spout extrusion performance and good stability for dough Make a ionic calcium enriched dough	Zhang, Lou, and Schutyser (2018)
	Vitamin E	Compare the influences of printing and post-printing heat treatment on the stability of vitamin E	Scerra, et al., (2018)
	Vitamin D	Make a vitamin D enriched printing material	Azam, Zhang, Bhandari, et al. (2018)
Probiotic bacteria and algae	*Lactis* BB-12	Study the feasibility of incorporation of probiotics into 3D printed mashed potatoes	Liu, Bhandari, and Zhang (2020)
	Lactobacillus plantarum WCFS1	Study the feasibility of 3D printing to produce cereal-based food structures	Zhang, Lou, and Schutyser (2018)
	Nostoc sphaeroides	Study the feasibility of incorporation of *Nostoc sphaeroides* into 3D printing	An et al. (2019)
Alive cells	*Valerianella locusta* cells	Proof a high amount of plant cells and air bubbles can be encapsulated into pectin-based bio-inks Produce a 3D-printed food similar to plant tissue	Vancauwenberghe et al. (2019)
	Daucus carota L. cells	Study a plant cell-laden hydrogel model extend the application range of plant-based food production to unique food texture	Park, Kim, and Park (2020)

food printing field. There are fewer studies that link the quality of internal structures of 3D printed foods to their texture properties and overall quality.

The infill structure of 3D printed objects could significantly affect their mechanical strength and stability. Mantihal et al. (2017) used a commercially available dark chocolate beans as raw material and produced three hexagon shapes: 1) cross bracing, 2) parallel bracing, 3) without bracing and then measured the force required to break them. The results showed that hexagonal structures produced by cross bracing were more stable (required higher breaking force) than those produced by parallel bracing. Severini, Derossi, and Azzollini (2016) designed a similar supporting structure to print cereal products using dough as the primary substrate. Support structures (parallel and cross) play an important role in preventing collapse of complex 3D structures. Mantihal, Prakash, and Bhandari (2019a) used Callebaut bittersweet dark, Cadbury dark chocolates together with plant sterol powders and magnesium stearate as food additives to design three complex infill structures, namely "honeycomb", "Hilbert" and "star". The "honeycomb" and "star" showed very high mechanical properties due to their vertical and horizontal integration. It is necessary to select an appropriate infill structure before printing to obtain a more stable 3D printed object.

Besides enhancing the stability of the 3D printed objects, the infill structure has other functions, such as protecting probiotics in the print material. Zhang, Lou, and Schutyser (2018) produced 3D printed dough containing probiotics using "honeycomb" and "concentric" structures with different surface area to volume ratios (9.20 cm^2/cm^3, and 7.25 cm^2/cm^3, respectively). The authors reported that the viability of probiotics increased and the baking process became faster when the surface area to volume ratio of the structure was higher. The "honeycomb" structure was more effective in protecting probiotics (baking at 145 °C), as the number of viable cells in "honeycomb" structure (10^6 CFU/g) was 2 log higher than that in "concentric" structure (10^4 CFU/g).

Several infill structures and their functions are shown in Table 2.

To investigate the effects of internal structure on the modification of texture properties of 3D printed food, infill density is another more important factor that should be mentioned. Huang et al. (2019) reported that the texture properties (gumminess and hardness) of 3D printed brown rice products were directly related to the infill density, i.e., he values increased with the increase in infill density (15%–75%). In order to improve the stability and mechanical strength of structure, suitable amount of deposition of mass and control of void fraction in the internal structure of 3D printed products was found essential. The judicious control of infill density helps to achieve these two important functions and then create a desired texture in 3D printed products.

The improvement of the texture properties and overall quality of printed foods by changing infill density has practical significances. Yang, Zhang, and Liu (2019) reported that when the infill density of 3D printed lemon juice gel was 100%, the filled material was bulged due to expansion. The filling up to 60%–80% produced significantly higher pore density while filling up to 90% produced desirable product. Liu, Bhandari, et al. (2018) reported that both Young's modulus and firmness (texture quality of 3D printed mashed potato) increased when infill density was increased from 10% to 70%. The paper by Mantihal, Prakash, and Bhandari (2019a) showed that the hardness of 3D printed chocolates was lower than that of traditionally produced chocolates even when the filling was 100%. The mass and density of 3D structure in chocolate can be controlled by controlling the infill density, which is not possible in melt casting method used in traditional chocolate making. They also conducted a sensory evaluation of texture-modified 3D printed chocolate to evaluate consumers' preferences (Mantihal, Prakash, and Bhandari 2019b). The results showed that there was no significant difference in preference between 3D printed samples (100% infill density) and casted commercial chocolate sample. Further, the participants significantly preferred the appearance of 3D printed chocolate with 25% and 50% infill density over the 100% and preferred the hardness of samples with 25% infill density. 3D printed chocolate can not only achieve the desired texture by varying the internal structure, but also improve their appearance and overall preferences.

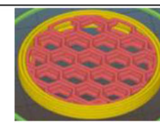
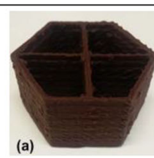
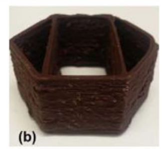
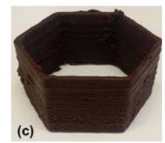
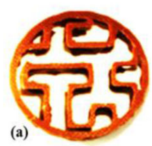
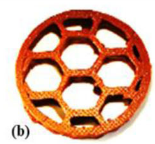
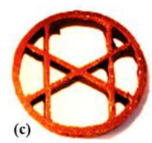
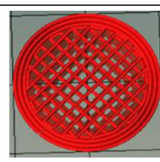
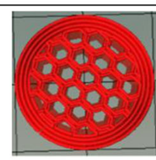
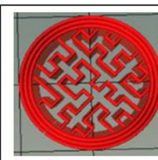
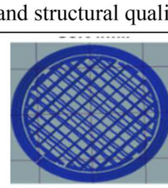
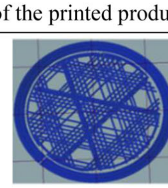
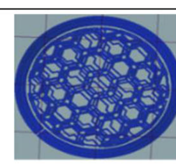
The above investigations only focused on the effects of infill structure or/and infill density on the texture properties of ready-to-eat 3D foods. To our knowledge, the texture modification of 3D foods requiring post-processing has also been reported. Dick, Bhandari, and Prakash (2019a) applied 3D printing technology in meat and studied the factors impacting the printability and post-processing stability of printed products. They found that the infill density affected the internal voids and post-processing stability and ultimately affected the texture of cooked products. Different infill structures also affected the stability of 3D printed meat products. "Honeycomb" and "linear" provided higher structural stability than "concentric" structure. They also studied the effects of fat content and infill density on texture and physical characteristics of 3D printed meat products, cooked with lean lard composite layer (Dick, Bhandari, and Prakash 2019b). They used lard and beef to create structures with increasing fat layers within each structure. These 3D printed products were conveniently cooked and preserved external and internal structures during cooking. The increased of infill density (50%, 75%, and 100%) increased the chewiness, hardness, and moisture retention and decreased the cohesiveness, and shrinkage but had no effect on fat retention.

Liu, Dick, et al. (2020) developed a unique air-fried 3D printed potato snack and found two new functions of internal structure, viz., control printing speed and decrease oil content. They observed that the printing speed can be controlled by changing the infill structure, including honeycomb, cubic, and rectilinear, among which, rectilinear infilled products gave the fastest one. Fracturability and hardness of such samples were significantly increased when infill density was increased (30%–70%). They finally produced one kind of product with multiple textural properties. After air-fried, the oil content of the product was 13.47%, significantly less than that of a common potato chips (39%) (Mai Tran et al. 2007). So, we speculate that 3D printing may be a new way to produce low-fat fried foods, which is an encouraging news for those who are looking for the foods to slim down.

4D Food printing

4D printing is an emerging technology, its concept was first introduced in 2013 (Miao et al. 2017). 4D printing uses the technology similar to 3D printing; however, it brings fourth dimension (transformation over time), which enables the printed objects to react with parameters such as humidity and temperature to change its shape, property, and functionality as desired (Javaid and Haleem 2019). As the fundamentals of this concept became more and more clear, its application has been increasing in many fields, such as medical, chemistry, material science, computer science, and engineering (Miao et al. 2017; Truby and Lewis 2016).

Table 2. Infill structures and their functions.

Purpose		Structure and their effects			References
Investigate the protecting effect of infill structure on probiotics in 3D printed mashed potatoes products	**Infill Structure**	 Concentric	 Honeycomb		(Zhang, Lou, and Schutyser 2018)
	Effects	The viable counts of probiotics in the "honeycomb" structure was 2 log higher than that in the "concentric" structure.			
Investigate the relationship between the physical properties of chocolate and the quality of the printed structures	**Infill Structure**	 cross-support	 Parallel support	 no support	(Mantihal et al. 2017)
	Effects	The 3D chocolate with "cross support" structure showed highest breaking strength.			
Investigate the effect of internal structure on the mechanical properties of 3D printed chocolate	**Infill Structure**	 Hilbert curve	 Honeycomb	 Star	(Mantihal, Prakash, and Bhandari 2019a)
	Effects	The "honeycomb" structure and "star" structure showed higher mechanical properties.			
Investigate the effect of infill density and infill structure on textural and structural quality of 3D printed mashed potatoes products	**Infill Structure**	 Rectilinear	 Honeycomb	 Hibert curve	(Liu et al. 2018b)
	Effects	Different infill structure did not have significant effect on textural and structural qualities of the printed products.			
Modify the texture of 3D Printed Air-Fried Potato Snack	**Infill Structure**	 Rectilinear	 Cubic	 Honeycomb	(Liu et al. 2020c)
	Effects	Products with "Rectilinear" structure showed the highest printing speed. "Rectilinear" and "Honeycomb" structure (infill 70%) showed higher fracturability and hardness.			

However, research in 4D food printing is still in its infancy and there are only few publications on 4D food printing.

Advantages of 4D food printing over 3D

The 3D printing technology is now quite intensely researched and successfully applied in some industrial cases. So far, 3D printed foods are rigid, and thus, their shapes cannot be easily changed using environmental factors such as temperature. The 4D printing technology can overcome this limitation of 3D printing of foods. 4D printed foods can change their characteristics and performance in a desired way when temperature of the surrounding changes (Javaid and Haleem 2019). The advantages of 4D food printing over 3D are listed below.

One of the most prominent advantage of 4D technology is that the 4D-printed model can respond to the changes in temperature, humidity etc as the time progresses. Moreover, the shape, sensory properties (color, taste, and flavor), and functionality of printed product can be changed as desired (Javaid and Haleem 2019). 4D printed foods can change

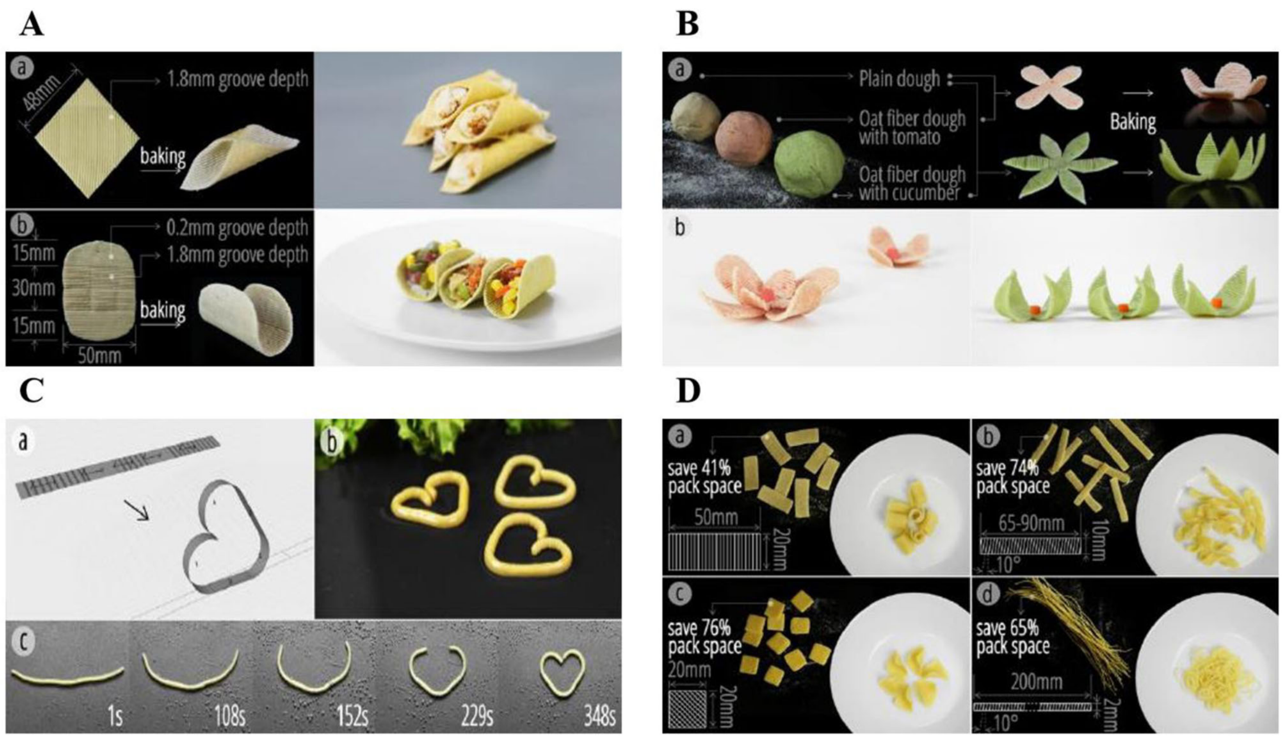


Figure 3. Dehydration-based examples through baking, including (a) the preparation process and final shape of self-wrapping tacos and self-wrapping cannoli and (b) the preparation process and final shape of self-folding cookies. Hydration-based examples through boiling, including (c) morphing heart and its actual transformation behaviors and (d) the shapes of four flat-pack hiking food, before and after cooking (Tao et al. 2019).

their sensory properties with time spontaneously or through the stimulation of water, temperature, pH, etc. These changes can bring desired sensorial characteristics and fun in meal. This feature is of practical significance to children with poor appetite. This technology offers greater options to innovate new products. It can also stimulate the imagination of engineers and designers, and help to implement new ideas to develop new products.

Recent advances in 4D food printing

To date, there is very less research available on "4D food printing". By searching keywords as "food" and "4D printing", we obtained only two papers published until 2020. However, to the best of our knowledge, there are other two articles produced 4D printed food in 2017 and 2019 but without calling it as such in title. Wang et al. (2017) first produced 3D printed food with shape transformation property. Edible 2 D films were produced from ordinary food materials (starch, cellulose, and protein), which was twisted or curled into 3D form by anisotropic swelling through cooking. They developed three types of films: 2D-to-3D film, self-wrapping films and temperature responsive strips and thus pioneered 4D food printing. The shape transformation in this 4D printed food was triggered by water when water-sensitive materials were used. Later, Tao et al. (2019) produced a flour-based food and found that baking (dehydration) could be used as a type of stimulus to trigger shape-changing for this new edible material. Figures 3a, b shows the preparation process and final shapes of baking-induced self-wrapping cannoli, self-wrapping tacos and self-folding multi-flavored cookies. They also found that boiling (hydration) is another shape-changing mechanism for this flour-based material to achieve shape-changing property. Figure 3c shows the final shapes of boiling-induced pasta and its actual transformation behaviors. Figure 3d shows the shapes of four flat-pack hiking food, before and after boiling.

Efforts to transform the color in 4D printed-food have also been reported. He, Zhang, and Guo (2020) used a dual-extrusion 3D printer printed to produce a colored food (Figure 4a), which spontaneously transformed color due to the presence of anthocyanin-rich purple sweet potato puree and mashed potatoes. The presence of anthocyanins made the mashed potato appear green at alkaline pH, purple-blue in neutral pH and red in acidic pH. When the mixed puree contained 27% alkaline-, 23% neutral- and 15% acid-mashed potato fractions, the printed products showed different colors over time. Ghazal et al. (2019) fabricated a 4D-printed health food (Figure 4b), which was a 3D-printed anthocyanin-potato starch gel that spontaneously changed color over time to improve visual appeal. The authors designed two samples, one of which changed color when a solution with different pH was sprayed onto the 3D sample. The printed sample can independently change color in response to an internal pH stimulus contained in this multi-material product. To achieve this, two types of gel system, namely anthocyanin-potato starch gel and lemon juice gel were prepared. These gels were then printed to obtain a multi-material sample, which spontaneously changed its color with time. These two articles mentioned 4D printed foods were developed by making use of property of anthocyanin that changes color as a function of pH stimuli. Anthocyanins also possess strong

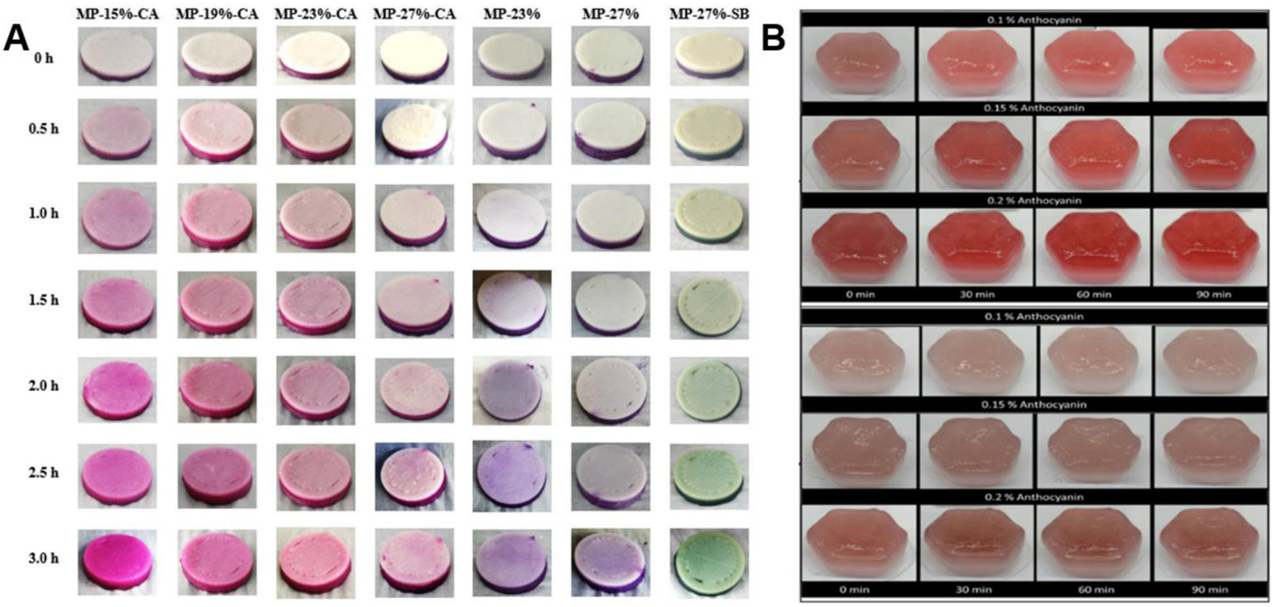


Figure 4. Four-D foods. Color changes of (a) 3D-printed mashed potatoes and (b) 3D-printed potato starch gel with different formulations over time after printing (He, Zhang, and Guo 2020; Ghazal et al. 2019).

free radical scavenging and antioxidant capacity (Wrolstad, Durst, and Lee 2005). Thus, this product not only possess interesting color changing feature, but also antioxidant function. These two works are also trend setters in terms of application of 4D technology in food industry and additive manufacturing.

Challenges and prospects of 4D food printing technology

Since 3D printing is the crucial element of 4D food printing, it faces all the challenges that 3D food printing faces. The 4D food printing is currently limited to changes in color, flavor, taste, and shape after printing. 4D food printing is an emerging technology with great promise. It is still in its infancy and requires technological advances in various aspects including software, hardware, and materials. The mechanisms involved in changes occurring over time and modeling also need to be advanced in order to fully utilize the potential of 4D food printing technology.

Concluding remarks and future prospects

3D printing technology can be instrumental in delivering customized food and personalized nutrition. The ability of using broad range of foods as printing raw materials and current advances in both software and hardware means that 3D printing can be used to produce foods to meet the needs of people of different occupations, age groups and life styles. 3D printing is not popularly applied in food industry and thus needs further research and development. Incorporation of bio-active compounds, development of microstructure in the 3D printed foods and their relationship with sensory attributes require further research.

Most 3D printers are of laboratory scale and only limited uses are in commercial settings. When the 3D printing technology matures, the list of printable materials will expand and the prices will come down, then there will be increased application of this technology in commercial manufacturing. It is also expected that 3D printing can be commonly used in household setting.

Increased adaptation of the 4D concept is expected to further increase the application of 3D technology. 4D research is expected to increasingly focus on edible water- and heat- and pH-sensitive materials as characteristics of 3D printed foods, which can be readily altered by changing humidity, temperature or pH. It is also expected for future research to focus on developing mechanisms to explain and quantify the changes in color and shape to further advance the concept of 4D printing. The increased application of 4D printing concept provides more options to innovate 3D printed foods.

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