3D Printing of Textured Soft Meat Analogues

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Abstract

Meat analogue is a food product mainly made of plant proteins. It is considered to be a sustainable food, and has gained a lot of interest in the recent years. Hybrid meat is a new type of meat analogue, which mixes plant protein and meat ingredients. It helps meat analogues express similarity with real meat, and also reduces the negative environmental impact.

Three-dimensional (3D) printing technology is becoming increasingly popular in food processing. 3D food printing involves modification of food structure, which leads to the creation of soft food. Currently, there is no available research on 3D printing of meat analogues. Therefore, this study was carried out to create plant and animal protein based formulations for 3D printing of hybrid meat analogues with soft texture.

This study was divided into three major sections. The first section was pre-printing experiments. Preliminary extrusion trials through a syringe were done using various materials, including different meat, plant protein samples and their combination, to finalize the most suitable material and formulations for further printing test using a 3D printer. Then rheology and forward extrusion tests were carried out on these selected samples to get basic understanding of their potential printability. In the second section, extrusion-based 3D printing was conducted to print various 3D shapes. The third section of the thesis presents the characterization of 3D printed products.

From the results of preliminary trials, pea protein isolate (PPI) was selected as the main plant protein source. For 3D printing tests, the addition of 20 % chicken mince paste (dry basis) to PPI based paste achieved better printability and fibre structure. In addition, the printing performance was standardized and was found to be optimal at 1.54 mm nozzle size, 10 mm/s printing speed and 100 % infill density. At these conditions, meat analogues with a layered structure could be designed through 3D printing.

Chicken mince and printed meat analogues had a similar moisture content of approximately 70 %. However, the protein content increased with an increase in the

amount of chicken in the 3D printed samples. The addition of chicken also hardened the texture of pre-printed products, but the printing process reduced the hardness. This demonstrated that printing is a suitable method to produce a soft meal. The results of light and scanning electron microscopy (LM & SEM) on cooked 3D printed samples showed that addition of chicken to plant protein matrix led to better fibre formation. PPI itself was not able to form any fibres merely by 3D printing and cooking in boiling water. Protein-protein interactions were also studied through the protein solubility test, which indicated that hydrogen bonding was the major bonding to contribute the structure formation in cooked and printed meat analogues. In addition, disulphide bonding was correlated with improved fibrous structures as observed through SEM.

This study investigated that meat analogues with softer texture were created through 3D printing. Due to the unique rheological properties of the printing materials, printing could only be carried out properly at a low speed (10 mm/s). High speed led to a poor shape forming capacity. Slight changes in the shape of printed products were observed after cooking however it did not affect their overall 3D structure. Further research is needed to develop an approach for high-speed printing by further optimizing formulation or printing parameters.

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Abbreviations

3D	Three dimensional
AOAC	Association of Official Agricultural Chemist
BSA	Bovine serum albumin
Eq	Equation
FAO	Food and Agriculture Organization of the United Nations
FDM	Fuse deposition modelling
GMO	Genetically modified organism
IDDSI	International Dysphagia Diet Standardisation Initiative
LM	Light microscopy
MPI	Ministry of Primary Industries
PPI	Pea protein isolate
RO	Reverse osmosis
RTE	Ready to eat
SEM	Scanning electron microscopy
SPC	Soy protein concentrate
SPI	Soy protein isolate
TPA	Texture profile analysis
TSP	Textured soy protein

Chapter 1. Introduction

Meat-based products are popular among consumers due to their unique taste, texture and nutritional values (Soto & Ortega, 2013). With the development of technology, meat is becoming more accessible to humans' daily diets. In contemporary society, various meat products have been processed and supplied all over the world. The demand of meat will continue to grow in the future (FAO, 2018). However, growing meat consumption is associated with increasing environmental concerns. To ensure sustainability, alternative diets or food sources have been suggested to decrease the average individual meat consumption (Aiking, 2011). Meat analogue is a type of food considered as a replacer by mimicking characteristics of meat.

Traditional meat analogues have been created and developed for many centuries. These meat analogues were generally made by vegetables or plants rich in protein, to deal with the low meat accessibility or due to religious reasons in different parts of the world (Tibbott, 2004; Riza, 2006; Joshi & Kumar, 2015). Due to the limitation of traditional processing technique, traditional meat analogues could not properly simulate sensory characteristics and texture of meat. Therefore, scientists have started researching new methods to improve the quality of meat analogues (Joshi & Kumar, 2015; Kyriakopoulou, Dekkers & van der Goot, 2019). Currently, the most common technology producing meat analogue is high-moisture extrusion. It can be used on various plant protein sources and produce different kinds of meat analogues (Riza, 2006). The extruded products tend to show higher similarity to meat, compared with conventional meat analogues. Other novel technologies like shearing and spinning have also been developed to further imitate the meat like fibres and microstructure (Boyer, 1954; Manski et al., 2007; Schiffman & Schauer, 2008). However, these methods have not been widely promoted to the industrial scale. Aside from meat like structure, nutritional value and other physical sensations of meat analogue also need to be improved.

Consumers' preference plays an important role in commercialisation of meat analogues. Currently, the challenges of developing meat analogues mainly include lower nutrition quality of plant proteins, lack of meaty sensations and high price (Kyriakopoulou et al., 2019). To improve nutritional and sensorial characteristics of meat analogues, one option is to add low value animal proteins to formulate a hybrid meat analogue. The hybrid meat would be rejected by vegetarianism but is also considered as a sustainable product (de Boer, Schosler & Boersema, 2013; Raphaely & Marinova, 2014). As a country specializing in animal food production, New Zealand should take this advantage to conduct research on hybrid meat. It is beneficial to attract global sustainable and a huge flexitarian market, while reducing the negative environmental impacts caused by food production (Hicks, Knowles & Farouk, 2018).

Additionally, it is worthwhile to develop some meat analogues, which are suitable for elderly. There are increasing numbers of elderly that need to be fed. However, the decreasing tooth strength with aging limits elderly's' food choices (Cichero, 2016). 3D printing is a novel technology, which could be introduced into food manufacturing to modify food structure and texture (Kouzani et al., 2017). In addition, it creates desirable food shapes and improves nutrient selection (Godoi, Prakash & Bhandari, 2016). Because of these, it has been used to produce some health care food products for elderly. Aged people would be benefited because they have more options, instead of simply consuming conventional pureed food. Scientists have already shown their interest in producing meat analogues from plant proteins through 3D printing. The printed plant-based materials with a steak shape has been shown to the public (Hitti, 2018). However, there is no available literature on evaluation of properties of printed meat analogue. The printing of hybrid meat analogue has not been mentioned by currently available research either.

Conventionally, soy was the most common material to produce meat analogues. Nevertheless, there are challenges to grow it in New Zealand due to the climatic limitations. As an alternative protein soure, pea has been successfully cultivated in New Zealand and sold to both domestic and overseas market (Plant & Food Research, 2014; Sutton et al., 2018). Moreover, pea has fewer food allergy issues and GMO concerns than soy (Osen, Toelstede, Wild, Eisner & Schweiggert-Weisz, 2014). The interest in research on pea protein has increased these years, especially for simulating chicken products (Raised & Rooted, 2019; Sunfed, 2019). Hence, pea protein and chicken were selected as the main materials to produce printed soft hybrid meats.

The main objectives of this project were: 1) Develop a printable formulation mainly made by pea protein and chicken mince paste. 2) Optimize the formulation to printing process based on rheology and extrusion tests. 3) Assess the printed meat analogue samples for texture profile, microstructure and molecular interactions.

Chapter 2. Literature review

2.1 The increasing requirement for meat

According to the United Nations (2017), the world population is over 7 billion currently and is estimated to reach 11 billion at the end of the 21st century. In addition, foods with high nutritional values, especially high protein value, are becoming increasingly popular among customers. The consumption of protein rich food such as meat or meat products is expected to be doubled in the next three decades (FAO, 2020). The growing global population and higher individual demand make meat supply face extreme challenges.

At the same time, the large amount of meat production is considered to negatively influence the environment (McMichael, Powles, Butler & Uauy, 2007). Firstly, Stehfest et al. (2009) claimed that around four fifths of anthropogenic land were used to raise and feed livestock. In addition, the water usage and greenhouse gas produced by livestock production were higher than plant-based food production. Ilea (2009) outlines that livestock farming contributes nearly 20 % of global greenhouse gas emissions. The reason was associated with the development of intensive livestock farming. Concentrated proteins derived from plant sources are chosen to feed livestock, which makes the ruminants' weight grow faster. Such processed feeding materials increase the methane emission from the production line of livestock. Moreover, Aiking (2011) indicated that the 1 kg of meat protein production requires 6 kg plant protein on average, which is not an efficient process. The environmental impact also varies in different meat production systems. For instance, poultry and pork productions generally cause fewer greenhouse gas emissions and require lower plant protein feeding compared to ruminant animals (Sabate & Soret, 2014).

The environmental impact of meat consumption, including climate change, is believed to further exacerbate hunger in the world. It is estimated that crop productivities of various common edible plants would decrease due to severe climate change. Because of this, food accessibility would face huge challenges in the future (Wheeler & Von Braun, 2013). Although it is controversial that global warming is directly caused by growing meat production, the increasing concerns about environmental issues lead consumers to pay more attention to sustainability of food products. For these reasons, it is necessary to develop some alternative methods to feed people in the future.

2.2 Future protein supply strategies

2.2.1 Exploration of new edible sources

In fact, there are various strategies focusing on future food supply. Diverse novel categories of edible materials, including animals, plants and microorganisms, have been explored (Boland et al., 2013; Anderson, Asche, Garlock & Chu, 2017). These edible materials would become alternatives to meat, based on their flavour, texture or nutritional values.

Seafood is considered as a substitute for land-based meat. It provides essential proteins for humans and saves land use. According to previous literature, seafood has already become an important animal protein source in many countries, especially in undeveloped coastal regions. The consumption of seafood grew very fast in recent decades (Anderson et al., 2017; Mustafa, Estim, Shapawi & Kinabalu, 2019). Compared with terrestrial animal products, one obvious advantage is that marine animals do not need large land areas. Meanwhile, the feed conversion ratios of seafood are generally lower than conventional livestock and poultry. Aqua animals which consume plankton are considered as eco-friendly food for humans (Langan, 2008; Mustafa et al., 2019). Similar to marine or aquaculture products, insects are another potential food that requires less land use and feeding energy. At the same time, insects are also a source rich in proteins. The feeding cost is verified to be low, which may reduce the pressure on the environment (Gahukar, 2011). Such benefits are believed to be a solution to deal with future food security. However, entomophagy (insect consumption) is not easy to promote in most nations for many reasons. People who are not suffering from hunger yet tend to show little attraction to consuming insects (Shelomi, 2016). Tucker (2014) indicated that their appearance constrains the promotion of insects as food. Instead of direct consumption, powdered and chopped insect proteins are more acceptable (Tan et al., 2015). Although it is possible to identify alternative edible sources, people's eating tradition or culture is hard to change. Preference for conventional meat would still be maintained in the long term.

2.2.2 Reducing meat waste

Another strategy to improve protein security is to reduce the waste from meat industry. The meat waste can occur through all supply chains (Hicks et al., 2018). In general, the waste is associated with human eating habits and meat processing (van der Goot et al., 2016). There is no doubt that modern industrial processing produces food with a longer shelf-life, which keeps numerous people safe from starvation. At the same time, however, current industrial processing also makes a huge food waste. Van der Goot et al. (2016) demonstrated that a large number of by-products are wasted, since they are considered as non-valuable by the food industries. The advancement in food processing is believed to add value to food by-products. For instance, Toldrá, Mora and Reig (2016) suggested that meat by-products such as blood, offal and bones could be utilized to feed livestock or produce functional ingredients in food productions. Hicks et al. (2018) also highlighted the importance of the efficient supply chain to reduce avoidable meat waste.

2.2.3 Meat alternatives

In addition to meat waste reduction in the supply chain, another opportunity is to utilize the low value meat or offal cuts in the production of meat analogues. Two types of meat alternatives or analogues are mentioned below.

2.2.3.1 Cultured meat

One kind of alternative meat product is called cultured meat, or *in vitro*-grown muscle tissues. Some scientists have successfully grown muscle tissues in the lab by utilizing stem cells from animals. This technology aims to assemble muscle fibres to create a meat-like object (Edelman, McFarland, Mironov & Matheny, 2005). The concept of cultured meat was generated early, but the methods have been recently developed. This method has already been successfully utilized to formulate beef burgers, which shows that cultured meat is possible as an alternative to conventional meat products. However, consumers tend to view cultured meat as an unnatural product (Siegrist, Sutterlin & Hartmann, 2018), which may affect their consumption choices.

2.2.3.2 Plant protein-based meat analogues

This is another type of meat analogue, which is mainly made by plant protein and other non-meat ingredients. Compared with cultured meat, plant-based meat is more acceptable since it is formulated of plant-based sources. Moreover, plant-based meat analogues is not a novel food concept. The formulation and processing of meat analogue can be dated to thousands of years, mainly in undeveloped areas, where people had difficulty purchasing and consuming meat. People in these areas tended to consume vegetable proteins to reach their nutritional demands (Kumar et al., 2017). For developed countries, meat analogues or replacers will also rise popularity, when meat shortages appear. Riaz (2006) shows that soy foods or restructured soy products became more popular in American and European markets around the 1970s, when meat products were not adequate to satisfy the increasing protein requirement of residents. Meat analogue processing technologies also rapidly developed at that time. In summary, an unsatisfying meat supply leads to promotion of meat analogues. Nowadays, some brands of meat analogues have been successfully commercialized, such as Beyond Meat, Impossible Foods, Quorn and Sgaia (Beyond Meat, 2018; Impossible Foods Inc 2019; Quorn, 2019; Sgaia, 2019). These brands are mostly founded in North America or Europe, and well known to global meat analogue consumers. In New Zealand, one of the most famous meat analogue brand is Sunfed meats, which produces meatless chicken chunks by using yellow pea (Sunfed, 2019).

2.3 Plant-based meat analogues

2.3.1 Trends for consumption of plant proteins

From a historic perspective, the wide consumption of vegetarian food could be associated with some religious factors. As demonstrated by Freedman, Chaplin and Albala (2014), vegetarianism in China became popular from the 4th to 6th centuries when Buddhism was introduced. Although it is not the major reason for vegetarianism in modern society, the tradition has been maintained to some extent till today. In addition, various meat substitutes made of soy and wheat were developed during that time. Such dishes were initially designed for Chinese Buddhists, who were not allowed to consume meat or other animal products.

In modern society, as mentioned above, numerous high protein plant sources are used to feed livestock. It is believed that unnecessary greenhouse emissions will be reduced if plant proteins are directly processed and consumed by humans (Ilea, 2009). Therefore, the consumption of plant proteins is considered as an environmentally friendly diet. Considering the low protein transformation rate, direct consumption of plant protein is also believed to be more economical (Aiking, 2011). Moreover, some people prefer to choose vegetarian or vegan diets to decrease the killing of animals (Ruby & Heine, 2011).

There is also evidence showing that vegetable proteins are good for humans' health. Based on meta-analysis, Dinu, Abbate, Gensini, Casini and Sofi (2017) showed that vegetarian diets reduce the possibility of catching ischemic heart diseases and cancer. Additionally, Marventano et al. (2017) identified that higher legume protein diets are less likely to lead to cardiovascular diseases. With the growing awareness on human health, more consumers are willing to change their diet. From a nutritional perspective, plant-based sources could also play the role of high protein food. Many protein-rich vegetables, such as soybeans, and processed products have already been consumed in human diets for centuries. These plants are not completely new protein sources. However, they can modify the diet while satisfying consumers' nutrition demands. Therefore, the intake of plant protein is predicted to be more popular.

Although many benefits of plant protein are shown in the literature, there are still some constraints to widely promote plant-based food consumption to people. One disadvantage of plant protein is the variation of composition of amino acids. It is proven that some essential amino acids are limited in one single category of plant food. For this reason, plant protein is labelled as 'low quality protein' compared with protein derived from animal sources (Young & Pellett, 1994). However, the combination of diverse plant proteins in the diet can solve this problem. From another perspective, Hoek et al. (2004) pointed out that food texture could significantly influence consumer preferences. Compared with meat or meat products, the texture of natural plant-based foods tends to be less desirable. Hence, the texturization of plant protein is highly recommended.

2.3.2 Development of textured plant-based meat analogues

2.3.2.1 Traditional meat analogues or substitutes

According to previous literature, there were many kinds of meat analogues made in Asian countries in history. Tofu is a type of product curded from soy beans. It is famous in East Asia and has been consumed for many centuries. But tofu expresses few similarities with meat in taste and texture. The reason it is considered as a meat replacer is because of special cooking methods or recipes (McIlveen, Abraham & Armstrong, 1999). Hence, tofu can be consumed as a meat analogue only when it has been well designed and cooked.

Another cake-like soybean analogue is called tempeh. It is a fermented soy product, which is originally created in Indonesia. Different from tofu, the formulation of tempeh also includes some by-products form other plants, such as peanuts and coconuts (Tibbott, 2004). Its low price and high protein content make it a popular meat analogue, especially in developing countries (Ashenafi & Busse, 1991). Due to the increasing interest in vegetarian diets, tempeh has also been loved by people in developed countries. Tibbott (2004) reported that tempeh gained extreme popularity in the North American market in the 1980s. Such new food provided more options to consumers who enjoy eating soy-based foods. Nonetheless, the trend slowed down recently. The reason is associated with the emergence of more novel processed soy products.

Seitan is a type of meat analogue, which is made from wheat gluten. Hence, it is also known as 'wheat meat'. Wheat gluten was commonly used in bakery products in Western countries to create the rubber like texture of bread. While in East Asia, it was conventionally use to provide a meat like chewing feeling. During the manufacturing of seitan, wheat glutenin and gliadin are derived from dough by rinsing and washing. The removal of starch and bran increases the elasticity of the dough (Inglett, 1974; Joshi & Kumar, 2015).

These traditional meat analogues are also consumed in current society, and considered to be sustainable diets. Rivera and Azapagic (2019) have shown that the replacement of meat ingredients by plant proteins in ready-to-eat (RTE) meals significantly reduced many negative environmental influences. Based on their assessment, the use of tofu, soy granules and seitan in meat showed different environmental impact. However, these traditional foods cannot perfectly simulate a meat-like texture or taste. They were popular in the past just because people could not afford to consume meat products. In contemporary society, they are no longer alternatives of meat since real meat can be easily purchased in the market. However, the processing methods of these foods provide some fundamental ideas for making new meat analogues. Soybean and wheat are still the main raw materials being used to formulate meat analogues.

2.3.2.2 Textured vegetable proteins (TVP)

TVP is a processed protein-based vegetarian food, which can be used in diet to replace meat. The chewiness of meat is simulated by creating a fibrous structure in plant proteins (Joshi & Kumar, 2015). TVP is mainly fabricated from soy beans, which are considered to contain existing high quantity and quality proteins. Soy beans are soaked, and the protein is extracted by extracting solvents, to produce a high-protein product. Different types of soy protein-based products are produced depending on further processing methods. Defatted soy flour, soy protein concentrate (SPC) and soy protein isolate (SPI), are three of the most common protein rich soy products that are used to produce TVP through texturization (Day, 2011; Kumar et al., 2015). According to the purpose, TVP can be extruded into different forms, including chunk, nugget, granule and shred. In addition, to imitate fibrous meat, TVP is also used to simulate some processed meat product, such as sausage, burger and nugget (Carvalho, Milani, Trinca, Nagai & Barretto, 2017; Hidayat, Wea& Andriat, 2018; Sharima-Abdullah, Hassan, Arifin & Huda-Faujan, 2018). As reported by Riaz (2011), TVP may not only play the role of meat replacer, but also could become an ingredient of breakfast cereals or frozen desserts.

Beside soy-based proteins, the texturization of other plant proteins are also applied and studied. Researchers have already develped TVP by using pea, peanut, lupin and mung bean (Rehrah, Ahmedna, Goktepe & Yu, 2009; Osen et al., 2014; Palanisamy, Topfl, Berger & Hertel, 2019; Samard & Ryu, 2019). In addition, chickpea, rice, spirulina, wheat gluten and plant-derived starches had also been added as minor ingredients to enhance the textural or sensory properties of meat analogues produced through high-moisture extrusion (Chiang, Loveday, Hardacre and Parker, 2019; Kyriakopoulou et al., 2019). With further exploration, more categories of plant sources are expected to be used to produce TVP.

2.3.2.3 Application of other alternative protein sources

Aside from legume or wheat proteins, mycoproteins can also be added to produce meat analogues. These proteins are extracted from edible filamentous fungi, which assist in building fibrous structures. Compared with conventional soy-based meat analogue, filamentous fungi do not generate an off-flavour. In addition, the texture and health benefits, such as fibrous structure and lack of cholesterol, are highlighted (Denny, Aisbitt & Lunn, 2008; Kim et al., 2011). The use of mycoprotein has already been successfully commercialized. There is a well-known brand named Quorn, which involves various meat analogues made by mycoproteins of a fungus species *Fusarium venenatum* (Wiebe, 2002; Joshi & Kumar, 2015; Quorn, 2019). However, the price of Quorn is generally higher than many meat products. It tends to be targeted as a high value food, which is insufficient to be universally promoted (Apostolidis & McLeay, 2016). In addition to Quorn, mushrooms are also considered to be a source of meat replacer. Mushroom based nugget and sausage analogues have been developed by researchers (Asgar, Fazilah, Huda, Bhat & Karim, 2010; Arora, Kamal & Sharma, 2017).

The similar physicochemical properties between soy and whey protein, like extrudability, provide the inspiration for processing dairy-based meat products. In fact, scientists already planned to use textured whey protein to simulate whole meat in the 1970s. Hydrated whey protein after extrusion even gains higher acceptance than TVP. However, one significant disadvantage of dairy based meat analogue is the high cost. For this reason, little research on dairy based meat analogue has been done in recent years (Onwulata & Huth, 2009). However, textured whey protein can still play the role of nutrient enhancer. It is used in meat patties or plant-based meat analogue to increase their nutritional value.

In addition, Azzollini et al. (2019) formulated a meat like texture by using coagula of lesser mealworm protein. However, the texture and mouthfeel of final products vary even though they are made from one single species of lesser mealworm. This finding suggests that insect or worm proteins need to be well characterized in the future.

2.3.3 Texturization and processing techniques

Processing techniques play a critical role to ensure the quality of meat analogues. With the development of processing techniques, some new plant-based meat analogues have been produced and promoted in the modern society. The novel processing techniques also follow the similar mechanism to restructure plant proteins. Differing from traditional methods, new knowledge and theories are added into the processing. Current common processing techniques mainly include mixing, extrusion, sharing and spinning.

2.3.3.1 Extrusion

In contemporary food industry, extrusion is the most popular processing method to form fibres of meat analogues. Indeed, extrusion has already been applied in various food processing to create an anisotropic structure. At the beginning, it was used to process low-moisture cereal-based food, like pasta and breakfast products. The use of extrusion in meat analogue processing can be dated to the 1960s, when Wenger laboratory developed small pieces of sponge-like and chewy extruded soy products. The processing of meat analogue with layered structure by extrusion started from the 1970s (Riaz, 2006; Manski, van der Goot & Boom, 2007). According to Riaz (2006), plant protein sources are mixed with moisture and blended before extrusion. A meat-like texture would appear during extrusion, when pressure, thermal energy and mechanical strength are added. The scheme of extruder and basic extrusion processing are presented in Figure 1. As illustrated, plant proteins as well as some other solid materials are combined in the mixer. Moisture is added when the dry materials are fed through the screw. Then high temperature is applied to denature the proteins, which modifies the structure of the food. Meat-like fibres are believed to be generated at this step. Finally, the sample is extruded and cooled until the fibres are stable (Liu & Hsieh, 2008).

Initially, meat analogues were commonly extruded with low moisture content. The moisture of those products was normally not higher than 30%. However, such extrusion methods poorly simulated meat texture. Consumers did not show much preference for low-moisture meat analogues (Riaz, 2006). The extruded plant proteins tend to show a sponge like texture if the moisture content is lower than 35% (Osen et al., 2014). Those products are normally consumed as meat extender since they could not simulate the actual structure of meat. Akdogan (1999) emphasized that moisture content was vitally important for plant protein food to form a meat like structure. For this reason, high-moisture extrusion has been invented and developed.

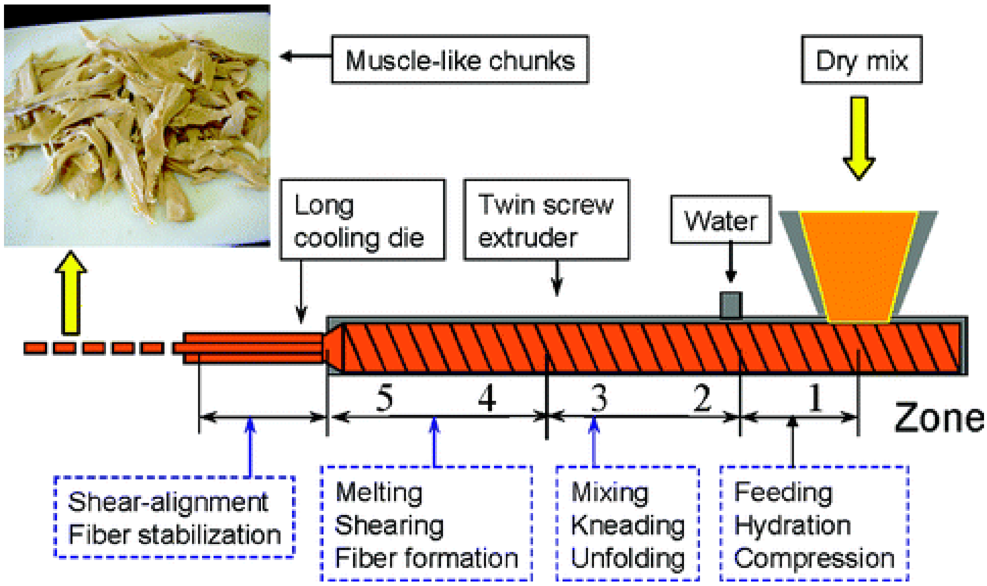


Figure 1. High moisture meat analogue extrusion process (Liu & Hsieh, 2008).

Compared with the low-moisture method, high-moisture extrusion tends to introduce a fibrous structure to meat analogues. Numerous literature has shown that desirable texture is only created under higher moisture content (Akdogan, 1999; Yao, Liu & Hsieh, 2004; Liu & Hsieh, 2008). The percentages of moisture in high-moisture extrusion normally vary from 40 to 80% (Liu & Hsieh, 2008; Chen, Wei & Yang, 2011). However, Lin, Huff and Hsieh (2000) identified that the texturization could not be completed if the moisture content was higher than 70%. The reason for improvement of texture is assumed to be that aggregation of proteins is decreased when the water content is higher. The interactions between disulphide bonds and hydrogen bonds or hydrophobic interaction are stimulated, which provide the unique three-dimensional network after extrusion (Chen et al., 2011).

In addition to modifying moisture, the formulation of meat analogue influences the texture as well. Literature has shown that the combination of different plant protein materials enhances the fibre formation. Chiang et al. (2019) investigated that addition of 30% wheat gluten contributes a desirable meat-like fibre to soy protein during extrusion. Furthermore, they outlined that the fibres are mainly created due to the cross-links formed between denatured proteins.

2.3.3.2 Shearing

The inspiration of shearing technology has come from the mechanism of rheometers. The fibrous and anisotropic structures of plant materials are formed by shearing strength in the well-designed devices (Manski et al., 2007). There are different types of devices available to produce meat analogues.

One shearing device with a concentric cylinder structure is named the Couette cell. The inner cylinder can rotate both concentrically and eccentrically, depending on the processing purpose (Peighambardoust, Van Brenk, Van der Goot, Hamer & Boom, 2007). There is another shear cell consisting of a double cone device. As Figure 2 shows, samples are positioned between two cones. The shearing strength is initiated when the bottom cone plate rotates. Then the microstructure of samples is modified (Habeych, Dekkers, van der Goot & Boom, 2008). The applications of both Couette cell and shearing cell for meat analogue processing have been reported. Krintiras, Göbel, van der Goot and Stefanidis (2015) identified that Couette cell was able to create obvious anisotropic fibres from the mixture of soy protein and wheat gluten at 30 rpm and 95 °C. Shear cell processing also formed fibrous structure from soy protein and pectin mixture. Differently, the optimal temperature of fibre formation was found to be between 120 and 140 °C (Dekkers, Nikiforidis & van der Goot, 2016) Aside from soy protein materials, shear cell was also used to produce meat analogue made from pea protein and calcium caseinate (Tian, Wang, van der Goot & Bouwman Schreuders, 2018; et al., 2019). Currently, the shearing technology is mainly based on laboratory level processing. The improvement of shearing equipment is needed before promoting it into industrial use.

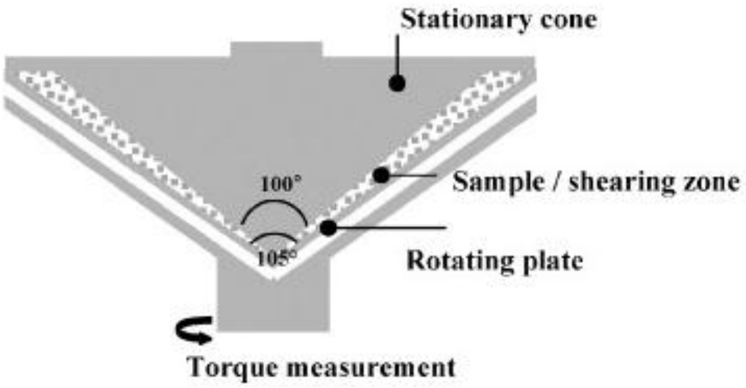


Figure 2. Schematic graph of cone-cone shear cell (Habeych et al., 2008).

2.3.3.3 Spinning

Spinning is a method which creates an alignment structure to imitate meat like fibres. There are two types of spinning method that have been researched. The first is wet spinning technique. During wet spinning, solutions containing plant proteins are selected to be the raw materials. The solution is pumped and squeezed through a spinneret to make streamlets. The aligned and stretched fibres are formed when the streamlets are solidified through a salt coagulating bath (Boyer, 1954). The structures of wet spun products vary because of the phase separation and solidification mechanisms. Fibres were formed where the dispersed phase was solidified while continuous phase stayed in solution. In contrast, the capillary and fibre filled gels are obtained in continuous and dispersed phases, respectively (Kyriakopoulou et al., 2017). The relatively new spinning method is called electrospinning. This method is shown to reduce the large waste of streams, compared with wet spinning. In electrospinning process, biopolymer solution containing protein is filled in and extruded from a syringe. By adding a high voltage, the solution forms a Taylor cone and jets straight for short time. Then it moves in a conical route and forms fibrils on a metal collector (Blanshard & Mitchell, 1988; Schiffman & Schauer, 2008). However, electrospinning requires a strict standard of protein polymeric solutions. Nieuwland et al. (2014) pointed out that most plant proteins, which have globular structures, are not suitable sources for electrospinning. To solve this issue, they tried using gelatine as a carrier of soy protein. Although gelatine successfully enabled globular protein to be spun, the formed fibres were quite soluble in water. The alignment of spun fibre also needed to be further researched. Therefore, there are still a lot of limitations to use electrospinning to produce meat analogues currently.

2.3.3.4 Fermentation-based processing

Fermentation is not a common processing method and has a limit availability of materials. It has been only used to produce mycoprotein-based meat analogues. According to previous studies, fermentation stimulates the cultivation of edible fungi, which assists to grow the mycelia mimicking meat like fibres. A mycoprotein-based suspension with mycelia is formed after fermentation (Wiebe, 2004; Kim et al., 2017). Normally, few secondary processing steps are needed to transform the mycoprotein suspension to meat-like products. For different fungus materials, fermentation is followed by different secondary processing to get final products (Wiebe, 2002; Kim et al., 2017). For these reasons, the feasibility of fermentation and secondary process in production lines are highly dependent on food characteristics.

2.3.3.5 Mixing and further processing

Textured or high moisture extruded meat analogue can be further processed by mixing with other ingredients to some specific type of products, such as mince, nuggets, sausage or burgers. The addition of TVP increases the fibrous structure, which expresses a more desirable texture. Sharima-Abdullah et al. (2018) showed that nugget containing 30% TVP and 10% chickpea flour express the closest values to real chicken nuggets in hardness, cohesiveness, springiness and chewiness. It also gained the highest score on texture based on a hedonic test. Chiang, Hardacre and Parker

(2020) produced sausages by using extruded soy meat analogues. However, the textural and sensory properties of meat analogue sausages differed from chicken sausage in their study. This indicates that more efforts were needed to increase the similarity between meat analogue sausages and real meat sausages. In general, further processing provides potential applications for textured meat analogues.

2.3.4 Consumers' acceptance and potential future market

2.3.4.1 Limitations of current meat analogues

From a nutritional perspective, plant-based meat analogues mainly simulate the protein level of real meat. Davies and Lightowler (1998) assessed the nutrient content of several typical meat analogues and compared them with lean beef. According to their finding, meat analogues generally provided carbohydrates which are not identified in lean beef. Tivall, which is made from wheat proteins, expressed the highest similarity to real beef in nutritional levels. Nevertheless, the absorption rate of iron in Tivall is much lower than haem iron in meat. The absorption of non-haem iron is suggested to be improved by taking food rich in Vitamin C.

Another reason which influences consumers' preference is the mouthfeel of meat analogues. A survey on person-related and product-related factors in consumer acceptance has been conducted. According to Hoek et al. (2011), most British and Dutch consumers show little interest in meat analogues. The main reason is assumed to be that meat analogue tends to express less attractive sensory properties than real meat products. Although this survey is not updated, it indicated the fact that the sensory acceptance of meat analogue should be improved. As shown by Sharima-Abdullah et al. (2018), the sensory score of meat nugget analogues made by chickpea and TVP were significantly lower than chicken nugget based on sensory tests. The overall acceptance is low, possibly because the chewiness, springiness and cohesiveness of chicken nuggets are not well simulated by using plant proteins. Moreover, the price of the new meat analogue products should be set at an acceptable level (Hoek et al., 2011; Tucker, 2014). Although plant protein-based foods are considered as cheap products compared to meat, the finely processed meat analogue products are expensive. The price of some brands, like Impossible Burger, is even higher than normal beef burgers (Joshi & Kumar, 2015; MPI, 2018). This phenomenon reduces consumers' preference to some extent. It is a big challenge to produce a meat analogue product with all desirable meat characteristics in an affordable price.

In general, there are many constraints for meat analogues to widely replace real meat. Nevertheless, ideas can also be found in previous research. In addition, the terminology problem of meat analogue should be highlighted. Davies and Lightowler (1998) recommend that plant-based meat products should be named uniquely. It would make meat analogues play a role of a novel food, instead of a merely replacer of real meat. This is because 'real' food is more popular than 'mock' products to the public. Similarly, Hoek et al. (2011) pointed out that meat analogue should be characterized as a type of novel protein-rich plant food. Hence, it is necessary to develop a new market.

2.3.4.2 Flexitarian market and Hybrid meat product

It is difficult for people to completely avoid consuming meat. Although people show favour for sustainable diets, not many people are willing to abandon consuming meat products. In the USA, more than four fifths of vegetarians and vegans broke their diet in recent years. The percentage of Americans who were vegetarians or vegans was only 2% in 2014 (Faunalytics, 2016). The reason was assumed to be that vegetable-based diets could not fit the consumers' satisfaction. However, there is a group of people defined as flexitarians. As described by Raphaely and Marinova (2014), the term 'flexitarian' refers to people who are willing to reduce meat or animal products consumption in diets due to various reasons mentioned above. Due to the growing trend of flexitarianism, new supply strategies of meat industries are suggested. As indicated by Hicks et al. (2018), it is wise for meat industries to promote meat-reduced meals, or meat substitutes, to maintain and attract flexitarian customers. For this reason, hybrid meat, which contains meat ingredients and non-meat protein, is considered as a suitable product for flexitarians. The addition of animal contents tends to improve the sensory acceptance of meat analogues. de Boer et al. (2013) demonstrated that hybrid meat would be a good choice for consumers who have lower food accessibility.

The food development strategy should also be based on the advantages of the producer countries. New Zealand is a country famous for its dairy and meat production. However, the potential opportunity for developing meat analogues can also be identified. As shown by MPI (2018), natural, organic, GMO-free and safety reliability are major characteristics of food from New Zealand. It suggests that New Zealand food producers should take advantage of the growing global flexitarian market. Thus, high-value hybrid meat produced by natural plant source and high-quality animal ingredients is recommended.

2.4 Food for elderly

2.4.1 The ideal properties of food for elderly people

In the past few decades, the average lifespan of people has continuously increased. This tendency dramatically altered the structure of the global population. The number of people over 60 years old is predicted to surpass 1 billion in the next two decades (Cichero, 2016). At the same time, there are various changes in human bodies because of aging. In geriatric conditions, many physical changes in the body modify eating behaviours. Compared with young adults, the decline in perception of hunger leads aged people to consume less food (Hays & Roberts, 2006). Oral health is another problem, which constrains food intake of elderly people. The loss of tooth strength by aging increases chewing difficulties (Walls & Steele, 2004). Because of these factors, some characteristics of conventional food products should be modified. The development of food suitable for aged people will be a future trend. Traditionally, pureed food with water is an ideal design for elderly. However, Cichero (2016) summarized that the increased moisture content could lead to nutrient dilution, which leads to challenges in consuming sufficient nutrition. Comprehensive studies have been done to produce easily-consumed texture while maintaining nutritional values.

2.4.2 Meat analogues in elderly diets

A potential consumer group of meat analogues would be people with special nutrient requirements which cannot be satisfied by current commercial food. The impact of increasing consumption of vegetarian foods is believed to benefit elderly people's health too. As summarized by Nicklett and Kadell (2013), higher fruit and vegetable intakes in the diet decreased the mortality rate for the elderly. Meanwhile, various geriatric problems, such as chronic diseases, cognitive impairment and aging associated disability could be prevented. The trend of consuming less meat has become popular in some countries already. In United States, around half of people over 65 started to reduce meat consumption in the recent decade (Harriman, 2018).

For meat analogues, fibre formation is one of the most important standards to assess the product quality. However, toughness tends to increase when the fibre is formed. As shown by Chiang et al. (2019), meat analogue is hardened by adding 30% wheat gluten, which ensures the ideal fibrous structure. This phenomenon is not appropriate for elderly. Therefore, it is necessary to design a formulation, which provides both soft texture and meat-like fibre.

2.4.3 Processing technologies or formulations for development of food for elderly

2.4.3.1 Conventional softening techniques

It is believed that special processing techniques can modify the texture of food. High pressure method, ultrasound, pulsed electric fields (PEF) are innovated techniques, which show potential to soften various common food products (Aguilera

& Park, 2016). The textural modification of meat products is particularly focused on in this section.

High pressure processing is a non-thermal technology, which is considered as an alternative to thermal preservation. Moreover, additional functions of high pressure have been identified, since it has influence on proteins in the food matrices. Previous studies have already shown that high pressure causes gelation, denaturation and aggregation of proteins, which modifies food structures (Knorr et al., 2011; Chen et al., 2018). The use of high pressure in meat structuring has also been studied. Tokifuji, Matsushima, Hachisuka and Yoshioka (2013) identified that 400 MPa pressure pre-treatment for 20 minutes contributed to a softer and less adhesive texture of pork gel. Before inducing high pressure, the pork gel was formed by adding water. Pork was ground and mixed with water at the ratio 1:1. By using this formulation, an easy-swallowing property was developed. Therefore, high-pressure as well as textural modification are helpful in meat processing for dysphagia diet. Likewise, these mechanical parameters have also worked on fish gels (Yoshioka, Yamamoto, Matsushima, Hachisuka & Ikeuchi, 2016).

There is research which has shown ultrasound treatment modifies the textural properties of meat. By sonication for different durations, various textural properties of beef were changed. The reason was assumed to be that both stability and functionality of collagen were changed. The hardness of beef was significantly decreased after 10 min sonication (Chang, Xu, Zhou, Li & Huang, 2012).

PEF is another non-thermal preservation technology, which inactivates microorganisms by damaging cell membranes. It is particularly suitable for liquid food preservation, but the use in meat was also researched. Aside from preservation, effects of PEF on other characters of meat was studied as well. It was shown that PEF improved the tenderness of meat (Wouters & Smelt, 1997; Warner, Ha, Sikes & Vaskoska, 2017). Nonetheless, the tenderization through PEF was only qualitatively recognized. Significant change in textural properties of beef after PEF treatment was not identified through textural profile analysis (O'Dowd, Arimi, Noci, Cronin & Lyng, 2013). Thus, a deeper understanding of the nature of meat tenderization using PEF is required.

2.4.3.2 Additives

Food additives help provide a softer texture as well. The addition of external proteins is one way to ensure enough protein in foods. Aside from direct formulation of meat analogues or extenders, plant proteins can also be applied as additives to form high-protein food matrices. Baugreet, Kerry, Botineştean, Allen and Hamill (2016) tested the effects of adding various plant protein on the general qualities of beef patties. Based on their investigation, the addition of rice protein significantly enhances the protein content. In addition, lentil flour was verified to soften the texture of beef patties. They presumed that the combination of rice protein and lentil flour would have positive influence on both nutrients and texture. However, such complementary effects need to be proved by further research.

Enzymatic treatment is another method to soften or tenderize meat. Proteases from fruits are considered as possible enzymes to modify the texture of meat. Eom, Lee, Chun, Kim and Park (2015) reported that the injection of collupulin (enzyme form papain) reduced hardness for about 90 % of chicken breast. Moreover, bromelain showed a stronger softening effect (95 %) on chicken breast and also significantly decreased the hardness of beef. These two enzymes did not alter the shapes of treated meat. It is also shown that freeze-thaw treatment increased the efficiency of enzyme impregnation (Shibata, Sakamoto, Nakatsu, Kajihara & Shimoda, 2010). The shape of texture softened food was also retained under freeze-thaw infusion. As shown by Nakatsu et al. (2012), freeze-thaw infusion could be used to treat fish products. They found that Pacific cod softened with a proper shape after freeze-thaw infusion. However, freeze-thawed Pacific cod showed a high adhesiveness value and low water retaining capacity, which suggested to a low swallowability.

2.4.3.3 Emerging structuring methods

The modified formulation and structuring technologies referred to above are mainly applied in commonly consumed food. There is little research particularly focusing on softening the texture of meat analogues. The uncertain feasibility to the unique food matrices of meat analogue is a limitation to these texture modifying methods. However, these methods give an inspiration to creating texture softened meat analogues. No matter what physical processing or additive treatment, the principles of food textural modification are all based on changing food structures. Hence, texture softened meat analogues can possibly be formulated by changing conventional structuring technologies.

There are some novel technologies modifying the structure of food associated with developing new food texture. Three-dimensional (3D) printing is a technology that softens the texture by reducing the particle sizes (Aguilera & Park, 2016). Compared with traditional pureed food or thickened liquid, 3D printed food expressed a more favourable appearance. The solid structure and the diverse food combination provide more choices for elderly (Kouzani et al., 2017). As reported by Michail (2016), 3D printing has been commercially used to produce texture softened food for elderly by a German company named Biozoon. The 3D printing of plant-based meat has already proven to be technically feasible. It has been reported that scientists designed a 3D printed steak by using rice and pea protein (Hitti, 2018). This inspires the new research area that uses 3D printing to produce meat analogues.

2.5 3D printing technology

3D printing, which is officially named as additive manufacturing, is an emerging technique to create a unique geometric shape by using three-dimensional computer-aided design (CAD) data (Rengier et al., 2010; Wang, Jiang, Zhou, Guo & Hui, 2017). The idea of 3D printing was generated in 1980s and has been developed for several decades (Hull, 1986; Gross, Erkal, Lockwood, Chen & Spence, 2014). Currently, 3D printing has been introduced to various areas, including construction, aircraft, medical, education and aerospace. Diverse materials such as plastic, metal, biopolymers and hydrogels, have been shown to be printable according to previous researchers (Gross et al., 2014; Kane & Bigham, 2014; Wang et al., 2017). Such technology increases the flexibility of manufacturing, which satisfies special consumer requirements and reduces production costs (Weller, Kleer & Piller, 2015).

The 3D structure is built through a layer-by-layer deposition. The material inks are feed into the 3D printers and squeezed from nozzles of printers. According to the CAD drawing, the nozzles move in X, Y and Z axis and release the material. The geometric shape is formed following a certain route set by the CAD file (Turner, Strong & Gold, 2014). This basic mechanism is shared by various 3D printing facilities, even though different 3D printers have different working principles.

In the past few years, numerous sorts of 3D printers have been designed for various purposes. Many 3D printers for home use have been developed. At the same time, comprehensive 3D models and model building software have been shared or sold by the designers. This provides the ability for customers to print products based on their individual needs (Weller, Kleer & Piller, 2015). To ensure the printability however, the preparation of 3D ink is vitally important. Materials with thermoplastic or thermosetting properties are the ideal substrates for 3D printing. By controlling the processing temperature, these materials express a good flow ability during printing while maintaining the shape after deposition (Wang et al., 2017). The difference in rheological properties of food inks limits the application of 3D printing technology.

2.5.1 3D printing in food manufacturing

3D printing of food has also been involved in previous and present studies. Food 3D printing is also called layered manufacturing, which is based on the nature of layer by layer processing (Wegrzyn, Golding & Archer, 2012). There is no significant difference between printing of food and other materials. As presented in Figure 3, the 3D model is initially designed based on the image of an ideal food structure. The model is loaded into a printing machine which controls the moving of nozzles. Food materials, which is also called food-inks, are deposited afterwards. The deposition could be further cooked according to the nature of the food (Sun, Zhou, Huang, Fuh & Hong, 2015). Utilizing 3D printing in food is becoming more and more popular recently. The first 3D food printer was created under funding by National Aeronautics and Space Administration in 2013 (Ibrahim et al, 2013). However, scientists had already done relevant experiments on the printability of food-inks even earlier. Hao et al. (2010) successfully printed chocolate by using a cold extrusion method. Their research provides an inspiration for 3D printing of various food materials. In the past few years, researchers have already noticed 3D printing of numerous foods, including baking dough, dairy products, starches, fish surimi and mashed potatoes (Yang, Zhang, Prakash & Liu, 2017; Liu et al., 2018; Liu, Zhang, Bhandari & Yang, 2018; Wang, Zhang, Bhandari & Yang, 2018; Chen, Xie, Chen & Zheng, 2019). Various examples imply that food 3D printing has gained extreme favour with researchers.

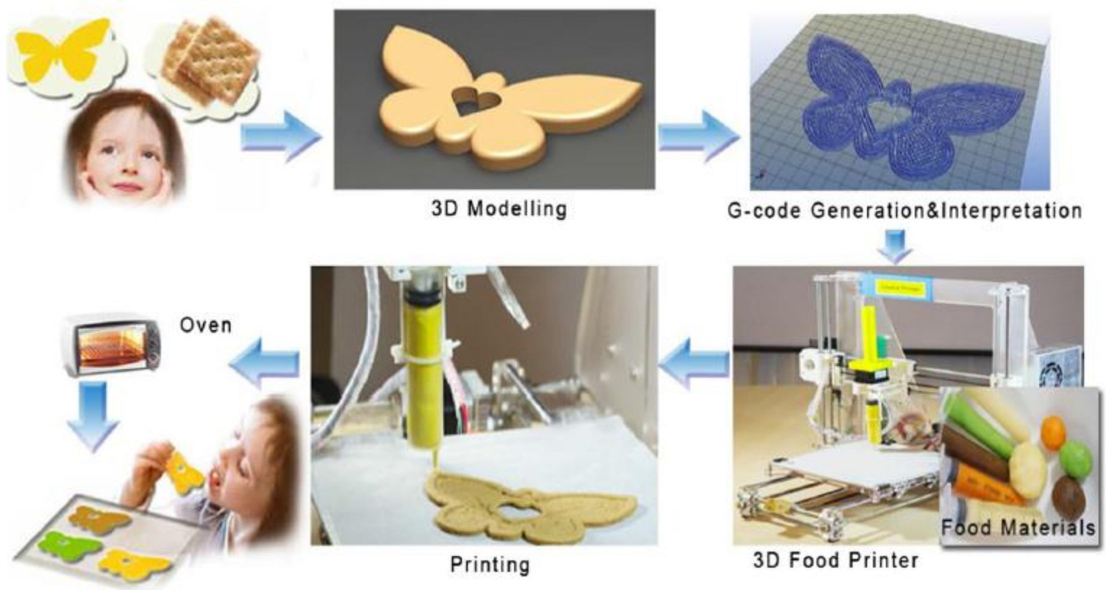


Figure 3. Brief processing line of 3D printed cookie (Sun et al., 2015).

The application of 3D printing is associated with future food market and supply strategies. One inspiration is to utilize 3D food printing in space missions. The quality of packed food is difficult to maintain during long space trips, while conventional refrigerating techniques require large power capacity. Thus, 3D printing is proposed as an alternative approach for manned missions (Joshi & Sheikh, 2015). For this huge project however, much more data is needed to test the feasibility. In fact, there are more potential uses of 3D food printing focusing on humans' daily life. Many innovations in food manufacturing may be completed by 3D printing. Different from conventional processing methods, 3D printing does not aim to decrease the cost of food manufacturing. Instead, such technology creates new properties and even develops novel categories of food (Wegrzyn et al., 2012). One application is developed by Natural Machine Llc. to extrude edible ingredients for food decoration (Godoi et al., 2016). As summarized by Turner and Lupton (2017), food 3D printing does not only contribute a desirable appearance, but also adds other values like healthcare, sustainability and waste reduction. For instance, Tran et al. (2017) utilized Fuse deposition modelling (FDM) method to print cocoa shell waste, which is a common by-product of the chocolate fabricating industry. The cocoa shell was powdered and extruded into filaments before printing. The shape could be well formed during layer-by-layer deposition. This finding highlighted the possibility of added-value processing of cocoa shell, which makes better use of food materials. Similarly, 3D printing may be used to create a desirable appearance for insect-based foods. Currently, insect ingredients have been used as additives in some research. However, the feasibility of 3D printing by using insect proteins as the major ingredients is not well studied yet (Lupton & Turner, 2018). Anyway, it suggests a potential use of 3D printing for designing sustainable diets. These advantages may lead a revolution in food product development.

2.5.2 Printing methods and printers

Currently, there are many types of food 3D printers available for lab use, household or commercial manufacturing. For different food printers, the printing mechanisms can be different. FDM, selective laser sintering and binder jetting are the three major methods for food printing (Liu, Ho & Wang, 2018). Each method has its advantages and limitations. The selection of printers and printing methods is based on the printing purpose and characteristics of the food ink.

2.5.2.1 Fuse deposition modelling (FDM) methods (extrusion)

FDM is the easiest printing method. It is also commonly known as extrusion-based printing. Food-ink with certain flow ability is filled in syringes and moved with nozzles in three-dimensional patterns and extruded to deposit layers. The accumulation of filament builds a layer-based structure in the food (Liu, Zhang, Bhandari & Wang, 2017). In contrast to conventional extrusion discussed in section 3.3.1, extrusion-based printing involves digital control and more personalized design of both food structure and nutrient concentrations. Moreover, food samples are normally not cooked during the printing step (Sun, Zhou, Yan, Huang & Lin, 2018).

Depending on different operating temperatures, FDM food printing can be divided into hot extrusion and cold (room temperature) extrusion (Sun et al., 2015). In a hot extrusion system, a filament type material is fed and melted by high temperature. Then the molten filament flows through a nozzle and deposits on the platform. The nozzle moves in X and Y axis direction before one layer has been deposited. After that, the nozzle slightly moves in the Z axis and starts to form the next layer. The shape of depositions will be stabilized by being solidified in a cooler environment (Dudek, 2013). For cold extrusion, temperature plays a minor role. Extrusion and deposition are done by the rheological properties of certain food inks.

As concluded by Sun et al. (2018), the major categories of extrusion printers involve: syringe–piston, compressed air and screw-based printers. The biggest differences among these printers are their structures, which are designed according to different intake methods. All these printers could be used to print viscous materials, such as biopolymers and food pastes. Different from biopolymers, printable food inks normally require a relatively large size of syringes and nozzles (Pusch, Hinton & Feinberg, 2018).

2.5.2.2 Selective laser sintering

Selective laser sintering, is suitable for food-inks in powder form. A 3D food printer based on a selective laser has already been designed by TNO Research. Such a printer is shown to successfully print sugar and some commercial products like NesQuik. These food materials are well-powdered and rich in carbohydrates or fats, which are easily fused by laser (Gray, 2010). The mechanism of laser food printing is further explained by Sun et al. (2015), powdered food materials are sintered by a selective laser to combine together to form a layer-based shape. As concluded by Liu et al. (2017), sugar, chocolate and some fat powders are suitable materials. This method is able to manufacture complex and unique food matrices. For this reason, selective laser sintering could be used to decorate food with special shape requirements. However, the availability of food materials is limited. It may be difficult to promote for common food printing. The use of a selective laser could be replaced by hot air, which is also a sintering source to heat and fuse food powders. The brief scheme and working principles of two sintering methods are illustrated in Figure 4.

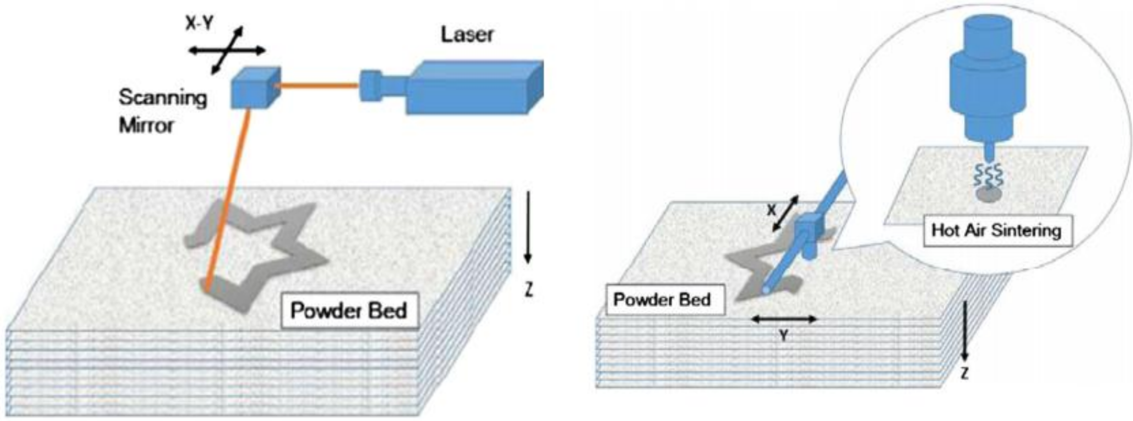


Figure 4. Scheme graph of selective laser sintering (left) and hot air sintering (right) (Sun et al., 2015).

2.5.2.3 Binder jetting

Binder jetting is another technique which is feasible for powdered food materials. Similar with sintering printing, the powder bed should be prepared before printing. Then a moisture mist is sprayed to wet and stabilize the powder bed. Afterwards, the binding agents are released from nozzles, which binds the powders in different layers to form shapes (Sun et al., 2015). Sugar is the most common materials since it is easy to crystalized during binder jetting. However, alternative materials such as cellulose have also been researched (Holland, Foster & Tuck, 2019). It was also shown that powder bed can be replaced by water-based solution. Nūfood is a new brand of food printer, which designed to jet binders into liquid solution. Gelling agents were dropped from printer into the water and 3D shapes are formed because of the gelation (Dovetailed, 2019). In general, food grade binders, which are natively viscous, play a significant role in beinder jetting system.

2.5.3 Feasibility of food ink for extrusion printing

Based on the description above, extrusion printing was selected to be the method of this project because it is suitable for paste and gel like food inks (Sun et al., 2018). Moreover, there are more available sources about extrusion printing that can be referred (Kim, Bae and Park, 2017; Lille, Nurmela, Nordlund, Metsa-Kortelainen &

Sozer, 2018; Wang et al., 2018; Chen et al., 2019; Krishnaraj, Anukiruthika, Choudhary, Moses & Anandharamakrishnan, 2019). Therefore, factors affecting printability and printing quality by extrusion printing are discussed in the following sections.

2.5.3.1 Rheological modification of food-ink

Similar to other materials, certain rheological properties of food-ink to print are required. As suggested by Liu et al. (2018), the ideal food ink is considered to be fluid or puree-like forms, which flow and extrude easily. Moreover, the food ink should express shear-thinning behaviour, which enables depositions to form special shapes (Lipton, 2017). Furthermore, viscosity, viscoelasticity and gelling properties of food ink needs to be studied. Based on rheological research, gel structure is known to be formed when the tan δ is lower than 1 and independent with frequency sweep in the linear viscoelastic region. The tan δ below 0.1 indicates a strong gel, which assists in stabilizing the printed shape. However, the firm gels tend to express negative flow behaviour that causes inconsistent printing. In contrast, weak gels ensure a uniform printing but weaker shape holding capacity (Vancauwenberghe et al., 2017).

Considering the diverse attributes of various foods, sometimes it is necessary to modify or redesign the formulation to reach printing requirements. Zhu, Stieger, van der Goot, and Schutyser (2019) reported that stability of printed aqueous-based foods was influenced by flow stress and zero shear viscosity. However, this effect did not show in lipid-based food inks. The finding indicated that food matrices also influenced the relationship between rheology and printability. Additionally, rheological properties can also be impacted by some physical factors. If a food material has a relatively low melting point, temperature would play an important role in the changes of food viscosity. Hao et al. (2010) also emphasized the influence of temperature on chocolate printing. In their research, they identified that the optimal temperature range was between 32 and 40°C, while the optimal viscosity of the chocolate was between 3.5 and 7 Pa·s.

It is known that food rheology is highly influenced by functional food ingredients. Therefore, food grade rheological modifying agents are powerful tools for 3D printing. Hydrocolloids are a type of additives that show the binding property. Common food hydrocolloids such as gelatine, xanthan and pectin were used by previous studies (Cohen et al., 2009; Vancauwenberghe et al., 2017). Binding agents can also be some normal food ingredients. Researchers studied the effects of various rheology modifying food ingredients, including starch, whey protein isolate, NaCl, on different food inks (Liu et al., 2017; Liu et al., 2018; Wang et al., 2018). The ingredients selection was all based on the conventional food formulation, which did not aim to introduce strange sensory attributes. No matter which kind of binding agents is selected, the addition amount must follow the relevant food safety regulations (Liu et al., 2018).

2.5.3.2 Printing parameters setting

Aside from rheological properties of food materials, printability is also influenced by the parameters of 3D Printers. Hao et al. (2010) demonstrated that printing parameters are significant in increasing the accuracy of food printing. Although they created desirable shapes of chocolate by focusing on the rheology, they further point out that infill and overhang problems should be overcome. They suggested that these issues could be solved by controlling printing parameters. As concluded by Wang et al. (2018), nozzle size, feeding rate, pressure, printing angle, nozzle length and printing height are all critical parameters which need to be controlled. Different combinations of these parameters should be confirmed depending on the printed food materials. The retraction ought to be considered too when choosing the extrusion-based printer. Retraction is a critical capability of 3D printers, which impacts the printability of food inks. This is because leakage of food ink should be prevented by stopping flow when the nozzle moves over a blank part of the pattern (Pusch et al., 2018). The parameter setting also varies depending on different types of printers. There is no standard for parameter setting which can be universally applied to print one category of food. Therefore, the configuration of the printer is vitally important for printing quality.

2.5.4 Assessment on quality of printed food products

2.5.4.1 Texture

As a textural modification process, the changes in textural profile of printed food by 3D printing need to be researched. Texture analysis on 3D printed food has been done by previous studies. The method to test texture of printed food is generally similar to conventional food by using texture profile analyser. Yang, Zhang, Bhandari and Liu (2018) tested texture of printed lemon gel in different formulations. They identified that hardness and gumminess of printed lemon gel were increased because of higher amount of starch. Similarly, Huang, Zhang and Bhandari (2019) found that small nozzle size created a softer texture of printed brown rice paste. Although these studies focused on different research perspectives, they all showed that texture of printed food was affected by various factors. To produce textural modified food, it is significant to know how formulation or printing processing influences food texture.

2.5.4.2 Sensory

Sensory properties are important standards of food product. The sensory tests of 3D printed food have been done by previous research (Krishnaraj et al., 2019; Mantihal, Prakash & Bhandari, 2019). Nevertheless, the taste of printed products was assumed from food materials or flavourings. There is little research that use 3D printing approach to create new flavours. The fast development of data mining and analysis technology ensure a deeper understanding on food flavour. By analysing comprehensive available data, Ahn, Ahnert, Bagrow and Barabasi (2011) constructed various models, which illustrated that compatible chemical flavour compounds were shared in different food ingredients in some proportions. They further highlighted that the flavour can be improved by pairing different flavour ingredients in a proper way. Based on this theory, Ozturk and Zeyrekce (2019) designed a new dessert by using food ingredients commonly consumed in Marmara Region, Turkey. The dessert was highly preferred by the local panellists. These investigations inspire 3D printing research to focus on the area of creating new flavours (Dovetailed, 2019). To reach this goal however, more data analysis and experiments on sensory of printed food need to be completed.

2.5.4.3 Microstructure

By observing the microstructure, it helps understand how food express the certain shape, appearance, texture and sensory properties. Microstructure of both pre-printed and post-printed food samples have been studied by researchers. Wang et al. (2018) showed that salt enhanced the particle aggregation of fish gel. This ensured the printed fish gel express the stable printing structure. For printed samples, Liu et al. (2018) identified that whey protein isolate helps create the smooth interface of food system. It increased the stability of printed mike powder. Their research focused on the influence of different ingredients on the structure of food. The relationship between printability and changes of microstructure was highlighted. Therefore, microstructure study is usually combined with food rheology study in 3D food printing. Understanding microstructure assists further analyse and explain the printability of food materials.

2.5.5 3D printing for meat products

According to Dick, Bhandari and Prakash (2019), meat is not a kind of natively printable product. The fibrous structures and unique rheological properties increase the difficulty of meat printing. For these reasons, modification of the rheology of meat is needed. Godoi et al. (2016) recommended that the viscosity of meat paste ought to be low before printing, which ensured an ideal flow in nozzle and convey devices. After printing however, the viscosity needed to be increased to a high level. This is because the shape of deposition could only be maintained in such condition. Therefore, viscosity enhancers play a significant role in the 3D printing of meat. Considering the purpose, feasible cold-set or heat-set binding agents should be chosen. As summarized by Dick et al. (2019), cold-set binders initiated the gelation of meat materials at a lower temperature. This ensured shape forming and retention of deposition by modifying the rheological properties. However, to maintain the desirable shape during cooking, heat-set binders are needed. Heat-set binders are normally derived from animal sources, such as plasm proteins, egg proteins and milk proteins. These functional proteins mainly alter the mechanical properties of the deposited products. Aside from the rheological properties, some limitations of various 3D printers should be considered. For syringe extrusion printer, the entrapment of air is another factor which limits the meat printing. The external air entrapped into paste requires higher compressing strength, which increases the difficulty of extrusion. Moreover, inconsistent flow is created which decreases the stability of the 3D shape of depositions (Lipton et al., 2010).

Meanwhile, shape retention of deposition is another important value for printed products. Different from chocolate and snacks, the shape retention of 3D printed meat is particularly important. This is because meat materials are generally not edible without cooking. To ensure food safety, cooking under high temperature is usually necessary. Another strategy is to use processed meat as the printing material. Kouzani et al. (2017) tried printing canned tuna at room temperature. According to their tests, the shape and sensory properties of their products seemed to be desirable. However, the denaturation of meat proteins also tended to reduce the fluidity, which limited the printability of samples. Therefore, pasted raw meat was recommended to be the material for printing. Shape maintenance during thermal cooking is important for printed meat products. Based on previous research, various binding agents are used to help maintain the shape. Lipton et al. (2010) showed that adding transglutaminase in the pre-extrusion step increased the heat-stability of printed turkey and scallop during high-temperature cooking. In addition, they utilized the sous-vide cooking methods, which decreased the negative impact on shape caused by extremely high temperature.

In general, the mechanisms of 3D printing of meat need to be better understood in the future. The lack of knowledge is a constraint of meat 3D printing. Therefore, the characterization of meat materials and printing parameters are highly recommended.

2.5.6 3D printing for plant-based meat analogue

Compared with meat materials, extracted plant proteins are generally easy to print. This is because plant proteins do not have complex fibrous structures. Even for textured vegetable proteins, the extrusion resistance is possibly lower because of the absent of collagen. The printability of plant proteins has already been shown by previous research. Chen et al. (2019) printed both simple and complex geometric shapes by using gels made from SPI. This showed that plant protein gel could play a role of printable inks. For this reason, plant protein gels are considered as a possible material to produce printed meat analogues.

The idea of printing plant-based meat has already been raised by previous researchers. Gray (2010) stated that algae protein is an ideal material to formulate a protein enriched meat-like product by 3D printing. However, the feasibility of this suggestion has not been verified by scientific experiments. In recent years, 3D printed meat analogue by plant proteins has been reported in news. A research team named Novameat has developed few types of printed plant-based foods by mimicking the appearance of meat steaks (Hitti, 2018; Novameat, 2020). However, no details about the texture of their products have been provided. The capacity of the formulation to work with different 3D printers is also not well described.

2.7 Importance and purpose of this study

Different from extrusion or shearing techniques, the characteristics of 3D printed meat analogues are not extremely well defined. Based on the discussion above however, the target product would be solid, fibrous and express soft texture suitable for elderly. For this reason, experiments were conducted to create a type of textured hybrid meat analogue from plant and animal protein.

Preliminarily, experimentation was done that involved preparation of pea protein isolate (PPI) and chicken mince. The contents of PPI and chicken mince, as well as some minor ingredients, varied in different formulations. The rheological properties and extrudability were trialled to know the printability of different formulations. This step aimed to understand the influence of formulations on the printability.

During the next phase of study, the 3D printing processing was conducted. The optimal formulation was determined according to the results of samples printed by using different nozzle sizes. The appearance and structure of both raw and cooked sample (cooking in boiling water) were compared. To further optimize the other printing parameters, printing speed and printing infill rate were set to print the optimal formulation.

Experiments involved the characterisation of printed and cooked meat analogues. It was hypothesised that 3D printing process will provide soft textured meat analogues. Therefore, texture profiles of both printed and non-printed samples after cooking were tested and analysed. In addition, their moisture and protein contents were determined. Microstructure and protein interactions were also studied to explain how 3D structure was formed during printing and the cooking process.

Chapter 3. Materials and methods

3.1 Materials

Pea protein isolate (PPI), containing 80% protein according to packaging, was purchased from Davis Food Ingredients, New Zealand (Figure 5). It was stored in a dry environment.

Figure 5. Pea protein isolate (PPI) used in this study.

Figure 6. Premium chicken mince used in this study.

Chicken mince (Figure 6) was purchased from a local market in Palmerston North (New Zealand). It was then blended with a Moulinex Food blender (Masterchef 650) and finely minced by a Silverson L4RT High shear mixer (Advanced Packaging System Limited, New Zealand) as much as possible into a paste like texture. Fresh chicken paste was used on the first day. The rest of the chicken paste was vacuum packed and stored in the freezer afterwards for one week. The required amount of frozen chicken paste for experiment was thawed in the refrigerator at 4 °C overnight before use.

Other minor materials include pre-gelatinized maize starch (Hi-Maize 1043; National starch and Chemical NZ Ltd., New Zealand), beef fat (Premium 100% pure beef dripping, Farmland foods, purchased from a local market, Palmerston North, New Zealand), soy lecithin (Hawkins Watts Ltd., New Zealand) and water. Other food ingredients used for preliminary experiments include wheat flour, vital wheat gluten and soy protein isolate (SPI). These were purchased from Davis Food Ingredients (Palmerston North, New Zealand). Soya chunks, pork mince, Farmhouse Pâté ChopChop chicken shred and canola oil were purchased from local market. Transglutaminase (Activa RM, Ajinomoto, Japan) was used as food grade enzyme.

Chemicals and regents used in this study included: dithiothreitol (DTT) (Sigma-Aldrich, New Zealand); di-potassium hydrogen orthophosphate (Sigma-Aldrich, New Zealand); potassium dihydrogen orthophosphate (Sigma-Aldrich, New Zealand); sodium dodecyl sulphate (SDS) (Fisher BioReagents, New Zealand) and Urea (Merck KGaA, Germany). All the materials used for production were food grade, while chemicals used for characterization were analytical grade.

3.2 Methods outline

The experiments were divided to three sections, including pre-printing, printing and post-printing experiments. The flow chart shown in Figure 7 illustrates the details of experiments conducted in this study.

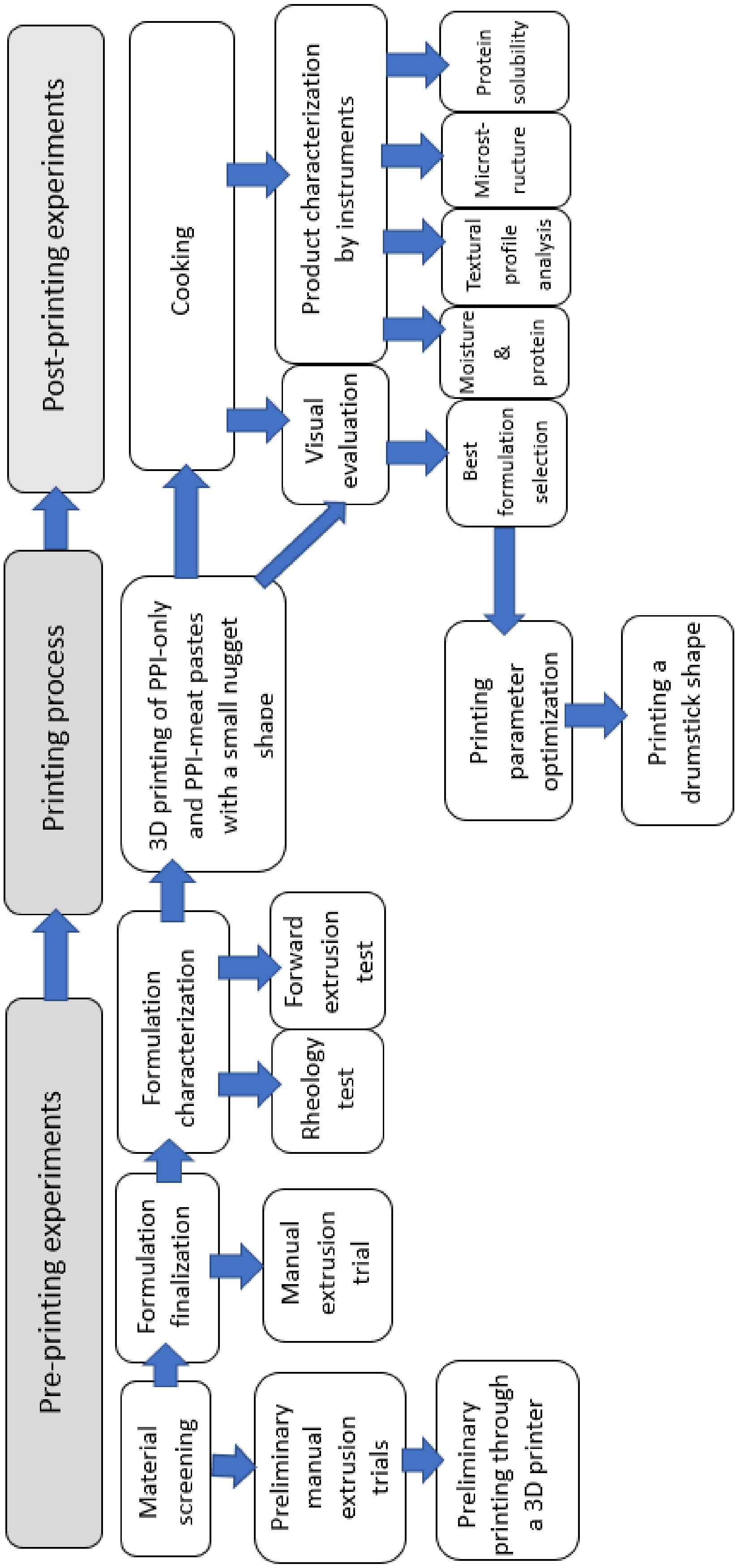


Figure 7. Design of experiment.

3.3 Pre-printing: preliminary experiments

Preliminary experiments aimed to find the basic extrusion related properties of the materials. The traditional trial-and-error printing design was not suitable for research, since it would take long time and lead to material wastage (Zhu et al., 2019). Thus, characterization of materials is important, as it helps to select the most suitable ingredients, design formulation and experiments. Only selected formulations were put through 3D printing processing.

3.3.1 Preliminary extrusion trial and material screening

Before conducting the printing experiments, various meat and plant protein-based sources were tested for their extrudability through a 60 mL polypropylene syringe (Discov3ry, Structur3d, Canada) with a 1.54 mm nozzle. Meat samples in the trials included pork mince, chicken mince, Farmhouse Pâté and ChopChop chicken shred. Firstly, these samples were directly filled in a syringe and extruded. Then, meat pastes were made with the help of a Moulinex food blender (Masterchef 650) for 2 min. 10 % (w/w) water was added if the paste was too dry. Pastes made from pork mince and chicken mince were further finely minced with the help of a Silverson L4RT high shear mixer (Advanced Packaging System Limited, New Zealand) at 4,000 rpm (556 × g) for 5 min. The manual extrusion performances of these meat samples in different forms were recorded and evaluated qualitatively.

Plant protein-based samples included SPI, PPI, vital wheat gluten, wheat flour and textured soy protein (TSP). TSP sample was made from soya chunks. Dried soya chunks were ground into a powder with help of a BCG200BSS coffee & spice grinder (Breville, New Zealand). Other plant protein sources were in a powder form initially. All plant protein powders were mixed with 70 % (w/w) water to form pastes through blending (Moulinex food blender, 2 min) and extruded by hand. There are two more formulations made from TSP powder. One formulation contained 25 % (w/w) TSP powder 75 % (w/w) water, and another contained 25 % (w/w) TSP powder, 5 % (w/w) pre-gelatinized maize starch and 75 % (w/w) water. The manual extrusion performances of all pastes were evaluated qualitatively.

After manual extrusion trials, chicken shred and PPI paste were selected (as per Section 4.1.1) to go through the printing trial by a large volume extrusion (LVE) 3D printer (NZ-3D Ltd., New Zealand). A hexagonal column with letters LVE on the top (Shown in Appendix I) was designed as the 3D model and printed with a 1.54 mm nozzle size and a speed at 15 mm/s.

3.3.2 Preparation of PPI and TSP based pastes for further manual extrusion trials

Pregelatinized maize starch was added at 20 and 25 % (Table 3) to PPI and TSP pastes. The formulations of PPI based pastes were also modified in the same manner as TSP pastes. The plant protein sources (PPI & TSP) were mixed with starch, canola oil, soy lecithin and water, and blended with Moulinex food blender (Masterchef 650) for 2 min into a paste. The details of formulations are shown in Table 1. For preparation of cooked sample, pastes were placed into a metal beaker and heated in a water bath at 80 °C for 5 min. Samples were cooked before manual extrusion through a syringe. Extrusion procedure was the same as mentioned in Section 3.3.1.

Table 1. Formulations of pea protein isolate (PPI) and textured soy protein (TSP) pastes for manual extrusion[1] trials.

Ingredients	**Percentage (% w/w)**							
	PPI1	PPI2	PPI3	PPI4	TSP1	TSP2	TSP3	TSP4
PPI	25	25	30	30	0	0	0	0
TSP	0	0	0	0	25	25	30	30
Starch	25	25	20	20	25	25	20	20
Canola oil	10	10	10	10	10	10	10	10
Soy lecithin	1	1	1	1	1	1	1	1
Water	39	39	39	39	39	39	39	39
[2]Cooking	N	Y	N	Y	N	Y	N	Y

[1]Extruded through a 60 mL polypropylene syringe (Discov3ry, Structur3d, Canada).

[2]In this row, N represents not cooked, and Y represents cooked. Cooking refers to pre-extrusion cooking in water bath.

In further trials, the effects of adding transglutaminase (TG) to both PPI and TSP pastes on fibre formation were evaluated, with TSP and PPI pastes prepared as described above. TG (0.5 %, w/w) was added to pastes and heated in a water bath at 40 °C and mixed (while heating) with a MicroMix Stick Blender (Robot Coupe, 220W) for 5 min. The pastes with TG were then stored in 4 °C for 16 hours overnight and thawed in a water bath set at 30 °C for 10 min before manual extrusion. Extruded samples were packed in thick polypropylene bags and sealed by an impulse hand sealer (Innovative Packaging, New Zealand). To avoid damaging the 3D shape, these polypropylene bags were not vacuum sealed. Samples in sealed bags were cooked in a boiling water bath for 10 min (standardized based on trials). Structures of both raw and cooked samples were visually viewed and assessed.

3.3.3 Formulation finalization: the mixture of chicken and PPI pastes

3.3.3.1 PPI based pastes

The effects of adding starch, fat, and their combination on PPI pastes was researched in this study. Beef fat was added as a plasticiser. Due to its sensitivity to temperatures, phase changing of fat happens in a mid-high temperature (Dreher, Blach, Terjung, Gibis & Weiss, 2020). This would ensure that samples exhibit proper flow behaviour during printing and also have shape maintaining capacity after printing. Dick, Bhandari and Prakash (2019) found that lard started to show fluid behaviour at temperatures > 27.9 °C, while it acted more solid-like at ambient temperatures.

Soy lecithin (1 %) was mixed and dispersed in water (69 %) with help of a MicroMix stick blender (Robot Coupe, 220W). Then water containing soy lecithin, PPI (24 %), maize starch (3.6 %) and beef fat (2.4 %) were added and mixed in a Moulinex food blender (Masterchef 650). All the ingredients were blended for 2 min to prepare a paste. The blended paste was transferred into metal beakers. As shown in Figure 8, the metal beaker containing pastes were placed in a pot containing boiling water and heated on a hotplate (MR 3001, Heidolph, Germany) for 10 min. The samples were further mixed during heating, using a Silverson L4RT high shear mixer (Advanced Packaging System Ltd., New Zealand) at 4,000 rpm (556 × g). The reason for cooking the pastes was based on the assumption that fibres are formed by shearing and high temperature (Habeych et al., 2008). The cooked paste was naturally cooled down to room temperature and used for further experimentations. Fresh pastes were prepared before each experiment.

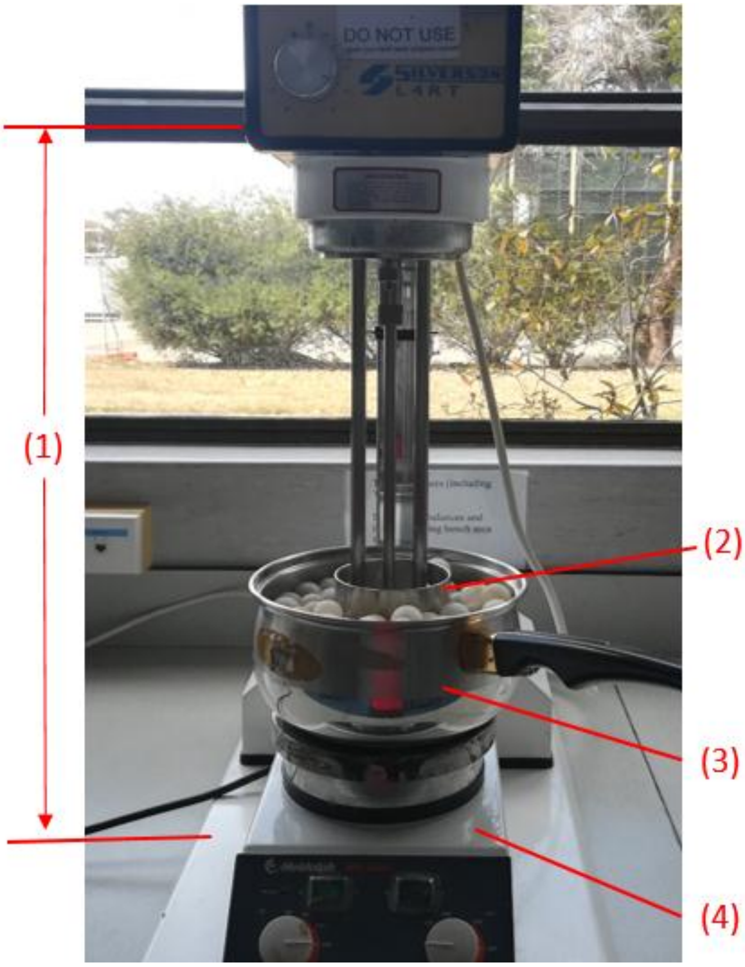


Figure 8. PPI paste being mixed by a high shear mixer during heating in a boiling water bath.
(1) Silverson L4RT High shear mixer (Advanced Packaging System Ltd., New Zealand). (2) A metal beaker with paste samples. (3) A pot with water. (4) Hotplate (MR 3001, Heidolph, Germany).

3.3.3.2 Preparation of PPI-chicken pastes

To identify the effects of adding chicken paste (prepared as mentioned in Section

3.1) on characteristics of PPI pastes, PPI-chicken pastes (PCP) were prepared. In PCP samples, the amount of added starch, fat, soy lecithin and total moisture was same as the PPI paste. The total dry matter in the chicken paste was aimed to replace PPI powder by 10, 20, 30 and 50 % respectively. When PPI paste was prepared, it was mixed with certain amount of raw chicken paste, as shown in Table 2 using a Moulinex food blender (Masterchef 650) for 2 min. The amount of chicken paste and water are based on moisture content analysis of the raw chicken pastes. The moisture of the chicken paste was 75.30 ± 0.23 % measured by hot air oven method (Section 3.6.1). The total moisture of these PCP samples was kept as 69 %, which is similar to PPI pastes.

Table 2. Pea protein isolate-chicken paste (PCP) formulations.

Ingredients	**Percentage (% w/w, wet basis)**			
	[1]10C	[1]20C	[1]30C	[1]50C
PPI	21.6	19.2	16.8	12
Starch	3.6	3.6	3.6	3.6
Fat	2.4	2.4	2.4	2.4
Chicken paste	9.6	19.2	28.8	48
Soy lecithin	1	1	1	1
Water	[2]61.8	[2]54.6	[2]47.4	[2]33

[1]10C to 50C represent 10 - 50 % chicken paste.

3.4 Pre-printing characterization of PPI and PCP pastes

3.4.1 Rheological properties

Rheological properties of PPI and PCP pastes were studied according to the methodology described by Wang et al. (2018) with slight modifications, using a dynamic rheometer (AR-G2, TA Instruments, USA). Temperature and frequency sweep experiments were performed on raw and cooked pastes, respectively. Steady shear viscosity tests were performed on cooked PPI pastes. A 40 mm parallel steel plate geometry was chosen to test of all samples, and a gap of 2 mm was set between two plates. The strain was set as 0.4%, which ensured all samples were in their linear viscoelastic region. The measurements on each selected sample were conducted in triplicate. Data were collected and analysed by software (TA Universal Analysis Version

4.5A, TA Instrument, USA).

3.4.1.1 Temperature sweeps

A temperature sweep test aims to find how the viscoelastic properties of experimental samples change with heating and cooling. Four different formulations were prepared: PPI control, PPI + starch (PS), PPI + fat (PF) and PPI + starch + fat (PSF). The proportions of starch and beef fat were set at 3.6 and 2.4 % (w/w, wet basis) respectively. They were added to partially replace PPI. Details of formulations are presented in Table 3. The preparation method was same as given in Section 3.3.3.1. The preparation of PCP was similar as described in Section 3.3.3.2. However, raw chicken pastes were blended with uncooked PSF paste in the proportions of 20 and 50 % (Table 4).

Pastes were loaded on the rheometer plate. The temperature was set at 25 °C at the beginning of the test and heated until 95 °C at the rate of 4 °C/min. After holding for 30 s at 95 °C, samples were cooled down from 95 to 25 °C at the rate of 4 °C/min. At the end of the test, cooled samples were held for 30 s at 25 °C. Storage modulus (*G′*), Loss modulus (*G″*) and *tan* δ at were recorded. Little amount of mineral oil (Bio-Rad Laboratories, New Zealand) was applied to the sample edges to minimize the moisture loss.

Table 3. Pea protein isolate (PPI) paste formulations for temperature sweep tests.

Ingredients	Percentage (% w/w, wet basis)			
	P control	PS	PF	PSF
PPI	30	26.4	27.6	24
Starch	0	3.6	0	3.6
Fat	0	0	2.4	2.4
Soy lecithin	1	1	1	1
Water	69	69	69	69

[1]Starch is pregelatinized starch (Section 3.3.3).

[2]P control represents pea protein isolate paste, PS represents PPI paste with starch, PF represents PPI paste with fat, PSF represents PPI paste with both starch and fat.

3.4.1.2 Oscillatory frequency sweeps

P control, PS, PF and PSF pastes were prepared as described in Section 3.4.1.1 and cooked in a boiling water for 10 min. PSF pastes with 20 and 50 % raw chicken paste (20C and 50C) were prepared in the same manner as described in Section 3.3.3.2. Chicken paste was not cooked because denaturation would decrease the flow ability of samples. Viscoelastic parameters like G′, G″ and tan δ were determined at 25 ± 0.1 °C, with angular frequency increasing from 0.1 to 100 rad/s. 10 points were recorded within each decade.

3.4.1.3 Shear flow behaviour tests

Samples used in shear flow behaviour tests were the same as mentioned in the frequency sweep tests. Tests were carried out at 25 ± 0.1 °C, while the shear rate was ramped from 0.1 to 100 s^{-1}. Shear-viscosity curves were obtained after testing, with 10 points shown within each decade.

3.4.2 Forward extrusion testing of PPI and chicken pastes

Rheological testing helps us to know the general rheological properties of experimental materials. However, it is also important to know the extrudability of materials during processing in an equipment (e.g. extrusion through). Therefore, the forward extrusion test was conducted with a textural analyser (TA.XT.plus, Stable Micro Systems, UK) with a 50 kg load cell, using the method described by Kim et al. (2017) and Zhu et al. (2019) with minor modification. A device consisting of a syringe and piston was set up on the texture analyser, which is shown in Figure 9. Four PPI pastes, 20C, 50C and chicken pastes were prepared as described in Section 3.1, 3.3.3.2 and 3.4.1.1. They were carefully scooped to fill into a 60 mL polypropylene syringe with a spatula. The syringe was placed vertically on a heavy-duty platform (HDP) with a hole in the centre. The test was conducted by a single compression with a 61 mm cylindrical probe. Compression force-time curve was obtained, and the maximum compression force was defined as the extrusion hardness of each food paste.

The compression speed for the forward extrusion test was calculated based on a range of equations. The relationship between compression distance and paste length is shown in Eq. 1.

$$\frac{\pi d_f^2}{4} \times compression\ distance = \frac{\pi d_n^2}{4} \times paste\ length \qquad \text{(Eq. 1)}$$

Where d_f is the diameter of the filament, which also equals to the syringe diameter in this study; d_n is diameter of the nozzle. *Paste length* refers to the length that paste extruded out from the syringe and nozzles.

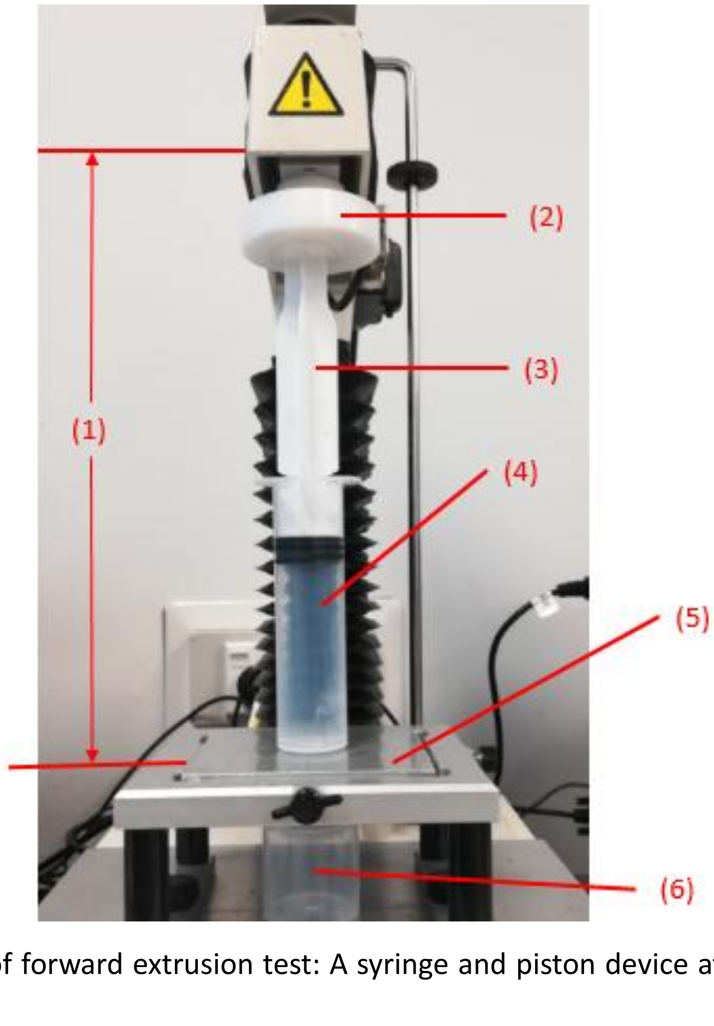


Figure 9. Design of forward extrusion test: A syringe and piston device attached to a texture analyser.
(1) The framework of textural analyser (TA.XT.plus, Stable Micro Systems, UK). (2) A 61 mm cylindrical probe. (3) A piston. (4) A syringe. (5) HDP/90 platform with a hole in the centre. (6) A container to collect the paste extruded out from syringe.

If the extrusion time is controlled, Eq. 1 can be modified to Eq. 2, which shows the relationship between compression speed and extrusion speed. If extrusion speed is set, then the compression speed could be calculated by Eq. 3.

$$\frac{\pi d_f^2}{4} \times Speed_c = \frac{\pi d_n^2}{4} \times Speed_e \qquad \text{(Eq. 2)}$$

$$Speed_c = Speed_e \times \left(\frac{d_n}{d_f}\right)^2 \qquad \text{(Eq. 3)}$$

Where $Speed_c$ is the compression speed; $Speed_e$ is the speed at which paste extruded out from the nozzle.

The diameter of syringe used in this study was 28.5 mm. The diameters of two selected nozzles were 1.54 and 2.16 mm (Figure 10). Thus, the compression speed was set as 0.04 mm/s for the 1.54 mm nozzle and 0.09 mm/s for the 2.16 mm nozzle. This referred to the extrusion speed of 15 mm/s for each nozzle size. The compression distance was 5 mm and the average compression force measured and calculated from triplicated observations.

Figure 10. Two nozzles used for 3D printing in this study: 1.54 mm diameter (left) & 2.16 mm diameter (right).

3.5 3D printing

3.5.1 Printer configuration

The printer used in this study was a newly assembled LVE 3D printer (Figure 11). It is a combination of a plastic filament 3D printer frame and an extruder unit. The frame of the printer belonged to Creality Ender-3 (Creality 3D, China). However, the extruder unit was created based on the of Pusch et al. (2019). Creality Ender-3 is a frugal 3D printer that is suitable for home and workshop use, and is able to print objects with the size 220 × 220 × 250 mm^3, with a maximum printing speed of 180 mm/s. It has been used in research by Culda, Muncut and Komjaty (2019).

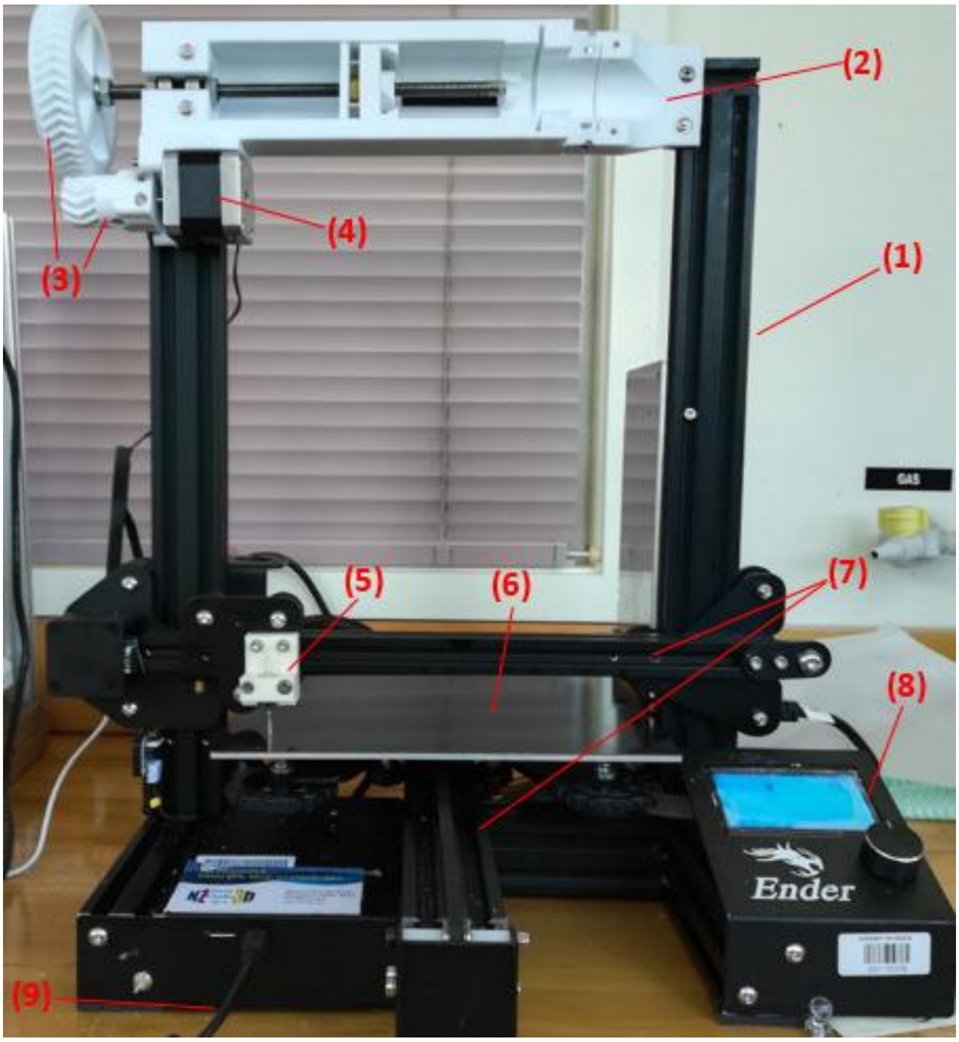


Figure 11. Photograph of LVE 3D printer used in this study.
(1) Framework of Ender-3 3D printer. (2) Extruder unit. (3) Gears. (4) Motor. (5) Nozzle holder. (6) Platform. (7) Conveyor belts, controlling the movement to x, y, z axis directions. (8) Operation menu. (9) USB connection to computer.

As described by Pusch et al. (2019), the low motor capacity of common paste extrusion printer constrains the printing speed. As for famous brands of 3D food printer, they have low printing flexibility. Such attributes are not suitable to print materials with little knowledge about their printability. Therefore, assembling an extrusion unit onto a printer with a high motor capacity was selected for this study.

3.5.2 Software setting

Software setting is an important procedure to conduct a 3D printing process. Two software named 3D Builder (Version 18.0.1931.0; Microsoft Co.) and Repetier Host (Version 2.1.4; Hot-World GmbH & Co. KG.) were used. 3D builder is a software where 3D models can be downloaded and designed according to people's preferences. In this study, all 3D models for experiments were downloaded from online sources, which are open to access for public, through 3D builder. Then they were loaded and sliced by Repetier Host.

Slicing is a step that translates the code of 3D models into printing paths. Repetier Host is a popular free software for slicing 3D models and conducting printing processes. By using Repetier Host, various printing parameters can be set or modified.

3.5.3 Printing process

PSF, 20C and 50C were selected for 3D printing based on their rheological properties. The pastes were prepared as described in Sections 3.3.3.1 and 3.3.3.2 and filled into syringes. A small nugget shape sample (Figure 12) was printed using a large volume extrusion (LVE) 3D printer. The effect of nozzle size (1.54 and 2.16 mm) on the printability (ability to form layers) was observed along with the appearance of the printed samples. Printing was carried out at ambient temperature, with a printing speed of 15 mm/s and 100 % infill density. Printed samples were cooked in sealed polypropylene bags in a boiling water bath as described in Section 3.3.3. The structures of both raw and cooked samples were visually evaluated. Fibre formation was particularly noticed.

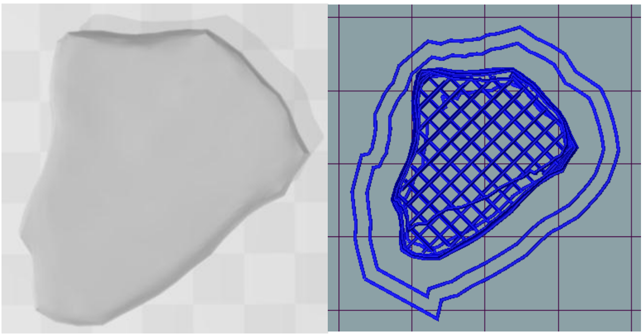


Figure 12. A 3D model of a chicken nugget shape downloaded through 3D builder (left) and sliced by Repetier Host (right).

3.5.4 Printing parameter optimization

3.5.4.1 Printing speed

By comparing the appearance and fibre formation of printed samples, 20C and 1.54 mm nozzle were selected as the best combination. Different printing speeds were tested on 20C and 1.54 mm nozzle in the next step. As shown in Figure 13, a 2 × 2 × 2 cm^3 cube model was designed and sliced. The selected sample was printed at four speed levels (10, 15, 20 and 25 mm/s). The final printed structure was visually inspected and assessed on their appearance.

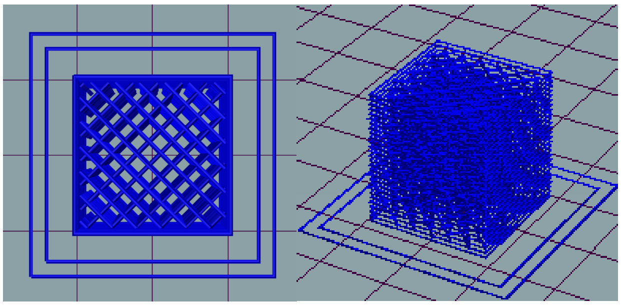


Figure 13. Sliced 3D model of a 2 × 2 × 2 cm3 cube with grid pattern infill (Sliced by Repetier Host).

3.5.4.2 Infill density

After optimizing the printing speed, different infill densities were tested. As presented in Figure 14, a 2 × 2 × 2 cm^3 cube model was sliced with three infill density levels (100, 60 and 20 %). The top and bottom layer were all in a solid infill (100 %). Food samples were printed at ambient temperature, with 1.54 mm nozzle and the optimal speed identified in 3.4.5.1.

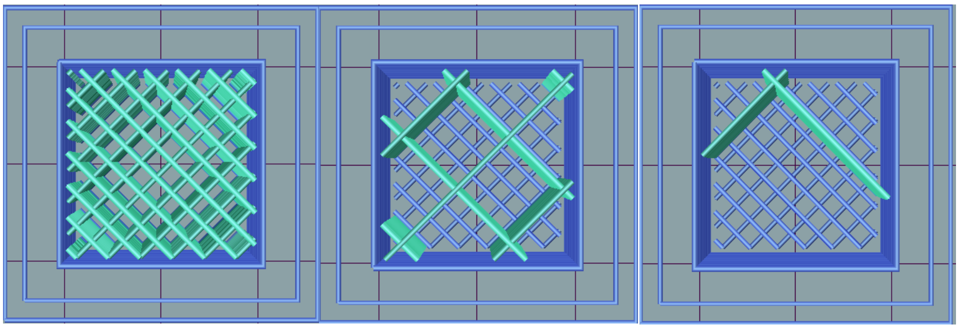


Figure 14. Sliced 3D model of 2 × 2× 2 cm3 cubes with grid pattern infill in different density 100 % (left), 60% (middle) and 20% (right) (Sliced by Repetier Host, the top layer is not shown).

3.5.5 Further printing process

After optimizing the printing parameters, the nugget shape was printed again with the optimized formulation and parameters. Also, the capability to print a complex 3D shape was tested. As shown in Figure 15, a chicken drumstick 3D model was sliced and printed.

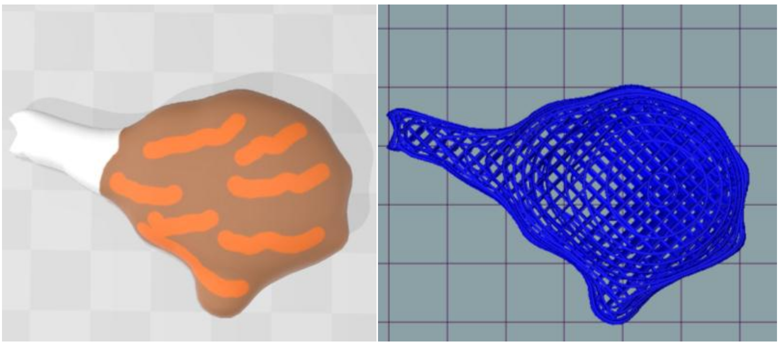


Figure 15. 3D model of a chicken drumstick shape, downloaded through 3D builder (left) and sliced by Repetier Host (right).

3.6 Characterization of printed products

3.6.1 Moisture and protein analysis

Printed PSF, 20C, 50C (formulations shown in Table 2 & 3) and raw chicken mince were cooked in boiling water as described in Section 3.3.3. The moisture contents of these samples were determined by hot air oven method, as described by Chiang et al.

(2019) with minor modification. Aluminium dishes were first labelled and weighed. Then 2 g of each sample was added onto the aluminium dishes, and the final weight was recorded. After weighing, samples were placed into the hot air oven (108 °C) and dried overnight. Dried samples were weighed and moisture content was calculated by Eq. 4

$$\% \text{ Moisture} = \frac{W_{total} - W_{dry}}{W_{total} - W_{dish}} \times 100 \qquad (\text{Eq. } 4)$$

Where W_{dish} was weight of an aluminium dish; W_{total} was total weight of fresh sample and aluminium dish; W_{dry} was total weight of dried sample and aluminium dish. Each experiment was replicated three times.

The Kjeldahl method was used to determine protein content (AOAC, 2005). each sample (0.5 g) was weighed and dissolved in a digestion tube with 2 Kjeltabs and 15 mL of sulphur acid. Samples were digested in the tube for about 16 hours overnight. Then all the digestion tubes were transferred to the digestor units (2006 Digestor, Foss TECATOR) and digested at 420 ˚C for 1 hour until colours disappeared. Afterwards, tubes were cooled to room temperature, waiting for distillation. At the same time, conical flasks with 25 mL of 4 % boric acid were prepared. Cooled tubes were positioned in the distilling unit (KjeltechTM 2100, Foss) and distilled for 4 min. Finally, conical flasks were titrated with 0.01 M hydrochloride (HCl) acid until the colour of the solution turned from green to grey. Each test was done in triplicate. The calculation of total nitrogen was shown in Eq. 5.

$$\% \text{ Nitrogen} = \frac{A \times B \times 14 \times 100}{1000 \times C} \qquad (\text{Eq. } 5)$$

Where A was the amount of HCl (mL) consumed; B was the molarity of HCL; C was the weight of tested sample.

The percentage of protein can be calculated based on the percentage of nitrogen. The crude protein content was calculated based on Eq. 6, where 6.25 is the nitrogen to protein conversion factor.

$$\% \text{ Protein} = \% \text{ Nitrogen} \times 6.25 \qquad \text{(Eq. 6)}$$

3.6.2 Textural profile analysis of chicken and printed analogues

In this section, hardness, springiness, cohesiveness and chewiness of both printed and non-printed samples were tested. The measurement followed the method described by Samard and Ryu (2019a), with slight modifications. Non-printed samples (including PSF, 20C, 50C and chicken mince) were cooked in the same way as described in Section 3.3.3 and cut into 2 × 2× 2 cm^3 cubes after cooking. The texture analysis method of printed samples was inspired by Yang et al. (2018). Small cubes of printed PSF, 20C and 50C samples were prepared and cooked by the method mentioned in Section 3.3.3. Considering the printing defects and cooking shrinkage, a bigger cube size (2.5 × 2.5 × 2.5 cm^3) was printed. Then the bigger cubes were cut into size 2 × 2 × 2 cm^3 before cooking. After cooking, both printed and non-printed samples were stored at 4 ˚C overnight and thawed at room temperature for 30 min before texture analysis.

All samples were positioned on the stage of the Texture Analyser (TA.XT.plus, Stable Micro Systems, UK). A 61 mm plate cylindrical probe and a load cell with 50 kg capacity were chosen, and the measurement was based on double compression test. Each sample was compressed twice with a 50 % strain. The speed of probe was 2 mm/s with 1 mm/s pre-test speed and 5 mm/s post-test speed. Gap time between the two compressions was 5 s. Hardness, springiness, cohesiveness and chewiness was calculated by software Exponent (version 6.1.16.0, Stable Micro Systems, UK).

3.6.3 Microstructure of chicken and printed meat analogues

Microstructures of chicken and and printed meat analogues (PSF, 20C and 50C) were observed and analysed as described below. All these samples were cooked as mentioned in Section 3.3.3 In the first stage, light microscopy (LM) was applied to have a brief understanding of microstructure. Then all samples were further analysed by using a scanning electrical microscope (SEM).

3.6.3.1 LM

One small piece (approximately 2 × 2 × 0.1 mm^3) of cooked chicken and cooked printed PSF, 20C and 50C were carefully taken by a spatula, and mounted on a flat glass slide. One drop of water Reverse osmosis (RO) water was added on the slide. The slide was covered by a cover slip and placed on a Lecia light microscope (Bio-Strategy, New Zealand). Each slide was viewed at 10× magnification. LM analysis aimed to observe the general structural arrangement of printed meat analogues. More details of microstructure were viewed through SEM.

3.6.3.2 SEM

Cooked chicken and printed meat analogues were fractured by hand. Fractured samples were then frozen at -30 ˚C overnight. The frozen samples then freeze-dried in a Cuddon FD18CT freeze drier (Cuddon Blenheim) at -40 ˚C for about 3 days. One tiny piece was taken from the surface, where had been fractured by hand, of freeze-dried samples was attached to an aluminium stub. 100 nm of gold sputter coater (Bal-tec SCD 050) was used to coat samples for 200 s. Structures of coated samples was observed by a FEI Quanta 200 Environmental scanning electrical microscope (Philips Electron Optics, The Netherland). The structure of each sample was viewed and photograghed at 200× and 400× magnifications, at an accelerating voltage of 25 kV.

3.6.4 Protein interactions (protein solubility test) of printed meat analogues

During meat analogue processing, unique structure formation is associated with protein-protein interactions. The formation of various covalent or non-covalent bonds among proteins contributes to the meat like structure (Liu & Hsieh, 2008). The determination of protein solubility in various solvent is a method to identify the various interactions in a protein system. The soluble protein contents in cooked and 3D printed meat analogues (PSF, 20C and 50C) samples were determined. Additionally, raw PSF paste without printing was selected as the control sample in this study.

3.6.4.1 Extraction solution preparation

According to previous literature, phosphate buffer (PB) with a neutral pH dissolve native protein, while SDS, DTT and urea destroy hydrophobic interactions, disulphide bonding and hydrogen bonding respectively (Uruakpa & Arntfield, 2006; Liu & Hsieh, 2008). Therefore, 0.1 M PB was used as the control extraction solution in this study (Table 4). KH_2SO_4 and K_2HSO_4 were added in different portions into water to prepare a buffer solution with pH 7.5. Afterwards, SDS, DTT and urea were weighed in the powder form and dissolved into PB to prepare PS, PD PU and PSDU at concentrations showed in Table 4. The solutions were dissolved with help of a magnetic stirrer (FB15001, Thermo Fisher Scientific, New Zealand) for 10 min at ambient temperature.

Table 4. Five extraction solutions prepared by combinations of different chemicals and regents.

Extraction solution	Description
PB	0.1 M phosphate buffer (Combination of KH_2SO_4 and K_2HSO_4 with pH7.5)
PS	PB + 1.5 g/100 mL SDS
PD	PB + 0.05 M DDT
PU	PB + 8M urea
PSDU	PB + 1.5 g/100 mL SDS + 0.05 M DDT + 8M urea

PB represents phosphate buffer; PS represents PB + sodium dodecyl sulfate (SDS); PD represents PB + dithiothreitol (DTT); PU represents PB + urea; PSDU represents PB + SDS +DTT + urea.

3.6.4.2 Protein solubilisation and centrifugation

Protein solubilisation was carried out as described by Chiang et al. (2019), with minor modifications. Printed PSF, 20C, 50C and non-printed raw PSF paste (0.5 g) (prepared as described in Sections 3.3.3.1 and 3.5.3) were weighed and dissolved with 10 mL of different extraction solutions in centrifugation tubes. To improve dissolving effect, samples in centrifugation tubes were located in a refrigerated incubator shaker (Infors HT Ecotron) and shaken for 30 min. Then they were further blended with help of a high-speed disperser (Ultra-Turrax® T25 Basic, IKA, Germany) at 14,000 rpm (4,390 × g) for 30 s. Before centrifugation, these samples were shaken for another 30 min in the refrigerated incubator shaker. Finally, samples were centrifugated in Heraeus™ Multifuge™ X3R centrifuge (Thermo Fisher Scientific, New Zealand) at 4,000 rpm (3,494 × g) for 10 min. Supernatant of each centrifuged sample was collected.

3.6.4.3 Bradford protein assay

Bradford protein assay, which is a rapid protein determination method, was used in this study (Kruger et al., 2009). At first, a standard absorbance curve was obtained by measuring protein content in bovine serum albumin (BSA) through SPECTROstar Nano microplate reader (BMG Labtech, Australia) at 595 nm. Based on the result, protein concentration was calculated by absorbance, which was shown in Eq. 7.

$$\text{Protein concentration (µg/mL)} = Absorbance/0.000225 \qquad \text{(Eq. 7)}$$

Supernatant (5 µL) of tested samples were pipetted into a microplate with 250 µL Bradford reagent (Thermo Fisher Scientific, New Zealand). Protein amount in supernatants was measured by a microplate reader (SPECTROstar Nano, BMG Labtech, Australia) at 595 nm. Finally, the percentage of soluble protein was calculated according to Eq. 8.

$$\%\ \text{Solubility} = \frac{\%\ soluble\ protein}{\%\ total\ Protein} \times 100 \qquad \text{(Eq. 8)}$$

Where % *total protein* was measured previously by Kjeldahl method shown in Section 3.6.1.

3.7 Statistical Analysis

The data presented in the results section are the mean values of at least triplicated measurements. Standard deviation (SD) is also presented in tables and figures as well. Texture profile analysis was replicated five times, while all other analyses were conducted in triplicate. Statistical significance was defined by a *p* value lower than 0.05. One-way analysis of variation (ANOVA) and Tukey's pairwise comparisons were conducted by Minitab (version 18.1, Minitab Inc., State College, PA) to analyse the significance of the data.

Chapter 4. Results and discussions

4.1 Pre-printing trials

4.1.1 General extrusion performance of materials (Manual and 3D printer)

The manual extrusion performance of meat samples is shown in Table 5. According to the results, chicken samples were easier to extrude than pork samples. Commercial products (including Farmhouse Pâté and ChopChop chicken shred) showed better extrusion performance than laboratory-made meat pastes.

Table 5. Extrudability (manual, through a syringe[1]) of different meat pastes.

Samples	Extrusion performance
Pork mince	Too thick, non-extrudable
[2]Pork mince (added 10 % water)	Stuck in nozzle due to big particles
[2,3]Pork mince (added 10 % water & finely minced)	Stuck in nozzle because some big particles like collagens cannot be removed
Farmhouse Pâté	Too thick
[2]Farmhouse Pâté (mixed with 10 % water)	Flowed well but non-deposition
[2]Farmhouse Pâté (add 10 % water & 5 % starch)	Could deposit and form simple shapes, but the shape was not stable (collapsed easily)
Chicken mince	Hard to extrude, extrusion was not consistent
[2]Chicken mince (blended)	Extrudable in 2.16 mm nozzle but needed a lot of strength, stuck in 1.54mm nozzle
[3]Chicken mince (finely minced)	Similar to blended samples
[2]Chicken mince (add 10 % water)	Splashed when extruded
ChopChop chicken shred	Not a paste form, non-extrudable
[2]ChopChop chicken shred (blended)	Extrudable, forms shape well, but not stable after extrusion

[1]A 60 mL polypropylene syringe, supplied by Discov3ry, Structur3d, Canada.
[2]Mixing or blending was carried out with Moulinex Food blender (Masterchef 650) for 2 min.
[3]mincing was carried out with Silverson L4RT High shear mixer (Advanced Packaging System Limited, New Zealand) at 4,000 rpm (556 × g) for 5 min

The extrusion performance of these plant protein pastes is shown in Table 6. As can be seen, plant protein pastes were generally easier to extrude than the meat pastes shown above. Of all the samples, PPI showed the best extrusion performance.

Table 6. Extrudability (manual, through a syringe[1]) of different plant protein pastes.

[2]Samples	Extrusion performance
Soy protein isolate (SPI) (30 %, w/w)	Formed shapes but not stable, extrusion not consistent, deposition was fragile
Pea protein isolate (PPI) (30 %, w/w)	Formed shapes, not consistent, deposition was fragile but better than SPI
Vital wheat gluten (30 %, w/w)	Too viscous, hard to extrude
Wheat flour (30 %, w/w)	Splashed when extruded
[3]Textured soy protein (TSP) (30 %, w/w)	Not soluble
TSP (25 %, w/w)	Too thin, splashed when extruded
TSP (25 %, w/w) + starch (5 %, w/w)	Too thin, splashed when extruded

[1]A 60 mL polypropylene syringe, supplied by Discov3ry, Structur3d, Canada.

[2]All samples were mixed with Moulinex Food blender (Masterchef 650) for 2 min. The percentage shown in the table is dry weight basis, the rest ingredient is water.

[3]TSP pastes were prepared as described in Section 3.3.1.

According to the result, blended chicken shred showed the optimal layered shape-forming capacity among all meat pastes. PPI paste was considered as the most suitable plant protein-based paste for shape-forming. Thus, chicken shred and PPI pastes were selected for printing trials on 3D printer. PPI was successfully printed to form a hexagonal column shape with 3D letters on the surface (see Appendix I). The high extrusion strength offered by the 3D printer reduced the extrusion difficulty compared to manual extrusion. However, chicken shred was not able to form any shape, which differed from the manual extrusion result. This might be because the high extrusion strength caused a high shear, which led to pastes spreading before forming shapes (Wang et al., 2018). Moreover, commercially processed meat samples tend to have complicated formulations. Their rheological properties might come from ingredient interactions created under industrial processing. Because details of processing are not available to the public, it is difficult to quantify and modify food formulations for research purposes. Thus, PPI paste was used for further trials because it is easy to alter its formulation. Aside from PPI, TSP powder was selected as another plant protein source for manual extrusion trials. Previous studies showed that fibre formation of soy protein was associated with the formation of protein interaction during texturization process (Lin et al., 2000; Liu & Hsieh, 2008). Thus, we hypothesized that TSP paste will exhibit a similar fibrous structure after extrusion through a syringe to that is shown after hydration (Figure 16). Due to a lower solubility of TSP powder in water, the TSP based formulation should be modified to form an extrudable paste.

Figure 16. Dry (left) and hydrated (right) textured soy protein (TSP) chunk.

4.1.2 Manual extrusion of PPI and TSP pastes with added starch

Manual extrusion through a syringe helped to identify the basic extrudability and shape forming capacity of pea protein isolate (PPI) and textured soy protein (TSP) pastes. According to the trials, both PPI and TSP pastes, without pre-extrusion cooking, did not form layered shapes. The pastes extruded from nozzles could not form a stable attachment to the platform. The flow was also inconsistent and could not be used to construct any 3D shapes. However, cooked PPI and TSP samples were able to be extruded stable and form 3D shapes (Figure 17). This could be because cooking at 80 ˚C enabled the maize starch (2.5 %, w/w) to gelatinize and provide a stable paste system by binding water (Eliasson, 1986). A paste with a suitable viscosity is a fundamental requirement to deposit and form shapes. Of all the samples, extruded PPI pastes showed more desirable outcomes than TSP pastes. They were able to form stable and solid nugget shapes (Figure 17 a & b). The difference could be because PPI has higher water solubility than TSP (based on trials), which helps form a smoother paste. The flow of PPI pastes was more consistent than TSP pastes. For TSP samples, TSP with 25 % starch could only deposit a single layer but did not add higher layers.

However, TSP with 20 % starch successfully formed a multi-layer structure (Figure 17 c & d). The higher amount of starch increased the dryness of the cooked TSP paste, which could be the reason why TSP with 25 % starch could not form shapes well. There was no fibre formation in either PPI or TSP samples. PPI pastes with 20 and 25 % starch samples were sticky and showed a soft cheese-like structure. This formulation might help to develop 3D printing of cheese analogues. For 3D printed meat analogues in this study, such a high amount of starch is not recommended. To develop desirable printed soft and fibrous products, more trials were conducted to seek for a suitable formulation.

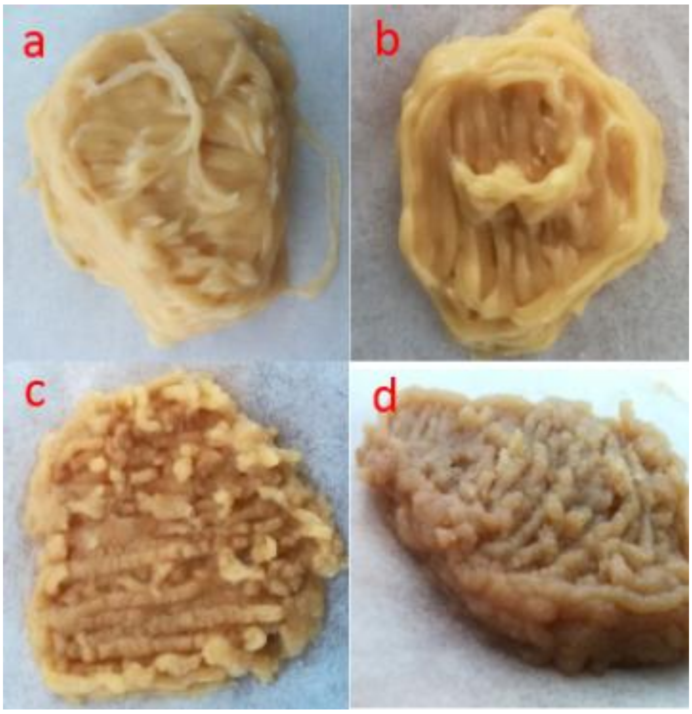


Figure 17. Extrusion performance of pre-cooked PPI and TSP pastes.
(a) PPI paste with 25 % starch. (b) PPI paste with 20 % starch. (c) TSP paste with 25 % starch. (d) TSP with 20 % starch.

4.1.3 Effect of adding Transglutaminase (TG) or chicken mince paste

TG has been used in food processing for various applications (Scarnato et al., 2017; Amirdivani et al., 2018). It is believed that TG modifies food texture by catalysing protein interactions, especially forming covalent crosslinks between glutamine and lysine (Amirdivani et al., 2018). Lipton et al. (2010) also used TG in 3D turkey meat printing. They found that adding TG effectively avoided shape destruction in turkey puree. They presumed that TG helped printed turkey puree with destructed macrostructure rebuild a new protein matrix to maintain structural stability during cooking. However, their result was only assessed qualitatively. How TG worked in printed food was not discussed.

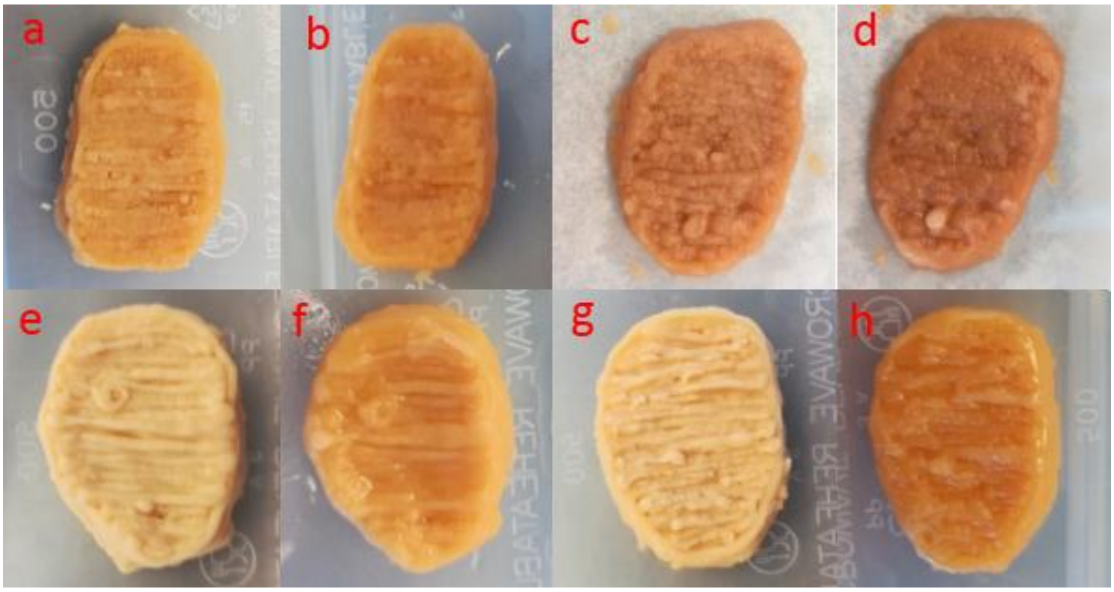


Figure 18. Manual extrusion performance of PPI and TSP pastes.
(a) PPI paste before post-extrusion cooking; (b) PPI paste after post-extrusion cooking; (c) TSP paste before post-extrusion cooking; (d) TSP paste after post-extrusion cooking; (e) PPI with 10 % chicken before post-extrusion cooking; (f) PPI with 10 % chicken after post-extrusion cooking; (g) PPI with 10 % chicken and TG before post-extrusion cooking; (h) PPI with 10 % chicken and TG after post-extrusion cooking.

As shown in Figure 18 (a, b, c & d), both PPI and TSP pastes with TG were able to be extruded and form a nugget shape. PPI samples expressed a better appearance than TSP samples. This could be because the smoother flow of PPI paste led to less difficulty of extrusion. Compared with extruded TSP in Section 4.1.1, the addition of TG stabilized the printed sample since the extruded sample was less crumbly than non-TG samples. It was agreed by Moreno et al. (2020) that TG reinforced the PPI gel by forming β-sheet aggregates in the gel network. Cooking darkened the colour of extruded samples but did not improve the fibre formation. None of the samples showed obvious meat-like (fibrous) structure either before or after cooking. Therefore, it shows that TG and heating did not improve a fibre formation effect on plant protein products in the trial. The hypothesis that TSP assists fibre formation cannot be proven. The reason could be that the water absorption capacity of TSP was lowered when it was ground into powder. Deformed TSP lost the ability of fibre formation during hydration. For this reason, TSP was eliminated from further experiments in this study. Although fibre formation was also not found in PPI samples, the proper flow made PPI worthy of consideration as a suitable plant protein source for extrusion.

Figure 19. Fibres in extruded PPI and 10 % chicken paste after cooking.

The colour of the extruded samples was lighter when the chicken was added into PPI paste (Figure 18 e, f, g & h). In addition, 10 % chicken paste was capable of forming slight fibrous structures after cooking in boiling water (Figure 19). Chicken paste with TG shows a more rigid structure, but there is no difference in fibre formation compared with non-TG samples. The surfaces of both TG and non-TG samples were oily after cooking, attributed to the presence of canola oil, which may have decreased the sample stability at high temperature. In meat analogues, oil droplets are usually dispersed from protein matrices. Fibre formation is inhibited when the oil content is too high (Gwiazda, Noguchi & Saio, 1987; Dekkers, Boom & van der Goot, 2018). However, flavour, juiciness, and tenderness of meat analogues can be improved when oil or fat is added (Kyriakopoulou et al., 2019). In the 3D printing process, the presence of lipid was considered as an added lubricant, which enhances the flow of the extruded materials (Lille et al., 2018). For these reasons, it may be useful to add a small amount of lipid in the formulation. Furthermore, Farjami and Madadlou (2019) demonstrated that the replacement of liquid oil by solid fat, reinforced gel stiffness of an emulsion-filled gel. Therefore, it was decided that plant oil be replaced with animal fat for further experimentation, aiming to potentially stabilize the printed products.

4.1.4 Manual extrusion of PPI and chicken pastes

Manual extruded PPI samples with different percentages of chicken are presented in Figure 20. According to the results of extrusion, extruded PPI with 50 % chicken (50C) paste showed the most obvious fibrous structure after cooking. There is little difference in fibre formation between PPI with 20 and 30 % chicken (20C and 30C) pastes. The flow consistency was reduced with the increase in chicken paste. Therefore, 20C, 50C and PPI paste without chicken were selected for the 3D printing process.

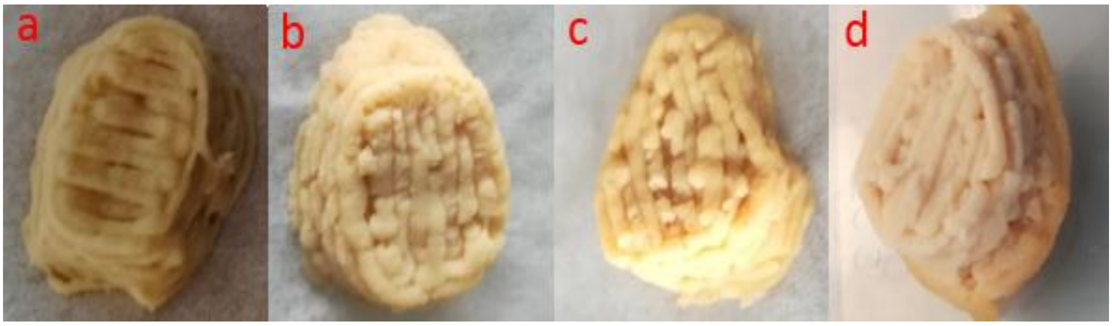


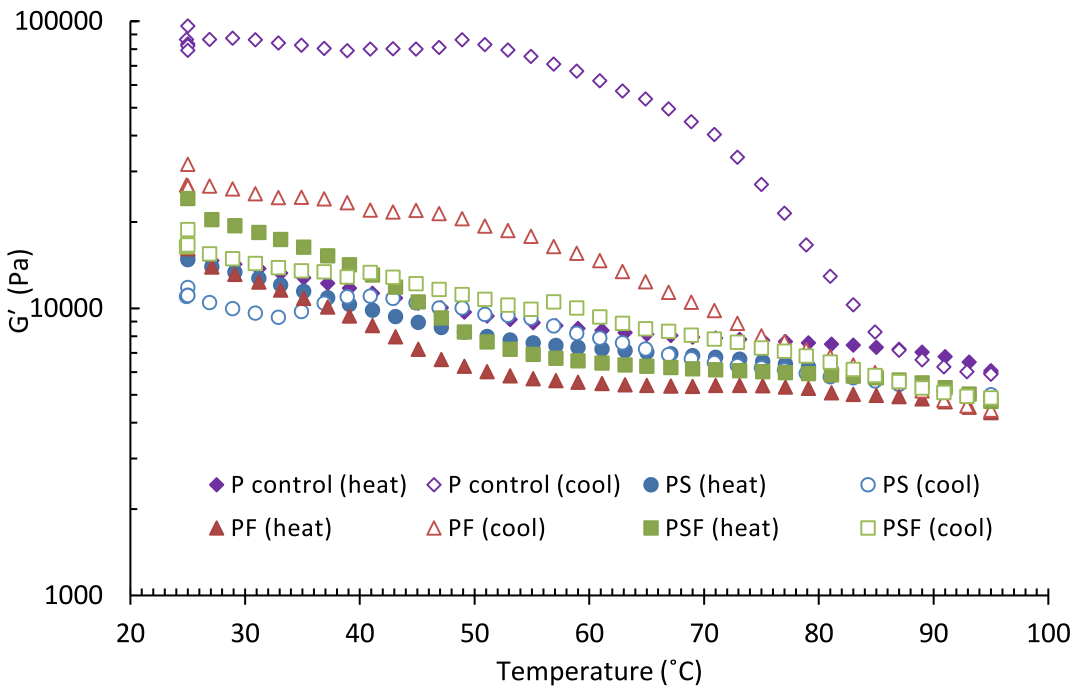
Figure 20. Manual extrusion performance of PPI and 10 % (a), 20 % (b), 30 % (c) and 50 % (d) chicken pastes.

4.2 Rheology

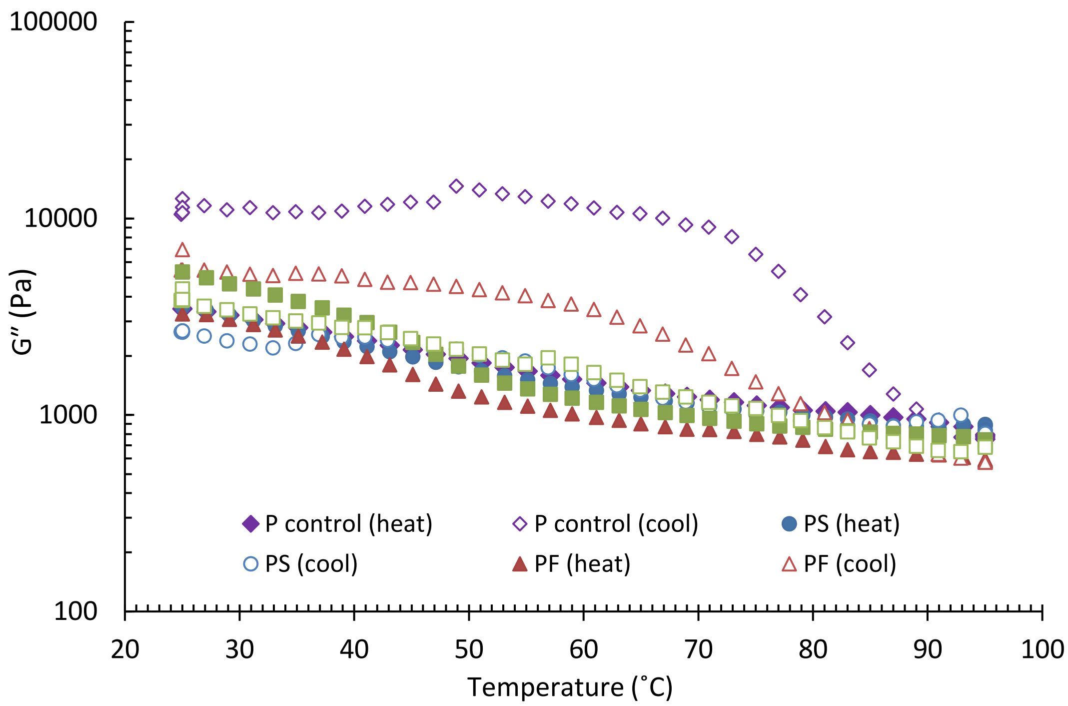
Rheology is used to investigate the flow and deformation of materials. It was aimed to study the changes in food properties during processing. The fundamental research on rheology started centuries ago but developed more quickly when instrumentation was applied (Zheng, 2019). The results from rheological analysis enable us to understand food characteristics and help to set or adjust processing conditions. The viscoelastic property change in samples during heating and cooling is reflected by the changes of rheological parameters. Rheological parameters, such as storage modulus (*G′*) and loss modulus (*G″*) were recorded. The relationship between *G′* and *G″* was described as a loss factor (*tan δ*), which equals *G″*/*G′*.

4.2.1 Temperature sweep

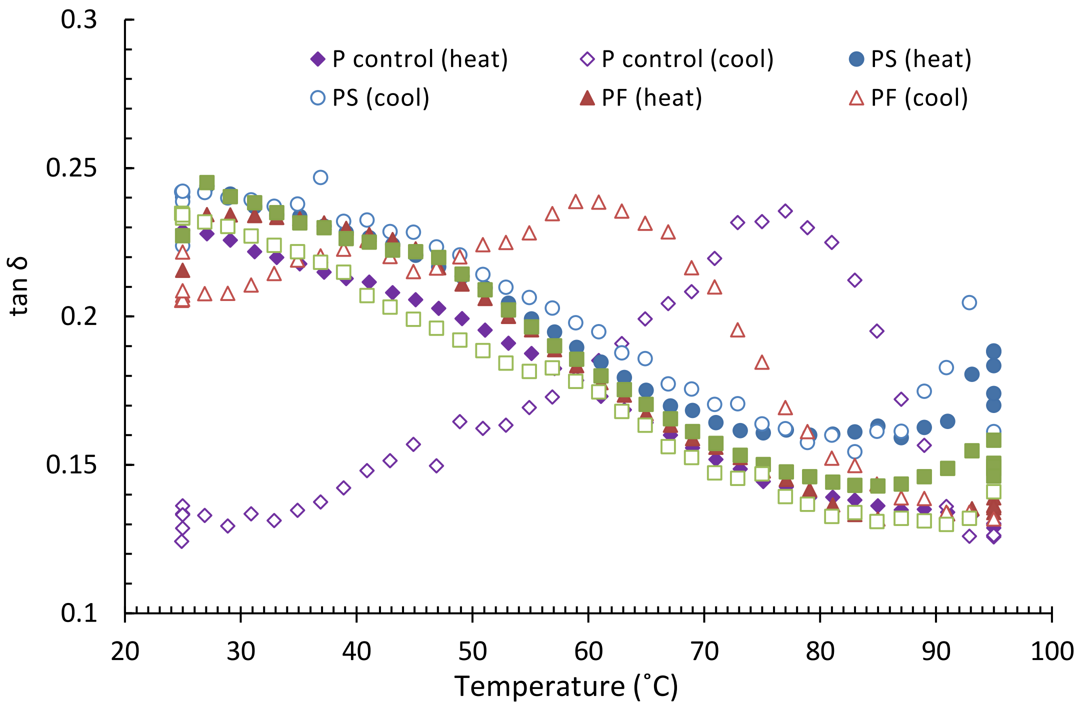
In temperature sweep tests, the comparison of *G′* and *G″* and *tan δ* of four PPI pastes is shown in Figure 21 a and b. As can be seen, both *G′* and *G″* of all samples decreased during the heating process. They dropped down rapidly before 55 °C and then slowly decreased after that. Change of rheological properties in protein-based food is normally associated with protein denaturation. According to Shand, Ya, Pietrasik and Wanasundara (2007), the denaturation temperature of non-globulin and globulin fractions in lab-prepared PPI were 67 and 85 °C respectively. However, there are no obvious changes on moduli at either temperature in Figure 21 (a). It was reported by Aryee, Agyei and Udenigwe (2018) that processing methods vary the characteristics of PPI products. The decreasing trend in moduli during heating is generally similar to the study by Moreno et al. (2020), in which PPI with a greater denaturation degree showed a continuous reduction during heating on *G′* and *G″*. This was explained to be the result of destruction of polar interactions, mainly leading to decreased moduli. Hence, the PPI used in this study is assumed to be denatured to some extent since being purchased. As demonstrated by Jiang et al. (2019), denatured protein shows a higher extrudability since most native proteins have poor shape-holding capacity. This could be the reason that PPI paste can be manually extruded in the trials.



(a)



(b)



(C)

Figure 21. Viscoelastic properties of four PPI pastes during heating (25 to 95 ˚C) and cooling (95 to 25 ˚C) at rate of 4 ˚C/min.

(a) Storage modulus (G') changes with temperature. (b) Loss modulus (G") changes with temperature. (c) Change on tan δ with temperature. In the figure, P control represents PPI paste; PS represents PPI Paste containing starch; PF represents PPI paste containing fat; PSF represents PPI paste containing both starch and fat. Result shown was the mean value of triplicated tests.

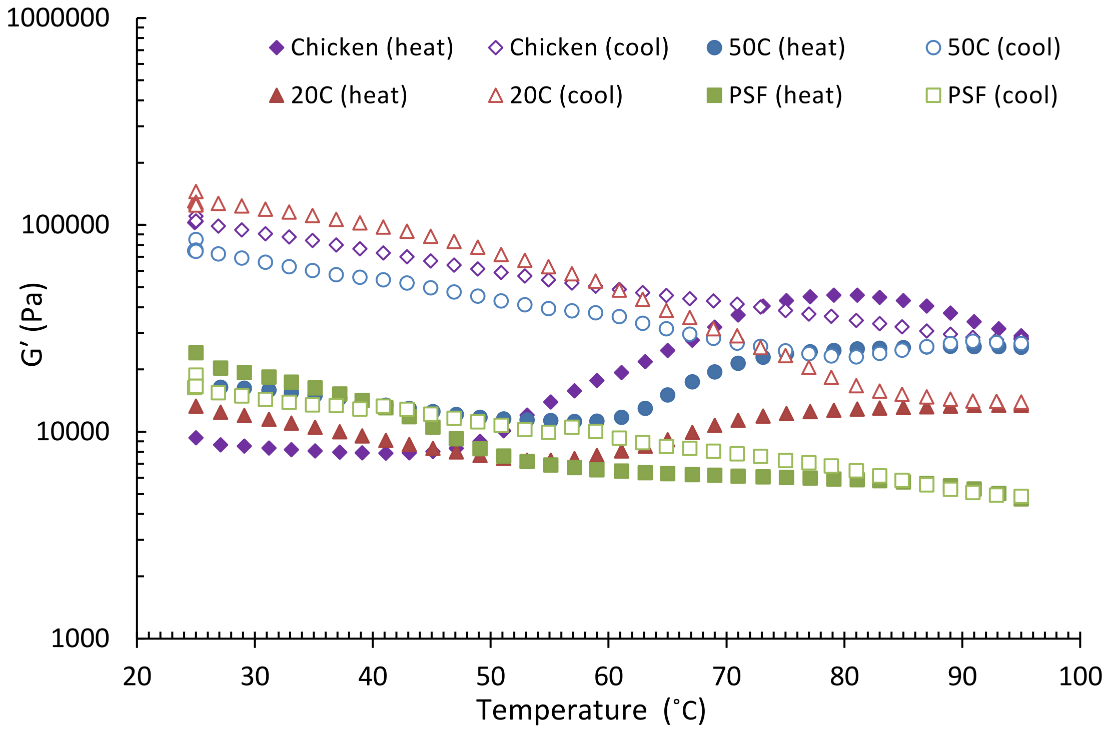
In the cooling procedure, *G′* and *G″* of the four pastes increased to different extents. A similar phenomenon was reported by Sun and Arntfield (2011b), who investigated the rheological properties of salt-extracted PPI at different temperatures. In their research, *G′* of all PPI samples increased steeply from 95 to 25 °C. The increasing curves of *G′* differ from this study. This could be caused by the different heating and cooling rates and sample compositions. As reported by O'Kane, Vereijken, Gruppen and van Boekel (2005), a slower cooling rate impacts the gelling properties of PPI by enhancing gel strength. Moreover, different formulations influenced rheological properties. As shown, *G′* and *G″* of samples with starch added (PS & PSF) returned to a slightly lower level than at the beginning of heating cycle. This finding suggested that starch limited the moduli change during heating and cooling. Therefore, cooling of PS and PSF can be considered as a roughly reverse process to heating. However, *G′* and *G″* of P control and PF were higher than initial values before heating. As can be seen, viscoelastic moduli of P control and PF rose from approximately 90 to 50 °C, while *G″* of these two samples increased dramatically from 90 to 70 °C.

Phase changes during heating and cooling did not exist, since *tan δ* of all these samples was lower than 1 at all temperatures (Figure 21 c). *Tan δ* of all these samples ranged between 0.1 and 0.25, which means samples were always predominantly elastic (Uruakpa & Arntfield, 2006). Before the heating process, the initial *tan δ* of all paste samples was between 0.2 and 0.25. During heating, all pastes showed generally a decreasing tendency in *tan δ*, indicating that the gel strength is reinforced. The reason was associated with the protein-protein interactions generated from temperature change, which contributed to a more elastic gel network (Sun & Arntfield, 2011b). *Tan δ* of P control and PF was lower than 0.15 at 95 °C. For PS and PSF however, tan δ rose slightly when the temperature was higher than 85 °C. During cooling, tan δ of PS increased dramatically at the beginning, dropping down afterwards and rising again when the temperature was below 83 °C. As for the PSF sample, *tan δ* steadily increased. The changes in *tan δ* of P control and PF during cooling were more complicated. *Tan δ* of PF increased drastically from 95 to 59 °C, and decreased smoothly afterwards. PS, PF and PSF samples did not change hugely during the entire heating and cooling process. *Tan δ* of P control rose rapidly and dropped down after 77 °C to nearly 0.13, which is much lower than the initial *tan δ*. This indicates that the cooling procedure increased the gel firmness of the P control sample.

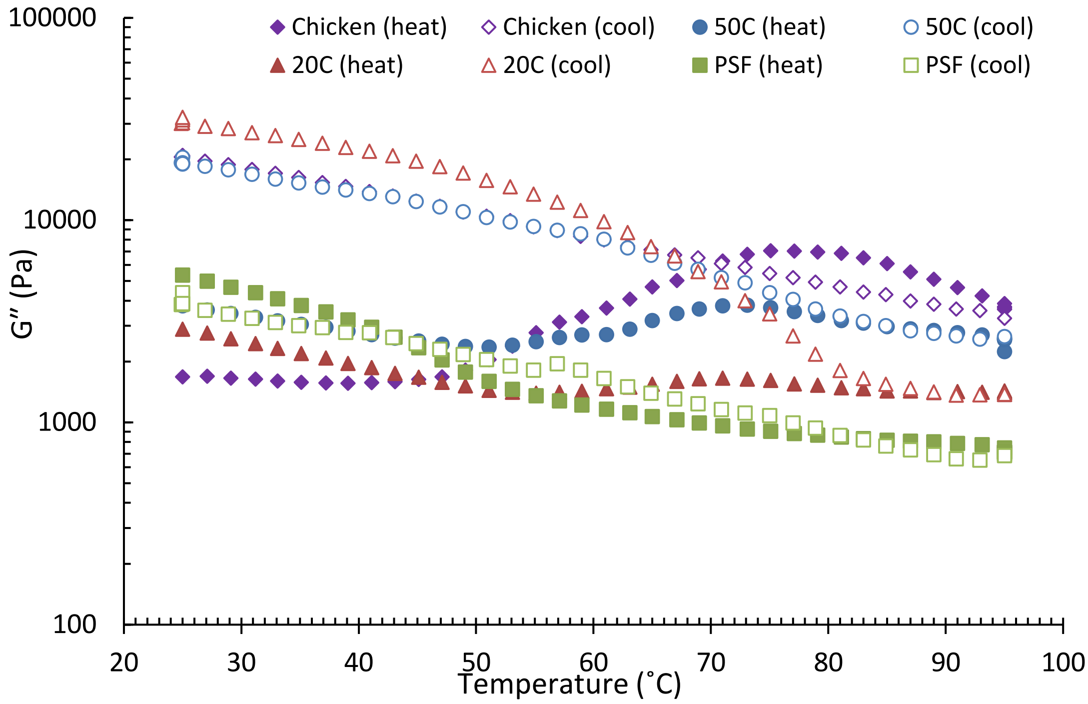
The effect of ingredient interactions among protein, starch and fat on food rheology during heating and cooling has been researched. Zhang and Hamaker (2003) showed that both whey protein and free fatty acids assisted sorghum starch to reach the second viscosity peak in the cooling stage. The presence of either whey protein or free fatty acids alone, did not exhibit such an effect. However, the main component in their experimental food profile is starch. More studies on plant protein-based food are needed to explore the interactions among protein, starch and fat. A deeper understanding of component interactions helps to design formulations with intentional rheological properties. For this study particularly, results show that starch added samples were suitable to be heated and cooled before printing. This is because they demonstrated a minor reduction in viscoelastic moduli and a similar tan δ, which potentially created a proper paste fluidity during printing.

For chicken added samples, the change of viscoelasticity is shown in Figure 22. During both heating (50 °C onwards) and cooling, chicken and chicken paste added samples showed an increased G′ over that of PPI paste alone. *G′* of chicken and chicken added samples increased steeply when the temperature was between 60 and 80 °C (Figure 22 a). This may have been caused by meat protein denaturation because a previous study showed that myoglobin proteins in poultry meat tend to denature when the temperature is higher than 55 °C (Guidi & Castigliego, 2010). A similar result was investigated by Rabeler and Feyissa (2018), in which the *G′* of chicken showed a rapid increase from 60 to 80 °C. The minor difference can be explained by the different protein contents in the meat samples. Tornberg (2005) indicated that denaturation by heating may have caused meat fibre contraction and connective tissue solubilization.

Such changes formed a denser network that enhanced *G′* of meat during heating. The variation of *G″* in the four samples showed a similar trend like *G′* in the entire process. However, *G″* of chicken and 50C reduced slightly from 75 °C onwards during heating. *G″* of 20C did not have any obvious change under heating after 50 °C. The increase in *G′* and *G″* during heating declined with the increasing amount of chicken paste, indicating that meat protein has more sensitivity to temperature than PPI at high temperature. Both *G′* and *G″* of chicken and chicken added samples increased steeply after the heating and cooling process (Figure 22 b).



(a)



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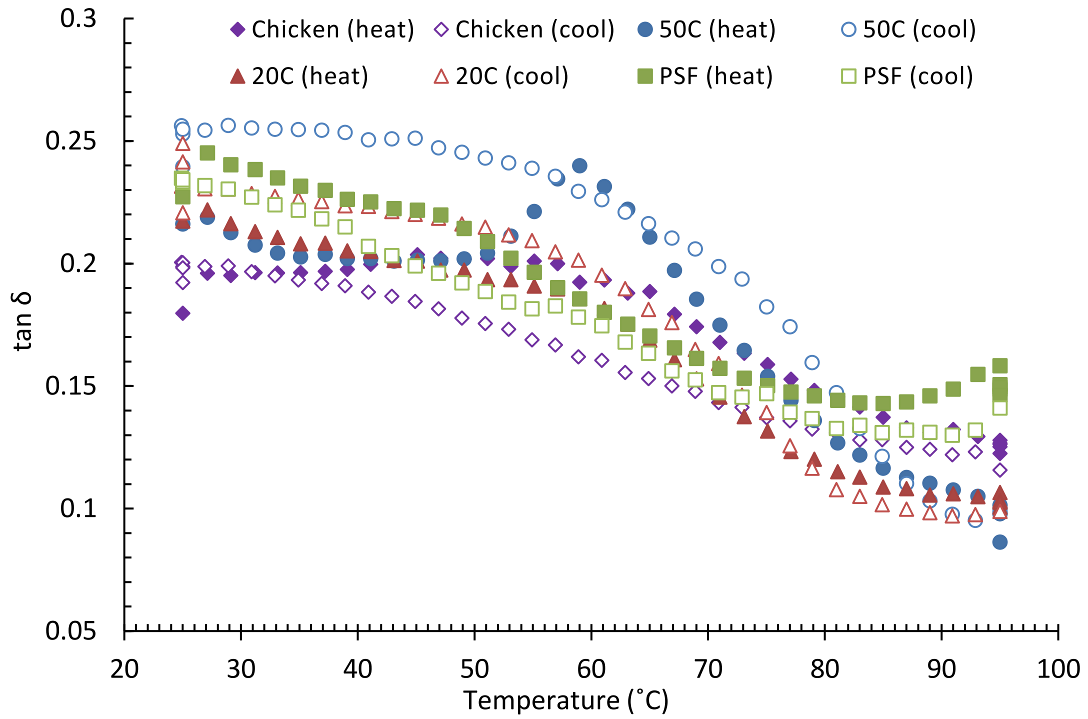


(c)

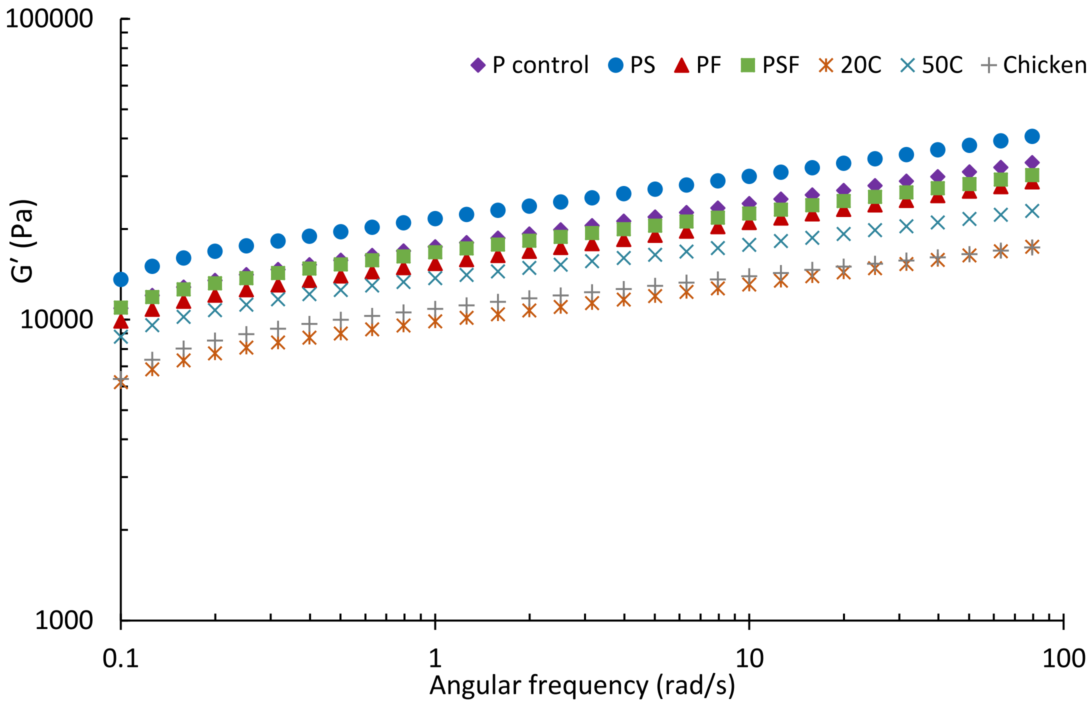
Figure 22. Viscoelastic properties of PPI and chicken mixture samples during heating (25 to 95 °C) and cooling (95 to 25 °C).

(a) Storage modulus (*G′*) changes with temperature. (b) Loss modulus (*G″*) changes with temperature. (c) Change on *tan δ* with temperature. In the figure, PSF represents PPI paste containing both starch and fat; 20C represents 20% chicken added into PSF paste; 50C represents 50% chicken added into PSF pastes. Result shown was the mean value of triplicated tests.

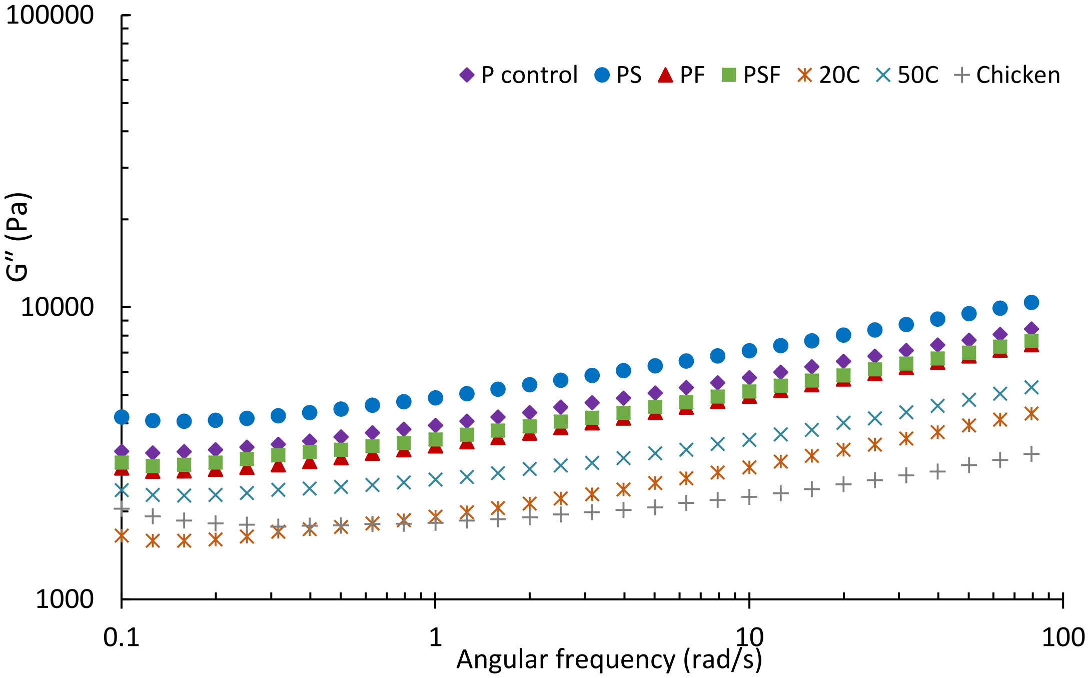
Compared with PSF and chicken, the extent of increase of *tan δ* after the entire process for the 20C and 50C samples were greater. It might demonstrate that interactions between plant and animal proteins simulate the change in *tan δ* during temperature change. Rheological properties of emulsion gel systems containing pea protein and animal protein have been studied by Graca, Raymundo and de Sousa (2016). Their investigation showed that *tan δ* of a food sample containing pea protein and collagen protein in a ratio of 50:50 increased to over 1 from 40 °C. It represented a breakdown of the original emulsion structure. The deformation was maintained until 80 °C when a new gel structure formed (*tan δ* < 1). This trend did not appear in samples containing either a higher amount of pea protein or purely collagen protein. It could explain why only 50C exhibits an increasing *tan δ* between 50 and 60 °C in this study (Figure 22 c).

4.2.2 Frequency sweep

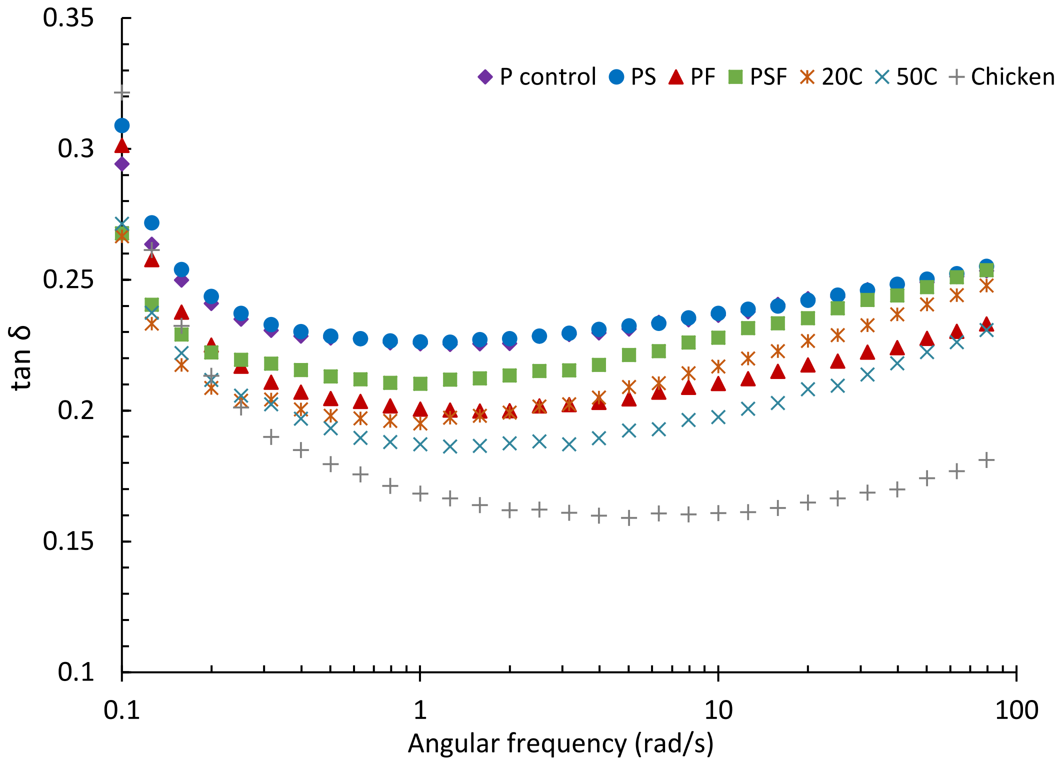
During frequency sweeps, *G′* and *G″* of four PPI pastes both progressively increased with the growing angular frequency (Figure 23). In addition, *G′* was always higher than *G″*, indicating that these pastes show a weak gel behaviour (da Silva & Rao, 2007). For both *G′* and *G″*, PS paste showed a higher value than other pastes during the whole frequency sweep test. PF showed the lowest *G′* and *G″* value, but there were no considerable differences compared with the P control and PSF pastes. Interestingly, cooked P control and PSF pastes did not exhibit high viscoelastic moduli in the temperature sweep after the heating and cooling cycle (Figure 21 a & b). This may be because cooked samples cooled at a different rate than in the conditions of the temperature sweep. Another possibility could be the moisture loss of samples during the temperature sweep. For starch added samples, moisture loss was limited because gelatinized starch absorbed water molecules (Tananuwong & Reid, 2004). Nunes, Raymundo and Sousa (2006) have researched the rheological properties of gels made by pea protein, maize starch and carrageenan. In their study, a high amount of starch increased *G′*, which agrees with the finding in this experiment. Therefore, starch is considered to create both high *G′* and *G″* values. Based on the discussion above, high *G′* and *G″* caused by adding starch decrease the fluidity (Wang et al., 2018). In contrast, addition of fat could enhance fluidity, which was agreed by Lille et al. (2018). This phenomenon may benefit the printing process.



(a)



(b)



(c)

Figure 23. Viscoelastic properties of four PPI pastes at angular frequency between 0.1 and 100 rad/s.

(a) Storage modulus (*G′*) changes with angular frequency. (b) Loss modulus (*G″*) changes with angular frequency. (c) Change on *tan δ* with angular frequency. In the figure, P control represents PPI paste; PS represents PPI Paste containing starch; PF represents PPI paste containing fat; PSF represents PPI paste containing both starch and fat; 20C represents 20% chicken added into PSF paste; 50C represents 50% chicken added into PSF paste. Result shown was the mean value of triplicated tests.

Similar to PPI pastes, *G′* of 20C, 50C and chicken was higher than *G″*, and both increased progressively during the frequency sweep (Figure 23 a & b). It shows that chicken added samples also behave like a weak gel. *G′* and *G″* of cooked PSF pastes were reduced by the addition of raw chicken paste, with 20 % chicken paste showing lower values than 50 % during the experiment. Raw chicken showed a higher *G′* than 20C but a lower value than 50C (Figure 23 a). *G″* of 20C was lower than chicken when the angular frequency was lower than 0.6 rad/s, but surpassed it afterwards (Figure 23 b). It demonstrates that the 20C sample had the highest fluidity among all three samples, which demonstrates a better flowability (Wang et al., 2018).

All seven pastes showed a decreasing tendency on *tan δ* below 1 rad/s (Figure 23 c). In this stage, *tan δ* of chicken paste showed the sharpest reduction. When the angular frequency is over 1 rad/s, *tan δ* of PPI and chicken added pastes increased slowly. For chicken paste, the *tan δ* value remained stable between 1 and 10 rad/s and rose slightly to a higher angular frequency. PF expressed the lowest *tan δ* among all PPI pastes after the frequency sweep. *Tan δ* of PSF showed minimal change before and after the whole process.

4.2.3 Shear flow behaviours

As phase change was not involved in during rheological testing in this study, shear-flow behaviour can be an important parameter to evaluate the sample's extrudability. According to Holzl et al. (2016), the viscosity of bio-ink for extrusion-based printing can range from 3×10^{-2} to 6×10^{4} Pa s. However, this is merely a basic requirement of general extrusion-based printing. To improve the extrudability and printability, the most suitable viscosity range of different materials need to be specified.

In this study, all samples were pseudoplastic materials, showing a shear-thinning behaviour (Figure 24). It indicates that all samples are suitable to extrude, which agrees the findings of Lipton (2017). The reduced viscosity caused by applied shear force enables food gels to be extruded from nozzles (Jiang et al., 2019). Among all PPI pastes, PS showed higher viscosity than the three other pastes (Figure 24). It indicates that starch increases the viscosity, which could be explained on the basis that cooked starch absorbs water and forms an intensive gel structure (Eliasson, 1986; Liu et al., 2018). However, adding both starch and fat did not negatively influence the flow behaviour. This might be because the addition of fat reduced the viscosity, as PSF and PF samples both showed low initial viscosity among all the PPI pastes. A similar finding was reported by Lille et al. (2018), namely that a food formulation with semi-skimmed milk powder was less viscous than with skimmed milk powder. In addition, the viscosity of the paste was negatively correlated with the increasing amount of raw chicken paste. Thus, higher amounts of chicken are considered to be beneficial for extrudability.

Four PPI pastes showed a higher viscosity under lower shear rates. With the increase in shear rate, the viscosity of PPI pastes dropped down sharply. When the shear rate is higher than 10 s^{-1}, PPI pastes (except the PS sample) become less viscous than chicken added pastes and chicken. The most drastic reduction exists in the shear-viscosity curve of the P control paste, showing that P control paste is easy to deform when a shear force is added. A slight infinite shear viscosity plateau is shown in PF when the shear rate was above 40 s^{-1}(Figure 24).

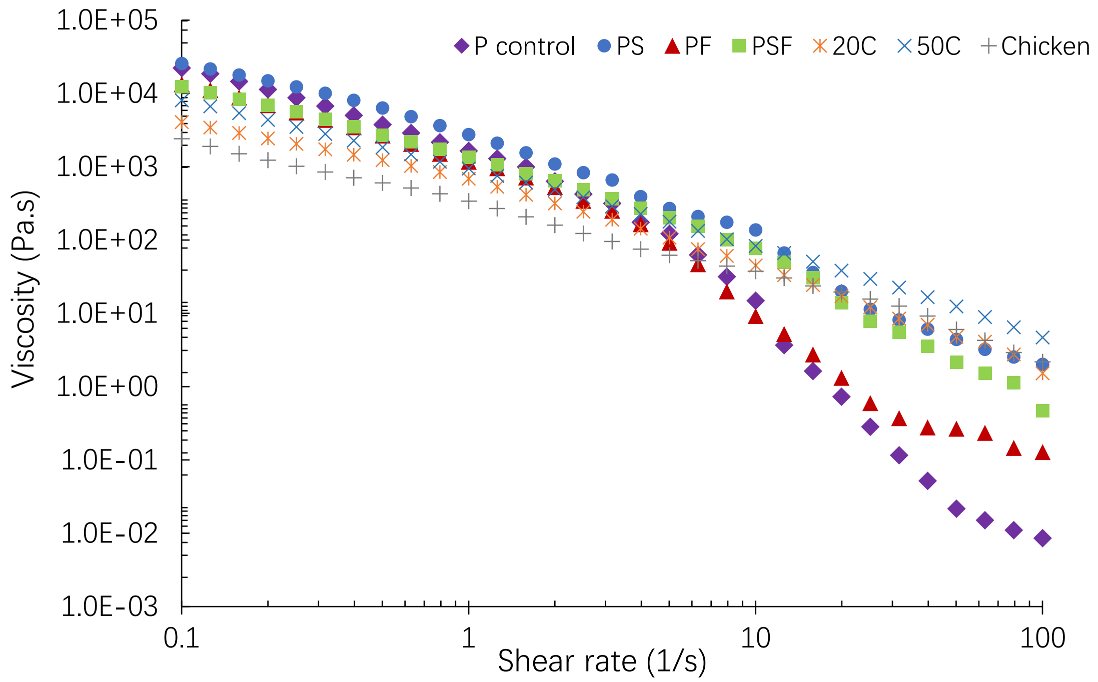


Figure 24. Apparent viscosity of samples at shear rate between 0.1 and 100 s^{-1}. Comparison of shear viscosity curve of PPI pastes, chicken and PPI-chicken pastes. In the figure, P control represents PPI paste; PS represents PPI paste containing starch; PF represents PPI paste containing fat; PSF represents PPI paste containing both starch and fat; 20C represents 20 % chicken added into PSF paste; 50C represents 50 % chicken added into PSF paste. Result shown was the mean value of triplicated tests.

Apart from shear-thinning behaviour, a high zero-shear viscosity was also considered as a suitable property for extrusion type printing (Faes, Valkenaers, Vogeler, Vleugels & Ferraris, 2015). Nevertheless, Wang et al. (2018) claimed that food material showed a poor printing performance if the zero-shear viscosity was too high. In their research, a food gel with zero-shear viscosity (viscosity at 0.1 s^{-1} shear rate) around

30,000 Pa s was extrudable but not capable of expressing a proper printing appearance. In this study, the zero-shear viscosity of PS and P control pastes are both close to 30,000 Pa s, which potentially means these two pastes are not suitable for a smooth 3D printing process.

4.3 Forward extrusion test

The extrusion force of various samples was measured by a forward extrusion test. As shown in Table 7, paste samples (except PS) showed a lower extrusion force from a 2.16 mm nozzle than a 1.54 mm nozzle. It was also shown by Zhu et al. (2019) that tomato puree exhibited lower extrusion stress through a bigger (1.2 mm) nozzle than a smaller (0.8 mm) one. These findings suggest that a bigger nozzle is easier for extrusion. Raw chicken paste was not able to be extruded from a 1.54 mm nozzle. The reason for this is that the presence of big particles constrains the flow (Figure 25), which leads to big variations in different tests. It suggests that mincing at 556 × g is not enough to break down some big muscle particles in chicken paste. Mincing in a higher rotation rate, or filtering out big muscle particles may be helpful to produce smooth flows. However, it would possibly lead to a low shape-forming capacity during extrusion (as preliminary trials). Hence, chicken paste was not suitable to directly use for 3D printing in this study. However, it can be added into PPI based pastes since 20C and 50C samples were able to extrude from both nozzle sizes. The P control paste exhibited the lowest extrusion force, demonstrating that it was easiest to extrude. The reason might be that the viscosity of the P control paste decreased dramatically at a high shear rate. PF showed the highest extrusion force of all the samples (Table 7).

Figure 25. Big particles in chicken blocked the 1.54 mm nozzle during forward extrusion test.

Table 7. The extrusion force of tested materials with 1.54 and 2.16 mm nozzle sizes.

[3]Samples	[1, 2]extrusion force (N)	
	Nozzle size	
	1.54 mm	2.16 mm
P control	57.74 ± 1.86[a]	49.91 ± 1.98[a]
PS	73.47 ± 4.30[b]	83.64 ± 2.18[d]
PF	141.10 ± 9.43[e]	98.80 ± 2.13[e]
PSF	87.88 ± 3.63[c]	62.13 ± 2.85[b]
20C	106.31 ± 3.06[d]	83.22 ± 2.50[d]
50C	84.27 ± 0.85[bc]	74.07 ± 0.54[c]
Chicken	N/A	67.73 ± 1.78[b]

[1] Results are shown as means ± SD (*n*=3).

[2] According to Tukey's pairwise comparison, different letters in each column show a significant difference ($p < 0.05$).

[3]P control represents PPI paste; PS represents PPI Paste containing starch; PF represents PPI paste containing fat; PSF represents PPI paste containing both starch and fat; 20C represents 20% chicken added into PSF paste; 50C represents 50% chicken added into PSF past.

A correlation between extrusion force (or extrusion stress) and rheological properties was shown by Zhu et al. (2019). They pointed out that the extrusion stress of food samples expressed a linear correlation with flow stress. A higher flow stress contributes to higher extrusion stress. However, there was no correlation between extrusion stress and viscoelastic properties. The reason was assumed to be that viscoelastic properties refer to the characteristics of a sample in a non-deforming stage, while flow stress and extrusion stress are both parameters related to deformation. Such correlation was suitable for various water-based food pastes, but not for oil-based food pastes. In addition, extrusion force was related to the materials’ printability, which is defined as the capacity of deposited materials to support their own weight (Godoi et al., 2016). Kim et al. (2017) showed that printability of hydrocolloid samples was positively correlated with the extrusion force. According to their finding, hydrocolloids with a higher methylcellulose concentration exhibited an increasing extrusion force, which simultaneously led to a lower deformation rate of a printed cylinder shape. Nevertheless, this correlation was not suitable for food samples with multiple ingredients. This is because interactions among ingredients creates a complex food matrix, which deserves further investigations. Currently, there is no available research that systematically assesses the relationship between extrusion force and printing performance. It is still necessary to correlate extrusion force and printing experiments, especially for food with numerous ingredients and complex structures.

4.4 Printing performance

4.4.1 Appearance of printed meat analogues in different formulations and nozzle sizes

Printed samples with a small chicken nugget shape are shown in Figure 26. In general, both PSF and chicken paste added samples formed more desirable shapes through a 1.54 mm nozzle, compared with a 2.16 mm. Since PSF, 20C and 50C all show lower extrusion force with 2.16 mm nozzle size, it may indicate that lower extrusion force results in a lower printability. This agrees with the finding of Kim et al. (2017). During printing through a 2.16 mm nozzle, the poor printing performance could be related to the shear rate and viscosity. As shown in previous research, shear rate in extrusion can be calculated as in Eq. 9 (Kern, Weiss & Hinrichs, 2018).

$$\dot{\gamma} = \frac{4Q}{\pi r^3} \qquad \text{(Eq. 9)}$$

Where $\dot{\gamma}$ is shear rate; *Q* is the volumetric flow rate, referred to extrusion rate in this study, the calculation is shown in Eq. 10; *r* is the radius of the nozzle.

$$Q = \pi r^2 \times Speed_e \qquad \text{(Eq. 10)}$$

Where Speed is the extrusion speed. Combining Eq. 9 and Eq. 10, the relationship between nozzle diameter and shear rate is shown in Eq. 11.

$$\dot{\gamma} = \frac{4 \times Speed_e}{r} = \frac{8 \times Speed_e}{d} \qquad \text{(Eq. 11)}$$

Therefore, increasing nozzle diameter with a controlled printing speed leads to a lower shear rate, resulting in a higher sample viscosity (Figure 24). It is assumed that higher viscosity causes poor extrusion behaviour. Although there is no available research that quantifies the relationship between shear viscosity and printability, similar investigations were reported by Wang et al. (2018) showing that fish surimi with a high viscosity would reduce the extrusion smoothness. In this study, the viscous extrusion flow from a 2.16 mm nozzle contributed to an inconsistent deposition line, and exhibited a less desirable appearance. Similar findings were reported by Yang et al. (2018), namely that a bigger nozzle tended to result in poorer printing quality. Wang et al. (2018) also tested printing performances through different nozzle diameters. In contrast to this study, however, they found that a printed sample from a smaller nozzle demonstrated poorer printing performance than from a bigger nozzle. The reason might be that printing through a small nozzle demanded a higher pressure, which caused an extremely low viscosity and a low shape-building capacity.

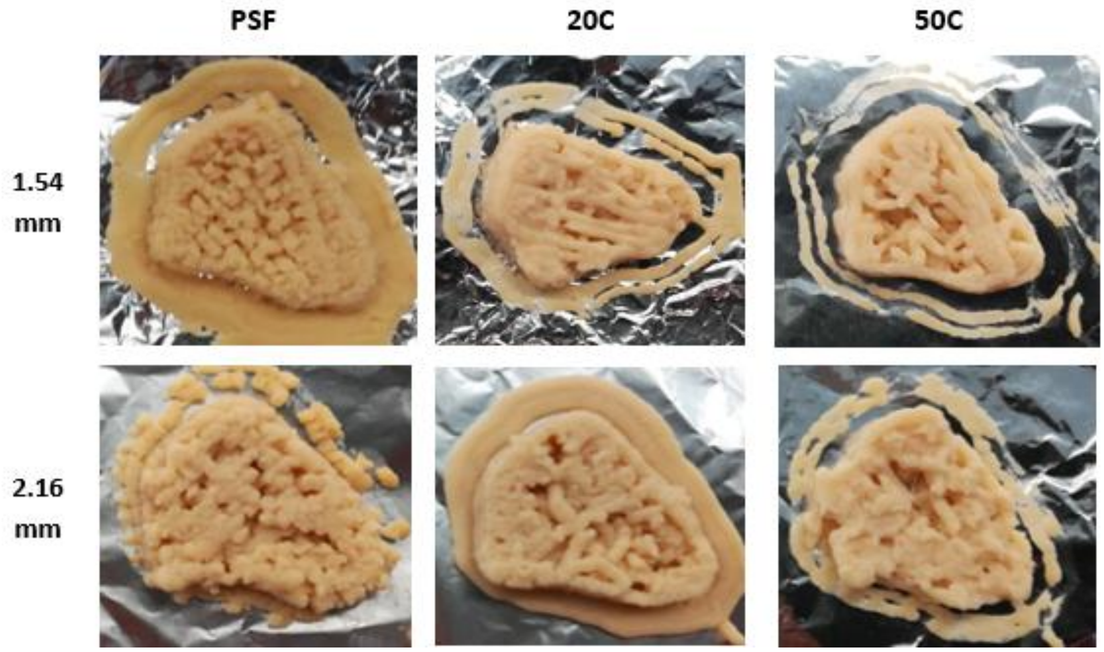


Figure 26. Printed meat analogues using 1.54 and 2.16 mm nozzles (15 mm/s, 100 % infill).

In the figure, PSF represents PPI paste containing both starch and fat; 20C represents 20% chicken added into PSF paste; 50C represents 50% chicken added into PSF paste.

The printing smoothness declined with the increasing amount of chicken paste. As presented in Figure 26, printed 50C samples through both nozzle sizes show many printing defects. It is associated with the hypothesis that a difference in smoothness of PSF paste and chicken paste may lead to a heterogeneous mixture and non-continuous flow during extrusion from nozzles. For meat paste samples, Dick et al. (2019b) recommended the use of nozzle sizes bigger than 2 mm to enable extrusion of some components containing big particles. A similar finding was shown in Section 4.3, namely that big particles in chicken blocked the 1.54 mm nozzle and stopped the extrusion process. Although 50C and 20C showed extrudability through a 1.54 mm nozzle, varied flow resistance of a chicken portion and a plant protein portion could cause a non-smooth extrusion. PSF paste was able to deposit bottom layers stably. Although printing defects also appeared on the surface layer, the general nugget shape based on the 3D model was formed. The reason could be that PSF paste has a more uniform structure than 50C and 20C samples.

4.4.2 Appearance and macrostructure of printed meat analogues after cooking

The comparison of three cooked samples is presented in Figure 27. Chicken paste added samples showed a more acceptable colour after boiling in water. The shapes of the printed samples were slightly damaged after cooking. Although heat-sealed bags were used to prevent damage of the shape, the edges of the printed samples were not protected perfectly. For future studies, new cooking methods causing less printing shape change are suggested. Lipton et al. (2010) tried cooking printed meat in a controlled vapour oven. The overall shape of printed turkey meat was protected from being damaged by the package. Nonetheless, cooking in a controlled vapour oven caused inward shrinkage of the meat, which made the shape bow upwards. To improve the printability of meat analogues, shape-maintenance after cooking is a challenge that needs to be overcome.

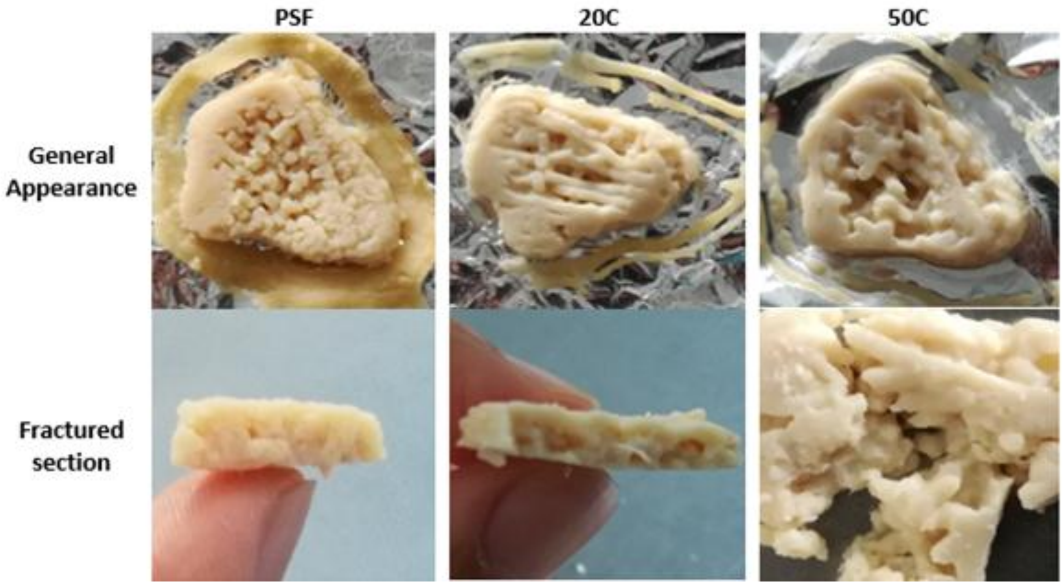


Figure 27. Printed meat analogues using 1.54 mm nozzle after cooking.
In the figure, PSF represents PPI paste containing both starch and fat; 20C represents 20% chicken added into PSF paste; 50C represents 50% chicken added into PSF paste. Printed samples were cooked in boiling water for 10 min.

Fibrous structure formation is a pivotal characteristic to assess the quality of meat analogue. As found from fractured sections, PSF sample was insufficient to form a fibrous structure without adding chicken (Figure 27). According to previous studies, the fibrous structure of PPI was formed in the thermal extrusion system above 120 °C (Osen et al., 2014). In a shearing processing system, the same conditions of temperature were required (Schreuders et al., 2019). To develop a printed product from a PPI based formulation without adding meat, the post-printing cooking method should be changed. This is because the temperature of the boiling water bath (100 °C) was inadequate to build fibrous structures. Pan-frying, microwaving and baking were suggested, since these methods enable cooking samples at a higher temperature. Moreover, addition of some ingredients such as wheat gluten would help fibre formation (Schreuders et al., 2019). Fibres were found in 20C and 50C, indicating that meat fibres were generally provided by chicken paste. More fibres were found in 20C than 50C sample because of its stable printed shape. This is associated with the proper extrusion and deposition performance of the 20C sample. Thus, 20C paste is considered as the optimal material for printing in this study.

4.5 Moisture and protein contents

Moisture and protein content are the two important standards to assess the quality of meat analogues. Moisture content has been recognized to influence the texture and structural characters of meat analogues (Lin et al., 2000; Yao et al, 2004; Riaz, 2006; Liu & Hsieh, 2008). Liu et al. (2000) highlighted that higher moisture contents led to a softer texture as well as an increasing protein solubility of high-temperature extruded meat analogues. As listed in Table 8, moisture content of all four cooked samples was around 70 %. Moisture content in PSF is lower than samples with chicken but there is no significant difference ($p < 0.05$). It has been widely recognized that high moisture content benefits fibre formation compared with low moisture extruded meat analogues (Riaz, 2006; Osen et al., 2014). However, the formation of fibrous structure is limited if the moisture content is too high. Yao et al. (2004) found that extruded meat analogues containing approximately 60 % moisture showed a desirable fibrous structure that was not observed in samples with around 70 % moisture. It may explain why the PSF sample has little fibre after printing and cooking. For 3D printing however, the reduction in the moisture content of PPI paste would decrease the flowability. PPI paste tends to become too dry if the moisture content is reduced to 60 %. On the other hand, the addition of other solid ingredients might cause the paste to be too sticky as described in Section 4.1.1. Cooked chicken mince contains 70 % moisture, which is similar to the result from boiled chicken breast meat in previous literature (Latifa et al., 2010; Chiang et al., 2019). In contrast to plant-based meat analogue, chicken meat naturally contains fibres, even in a higher level of moisture content. Therefore, fibres appeared in printed and cooked 20C and 50C samples.

For meat analogues, protein content is relevant to its nutritional value. In this study, protein content was increased in the sample containing a higher amount of chicken (Table 8). Protein in the boiled chicken sample is nearly 27 %, which is significantly higher than other samples ($p < 0.05$). Protein content was reduced with the decreasing amount of chicken in samples. It indicates that hybrid meat analogues improve nutritional values of food.

Table 8. Moisture and protein contents of printed meat analogues and cooked chicken.

[1]Samples	[2,3]Moisture (%)	[2,3]Protein (%)
PSF	69.13 ± 0.40[a]	19.19 ± 0.30[a]
20C	70.45 ± 0.43[a]	20.73 ± 0.23[b]
50C	70.04 ± 0.43[a]	22.88 ± 0.29[c]
Chicken	70.20 ± 0.90[a]	26.95 ± 0.48[d]

[1]PSF represents PPI paste containing both starch and fat; 20C represents 20% chicken added into PSF paste; 50C represents 50% chicken added into PSF paste.
[2]Results are shown as means ± SD (n=3).
[3]According to Tukey's pairwise comparison, different letters in each column show a significant difference ($p < 0.05$).

4.6 Texture profile analysis

4.6.1 Hardness

Instrumental hardness of food products refers to the force to break food samples by molar teeth (Lyon, Lyon, Davis & Townsend, 1980). Since 3D printing aims to soften the texture of meat analogues, hardness of printed meat analogues is presented in Figure 28. As can be seen, all meat analogue samples have significantly lower hardness than cooked chicken mince ($p < 0.05$). The hardness of cooked chicken mince in this study is similar to the hardness of chicken breast sausage from Chiang et al. (2020). Although the nutrient profile of chicken breast sausage differs from our cooked chicken mince, both samples showed a similar moisture content. Thus, it shows moisture content has a vitally important influence on the hardness of food made from similar ingredients.

The hardness was reduced with the increasing amount of pea protein paste. Due to all samples containing roughly the same level of moisture, the different values of hardness should be caused by the varying formulations. As stated by previous researchers, pea protein tends to form a soft gel (Osen et al., 2014; Schreuders et al., 2019). This could be the reason why the hardness of cooked PSF is approximately 10 times lower than cooked chicken. In addition, the hardness of 50C is lower than the average hardness of cooked chicken mince and PSF. This may be because blending process damages the network-forming property of chicken. Chiang et al. (2020) also reported that the destruction of some inter-molecular strength during meat grinding would reduce the hardness of meat products. Another reason could be also related to the interactions between chicken protein and pea protein, which would soften the food texture. To prove this assumption, further research on interactions between these two proteins is needed.

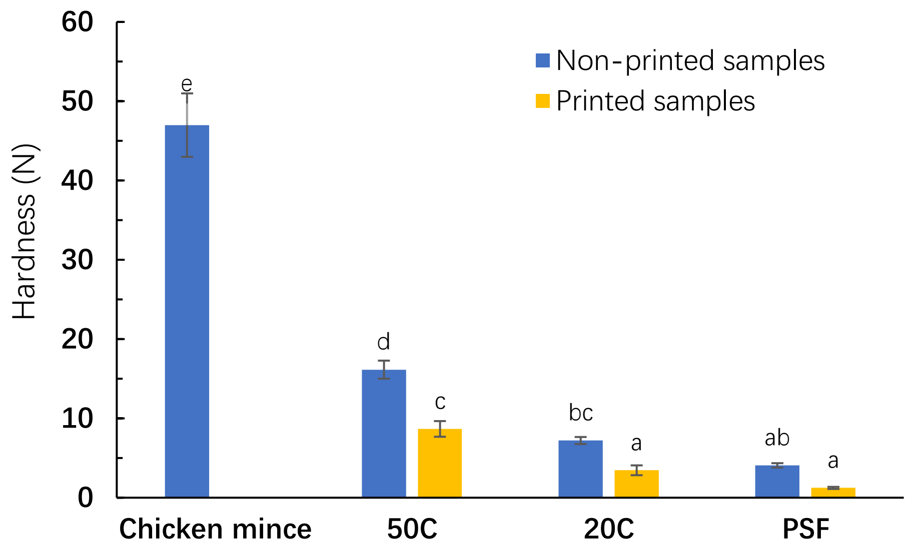


Figure 28. Hardness of printed and non-printed samples after cooking in a boiling water bath.

Each bar shows the means ± SD (*n*=5). All results were obtaind from Exponent (Appendix II). Data with different letters for hardness of samples are significantly different ($p < 0.05$) based on Tukey test. PSF represents PPI paste containing both starch and fat; 20C represents PSF paste with 20% chicken; 50C represents PSF paste with 50 % chicken.

The 3D printing process approximately halves the hardness of non-printed meat analogues. The hardness reduction is related to the space between deposition lines in the printing process. The restructured food material contributes a less intensive matrix compared with non-printed samples. Watanabe et al. (2018) recommended that the hardness of a soft diet should be lower than 40,000 N/m^2, associated with food in level 4 (pureed food) in the Dysphagia Diet Pyramid designed by the International Dysphagia Diet Standardisation Initiative (IDDSI). For a 2 × 2 × 2 cm^3 meat or meat analogue cube, such hardness roughly equals 16 N in the double compression TPA tests. Therefore, chicken and non-printed 50C were not considered as a soft diet. Meanwhile, all printed samples reached the basic requirement of soft food. It shows that 3D printing is a way to prepare a soft meal, which could potentially be used for health care. For this purpose, more mastication tests of 3D printed meat analogues need to be done in future studies.

4.6.2 Springiness, cohesiveness and chewiness

Other textural properties, including springiness, cohesiveness and chewiness, were listed in Table 9. These characteristics of meat analogues are also important as they are often referenced in previous research. Cooked chicken mince showed higher value than printed meat analogues in all three profiles. These values were also similar to chicken breast sausage (Chiang et al., 2020). Meanwhile, these textural values of non-printed meat analogues decreased with the lower amount of chicken. This indicates that the addition of chicken positively influences springiness, cohesiveness and chewiness.

It has been reported in previous research that 3D printing processes soften food texture (Le Tohic et al., 2018; Huang et al., 2019; Mantihal et al., 2019). However, the effect on other textural properties varied depending on printing materials. As shown by Le Tohic et al. (2018), 3D printing did not significantly affect the springiness or cohesiveness of cheese. In this study however, springiness, cohesiveness and chewiness were all changed by 3D printing to some extent (Table 9). Springiness, in food texture analysis, could also be described as elasticity, which is measured as the height recovery degree of food. Cohesiveness refers to the level of difficulty of breaking down food internal structure (Yang, Choi, Jeon, Park & Joo, 2007). The 50C sample showed an increased springiness and a decreased cohesiveness after printing. It is associated with the high void rate in printed 50C caused by non-smooth printing behaviour. In contrast, the printing process reduced springiness and improved cohesiveness of 20C, which might be because of the lower void rate. As to the PSF sample, both springiness and cohesiveness were significantly changed after printing ($p < 0.05$). It might be because the printing process restructured food material like cooked PSF paste, which is pastier and less solid. Chewiness is an important textural property of solid food. It is defined as the chewing strength required to break food into easy-swallowing pieces, and measured by hardness × cohesiveness × springiness (Lyon et al., 1980). The chewiness values are generally related to hardness of samples, since a hard sample also exhibits a high chewiness. However, the chewiness of printed PSF is higher than non-printed PSF. In addition, printed PSF shows a huge variation in chewiness, suggesting that printed PSF should be analysed under a lower load force.

Table 9. Springiness, cohesiveness and chewiness of printed and non-printed samples after cooking.

[1]Samples		[2,3]Springiness (%)	[2,3]Cohesiveness (%)	[2,3]Chewiness (N)
Chicken mince		87.31 ± 2.53^{d}	48.21 ± 1.57^{d}	19.82 ± 2.36^{d}
50C	control	72.23 ± 8.82^{cd}	33.55 ± 2.61^{c}	3.93 ± 0.75^{c}
	printed	85.17 ± 10.89^{d}	30.85 ± 3.02^{bc}	2.26 ± 0.34^{bc}
20C	control	55.10 ± 2.90^{bc}	24.58 ± 1.43^{ab}	0.98 ± 0.11^{ab}
	printed	38.79 ± 6.98^{ab}	20.98 ± 3.12^{a}	0.27 ± 0.05^{a}
PSF	control	29.95 ± 5.32^{a}	20.60 ± 0.95^{a}	0.25 ± 0.06^{a}
	printed	68.80 ± 27.2^{cd}	31.86 ± 9.86^{bc}	0.29 ± 0.21^{a}

[1]PSF represents PPI paste containing both starch and fat; 20C represents 20% chicken added into PSF paste; 50C represents 50% chicken added into PSF paste.All samples were cooked in boiling water for 10 min.

[2]Results were means ± SD (*n*=5), obtaind from Exponent (Appendix II).

[3]According to Tukey's pairwise comparison, different letters in each column show significant difference ($p < 0.05$).

4.7 Microstructure of cooked chicken and printed meat analogues

4.7.1 Light microscopy (LM)

The microstructure of printed meat analogue and cooked chicken mince viewed by LM is shown in Figure 29. As can be seen, PSF shows a network consisting of crowded small globes (Figure 29 a). It is assumed to be the aggregation of pea protein, since PPI was the main ingredient. However, this structure showed a similarity with gelatinized maize starch, in which starch granules absorb water and form an intensive network of swelled particles (Ratnayake & Jackson, 2006). It is difficult to clarify the structural details of PSF through LM. More information was observed by SEM and is discussed in Section 4.7.2.

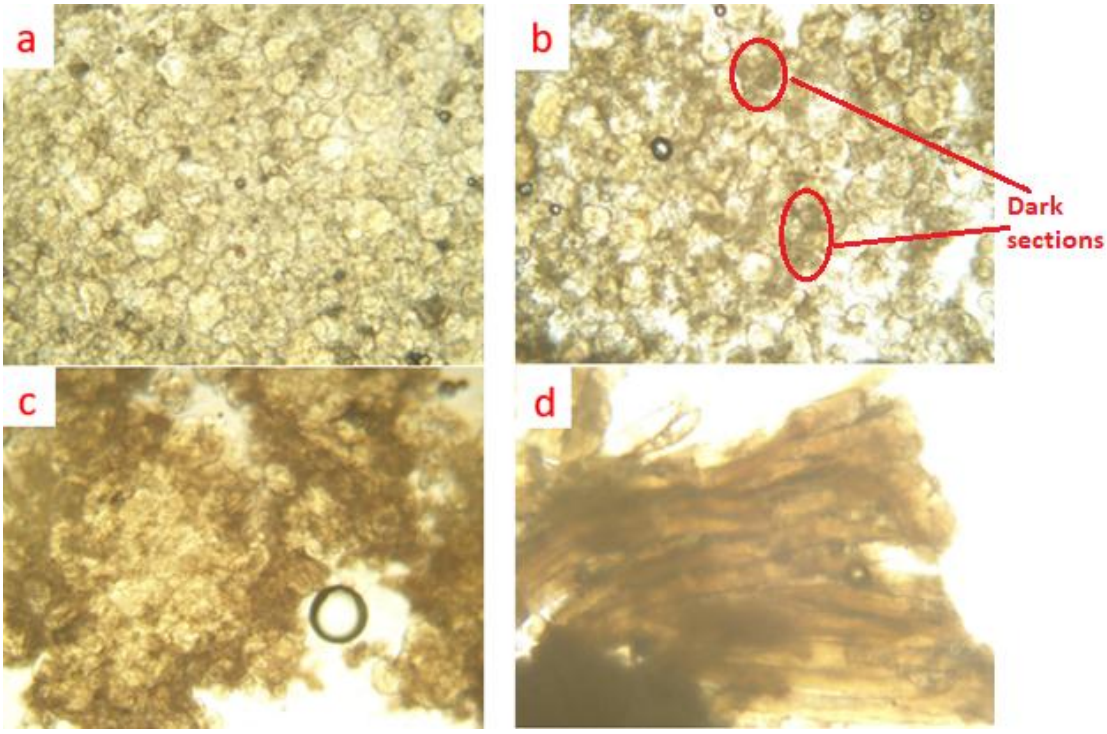


Figure 29. Microstructure of cooked printed meat analogues (PSF (a), 20C (b), 50C (c)) and chicken (d) viewed by LM at 10× magnification.

In the figure, PSF represents PPI paste containing both starch and fat; 20C represents 20% chicken added into PSF paste; 50C represents 50% chicken added into PSF paste. Both printed samples and chicken mince were cooked in boiling water for 10 min.

The dark sections between globes in Figure 29 (b) and (c) are assumed to be chicken muscle particles. With the higher amount of chicken paste, the dark section becomes bigger and widely distributes in the plant protein matrix. It indicates that the blending process did not completely mix chicken paste and PSF pastes into a continuous phase. The dark sections were aggregated in Figure 29 (c), showing that chicken portions became accumulated and formed a unique matrix. It could be the reason that the 50C sample did not exhibit a smooth extrusion behaviour, resulting in a larger number of printing defects. For cooked chicken mince, the fibrous structure is obvious, shown in Figure 29 (d). It shows that chicken fibre was not completely broken down during the processing of chicken mince. Fibres were able to form after cooking in boiling water.

4.7.2 Scanning electron microscopy (SEM)

Figure 30 illustrates the microstructure of printed meat analogue and boiled chicken mince in 200× magnification by SEM. Printed PSF showed a highly aggregated structure with globular particles inside (Figure 30 a). The aggregation is assumed to consist of PPI, starch and fat. Feng, Wang, Li, Zhou and Meng (2018), found similar aggregation constructed by a starch and pea protein network through SEM. Although starch was the major ingredient in their study, they demonstrated that an increasing amount of pea protein began to establish a continuous pea protein matrix. The aggregation can also be investigated in a printed 20C sample. However, the globular particles are not shown (Figure 30 b). Some meat fibre-like structure is shown in the printed 50C sample, which is found inside the plant protein-based matrices (Figure 30 c). As can be seen from Figure 30 (d), the microstructure of cooked chicken mince showed an alignment of chicken fibres. This alignment is different to the three former samples. The fibrous structure of cooked chicken mince was very similar to chicken meat under SEM in previous studies (Chiang et al., 2019; Samard & Ryu, 2019a). It verifies the assumption that chicken mince exhibits the fibres after cooking.

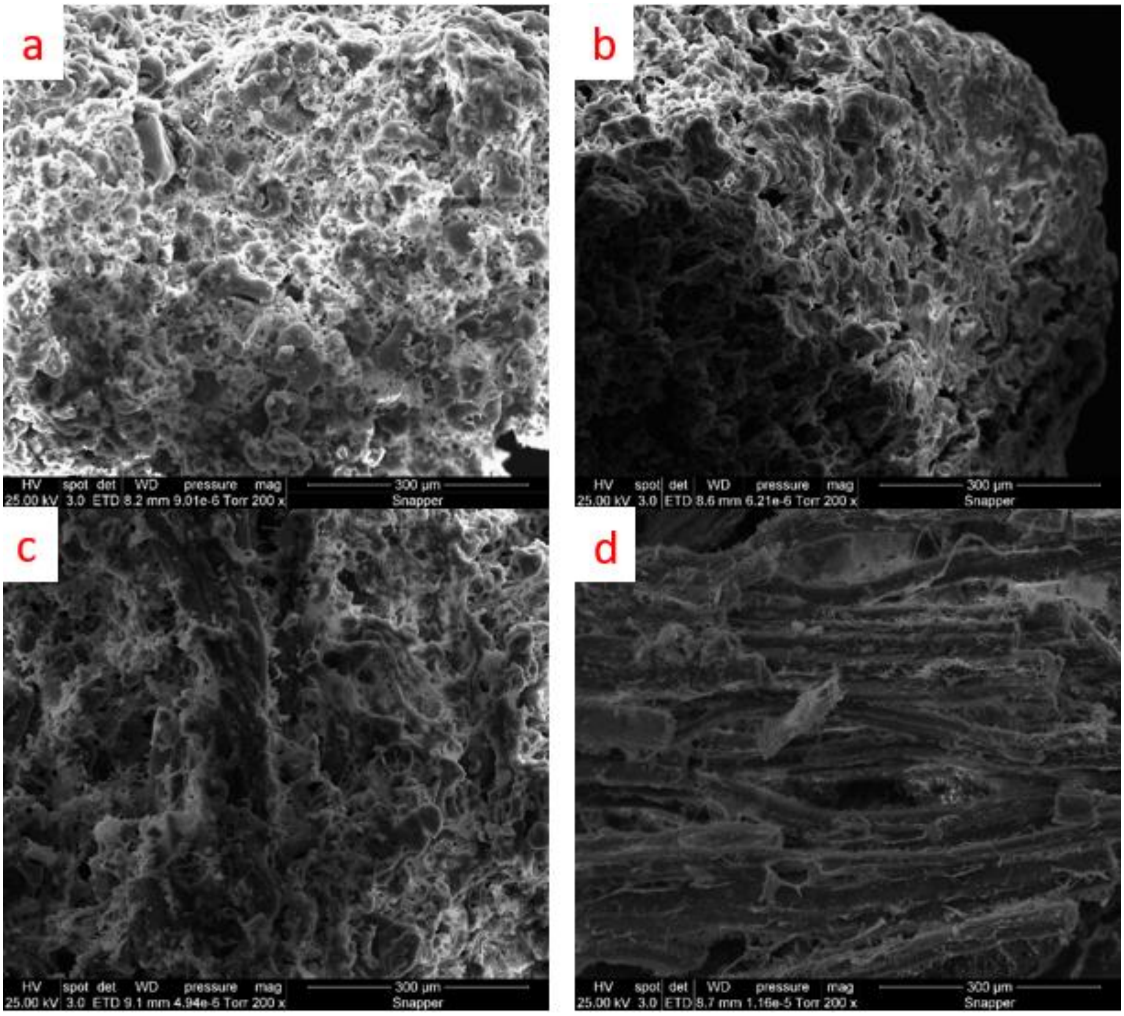


Figure 30. Microstructure of cooked printed meat analogues (PSF (a), 20C (b) & 50C (c)) and chicken mince (d) viewed by SEM at 200× magnification.

In the figure, PSF represents PPI paste containing both starch and fat; 20C and 50C represents 20 and 50 % chicken added into PSF paste. Both printed samples and chicken mince were cooked in boiling water for 10 min.

To observe more details in microstructure, micrographs taken at 400× magnification are shown in Figure 31. There is no apparent visual difference in fibres in PSF and 20C samples under different magnifications (Figure 31 a & b). The 20C sample exhibits a layered structure at 400× magnification, but no fibrous structure was observed. When compared at 200× magnification level, the fibrous structure in the 50C samples was clearer (Figure 31 c). It shows an aligned fibre, which is similar to the chicken fibres in Figure 31 (d). These findings suggest that the large proportion of chicken paste in the 50C samples assists in forming obvious fibres during cooking.

However, such structure also negatively influenced the extrusion smoothness, resulting in a poor printing performance with few fibres presented in macrostructure.

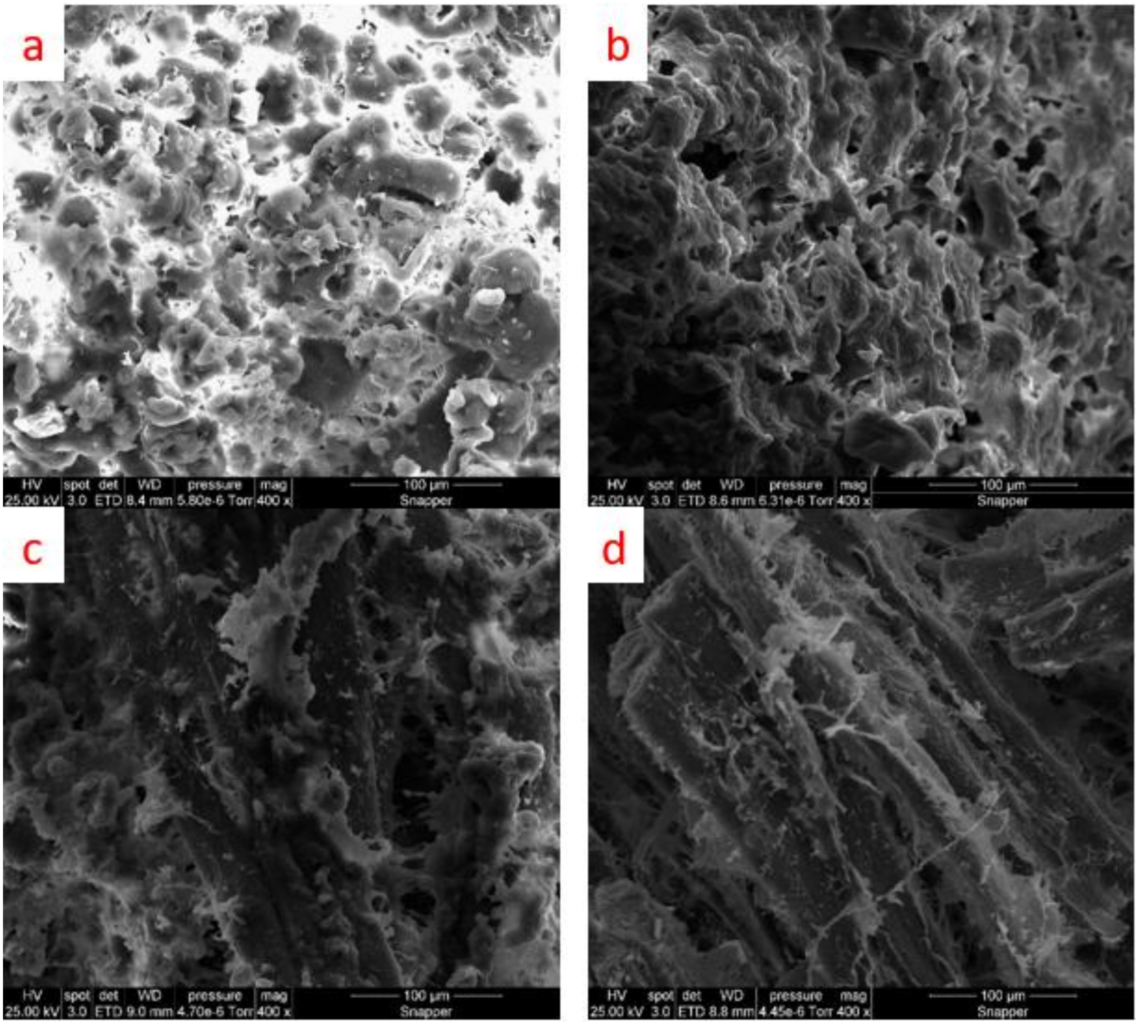


Figure 31. Microstructure of cooked printed meat analogues (PSF (a), 20C (b), 50C (c)) and chicken mince (d) viewed by SEM at 400×magnification.

In the figure, PSF represents PPI paste containing both starch and fat; 20C and 50C represents 20 and 50 % chicken added into PSF paste. Both printed samples and chicken mince were cooked in boiling water for 10 min.

According to microstructures of all four samples, it is presumed that fibre is created by chicken directly. PPI or other ingredients would not provide fibre through the preparation and printing process in this study. To print a fibrous non-meat product, other potential plant-based fibre forming agents need to be added. More research on fibre forming mechanisms is required.

4.8 Protein solubility

The content of soluble protein in printed meat analogues and control PSF paste is shown in Figure 32. Each column represents the protein solubility of one particular extraction solution. As shown in the figure, protein exhibits the lowest solubility in PB compared with all extraction solutions. It indicates that protein interactions exist and support the structure of samples. Even though the PSF paste showed a significantly higher solubility than the other three samples in PB ($p < 0.05$), it is still lower than 5 %. Such low solubility further proves that the PPI used in this study had been denatured before the experiments, since only native plant protein can be dissolved in a phosphate buffer (Lin et al., 2000). It is obvious that soluble protein in PU is higher than in PS and PD, demonstrating that proteins are more soluble in urea than SDS and DTT. It is believed that a urea solvent destroys hydrogen bonds (Uruakpa & Arntfield, 2006). Thus, hydrogen bonding is considered as the major interaction between proteins in all samples. Previous research also showed that hydrogen bonds were the major force in pea legume protein gel (O'Kane, Happe, Vereijken, Gruppen & van Boekel, 2004).

Protein-protein interactions have been recognized as a mechanism of fibre formation. The formation of fibrous structure is associated with the formation of hydrophobic interactions, disulphide bonds among proteins, or the combination of both bonds (Dekkers et al., 2018). In high-temperature extrusion processing, the effect of the disulphide bond on fibre formation was particularly emphasized. Extruded meat analogues tended to show a high protein solubility in DTT solvents, and DTT is considered as an agent which breaks disulphide bonding (Liu & Hsieh., 2008; Chiang et al., 2019). In this study, the disulphide bond is also considered as the main protein-protein interaction building fibrous structures. This is because chicken added samples, which have a fibrous structure, showed lower protein solubility in overall extraction solutions except in PD. However, PSF paste and printed PSF did not show significantly different ($P < 0.05$) protein solubility in PD, interpreted as showing that printing and cooking did not help form fibres. It has been already discussed that fibres in printed samples were mainly provided by chicken paste. Similar to results from Chiang et al. (2019), protein solubility of all samples in PSDU is greater than the sum solubility in PS, PD and PU. It indicates that the structure of PSF paste and printed meat analogues is not only supported by hydrophobic interaction, disulphide bond and hydrogen bond, but also their combinations.

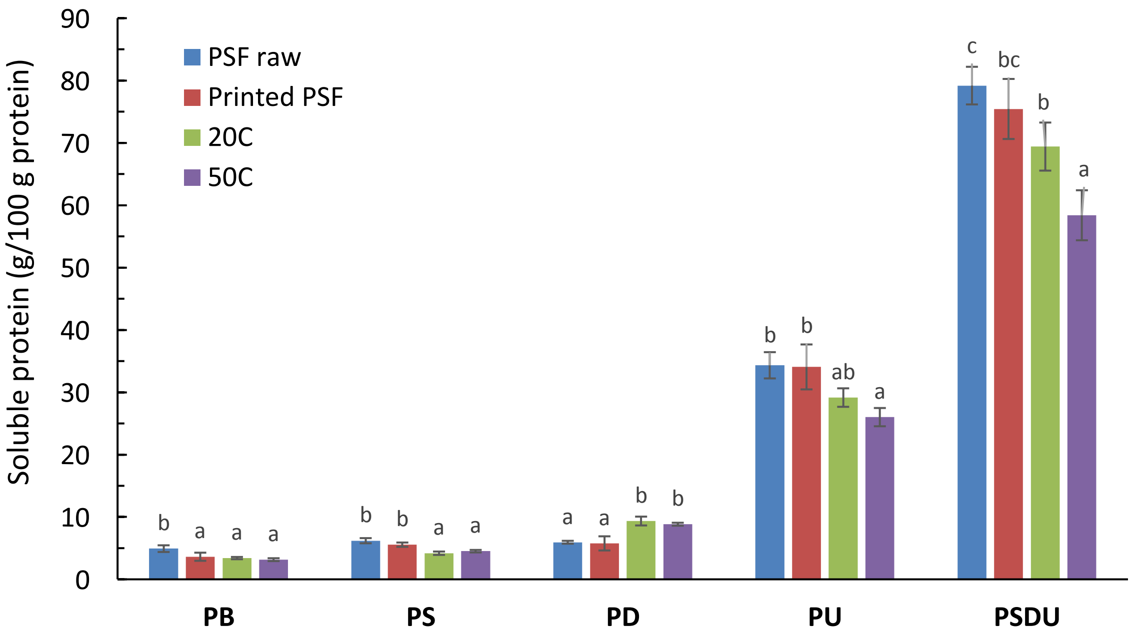


Figure 32. Soluble protein percentages in PSF paste, Printed PSF, 20C and 50C in five extraction solutions.

Each bar shows means ± SD (*n=3*). Data with different letters for each extraction treatment are significantly different ($p < 0.05$). In the figure, PSF raw represents PPI paste containing starch and fat without cooking; Printed PSF represents PPI containing starch and fat after cooking; 20C represents 20% chicken added into PSF paste; 50C represents 50% chicken added into PSF paste. PSF cook, 20C and 50C sample were printed and cooked in boiling water for 10 min. PB represents phosphate buffer; PS represents PB + SDS; PD represents PB + DTT; PU represents PB + urea; PSDU represents PB + SDS +DTT + urea.

4.9 Printing parameter optimization

4.9.1 Printing speed optimization

Figure 33 shows the appearance of four printed cube shapes from 20C sample in different printing speeds (10, 15, 20 and 25 mm/s). In general, increasing the printing speed reduced the printing precision, leading to an undesirable appearance with higher printing defects in different degrees. Slower (10 and 15 mm/s) printing constructed a cube shape and retains stability after printing. Compared with 10 mm/s, 15 mm/s printing presents more non-smooth deposition lines in its front view. Printing with 20 mm/s shows a better top view but the cube shape slightly collapsed after printing. As to 25 mm/s printing, both the top view and the front view exhibited a large number of printing defects. When considering printing quality, however, 10 mm/s proves to be the optimal speed. However, it is also worthwhile to develop a high-speed printing method in future research. One significant benefit of high-speed printing is time saving. Increasing from 10 to 25 mm/s, drastically reduces printing time (Figure 34).

Although 3D printing technology is also called rapid prototyping, the food printing process is considered to be time-consuming. The printing speeds of food materials have been normally lower than 50 mm/s, which is much slower than printing many non-food materials (Wegrzyn et al., 2012; Yang et al., 2018; Derossi, Paolillo, Caporizzi & Severini, 2020). For instance, the printing of concrete materials could reach the speed of around 250 mm/s (Diggs-McGee, Kreiger, Kreiger & Case, 2019). For this reason, small sizes of food materials were usually printed to save processing time. The LVE type 3D printer enables a printing speed of about 80 mm/s (Pusch, Hinton & Feinberg., 2018). In this study however, the printing speed of food pastes was limited to a low level because of the poor printing precision at mid-high speed. In one of the most up-to-date pieces of research, Derossi et al. (2020) successfully printed cereal dough with a printing speed at 200 mm/s. They highlighted that high-speed printing failed when following conventional printer settings. Instead, they adjusted the filament diameters and retraction distance, which were not common parameters mentioned in previous studies on food printing. By setting suitable filament diameters and retraction distances, printed dough with desirable appearance was created. Further studies are recommended to develop more high-speed printing approaches for different food materials, including meat or plant protein pastes.

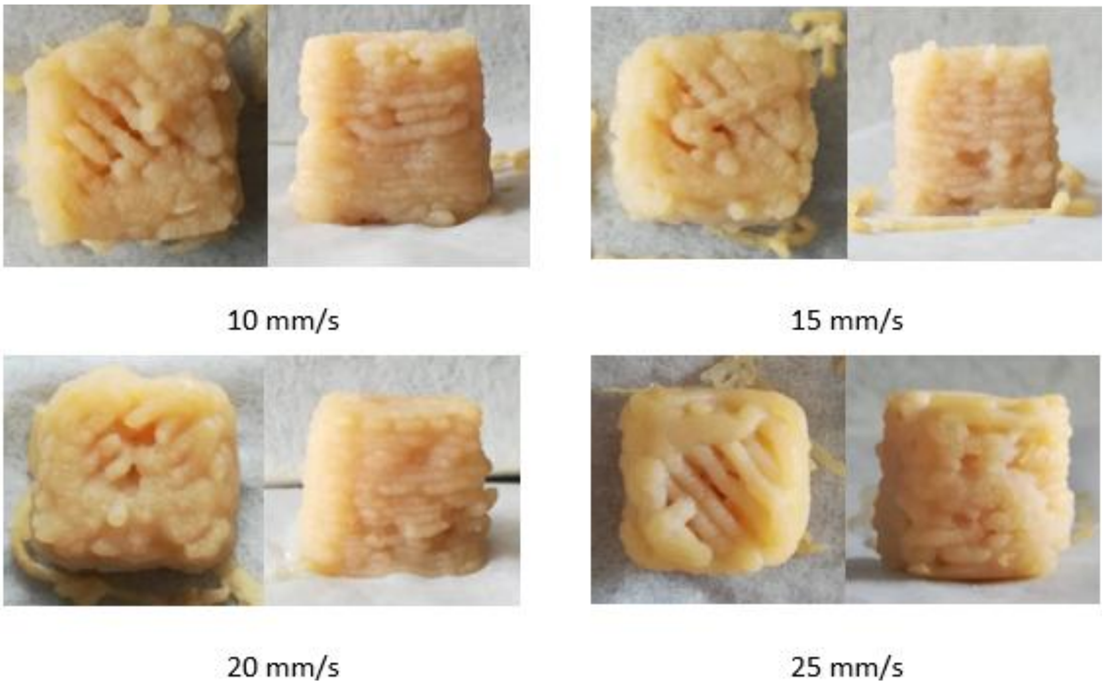


Figure 33. Printed 20 % chicken analogues at different printing speed (1.54 mm nozzle, 100% infill density).
In the figure, 10, 15, 20 and 25 mm/s shown in the figure represent printing speed in experiments.

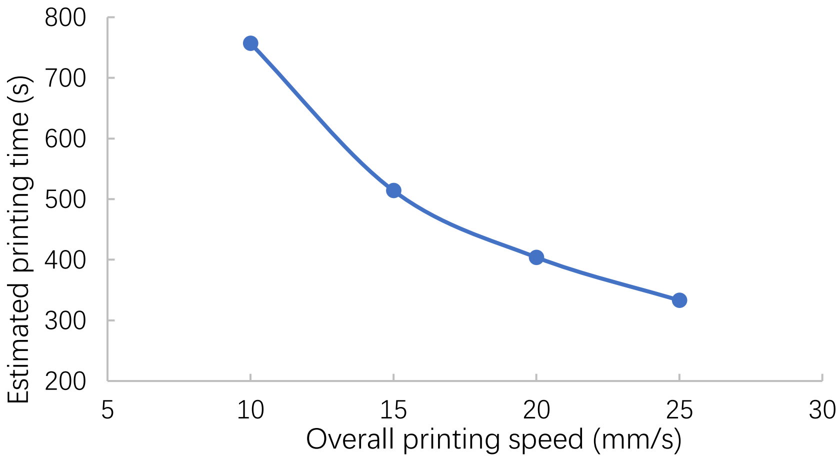


Figure 34. Printing speed-time curve of 20C (1.54 mm nozzle, 100 % infill density; estimated by Repetier-host).

4.9.2 Infill density optimization

In 3D printing, infill is defined as the non-solid portion which is inside the outer shell of a printed product. Another method of saving printing time is by adjusting the infill density (Figure 35). In addition, printing material wastes can also be reduced (Cheng, Zhang, Chen & Tseng, 2018). Huang et al. (2019) showed that infill density did not affect the printability of printed brown rice paste. Moreover, lower infill density decreased the hardness due to the increasing void rates. A similar result was outlined by Mantihal et al. (2019), namely that printed dark chocolate was able to form a stable shape in infill densities ranging from 5 to 100 %. These findings point to the possibility of saving printing time by reducing the infill density.

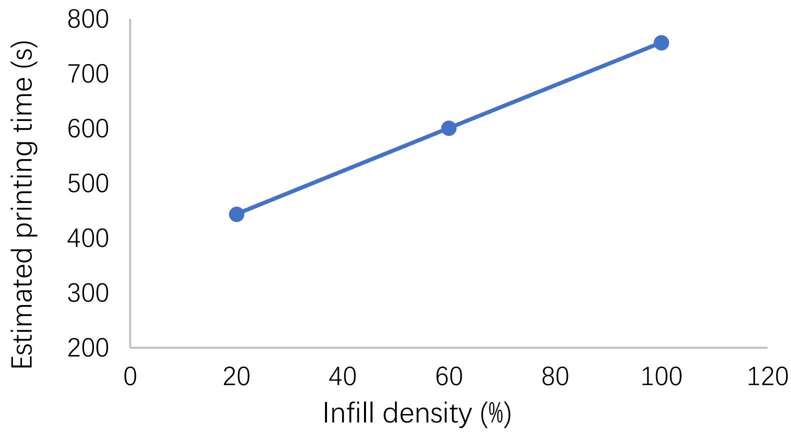


Figure 35. Printing infill-time curve of 20C (1.54 mm nozzle, 10 mm/s printing speed; estimated by Repetier-host).

In this study, printed cube shapes of 20C sample with different infill densities (100, 60 and 20 %) were shown in Figure 36. As can be seen, the 20 % infilled sample collapsed easily and failed to build a proper cube shape. It indicates that 20 % infill density is insufficient to support the outer shell, leading to a poor shape-forming capacity. 60 % infill density was enough to form a cube-like shape. However, it looked crooked in the front view after printing, demonstrating that reducing the infill density decreases the printing stability. In summary, reducing infill density was not suitable for printing a 20C sample. The optimal infill density should be 100%. The results differ from Huang et al. (2019) and Mantihal et al. (2019), indicating that the relationship between printability and infill density also depends on the characteristics of food inks. Food inks in both studies formed stable outer shells. That is why the infill density did not significantly influence the printability in their studies. The infill density could be reduced only if the stability of outer shells is ensured. The printing of a hollow cylinder or square column is a method which would help understand the stability of a printed outer shell. This method has been used by Zhu et al. (2019) to test the printing stability of tomato puree. It could be simulated and applied in a future experiment to create stable outer shells of printed products by using plant protein materials and chicken.

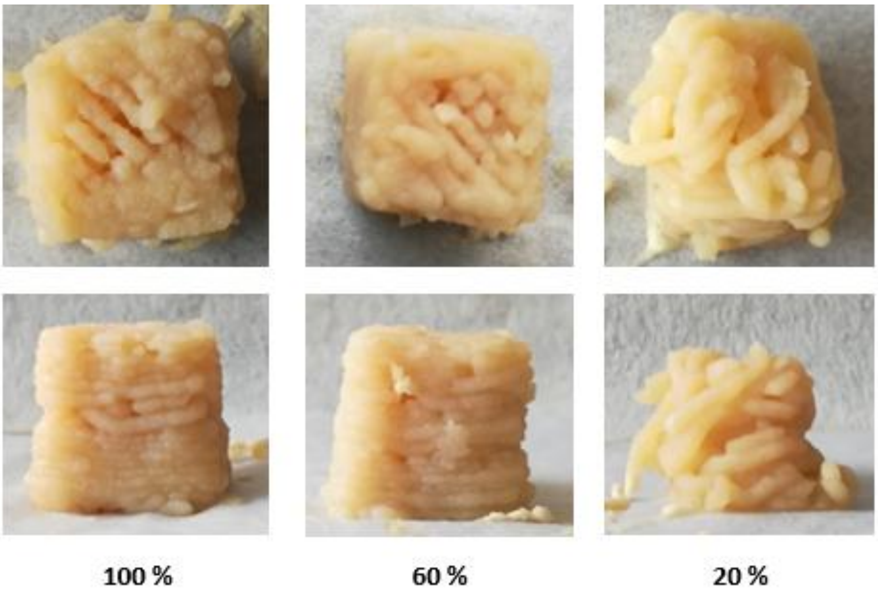


Figure 36. Printed 20C meat analogues at different infill densities (1.54 mm nozzle, 10 mm/s).

In the figure, 100, 60 and 20 % shown in the figure represent the infill density of printing objects.

4.9.3 Further printing process with optimized parameters

According to the discussion above, a chicken nugget geometric shape was printed from a 20C sample through a 1.54 mm nozzle with 10 mm/s and 100 % infill density (Figure 37). Compared with the printed 20C sample in Section 4.4.1, there were fewer broken deposition lines since the paste was extruded smoothly.

Figure 37. Printed 20C meat analogue with a nugget shape at optimized parameters (1.54 mm nozzle, 10 mm/s & 100 % infill).

By using the optimized printing parameters, a chicken drumstick model was also printed (Figure 38). Slight printing defects existed in the printed product, but the general shape was able to be formed. There was some apparent damage to the shape after cooking. This is because a heat-sealed bag is not able to prevent shape damage to printed samples with a complex structure. The development of a post-printing processing method minimizing shape damage to 3D printed meat analogues is needed.

Figure 38. Printed 20C meat analogue with a drumstick shape at optimized parameters (1.54 mm nozzle, 10 mm/s & 100 % infill).
Raw (left), cooked in boiling water for 10 min (right).

Chapter 5. Conclusion and recommendations

5.1 Conclusion

This study investigates to print 3D meat shapes (small nugget and chicken drumstick) by using plant-only and plant-meat-based formulations. PPI combined with maize starch, beef fat and water, expressed suitable rheological properties for extrusion-based 3D printing. Both PPI paste and PPI chicken paste showed a weak gel behaviour according to rheology tests. The addition of maize starch increased the viscosity of food paste, while it minimized the moduli change during temperature sweep. The combination of chicken and PPI-based paste reduced the viscosity change with the increasing shear rate. The addition of raw chicken paste to cooked PPI–based paste was recommended since it provided a suitable flow behaviour. Forward extrusion tests helped understand the general extrusion difficulties of different samples. However, the correlation between the extrusion force and other characteristics was not identified in this study.

Printed meat analogues showed a softer texture compared with conventionally cooked chicken meat. The hardness of all printed meat analogues reached the standard of soft meal as defined by IDDSI, which potentially benefits elderly and dysphagia patients. However, the fibrous structure was not clearly observed in PPI only sample. It is recognized that fibres in printed hybrid meat analogues are contributed by native chicken muscle fibres. The evidence was shown by various microscopies that samples with a high amount of chicken exhibit well aligned fibres. Nevertheless, the addition of a high amount of chicken decreased the extrudability, which also negatively influenced the printing performance, resulting in less fibre shown in the macrostructure. The printing performance would potentially affect the springiness and cohesiveness of printed products. Therefore, the addition of 20 % chicken was selected for an optimal formulation. In addition, a 1.54 mm nozzle diameter with a 10 mm/s printing speed and a 100 % infill density was found to be the optimal combination to conduct the printing process in this study. Cooking in nonvacuum sealed bag in a boiling water bath prevented the changes in the shape of printed samples. However, the edge of the printed meat analogues was still slightly damaged due to the contact with the sealing bags. Through protein solubility assay, it is known that the hydrogen bonding is the major protein interaction that helps support the frame structure of printed meat analogues. The disulphide bonding is considered as the main interaction related to fibrous structure. Therefore, hydrogen bonding, disulphide bonding and hydrophobic interactions play an important role during the production of meat analogues.

5.2 Recommendations for future works

Current study shows that plant-based meat analogues made of pea protein isolate (PPI) exhibit a proper printability. However, the optimal printing speed found in this study is slow. Thus, it is important to develop some methods which ensure printing precision with a high printing speed. The methods would include but are not limited to modifying printing parameters, printer configurations and CAD models of products. Overcoming food printing speed constraint would assist to promote 3D printing technology to processing industries.

Premium chicken mince was used in this study, which aimed to test the extrudability of hybrid paste with both plant and animal proteins. For any future research, the use of low-quality chicken in the formulation is suggested, which would fit the propose of reducing food waste. It is also recommended to explore feasible and new plant protein sources which would contribute to the flexibility of utilising more plant based printable sources. The effect of adding food grade binding agents to modify rheological and gelling properties is also recommended. Furthermore, a deeper understanding of fibre formation mechanisms and food microstructure is needed, which would help to select suitable ingredients and identify the optimal printing parameters and cooking method.

As a food product, sensory properties are always important. It is highly recommended to conduct a sensory test for 3D printed meat analogues. The similarity of printed meat analogue to real meat or meat-based paste should be focused on. The panel members for sensory analysis may also include persons with mastication issues. The feedback of sensory panels would provide useful advice to improve the formulation. In addition, digestion issues should be considered. Therefore, it is meaningful to carry out experiments on digestibility of printed meat analogues. Aside from these intrinsic characteristics, it is also important to understand consumers' opinions on meat analogues, hybrid meat and 3D printed food.

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Appendices

I. Experimental pictures and descriptions

i. Manual extrusion performance of samples

As described in section 3.3.1, canned chicken and Pea protein isolate (PPI) paste expressed the most desirable deposition after manual extrusion. The depositions of these two samples are shown in Figure Appendix IA. As can be seen, both of them form 3D shapes based on the accumulation of deposition lines. Thus, these two samples were selected for the printing feasibility tests through a 3D printer.

Figure Appendix IA. Simple depositions of canned chicken (left) and PPI paste (right)

ii. Printing feasibility tests on a 3D printer

Printing feasibility tests of canned chicken and PPI paste were carried out through a large volume extrusion (LVE) 3D printer. Such LVE printer was tested previously that it was able to print food products like Nutella (Figure Appendix IB).

Figure Appendix IB. Printed Nutella with a hexagonal column shape.

The same hexagonal column shape model (Figure Appendix IC) was applied and sliced through Repetier-host to print canned chicken and PPI paste. The results are shown in Figure Appendix ID. As can be seen, canned chicken splashed and could not deposit properly during printing. However, PPI paste was able to form a 3D hexagonal column. It indicates that PPI paste was more suitable to be printing material compared with canned chicken.

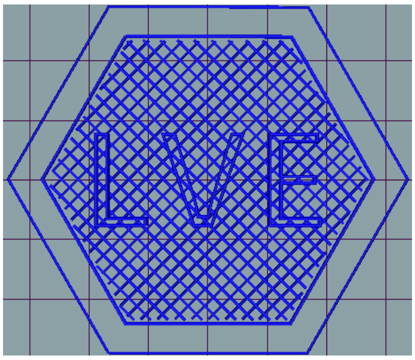

Figure Appendix IC. A 3D model of hexagonal column with letters LVE on the top, sliced by Repetier-host.

Figure Appendix ID. Printing performance of canned chicken (left) and PPI paste (right) through a LVE 3D printer.

II. Graph of texture profile analysis (TPA) obtained from Exponent

The time-force curve was obtained from software Exponent (Figure Appendix II). As can be seen, different areas were divided by 6 lines in the graph. Hardness was measured as the peak force in the graph. Springiness, cohesiveness and chewiness were calculated based on formulas below.

$$\text{Springiness} = \text{Distance 2} / \text{Distance 1}$$

$$\text{Cohesiveness} = \text{Area 2} / \text{Area 1}$$

$$\text{Chewiness} = \text{Hardness} \times \text{Cohesiveness} \times \text{Springiness}$$

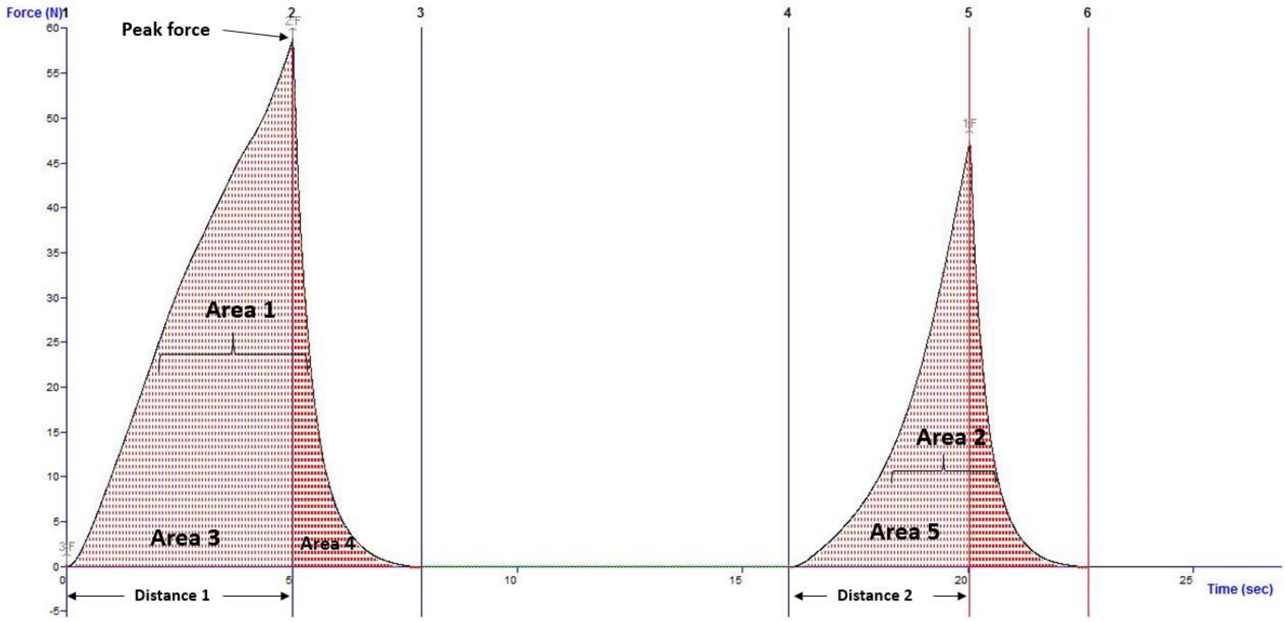

Figure Appendix II. Graph of the texture profile analysis (TPA) of the tested food sample, obtained from Exponent.

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