UNIVERSITAT POLITÈCNICA DE CATALUNYA BARCELONATECH

Personalized meal by using different proteins based on 3D printing

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ABSTRACT

Currently, the use of 3D printing technology in the food sector is being considered, which can improve or displace some of the traditional methods of food manufacturing. The 3D printing market has grown significantly and is poised to grow in line with the digitization and robotization of manufacturing. This technology can allow relocation of food preparation and personalize diets adapting them to the personal needs of everyone (malnutrition, swallowing problems, diets for the elderly, athletes, children, pregnant women...). On the other hand, we find ourselves with the problem of the scarcity of resources due to population growth and obtaining food through current production systems that will need to be revised to reduce the carbon footprint and energy consumption. Another factor to consider is also the waste generated during production, transformation and consumption. The use of personalized nutritional formulations from printable ingredients could be a solution, although it is still a challenge.

The main objective has been to design different formulations of protein-enriched mashed potatoes for personalized diets. For this, different formulations of mashed potato enriched with soy protein (SPAH) were designed at different concentrations (3%, 5% and 7%) together with 0.2% agar to study their ability to be printable for using by people with dysphagia. In addition, the effect of different proteins (soy, cricket and egg albumin) at concentrations of 3% and 5% in the formulation was evaluated through the evaluation of their rheological characteristics (viscosity, thixotropy, creep and viscoelastic behavior), textural characteristics (TPA (Texture Profile Analysis), force of extrusion and back-extrusion), ability to be printed and sensory characteristics (hedonic and sensory evaluation).

Of all the formulations tested, it was obtained that the best capacity to be printed was achieved in the sample with 5% soy protein and 0.2% agar, which presented values of yield stress (1489 Pa) and storage modulus (1352 Pa. s). All the samples of puree enriched with SPAH presented a noticeable increase in viscosity and elastic limit, which favors its impression. According to the IDDSI (International Dysphagia Diet Standardization Initiative) test, all samples (with SPAH and agar) were suitable for use in people with dysphagia problems. On the other hand, it was observed in all the formulations used that not only the rheological properties such as yield stress and viscoelastic behavior, but also the texture test, such as force of extrusion and back-extrusion, can be correlated with printability. The force of extrusion test with firmness and consistency results in a minimum range of (0.03 N) and (0.40 N·s) respectively, allow favorable 3D printing. Of the formulations used, those containing soy and cricket protein with values of firmness and consistency between the range of (0.03 – 0.04 N) and (0.40 – 0.52 N·s), respectively, presented great stability even for complex shapes. while the formulations with egg albumin could not achieve this stability due to the fact that the interlocking networks were very weak, presenting significantly lower rheological and textural values. The puree enriched with cricket protein and SPAH showed good mechanical properties for printing and self-supporting, but SPAH protein was not acceptable due to the taste and odor induced by the acid hydrolysis process, but in most cases the attributes sensory (firmness, thickness, smoothness, rate of breakdown, adhesive, and difficulty swallowing) were positively correlated with instrumental parameters and 3D printing. In conclusion, in this thesis, it has been shown that the enrichment of mashed potatoes with different proteins (soy, cricket) for 3D printing is feasible. This allows the customization of fortified foods for people with special needs.

Keywords: Personalized diet; fortified food; nutrition; protein; rheology; texture; IDDSI; dysphagia; sensory evaluation

RESUMEN

El mercado de la impresión 3D ha crecido significativamente y está preparado para crecer en línea con la digitalización y la robotización de la fabricación. Su uso en el sector alimentario puede dar lugar a grandes cambios tanto desde el punto de vista tecnológico como en el de fabricación. El uso de esta tecnología podría permitir la deslocalización de la preparación de los alimentos y la realización personalizada de dietas, adaptándolas a las necesidades personales de cada consumidor (desnutrición, problemas de deglución, dietas para personas mayores, deportistas, niños, mujeres embarazadas...). Por otro lado, nos encontramos con problema de escasez de recursos por el crecimiento continuado de la población y que la obtención de alimentos a través de los sistemas de producción actuales comporta importantes efectos sobre el medioambiente, especialmente relevantes, sobre la huella de carbono y a nivel de consumo energético. Otro factor a considerar también son los residuos generados durante la producción transformación y consumo. El uso de formulaciones nutricionales personalizadas a partir de ingredientes imprimibles podría ser una solución, aunque no deja de ser un desafío.

El objetivo principal ha sido diseñar diferentes formulaciones de pure de patata enriquecido con proteína para dietas personalizadas. Para ello, se diseñaron diferentes formulaciones de puré de patata enriquecido con proteína de soja (SPAH) a diferentes concentraciones (3%, 5% y 7%) conjuntamente con agar al 0,2% para comprobar su aptitud a ser imprimibles para su uso en personas con disfagia. Además, se evaluó el efecto de diferentes proteínas (soja, grillo y albúmina de huevo) a concentraciones del 3% y 5% en la formulación a través de la evaluación de sus características reológicas (viscosidad, tixotropía, fluencia y comportamiento viscoelástico), texturales (TPA (Texture Profile Analysis), fuerza de extrusión y retroextrusión), capacidad de ser impresa y sensoriales (evaluación hedónica y sensorial).

De todas las formulaciones ensayadas, se obtuvo que la mejor capacidad para ser impresa se logró en la muestra con un 5 % de proteína de soja y un 0,2 % de agar, que presentó valores de fluencia (1489 Pa) y módulo de almacenamiento (1352 Pa·s). Todas las muestras de puré enriquecidas con SPAH presentaron un incremento notorio de la viscosidad y de límite elástico, lo que favorece su impresión. Según la prueba IDDSI (International Dysphagia Diet Standardisation Initiative), todas las muestras (con SPAH y agar) eran adecuadas para su uso en personas con problemas de disfagia. Por otro lado, se observó en todas las formulaciones utilizadas que no solamente las propiedades reológicas como fluencia y comportamiento viscoelástico, sino que también la prueba de textura, como fuerza de extrusión y retroextrusión, pueden ser correlacionadas con la capacidad de impresión. La prueba de fuerza de extrusión con resultados de firmeza y consistencia en un rango mínimo de (0,03 N) y (0,40 N·s) respectivamente, permiten la impresión 3D favorable. De las formulaciones utilizadas las que contenían proteína de soja y grillo con valores de firmeza y consistencia entre el rango de (0,03 – 0,04 N) y (0,40 – 0,52 N·s) respectivamente presentaron gran estabilidad incluso para formas complejas, mientras que las formulaciones con albúmina de huevo no pudieron lograr esta estabilidad debido a que las redes de entrelazamiento eran muy débiles, presentando valores reológicos y texturales significativamente inferiores. El puré de patata enriquecido con proteína de grillo y SPAH mostraron buenas propiedades mecánicas para la impresión y autosoporte, pero la proteína SPAH no fue aceptable debido al sabor y olor inducido por el proceso de hidrólisis ácida, pero en la mayoría de los casos los atributos sensoriales (firmeza, viscosidad, suavidad, fracturabilidad, adhesividad, y dificultad para la deglución) se correlacionaron positivamente con los parámetros instrumentales y la impresión 3D. En conclusión, en esta tesis se ha demostrado que es viable el enriquecimiento del puré de patata con diferentes proteínas (soja, grillo) para su impresión en 3D. Lo que permite la personalización de alimentos fortificados para personas con necesidades especiales.

Palabras clave: Dieta personalizada; alimentos fortificados; nutrición; proteína; reología; textura; IDDSI; disfagia; evaluación sensorial

RESUM

El mercat de la impressió 3D ha crescut significativament i està preparat per créixer en línia amb la digitalització i la robotització de la fabricació. El seu ús al sector alimentari pot donar lloc a grans canvis tant des del punt de vista tecnològic com en el de fabricació. L'ús d'aquesta tecnologia podria permetre la deslocalització de la preparació dels aliments i la realització personalitzada de dietes adaptant-les a les necessitats personals de cada consumidor (desnutrició, problemes de deglució, dietes per a gent gran, esportistes, nens, dones embarassades...). D'altra banda, ens trobem amb un problema d'escassetat de recursos pel creixement continuat de la població i que l'obtenció d'aliments a través dels sistemes de producció actuals comporta importants efectes sobre el medi ambient, especialment rellevants, sobre la petjada de carboni i a nivell de consum energètic. Un altre factor que cal considerar és també els residus generats durant la producció transformació i consum. L'ús de formulacions nutricionals personalitzades a partir d'ingredients imprimibles podria ser una solució, encara que no deixa de ser un desafiament.

L'objectiu principal ha estat dissenyar diferents formulacions de puré de patata enriquit amb proteïna per a dietes personalitzades. Per això, es van dissenyar diferents formulacions de puré de patata enriquit amb proteïna de soja (SPAH) a diferents concentracions (3%, 5% i 7%) conjuntament amb agar al 0,2% per comprovar la seva aptitud a ser imprimibles per al seu ús en persones amb disfàgia. A més, es va avaluar l'efecte de diferents proteïnes (soja, grill i albúmina d'ou) a concentracions del 3% i 5% en la formulació a través de l'avaluació de les seves característiques reològiques (viscositat, tixotropia, fluència i comportament viscoelàstic), texturals (TPA (Texture Profile Analysis), força d'extrusió i retroextrusió), imprimibilitat i sensorials (avaluació hedònica i sensorial).

De totes les formulacions assajades, es va obtenir que la millor imprimibilitat es va aconseguir a la mostra amb un 5% de proteïna de soja i un 0,2% d'agar, amb de fluència de (1489 Pa) i de mòdul d'emmagatzematge de (1352 Pa·s). Totes les mostres de puré enriquides amb SPAH van presentar un increment notori de la viscositat i de límit elàstic, cosa que n'afavoreix la impressió. Segons la prova IDDSI (International Dysphagia Diet Standardisation Initiative), totes les mostres (amb SPAH i agar) eren adequades per al seu ús en persones amb problemes de disfàgia. D'altra banda, es va observar en totes les formulacions utilitzades que no només les propietats reològiques com fluència i comportament viscoelàstic, sinó que també la prova de textura, com força d'extrusió i retroextrusió, poden ser correlacionades amb la capacitat de impressió. La prova de força d'extrusió amb resultats de fermesa i consistència en un rang mínim de (0,03 N) i (0,40 N·s) respectivament, permeten la impressió 3D favorable. Les formulacions utilitzades les que contenien proteïna de soja i grill van presentar valors de fermesa i consistència entre el rang de (0,03 – 0,04 N) i (0,40 – 0,52 N. S) respectivament, i van presentar gran estabilitat fins i tot per a formes complexes, mentre que les formulacions amb albúmina d'ou no van poder assolir aquesta estabilitat pel fet que les xarxes d'entrellaçament eren molt febles, presentant valors reològics i texturals significativament inferiors. El puré de patata enriquit amb proteïna de grill i SPAH van mostrar bones propietats mecàniques per a la impressió i autosuport, però la proteïna SPAH no va ser acceptable a causa del sabor i l'olor induït pel procés d'hidròlisi àcida, però en la majoria dels casos els atributs sensorials (fermesa, viscositat, suavitat, fracturabilitat, adhesivitat, i dificultat per a ser empassat) es van correlacionar positivament amb els paràmetres instrumentals i la impressió 3D. En conclusió, en aquesta tesi s'ha demostrat que és viable l'enriquiment del puré de patata amb diferents proteïnes (soja, grill) per imprimir-les en 3D. Això permet la personalització d'aliments fortificats per a persones amb necessitats especials.

Paraules clau: Dieta personalitzada; aliments enriquits; nutrició; proteïnes; reologia; textura; IDDSI; disfàgia; avaluació sensorial

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Chapter 1. Introduction

1.1. Swallowing difficulties

The number of studies for implementation and evaluation of food for the elderly and people with dysphagia is raising a lot knowing that the population ages 65 and over is expected to grow very rapidly in all parts of the world. Over the next decades, the elderly population is projected to grow much more quickly than the total population in all parts of the world. At the global level, this increase is from just under 800 million in 2011 (representing 11% of the world population) to just over 2 billion in 2050 (representing 22% of the world population) (Noroozian, 2012). As the number of older people continues to rise, the provision of improved healthcare to the elderly both in hospitals and in the community is imperative. Often, the focus of nutrition in older adults is a healthy diet and exercise to minimize the risk of developing lifestyle diseases (such as cardiovascular disease, and type 2 diabetes mellitus). However, there is a large body of evidence to indicate that protein-energy malnutrition is a common problem in this age group, including in the hospital, nursing homes, and community settings (Agarwal *et al.*, 2013). The risk of malnutrition is increased in the elderly because of insufficient food intake and debilitating diseases. It not only increases the susceptibility to the development of diseases but also decreases the quality of life in the absence of proper intervention (Abdollahzade *et al.,* 2018). Malnutrition is considered a frequent and serious problem in the elderly (Meijers et al., 2010).

As mentioned before with the trend of population aging, the elderly population suffering from chewing/swallowing problems is increasing rapidly, as well. This is because the elderly often experiences loss of teeth, decreased strength of oral or tongue muscles, and weakness of other physiological functions. Dysphagia can easily cause coughing, choking, or swallowing initiation difficulty because of the food residue left in the oral cavity (Alagiakrishnan *et al.*, 2013). According to the most conservative estimates, this problem affects approximately 8% of the world's population (Cichero *et al.*, 2013). Dysphagia can be a consequence of a multitude of neurological, muscular, and structural pathologies or can even be drug-induced. It can lead to very serious complications, including malnutrition and dehydration as well as severe respiratory problems, such as aspiration pneumonia, resulting from the aspiration of food or fluids into the airways. It leads patients to dehydration, malnutrition, and aspiration pneumonia, besides depression and deterioration in the quality of life (Shaker, 2006).

Disorders of feeding and swallowing are serious and potentially fatal problems in children. Among infants, particularly those born prematurely, aspiration due to dysphagia may lead to severe pulmonary compromise, and impaired oral and pharyngeal function may rapidly result in failure to thrive. In other children, avoidance behaviors and inadequate intake may lead to chronic malnutrition. Prompt and thorough evaluation of swallowing disorders is therefore critical if complications are to be avoided (Darrow & Harley, 1998). Children with swallowing problems are at risk for malnutrition, behavioral delay, and stressful interaction with caregivers and are at higher risk of post-operative complications (Arvedson, 1998).

To provide all types of people with swallowing difficulties and people with dysphagia patients with safe food, currently, the dysphagia-oriented diet is usually in pureed/mashed or thick liquid state, which is poor attractive and not easy to provoke appetite. As a result, chew or swallow impaired patients are easy to experience nutritional deficiency and weight loss due to less food choice and consumption (Tokifuji *et al.*, 2013). Therefore, the development of dysphagia-oriented safe foods with adequate nutrition and appetizing appearance/texture is a great challenge (Bannerman & McDermott, 2011). There is a great demand for the development of appetite-provoking foods in terms of appearance, flavor, and texture, meanwhile with enough nutrition and safe to swallow.

1.2. 3D food printing

Three Dimensional Food Printing (3DFP), also known as Food Layered Manufacture (Wegrzyn *et al.*, 2012), is a digitally controlled, robotic construction process that can build up complex 3D food products layer by layer (Huang *et al.*, 2013). The first-generation food printer concept designs were introduced to the general public more than 10 years ago (Yang *et al.,* 2001). Food printing is a digital food fabrication process integrating 3D printing and digital gastronomy technique to manufacture food pieces. It allows users to design and fabricate food with customized color, shape, flavor, texture, and even nutrition. As a result, our eating experiences can go beyond taste to encompass all aspects of gastronomy such as food preparation, culture, economy, physics, and chemistry (van Bommel & Spicer, 2011). However, adapting the 3D-printing technology in the food sector has generated new challenges, as food materials often consist of many different components with distinct physicochemical properties (Godoi *et al.,* 2016). Several techniques are available for 3D-printing of food including extrusion-based printing, selective laser sintering, binder jetting, and inkjet printing (Liu *et al.*, 2017). This research is done by extrusion-based printing, as this is the most commonly studied method for 3D-printing of food (Liu *et al.,* 2018b). Extrusion-based printing involves a liquid or a semi-solid material being extruded through a nozzle, moving in x-, y-, and z-direction, building up a structure layer-by-layer. One of the advantages of using extrusion-based printing is that a wide range of food materials can be simultaneously extruded to create an entire meal (Lanaro *et al*., 2017). However, the method requires a material with the capability to easily extrude out of the nozzle tip and at the same time be able to support the weight of the next printed layers without deformation (Liu *et al.*, 2018). Printability relates to the ability of a material to make a 3D-object by layer-by-layer deposition and to support its structure once printed (Godoi *et al.*, 2017) . Thus, printability is determined by the physicochemical and rheological properties of a given material among other parameters. Many foods do not have the right properties to be printed without being processed before printing, and quantification of food ingredients' printability is thus an important factor for the development of 3D-printing of food. As printability is affected by several factors including food additives and rheology behavior of the food system quantifying the printability of food ingredients is a complex task. The knowledge about rheological properties of food formula and the optimization of printing parameters are key factors for successfully creating advanced 3D-structures (Lipton *et al*., 2010). The printability of a material is highly dependent on the properties of the food system and the 3D-printer parameters used. Thus, it is necessary to optimize the printability of every new food matrix for 3D-printing. Adjusting the food matrix to achieve a higher quality of the printed structure can be successfully carried out based on rheological properties such as storage modulus, yield stress, consistency and flow behavior index (Yang *et al.,* 2018). 3DFP is being widely investigated due to key advantages such as customized food designs and personalized nutrition. It is expected to contribute towards the design and development of innovative food products available for a wide market with diverse consumers' needs and demands. Personalized nutrition that refers to tailored nutritional recommendations aimed at the promotion, maintenance of health and prevention against diseases and people with special needs (Betts & Gonzalez, 2016).

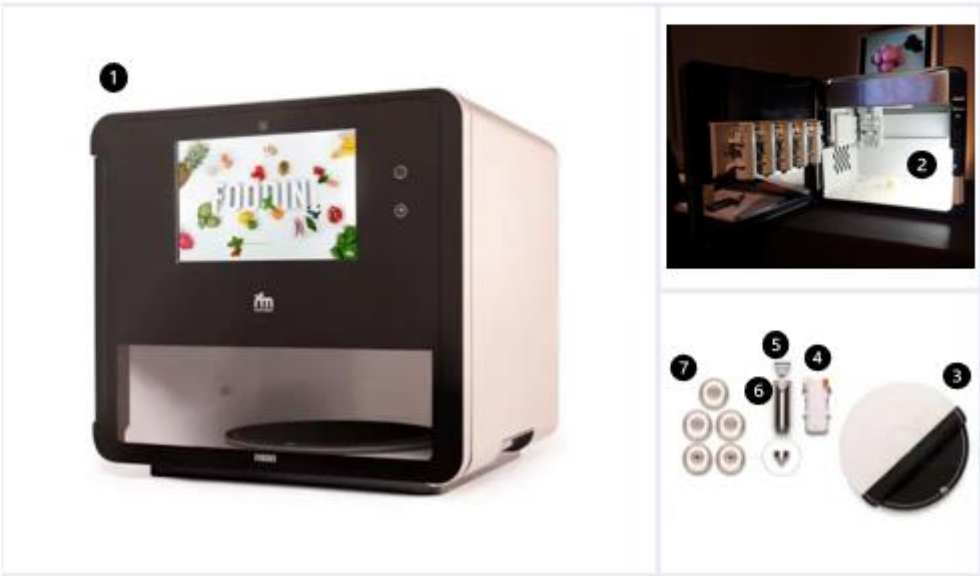


Figure 1. 1. Foodini 3D printer.

Note: 1- 3D printer, 2- 3D printer inside, 3- Dish & silicon mat, 4- Capsule holders, 5- Capsule tags, 6- Capsule bodies, 7- different nozzles

1.3. Novel uses of 3DFP and food personalization

Increasing demand for modern healthy foods due to changing consumers' attitudes toward healthy lifestyles and industrial strategies in line with the principles of sustainable development are driving the research towards the progress of innovative functionalized products (Bachmann, 2001). The food-related fields of production, transformation, distribution, and consumption contribute massively to non-sustainable environment and food systems. Technological advances in food production alone are unlikely to generate the significant conversions required to build a more sustainable food landscape. One of the multiple possibilities offered by 3DFP technology is its capacity to reduce food waste by reusing materials that would otherwise be discarded.

Furthermore, in the future, the widespread availability of 3D food printers would be helpful to improve environmental sustainability (Davies, 2014).

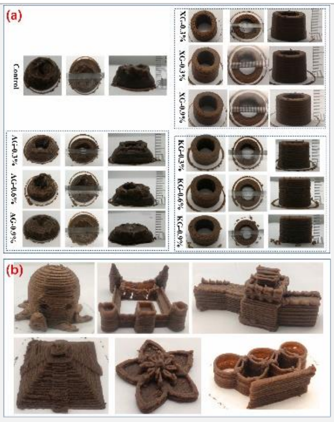
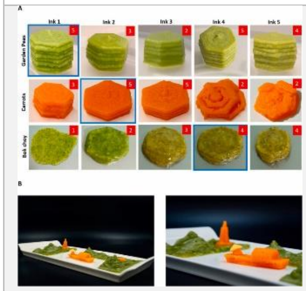
A practical application of 3DFP technology is food customization for an individual's health-nutritional needs, including medicinal and nourishment requirements. In the last decade, personalized medicine, nutrition control, and some therapeutic approaches have been changing. The evolution of technological developments associated with food engineering, food processing, and consumption patterns is evolving. Moreover, 3DFP is developing an enormous market potential with a customized approach to tailoring food to individual needs and for personalized nutrition. The organoleptic acceptability and neurotherapeutic possibilities offered by 3D products are related to their geometric complexity, extended shelf life, and mass customization. These are critical areas that provide the advantages of 3DFP over other techniques (Baishakhi *et al.,* 2019). For the entire creation of customized foods, it is necessary to use materials that are large enough to satisfy all consumer requests or small materials that can be combined at varying proportions. Consequently, food printing is a method that requires food distribution in a personalized way to satisfy nutritional needs. 3D printing can provide a personalized diet guidance that is individualized for different persons according to their health, body condition, specific necessities, and preferences (Escalante-Aburto *et al.,* 2021).

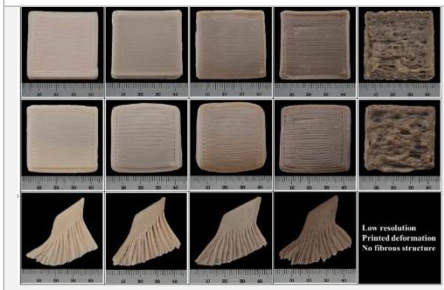
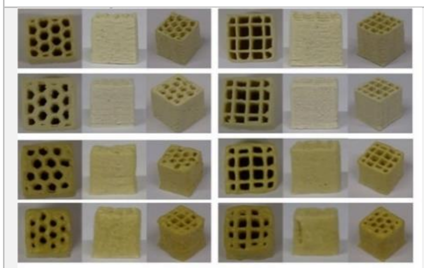
1.4. Treatment of swallowing disorders

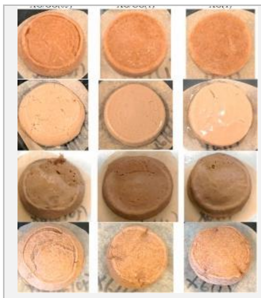
Dietary illnesses, such as digestive problems (from mouth to anus), allergies, and intolerances, lead to unnecessary hospitalizations due to the lack of adherence to dietary and pharmaceutical treatments (Lipton *et al.,* 2017). The personalization of 3DFP could meet the nutritional recommendations related to restricted food regimens, probably reducing complications and hospitalizations. In addition, 3D technology has also been applied to design foods for people with swallowing disorders (Gudjónsdóttir *et al.,* 2019). It can help people with dysphagia and those who support them. It also represents a new area for collaboration between food engineering and health. The development of these products for populations with specific disorders such as dysphagia should contemplate the provision of safe and enjoyable meals. Individually tailored food to cover special nutritional needs must consider the consumers' age, health status, allergies or intolerances, and comorbidities, among others (Hemsley *et al*., 2019). Perhaps, food consistency and texture are the most crucial issues to consider while developing 3D-printed foods for patients with dysphagia. Inappropriate food textures can cause choking and death in people with swallowing diseases due to unsupervised consumption (Hemsley *et al*., 2015). In the same context, the improvement of inks to produce 3D-printed foods to treat dysphagia disorders has been studied to develop personalized 3D-printed foods that meet the International Dysphagia Diet Standardization Initiative categories. These 3D-printed foods can be distributed or prepared in hospitals, nursing homes, and daycare centers that attend to elderly populations and patients with related pathologies. Promising results were obtained above all, textural properties and qualitative measurements are helpful tools to specify 3D-printed foods models because they involved evaluations of objective parameters and also from individuals with dysphagia (Pant *et al.*, 2021).

Protein is an indispensable nutrient required by the human body, and it is widely present in various foods. But nowadays, people have few options to get high-quality protein mainly for people having swallowing difficulties or the case of children that dislike some protein-rich foods. For this reason, getting enough protein is very important for these two groups of people. Therefore, the use of 3D printing technology to customize food nutrition has become very meaningful and necessary. In 3D food printing, a good printability of printing ink is a prerequisite for the application and development of this material. In extrusion-based 3D printing, the rheological properties of the printing ink are critical to successful printing, and this depends on the ink formulation (Zhu *et al*., 2019). Currently, a way of reducing the risk of choking and aspiration is by means of texture-modified diets. consisting of thickened fluids, and minced, pureed, or bite-sized foods, which can be unpleasant and distressing when eating among others. 3D printing enables the creation of foods with an appealing appearance and personalized nutrition based on individual requirements and personalized diets. Different people have different dietary needs, and people's dietary guidance can be tailored to them specifically in a reasonable and science-based way (Vasseur et al., 2021). The extrusion-based printing technique is very suitable for the elderly and people with swallowing difficulties, table 1.1 shows many products that have already been developed by this method.

Table 1. 1. Studies of food development for dysphagia by 3D printing in last 2 years.

3D printing Design	Base	Hydrocolloid	References
	Black fungus	*k*-carrageenan gum, xanthan gum, and arabic gum.	(Xing *et al.*, 2022)
	Potato puree	Soy protein Agar	(Mirazimi *et al.* , 2022)
	Garden pea, carrot, and bok choy	Xanthan gum, kappa carrageenan, and locust bean gum	(Pant *et al.*, 2021)

	Shiitake mushroom	Arabic gum, Xanthan gum, and k-carrageenan gum	(Liu *et al.*, 2021)
	Chicken surimi	Mealworm protein isolate	(Chao *et al.*, 2022)
	Pork paste	Xanthan gum and Guar gum	(Dick *et al.*, 2020a)
	Protein, Starch, and Fiber extracted from faba beans		(Paucean *et al.*, 2022)
	Banana	Pea protein isolate	(Kim *et al.*, 2021)

	Beef paste	Xanthan gum and Guar gum	(Dick *et al.,* 2021)

Chapter 2. Objectives

2.1. Objectives

In the present work, we prepared different formulations with different proteins (soy protein acid hydrolyzed (SPAH), cricket protein, and egg albumin) and SPAH with agar mixtures to print various geometries by an extrusion-based 3D printer and evaluation of the rheological, textural, and sensorial properties.

To achieve this objective, the following intermediate steps were set:

1. Characterize the suitable printed food for people with dysphagia.

2. Investigate the rheological properties and 3D printability of different mixtures.

3. Understand the effect of sieving on the characteristic of the puree products before and after printing.

4. Evaluate the texture characteristics of puree food with new methods.

5. Preparing the nutritional printed formulations to persuade people about the convenience of a personalized diet by 3D food printing technology.

6. Understand the composition of different proteins on printability with physicochemical analysis.

7. Examine the sensorial evaluation of different formulations.

8. Find the correlation between instrumental and sensorial evaluation for 3D printed fortified puree.

2.2. Working plan

According to the objectives, different tasks were considered for this thesis to investigate which are shown below:

1

- **Task 1:** Design a puree potato enriched with soy protein for dysphagia patients by using 3D printing
- **Objective 1:** Characterize the suitable printed food for people with dysphagia.
- **Objective 2 :** Investigae the rheological properties and 3D printability of different mixtures.
- **Objective 3:** Understand the effect of sieving on the characteristic of the puree products before and after printing.

2

- **Task 2:** Prepare a personalized meal by using soy, cricket, and egg albumin protein based on 3D printing
- **Objective 4:** Evaluate the texture characteristics with new methods.
- **Objective 5:** Prepare the nutritional printed formulations to persuade people about convenience personalize diet by 3D food printing technology.
- **Objective 6:** Understand the composition of different proteins on printability with physicochemical analysis.

3

- **Task 3:** Study the correlation between the instrumental and sensory evaluation of fortified 3D printed potato puree
- **Objective 7:** Examine the sensorial evaluation of different formulations.
- **Objective 8:** Find the correlation between instrumental and sensorial evaluation for 3D printed fortified puree.

2.3. Experiment setup

The experimental setup is shown in figure 2.1. A set of experiments were conducted to accomplish the objectives proposed in the working plan.

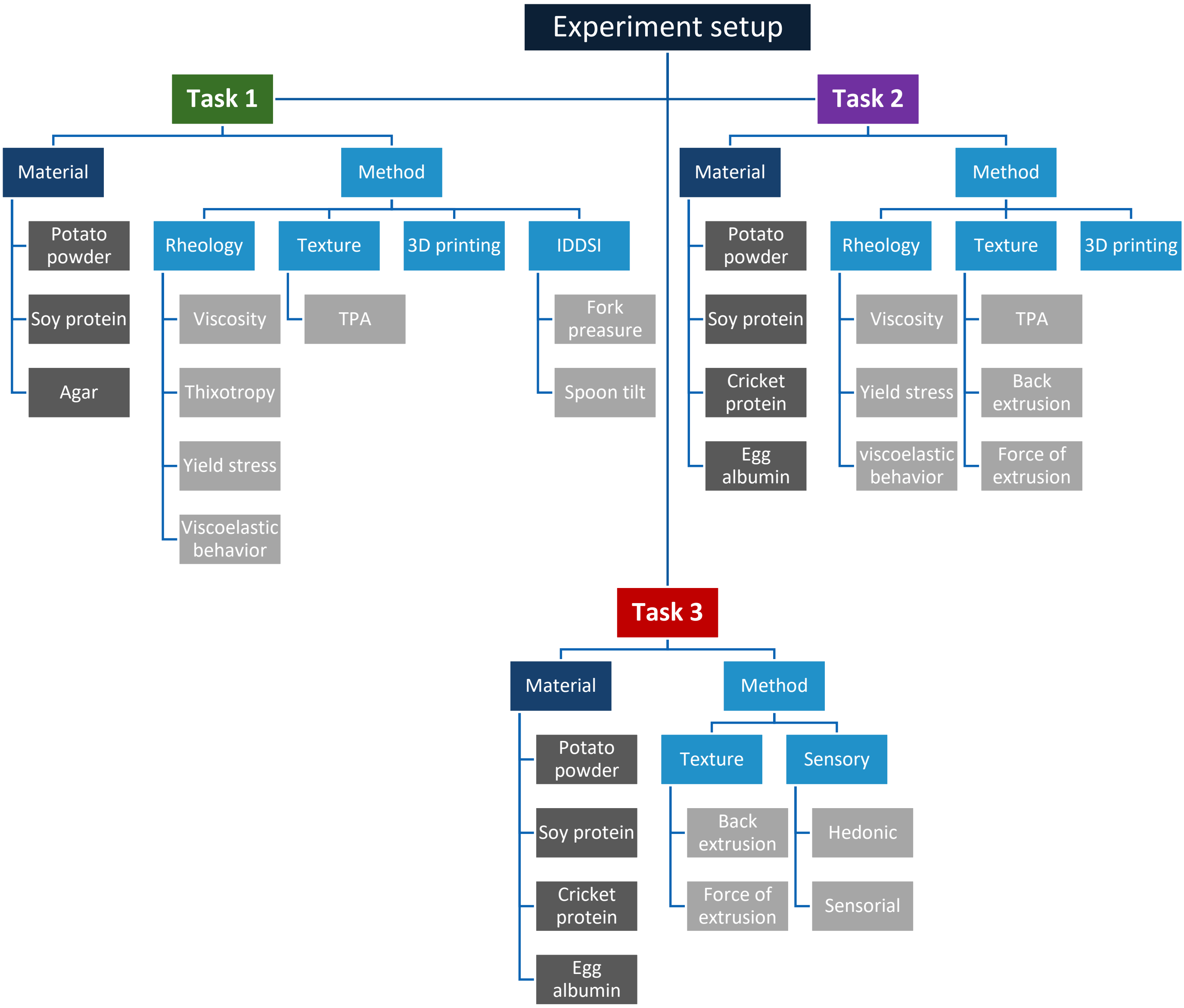


Figure 2. 1. Experiment setup diagram summarizing the data analysis methodology.

Task 1: Effect of protein addition on mashed potato for rheology and 3D printing. Comparison with specific tests for dysphagia.

Task 2: Effect of the addition different proteins on the rheology, 3D printing and texture in potato puree.

Task 3: Effect of protein nature on sensory characteristic.

Chapter 3. Enriched puree potato with soy protein for dysphagia patients by using 3D printing

This chapter is based on the article:

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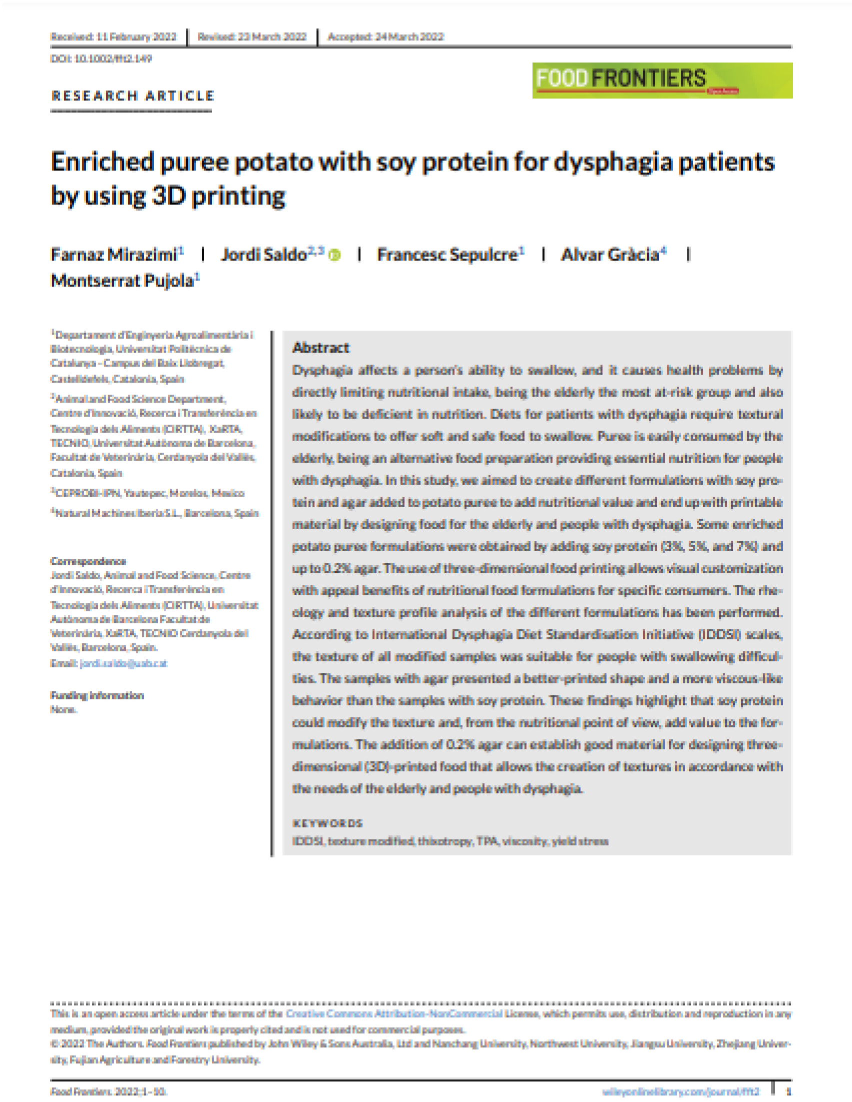

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FOOD FRONTIERS

RESEARCH ARTICLE

Enriched puree potato with soy protein for dysphagia patients by using 3D printing

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Abstract

Dysphagia affects a person's ability to swallow, and it causes health problems by directly limiting nutritional intake, being the elderly the most at-risk group and also likely to be deficient in nutrition. Diets for patients with dysphagia require textural modifications to offer soft and safe food to swallow. Puree is easily consumed by the elderly, being an alternative food preparation providing essential nutrition for people with dysphagia. In this study, we aimed to create different formulations with soy protein and agar added to potato puree to add nutritional value and end up with printable material by designing food for the elderly and people with dysphagia. Some enriched potato puree formulations were obtained by adding soy protein (3%, 5%, and 7%) and up to 0.2% agar. The use of three-dimensional food printing allows visual customization with appeal benefits of nutritional food formulations for specific consumers. The rheology and texture profile analysis of the different formulations has been performed. According to International Dysphagia Diet Standardisation Initiative (IDDSI) scales, the texture of all modified samples was suitable for people with swallowing difficulties. The samples with agar presented a better-printed shape and a more viscous-like behavior than the samples with soy protein. These findings highlight that soy protein could modify the texture and, from the nutritional point of view, add value to the formulations. The addition of 0.2% agar can establish good material for designing three-dimensional (3D)-printed food that allows the creation of textures in accordance with the needs of the elderly and people with dysphagia.

KEYWORDS
IDDSI, texture modified, thixotropy, TPA, viscosity, yield stress

Abstract

Dysphagia is affecting a person's ability to swallow, and it causes health issues and problems by directly limiting nutritional intake. The elderly are the most at-risk group suffering from dysphagia also likely to be deficient in nutrition. Diets for patients suffering from dysphagia require textural modifications to provide them with soft and safe to swallow food. Puree is a common food easily consumed by the elderly that can be an alternative food preparation providing essential nutrition for the elderly with dysphagia. In this study, we aimed to create different formulations with soy protein and agar added to potato puree to add nutritional value and end up with printable material by designing food for elderly and dysphagia-suffering people. Some enriched potato puree formulations were obtained by adding soy protein (3%, 5%, and 7 %) and up to 0.2% agar. The use of three-dimensional food printing allows visual customization with appealing benefits of nutritional designed formulations for specific consumers. The rheology and texture profile analysis of the different formulations has been examined. According to IDDSI scales, the texture of all modified samples was suitable for people with swallowing difficulties. Whereas the samples with agar presented a better-printed shape and a more viscous-like behavior than the samples simply with soy protein. These findings highlight that soy protein could modify the texture and, from the nutritional point of view, add value to the formulations. The addition of 0.2% agar can establish good material for designing food 3D printed that allows the creation of textures in accordance with the needs of the elderly and people with dysphagia.

Keywords: IDDSI, texture modified; thixotropy; TPA; viscosity; yield stress

3.1. Introduction

DYSPHAGIA is an age-related disease and one of the dominant problems for the elderly. Dysphagia is directly linked to nutritional deficiency and expansion risk of pneumonia (Sura *et al.*, 2012) for its impairment of the swallow function. The topic of dysphagia in the elderly has become an increasingly important concern (Aslam & Vaezi, 2013). Approximately one-quarter of elderly patients in care centers had dysphagia whereas almost half were malnourished (Bomze *et al.*, 2021). According to Sitzmann (1990), dysphagia should be viewed as a systemic disease associated with severe malnutrition, with a 13% risk of mortality. Dysphagia can be managed by prescribing texture-controlled diets that seek to modify the consistency of foods and/or drinks by changing the rate at which food is transported through the pharynx and thus decreasing the risk of aspiration (Quinchia *et al.*, 2011). For solid kinds of food, texture is an important factor to be considered because textural changes occur more drastically during oral processing in solid foods than in liquid foods. Rheological properties have a key role to understand about swallowing process, being of paramount importance to be aware of dysphagia (Gallegos *et al.*, 2012).

Protein and starch are used in the formulation of many foods because of textural characteristics and structural gelation behavior. The texture and stability of protein – polysaccharides interactions depend on the properties of protein and polysaccharides But in addition, their interaction will increase the strength (Hemar *et al.*, 2002). In recent years we can find an increasing number of studies on the interaction between starch and polysaccharides in academic and industrial sectors (Quan *et al.*, 2020). Adding Soy protein to potatoes is not just to create a formulation with high nutritional value and have a healthy product, moreover is to enrich a better physiochemical, functional, and sensory characteristic potato product for the elderly and people with dysphagia (Alvarez *et al.*, 2012).

Since just a while ago, 3D printing has gotten high consideration for customized food and personalized meals, and the use of this technology has become very important for people with specific nutritional requirements such as the elderly and people with swallow difficulties (Pérez *et al.*, 2019; Pant *et al.*, 2021). Adapting 3D printing to the food sector induced a new challenge because most food materials often consist of different ingredients with distinct physicochemical properties (Godoi *et al.,* 2016).

Soy protein has multiple health benefits, and because of its high nutritional value and desirable functionality has been included in a wide variety of formulated foods (Kinsella & Whitehead, 1989). Many studies show that consuming soy protein might be the reason for lower incidences of certain diseases (Hagen *et al.*, 2009), and even it is recognized that soy protein at the same time as a low-fat diet can decrease the risk of certain cancer (Nestel, 2002; Lille *et al.*, 2018). Agar is obtained from seaweed and due to its characteristic physicochemical, mechanical, and rheological properties can modify food texture and is useful to control the rheological properties in the design of foods for the elderly. The most important function of agar at low concentration is as a thickening agent (Nishinari *et al*., 2016).

The overall objective of this work was to determine the effect of soy protein and agar on the physicochemical properties of puree potato. The specific objectives of this research were to study the rheological measurements, to characterize the suitable range of formulation ingredients for people with dysphagia, and to create printable nutritional formulations to investigate possible opportunities to personalize food for the elderly with 3DFP and to understand the effect of sieving on the characteristic of the puree products.

3.2. Materials and methods

3.2.1 Sample preparation

Dehydrated potato puree (Maggi, purchased in a local supermarket) has the following nutritional values: energy 348 Kcal, fat 0.8 g, carbohydrates 75 g, fiber 6.8 g, proteins 7.4 g, and salt 0.06 g. Soy Protein acid hydrolysate (SPAH) food-grade was procured from Sigma-Aldrich (Nitrogen analysis ≥12% total= 75% protein, calculated applying a factor of 6.25 g prot/g N. Powdered Agar (food grade) was purchased from Panreac AppliChem.

For all the food formulations the same base (100 mL distilled water plus 17 g potato powder) was used and differed in soy (SPAH) and agar amounts were added regarding the following codes: S3 (3 g Soy), S6 (6 g Soy), S9 (9 g Soy), S3A (3 g Soy and 0.2 g Agar), S6A (6 g Soy and 0.2 g Agar) and S9A (9 g Soy and 0.2 g Agar).

Samples were prepared by boiling 50 ml distilled water to dissolve agar and added with 50 ml distilled water (25 °C) where the corresponding SPAH was incorporated. Subsequently, all samples were placed in an oven at 90 °C and kept for 30 minutes to denature the protein and, as the last step, the dehydrated potato powder (17 g) was added.

The samples were kept overnight (22-24 hours) in a fridge set at 2 °C and all samples were placed in an incubator at 20 °C for two hours before starting the rheological measurements.

3.2.2. Rheological measurements

The rheological measurements were carried out in a rheometer (Rheostress RS1, version 127, Barcelona, Spain) controlled with commercial computer software (HAAKE RheoWin 3 Job and Data Manager Software). Samples were analyzed for their viscoelastic properties using 35-mm plate-plate geometry with a 1 mm gap. After placing the sample and trimming off the excess, it was let to rest for 2 minutes before starting the assay. The temperature was kept at 20.0 ± 0.1 °C during all the assays. A preliminary test was conducted to identify the deformation limits for the linear viscoelastic region.

3.2.3. Frequency sweep test

Oscillatory tests were performed to explain the behavior of material from 0.1 to 10 Hz to understand viscous or elastic dominating properties. Each sample from each of the 16 different formulations and 3 repetitions was measured independently 3 times. Rheological properties can be evaluated by the storage modulus (G′) and loss modulus (G″).

3.2.4. Rotational rheological measurements, thixotropy, and yield stress

The shear rate raised logarithmically from 0.1 to 10 s^{-1} during the first 30 s, kept constant at 10 s^{-1} for 30 s, and finally was cut down logarithmically again to 0.1 s^{-1} over 30 s. For each sample were recorded the viscosity (η) and the shear stress (τ), along with the yield stress (Tabilo-Munizaga & Barbosa-Cánovas, 2005).

3.2.5. 3D Food Printing Conditions

All formulations were 3D printed using a commercial extrusion-based 3D food printer (Foodini®, Natural Machines Iberia S.L., Barcelona, Spain). Samples were printed using a nozzle with a diameter of 1.5 mm, the print speed was set at 3500 mm/minutes, the first ingredient holds at 4.2 mm, the first layer nozzle height was 1.4 mm and at the moment is not possible to control the temperature with Foodini machine, so all the samples printed in a room temperature at 20 °C. Before printing, all the samples were sieved to avoid the presence of lumps that may affect the printing process. One spoon was used to press the samples through the sieve 250-μm (mesh sieve). Then, the stainless-steel printing capsules (100 mL) of Foodini were manually filled with the sieved samples. Food samples must be well pressed down inside printing capsules to avoid the presence of air bubbles.

Each formulation was printed 3 times, either in the form of a pentagon prism for TPA assay or as some complex shapes such as snowflake, mountain, and honeycomb.

3.2.6. IDDSI tests (International dysphagia diet standardization initiative)

IDDSI divided modified food into 8 levels (0–7) and present a combination of tests to confirm to which level texture is modified food for people with swallow difficulties (Cichero *et al.*, 2017). The fork pressure test and spoon tilt test were performed on the sieved samples. The fork test was done by pressing the fork by a thumb until it blanched (pressure of ~17 kPa), resembling the tongue pressure while swallowing. The spoon tilt test was completed for all formulations for experimenting with adhesiveness and cohesiveness (Steele *et al.*, 2014).

3.2.7. Statistical Analysis

Statistical analyses of the data were conducted on Minitab 18 (Minitab link. Coventry, UK). Data concerning Rheology and textural characteristics were tested for significant differences ($p < 0.05$) using analysis of variance, general linear model, and Tukey's comparison test.

3.3. Results

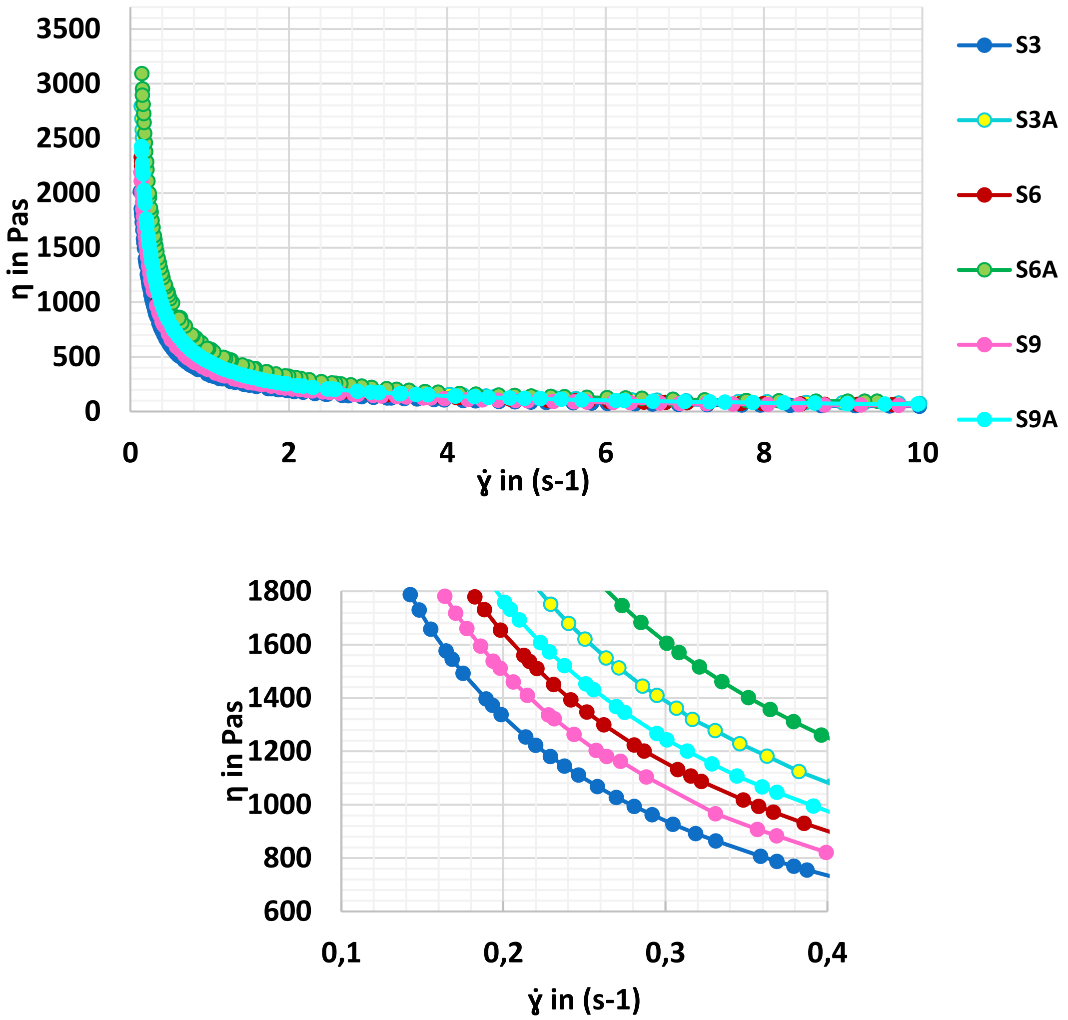
3.3.1. Viscosity

All soy-enriched potato puree samples exhibited a non-Newtonian behavior as can be seen by the exponential decay of the viscosity with the increase of shear strain in Figure 3.1.

Figure 3.1 shows that SPAH and agar had a significant ($p < 0.05$) effect on increasing the viscosity. The samples with different concentrations of SPAH had increased the viscosity at 0.3 s^{-1} shear rate from 3% (S3, 925 Pa·s) to 5% (S6, 1205 Pa·s), but from 5% (S6) to 7% (S9) 1104 Pa·s rate decreased.

To evaluate the effect of sieving the formulation, a step necessary to 3D print it, the rheological properties were evaluated before and after sieving. Sieving slightly decreased the viscosity of samples with agar (Figure 3.1). For instance, the formulation S3A changed its apparent viscosity at 0.3 $^{s-}1$ from 1409 Pa·s to 1289 Pa·s after sieving and also happen to S6A (from 1606 Pa·s to 1458 Pa·s) and S9A (from 1244 Pa·s to 1164 Pa·s). Conversely, the sieving process did not change the viscosity of the formulations without agar, irrespectively their protein content.

(A)


3500
3000
2500
2000
1500
1000
500
0
η in Pas
0
2
4
6
8
10
γ̇ in (s-1)
S3
S3A
S6
S6A
S9
S9A
1800
1600
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(B)

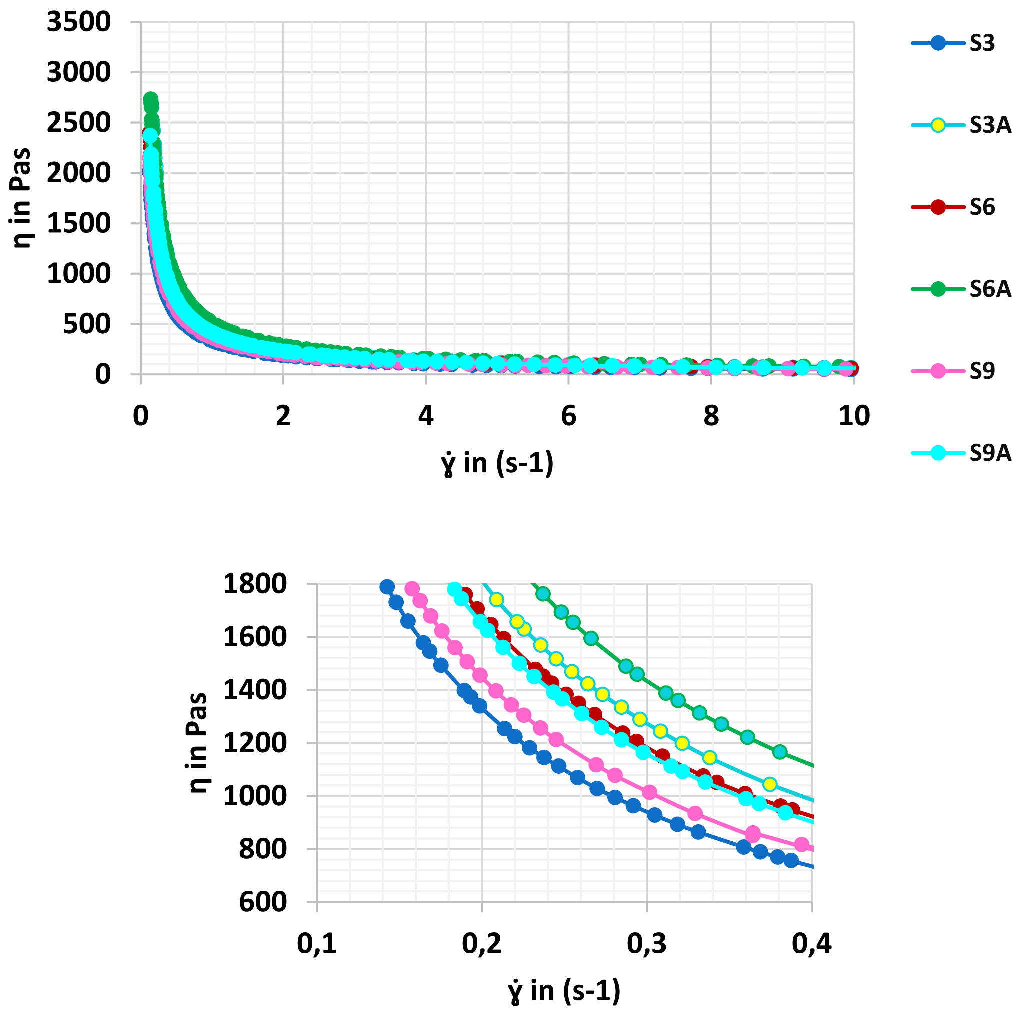


Figure 3. 1. Rheological characterization of food formulations, Viscosity versus Shear rate. Each curve represents the average of three replicates per sample. A) Viscosity before sieving and B) Viscosity after sieving.

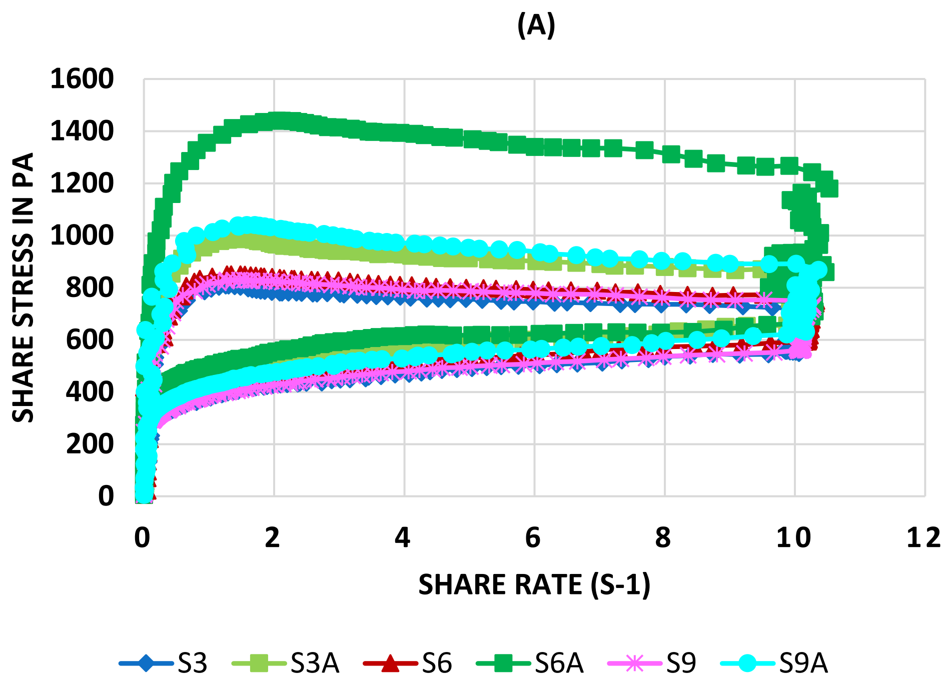
Note: S3 (3 g Soy), S6 (6 g Soy), S9 (9 g Soy), S3A (3 g Soy and 0.2 g Agar), S6A (6 g Soy and 0.2 g Agar) and S9A (9 g Soy and 0.2 g Agar).

3.3.2. Thixotropy

All formulations were studied varying the shear rate to characterize the effects of including SPAH and agar, and to depict the effect of sieving. The results showed hysteresis loops, indicating that all formulations exhibited thixotropic behaviors.

Flow curves obtained with controlled shear stress for all formulations, before and after sieving, are presented in Figure 3.2. The samples showed behavior that differs by the effect of sieving, with a reduction in the shear stress, but more noticeably in the hysteresis area for formulations with agar with a decrease that means less energy needed to destroy the internal structure of the material responsible for the flow time dependence (Tárrega *et al.,* 2004) in the samples that have been previously sieved.

Formulation with 6 g SPAH and 0.2 g agar (S6A) exhibited the highest degree of thixotropy, with the largest hysteresis area indicating stronger the thixotropic properties required the highest energy to break down the internal structure, with high resistance to time-dependent flow and high levels of internal viscosity and stability. However, upon decreasing the shear rate in the last part of the test, all formulations showed the capacity to reform the damaged internal network and to recover their viscosities.



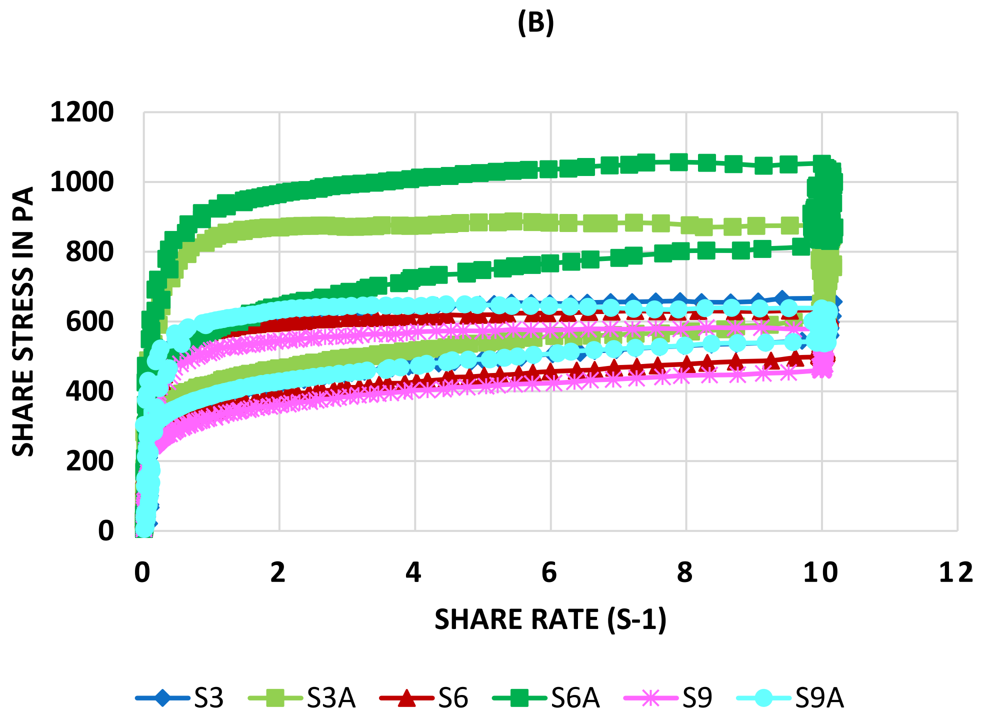


Figure 3. 2. Rheological characterization of food formulations. Shear stress versus shear rate, each curve represents the average of three replicates per sample. A) Thixotropy before sieving and B) Thixotropy after sieving.

Note: S3 (3 g Soy), S6 (6 g Soy), S9 (9 g Soy), S3A (3 g Soy and 0.2 g Agar), S6A (6 g Soy and 0.2 g Agar) and S9A (9 g Soy and 0.2 g Agar).

3.3.3. Yield stress

Many methods have been applied to characterizing the yield stress of food systems (A. Sun & Gunasekaran, 2009). To choose the model that best fits our data, different mathematical equations or models (Power Law, Herschel-Bulkley, Casson, and Bingham models) were tested to select the best performer. Bingham was determined to be the best model to fit the flow characteristics of the samples, with a high coefficient of determination ($R > 0.98$). The Bingham model is described by the equation $\tau = \tau_0 + \eta_p \dot{\gamma}$, where τ (Pa) is the shear stress, τ_0 (Pa) is the yield stress, η_p (Pa·s) is the viscosity and $\dot{\gamma}$ (s^{-1}) is the shear rate.

S6A presented the highest yield stress among the tested formulations (Table 3.1). This high yield can be explained by the effect of both agar and SPAH in the starch internal microstructure, contributing to the elasticity of the network of the potato starch puree and consequently generating a starch internal microstructure that was more resistant to deformation in the shear stress region where the sample is fully elastic.

The yield stress decreased for all sieved samples, showing the increase in the easy-flowing of the materials after passing through a sieve and breaking the bonds and strengths of the structure, especially for the formulations with agar.

Table 3. 1. Rotational rheological measurements, thixotropy, and yield stress, before and after sieving. The yield stress was obtained according to Bingham model $\eta = \eta_\rho + \tau_0/\dot{\gamma}$. The numbers represent the average of three replicates per sample.

Samples	Yield point (Pa)	Thixotropy (Pa·s)	η_ρ (Pa·s)	τ_0 (Pa)	R^2
			BEFORE SIEVING		
S3	638^{d}	4246^{c}	155^{c}	405^{b}	0.99
S6	757^{cd}	5756^{bc}	262^{bc}	453^{ab}	0.99
S9	841^{cd}	5482^{bc}	164^{c}	461^{ab}	0.99
S3A	1084^{b}	7971^{b}	386^{ab}	488^{ab}	0.98
S6A	1489^{a}	15345^{a}	549^{a}	602^{a}	0.98
S9A	950^{bc}	6854^{bc}	211^{bc}	537^{ab}	0.99
			AFTER SIEVING		
S3	623^{c}	3648^{bc}	76^{b}	405^{b}	0.99
S6	743^{bc}	3880^{bc}	128^{b}	406^{ab}	0.98
S9	639^{c}	2537^{c}	85^{b}	409^{ab}	0.99
S3A	881^{b}	6414^{ab}	234^{a}	456^{a}	0.98
S6A	1081^{a}	7360^{a}	315^{a}	475^{a}	0.98
S9A	707^{c}	4888^{abc}	123^{b}	422^{ab}	0.99

Note: S3 (3 g Soy), S6 (6 g Soy), S9 (9 g Soy), S3A (3 g Soy and 0.2 g Agar), S6A (6 g Soy and 0.2 g Agar) and S9A (9 g Soy and 0.2 g Agar). The results with different letters in the same column are different with a significance level of $p < 0.05$

3.3.4. Viscoelastic behavior

The shallowing process is determined by the interactions between the major molecules in thickened fluids and their effects on their extensional properties. These interactions can be elucidated by studying the viscoelastic properties of the food products (Hadde and Chen, 2018; Nishinari *et al.*, 2019). As one of the requirements for dysphagia patients is safe swallowing, the characteristic and gel properties of starch have an important role in the final product quality (Sungsinchai *et al.*, 2019).

Viscoelastic behavior is explained by the level and nature of the leached material and the molecular interactions upon starch granule disintegration in a three-dimensional network structure (Alcázar-Alay & Meireles, 2015). The storage or elastic modulus G' measures the recovered or accumulated energy in each deformation cycle and determines the elastic behavior of the samples. The loss or viscous modulus G" describes the loss of energy or dissipated energy in each deformation cycle, which describes the viscosity behavior of the material. Table 3.2 shows that all formulations exhibit larger G' than G'', indicating the presence of an internal network arrangement and a gel-like structure, as happens in starch-rich preparations.

The storage modulus measured at 10 Hz significantly changed with the amount of SPAH included in the formulation. It increased from 602 Pa in S3 to 850 Pa in S6 but significantly decreased to 719 Pa in S9. Similarly happened to G", with an increase from 107 Pa in S3 to 137 Pa in S6 and a significant decrease to 122 Pa in S9.
The effects of agar on increasing the elastic and viscous moduli of potato puree.
For formulation S6A, G` is higher than that corresponding to the other samples over all the range of frequencies studied.
After sieving, all formulations show weaker structures especially samples with agar. Among all the formulations, S6A has the highest elastic modulus before (1352 Pa at 10 Hz) and after (1090 Pa) sieving and also viscous modulus before (226 Pa at 10 Hz) and after sieving (184 Pa at 10 Hz).

Table 3. 2. Frequency sweep rheological measurements. Storage modulus and loss modulus are reported at 10 Hz. The numbers represent the average of three replicates per sample.

Samples	G´ (Pa)	G” (Pa)
BEFORE SIEVING		
S3	602^{e}	107^{c}
S6	850^{cd}	137^{c}
S9	719^{de}	122^{cd}
S3A	1089^{b}	185^{b}
S6A	1352^{a}	226^{a}
S9A	974^{bc}	168^{b}
AFTER SIEVING		
S3	599^{c}	105^{c}
S6	714^{bc}	123^{bc}
S9	613^{c}	117^{c}
S3A	977^{ab}	165^{ab}
S6A	1090^{a}	184^{a}
S9A	529^{c}	93^{c}

Note: S3 (3 g Soy), S6 (6 g Soy), S9 (9 g Soy), S3A (3 g Soy and 0.2 g Agar), S6A (6 g Soy and 0.2 g Agar) and S9A (9 g Soy and 0.2 g Agar). The results with different letters in the same column are different with a significance level of $p < 0.05$.

3.3.5. 3DFP test and structure and correlation with rheology

Some requirements are necessary to find out the best formulation for any food product for 3DF printing that is such a food type, hydrocolloids, type of printer, etc. Each type of food has different physical and chemical interactions with the hydrocolloids (Pant *et al.*, 2021).

Different shapes were applied to see the printability of the formulations simply with SPAH and SPAH with agar and to test the visual performance when attempting complex structures as presented in Figure 3.3 All formulations were able to form stable self-supporting structures for simple shapes. The pentagon prism shape for all food formulations was able to keep the structure for more than 15 minutes. Honeycomb shapes were able to form a stable self-supporting. All the printing materials could build the geometry of the design of a pentagon and honeycomb while displaying abilities to print snowflakes as well.

The complicated mountain shape collapsed during printing in the formulations fortified simply with SPAH as the material was not able to tolerate the load of many layers. The formulations with agar, however, had the strength to tolerate the weight of many layers, a fact that can be explained by the addition of agar which could restrain the mobility of starch chains (Wu *et al.*, 2009).

Some researchers (Lewis *et al.*, 2006; Zhang *et al.*, 2015a; Liu *et al.*, 2018) found that printed shape retention was closely related to the G′ and τ_0 of materials and to develop periodic structures through 3D printing and found that materials with higher G′ and τ_0 showed better shape retention, the τ_0 shows the minimum force necessary for material extrusion and G` reflect the mechanical strength of mixtures, which is critical for supporting subsequently deposited layers and maintaining printed shape. The samples with only SPAH had low τ_0 (Table 3.1) and G′ (Table 3.2), but the addition of agar led to an increase both in τ_0 and G′. The formulations with only SPAH did not possess enough mechanical strength for complex shapes with many layers thus the deformation and poor resolution supporting structure observed in formulations without agar for complex printed objects (Figure 3.3). The mixture with agar and SPAH had higher τ_0 and G′ and was strong enough to support the deposited layers and hold the printed structures.

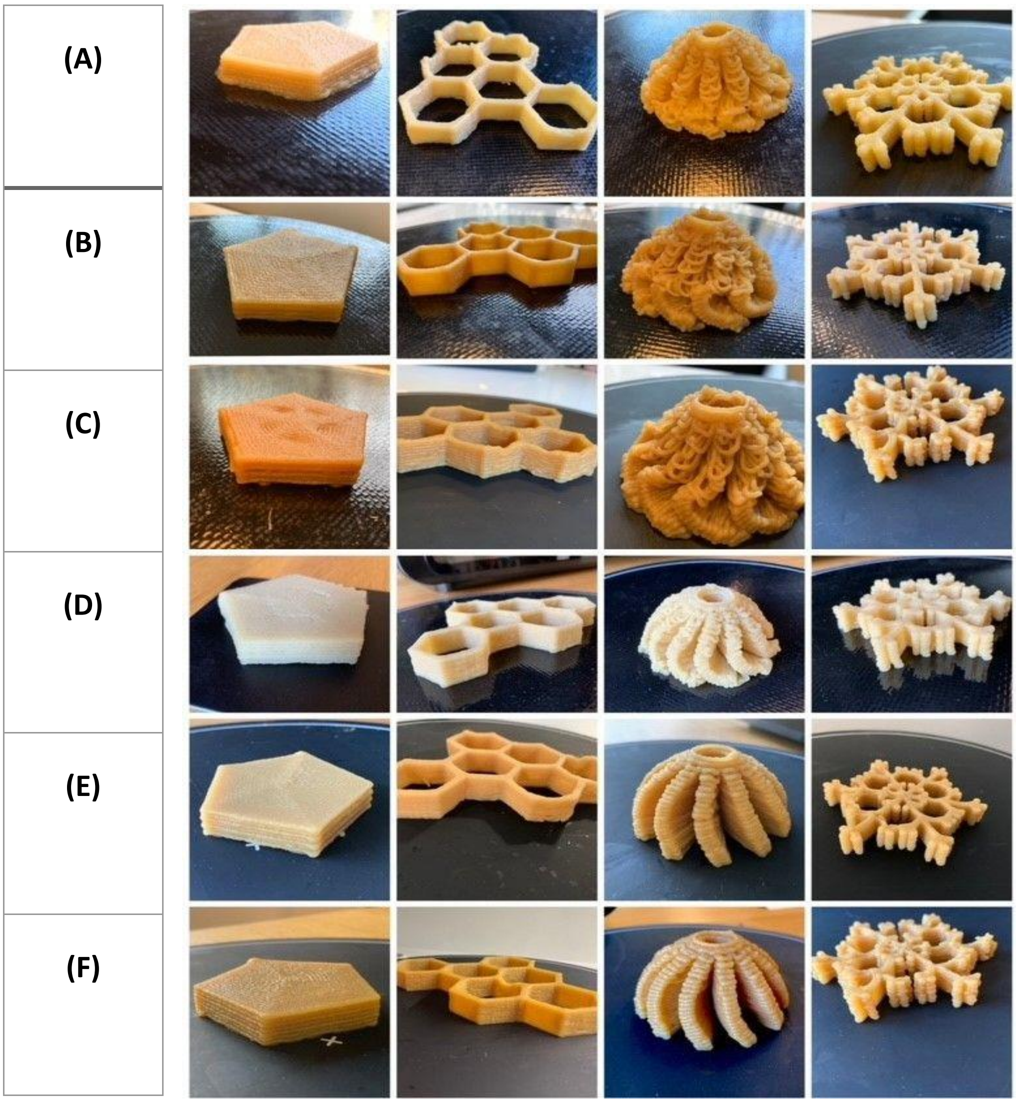


Figure 3. 3. 3D prints. Representative image of 3D printed shape with six formulations.

Note: (A)S3 (3 g Soy), (B) S6 (6 g Soy), (C) S9 (9 g Soy), (D) S3A (3 g Soy and 0.2 g Agar), (E) S6A (6g Soy and 0.2 g Agar) and (F) S9A (9 g Soy and 0.2 g Agar).

3.3.6. Texture properties: TPA

Formulations only with SPAH had lower hardness values than those that also contain agar and were not significantly different according to the amount of SPAH (Table 3.3). The formulation S6A showed significantly the highest hardness values. This was the formulation that had the best 3D shape.

High adhesiveness is a very important property of semi-solid food that needs more effort in pharyngeal swallowing in older adults (Park *et al*., 2020). The formulations S6A and S9A had the highest values for adhesiveness pointing to a good performance in the design of formulations for dysphagia suffering people.

Resilience is another attribute that indicates a food's ability to resist deformation and the samples with just SPAH had lower resilience values in coincidence with the worse performance on 3D printing tests and its difficulty to keep the designed shape.

The structural strength of internal bonds is measured as cohesiveness, which holds the food matrix together in a bolus and prevents it from disintegrating into fragments during swallowing (Sharma *et al.*, 2017). Formulations S6 and S9 presented the highest and lowest cohesiveness, respectively, among all the tested samples.

Gumminess is explaining the amount of work needed to make a food sample ready to swallow. Sample S6A had significantly higher values for gumminess compared to the rest.

The hardness, resilience, and chewiness were increased with the addition of agar, which could support the designed structure during the 3D printing process.

Table 3. 3. The average values of TPA for six formulations from three replicates.

Samples	Hardness (N)	Adhesiveness (N·s)	Resilience (-)	Cohesiveness (-)	Gumminess (N)
S3	1.01^{b}	-34.3^{a}	0.005^{bc}	0.007^{ab}	0.80^{ab}
S6	1.31^{b}	-46.9^{b}	0.006^{bc}	0.009^{a}	1.17^{ab}
S9	1.20^{b}	-28.8^{a}	0.004^{c}	0.003^{c}	0.43^{b}
S3A	2.02^{ab}	-29.8^{a}	0.014^{a}	0.005^{bc}	1.01^{ab}
S6A	5.49^{a}	-71.2^{c}	0.013^{ab}	0.006^{abc}	3.54^{a}
S9A	3.31^{ab}	-64.4^{c}	0.011^{abc}	0.006^{abc}	2.11^{ab}

Note: S3 (3 g Soy), S6 (6 g Soy), S9 (9 g Soy), S3A (3 g Soy and 0.2 g Agar), S6A (6 g Soy and 0.2 g Agar) and S9A (9 g Soy and 0.2 g Agar). The results with different letters in the same column are different with a significance level of $p < 0.05$.

3.3.7. Fork pressure & spoon tilt test

All the formulations could slide off the spoon on tilting and showed that they will not stick to the oral cavity. Fork pressure tests were done with all formulations and show the indent pattern (Figure 3.4).

According to these tests, all formulations terminated to be transitional foods. Howsoever all printed structures may change meantime change with water/saliva in the mouth and the time for slide-off from spoon test is required further study to accept the food as a dysphagic diet food.

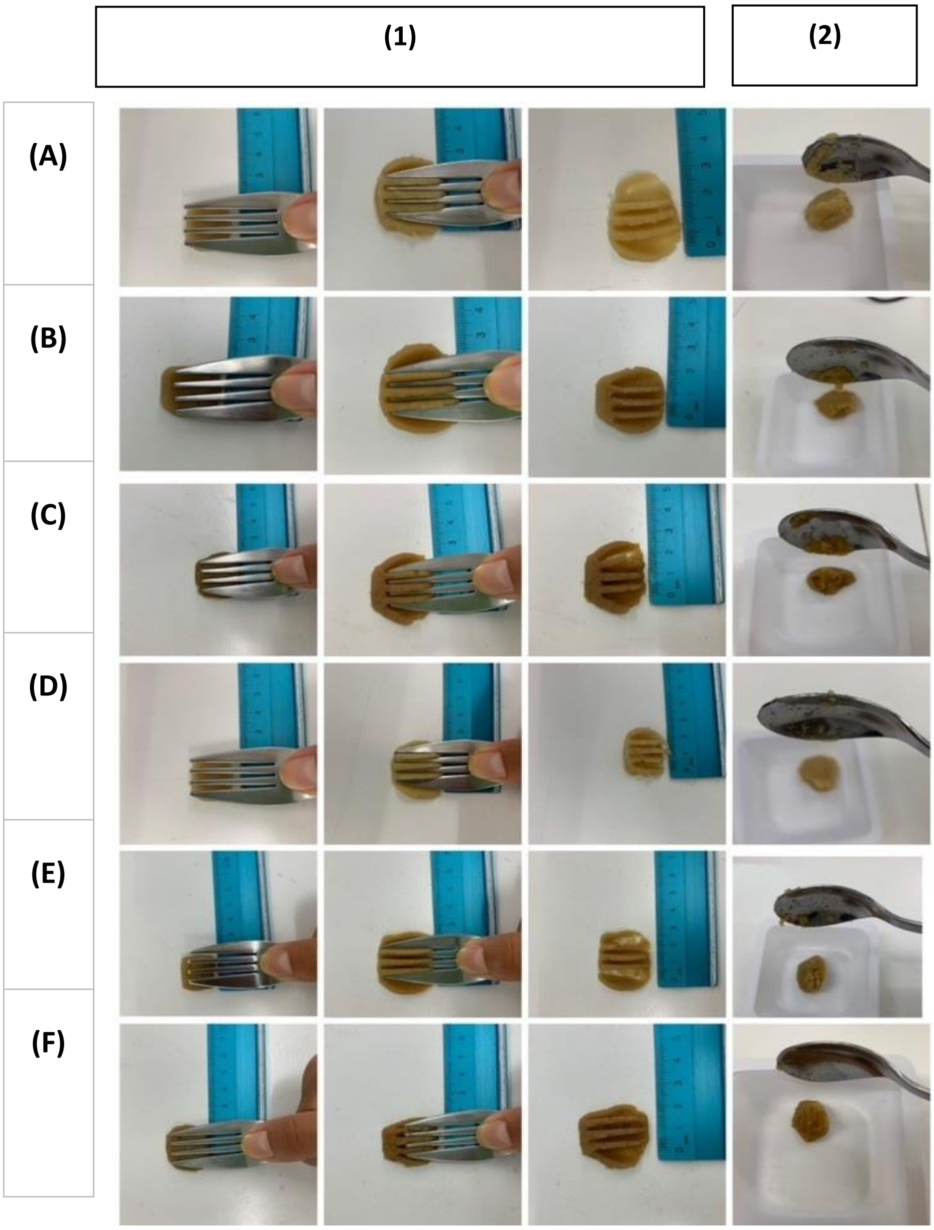


Figure 3. 4. IDDSI (International Dysphagia Diet Standardization Initiative, 2019). 1) Fork pressure test on soft and bite-sized 3D printed samples and 2) Spoon tilt test.

Note: (A) S3 (3 g Soy), (B) S6 (6 g Soy), (C) S9 (9 g Soy), (D) S3A (3 g Soy and 0.2 g Agar), (E) S6A (6g Soy and 0.2 g Agar) and (F) S9A (9 g Soy and 0.2 g Agar).

3.4. Discussion

The number of elderly and people with swallowing difficulties is increasing all over the world so personalization diet and understanding of the correct range of texture and rheological properties are necessary. There are studies that show 3D printing is capable to convert mashed and not attractive dysphagia diets into appetizing foods with an appealing appearance (Diañez *et al.*, 2021; Xing *et al.*, 2022).

The addition of SPAH and agar in the given range used in this work had a significant effect on the rheological and textural properties.

All the rheological parameters (viscosity, thixotropy, yield stress, and viscoelastic behavior) measured on the formulations without agar significantly increased with rising the soy concentration from 3% to 5% but from 5% to 7% had no significant differences that this can be explained by insufficient water in the formulation caused by the high concentration of solids. This phenomenon has been previously reported, with a decrease in thickening for the highest concentrations of added soy protein (Alvarez *et al.*, 2012).

The formulations with agar showed higher values on the rheological measurements as compared to the ones without. Agar can improve the mechanical strength due to the strong interaction of hydrogen bonding and the formation of hydrophobic aggregates (Wongphan & Harnkarnsujarit, 2020). Starchy foods' texture and appearance can be improved by mixing starch with some hydrocolloids as both share some key behavior as both being polysaccharide molecules (Bemiller, 2011).

The formulation with the highest values for all measured parameters has been S6A. This can be explained by the formation of a strongly interconnected network due to the effect of having both interactions, where a major part of the granules are strongly connected by the high molecular weight and length of the agar molecule (agar-starch), (agar-SPAH) and (SPAH-starch) interactions and the formation of (agar-agar) gel-like interactions. These interactions would result in a more elastic, structured, and gel-like microstructure than in the case of the samples with SPAH alone. Our finding highlights that the physiochemical properties of the samples with agar changed by the effect of sieving as agar could build different bonds with starch and proteins. The addition of agar improved the mechanical properties, but after passing through a sieve physiochemical properties decreased by breaking hydrogen bonds that give strength to the three-dimensional gel network. Passing the samples through the sieve before printing is a common practice in 3D printing, recommended for avoiding blocking the nozzles of the printer (Kim *et al.*, 2018; Huang *et al.*, 2020; Pant *et al.*, 2021). Our results showed sieving process has a significant effect on the rheological properties of samples containing hydrocolloids, and it is important to consider this effect for the food products addressed to the aging population and people with dysphagia which could be a future work.

SPAH addition increased the viscosity and mechanical strength of all food formulations. The resultant self-supporting capacity of 3D printed was very good for simple shapes and after adding agar self-supporting capacity of 3D printed samples increased and were suitable for printing complex shapes because of increasing viscosity and viscoelastic properties. Formulation S6A was available to perfectly support the extruded materials for a complex shape, and the layers remain strong during the printing process.

All samples were classified as level 4 puree dysphagia diet within IDDSI framework.

Overall, the S6A formulation (6 g soybean and 0.2 g agar) showed fine 3D printability with an attractive and acceptable appearance for dysphagia diets. Therefore, this study has developed a new way to design dysphagia diets using emerging 3D printing technology. Offering more appetizing foods and with a texture suitable for the elderly and people with mastication and shallowing problems.

3.5. Conclusion

The current study provides a new way of creating food texture for the elderly and people with dysphagia. The SPAH could modify the texture and from the nutritional point of view add value to the formulations with potato puree. The use of agar in the formulation can establish good printable material for 3D printing.

Our results showed that the rheological properties (viscosity, yields stress, and thixotropy) before and after sieving and printing changed and it is an important factor to consider, especially for patients with swallow problems

The addition of SPAH and agar in the given range used in this work had a significant effect on the rheological and textural properties of potato puree. SPAH addition increased the viscosity and mechanical strength of samples, and the resultant self-supporting capacity of 3D printed samples for simple shapes. After adding agar, the self-supporting capacity of the 3D printed samples was suitable for printing complex shapes because of increasing viscosity and viscoelastic properties. All samples were classified as level 4 minced and moist dysphagia diets within IDDSI framework.

Generally, formulation S6A (Soy 6 g and agar 0.2 g) showed fine 3D printability with an appealing appearance and were acceptable for dysphagia diets.

Chapter 4. Preparing a personalized meal by using soy, cricket, and egg albumin protein based on 3D printing

This chapter is based on the article:

Mirazimi, F.; Saldo, J.; Sepulcre, F.; Gràcia, A.; Pujolà, M. Preparing a Personalized Meal by Using Soy, Cricket, and Egg Albumin Protein Based on 3D Printing. Foods 2022, 11, 2244. https://doi.org/ 10.3390/foods11152244

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Article

Preparing a Personalized Meal by Using Soy, Cricket, and Egg Albumin Protein Based on 3D Printing

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Abstract: Recently, personalized meals and customized food design by means of 3D printing technology have been considered over traditional food manufacturing methods. This study examined the effects of different proteins (soy, cricket, and egg albumin protein) in two concentrations (3% and 5%) on rheological, textural, and 3D printing characteristics. The textural and microstructural properties of different formulations were evaluated and compared. The addition of soy and cricket protein induced an increase in yield stress (τ_0), storage modulus (G'), and loss modulus (G'') while egg albumin protein decreased these parameters. The textural analysis (back extrusion and force of extrusion) demonstrated the relationship between increasing the amount of protein in the formula with an improvement in consistency and index of viscosity. These values showed a straight correlation with the printability of fortified formulas. 3D printing of the different formulas revealed that soy and cricket proteins allow the targeting of complex geometry with multilayers.

Keywords: protein; fortified food; nutrition; texture; rheology; 3D food printing

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1. Introduction

3D food printing is an example of digitalization in the food sector, which is a popular means of developing the capability of the supply and manufacturing chain [1]. 3D food printing is able to formulate personalized nutrition control and customized food design, and is a potential technology to reconfigure a customized food and prototyping tool to facilitate new food product development [2]. Using 3D printing to personalize food nutrition is a field of high importance in research. Food customization is an advantage of 3DFP (3D Food Printing) technology for providing for an individual's health-nutritional needs, including for athletes, pregnant women, older adults, and people with dysphagia. Recently, nutrition control, personalized medicine, and some therapeutic approaches have changed. The technological developments associated with food engineering, food processing, and consumption patterns are evolving. Moreover, 3DFP applications are growing to fulfill individual needs and for personalized nutrition [3].

For adults, dietary guidelines suggest an acceptable macronutrient distribution range of 45–65% from carbohydrates, 20–35% from fat, and 10–35% from protein, with a recommended dietary allowance of 46 and 56 g/d or 0.8 g/kg body weight of protein [4].

Soenen et al. [5] demonstrated that a high-protein diet can help with body-weight loss and weight maintenance. This also resulted in a larger reduction of body weight and fat mass, and thereafter, since they promote a sustained level of satiety, sustained energy expenditure, sparing of fat-free mass, and increased fat oxidation [6,7].

Abstract

Recently, personalized meals and customized food design by means of 3D printing technology have been considered over traditional food manufacturing methods. This study examined the effects of different proteins (soy, cricket, and egg albumin protein) in two concentrations (3% and 5%) on rheological, textural, and 3D printing characteristics. The textural and microstructural properties of different formulations were evaluated and compared. The addition of soy and cricket protein induced an increase in yield stress (τ_0), storage modulus (G'), and loss modulus (G'') while egg albumin protein decreased these parameters. The textural analysis (back extrusion and force of extrusion) demonstrated the relationship between increasing the amount of protein in the formula with an improvement in consistency and index of viscosity. These values showed a straight correlation with the printability of fortified formulas. 3D printing of the different formulas revealed that soy and cricket proteins allow the targeting of complex geometry with multilayers.

Keywords: Protein; fortified food; nutrition; texture; rheology; 3D food printing

4.1. Introduction

3D food printing is an example of digitalization in the food sector, which is a popular way of developing the capability of the supply and manufacturing chain (Lille *et al.*, 2018). 3D food printing is able to formulate personalized nutrition control and customized food design, and is a potential technology to reconfigure a customized food and prototyping tool to facilitate new food products (Sun *et al*., 2015). Using 3D printing to personalize food nutrition is a field of high importance in research. Food customization is an advantage of 3DFP (3D Food Printing) technology for providing for an individual's health-nutritional needs, including for athletes, pregnant women, older adults, and people with dysphagia. Recently, nutrition control, personalized medicine, and some therapeutic approaches have changed. The technological developments associated with food engineering, food processing, and consumption patterns are evolving. Moreover, 3DFP applications are growing to fulfill individual needs and for personalized nutrition (Baishakhi *et al.,* 2019).

For adults, dietary guidelines suggest an acceptable macronutrient distribution range of 45–65% from carbohydrates, 20–35% from fat, and 10–35% from protein, with a recommended dietary allowance of 46 and 56 g/d or 0.8 g/kg body weight of protein (Trumbo *et al.*, 2002).

Soenen *et al*., (2012) demonstrated that a high-protein diet can help with body-weight loss and weight maintenance. This also resulted in a larger reduction of body weight and fat mass, and thereafter, since they promote a sustained level of satiety, sustained energy expenditure, sparing of fat-free mass, and increased fat oxidation (Lejeune *et al.,* 2005; Weigle *et al.*, 2005).

Soy protein is a very well-known protein rich in essential and non-essential amino acids and has been extensively studied to demonstrate that it can improve physicochemical properties (Zhou *et al*., 2015). Soy protein consumption has been associated with many beneficial health effects (Frigolet *et al.,* 2012). Some studies show that soy protein can reduce the circulating levels of total and LDL-cholesterol, modify the taxonomy of the gut microbiota, and improve insulin sensitivity (Torres *et al.,* 2006; Panasevich *et al.*, 2017). The addition of soy protein increased mechanical strength and the viscosity of food products and can help the self-supporting ability of 3D-printed food *(*Mirazimi *et al.,* 2022).

Edible insects can act as an enriching additive when added to food (Duda *et al*., 2019). They are very rich in protein, essential amino acids, vitamins, and minerals, and have recently been attracting the attention of the food industry and researchers for their use in different formulations with different characteristics (Belluco *et al*., 2013; Rumpold & Schlüter, 2013). Nowadays many studies show that crickets (*Acheta domesticus*) have high nutritional value and consumption is safe and is not associated with side effects. This increasingly improves functionality in food formulations and, as an alternative protein, makes them suitable for many food products (Hall *et al.*, 2017).

Egg white contains many functionally important proteins, such as ovalbumin, ovotransferrin, ovomucoid, ovomucin, and lysozyme (Abeyrathne *et al.,* 2013). Ovalbumin has been used extensively in food technology because of its foaming, emulsification, and binding adhesion (Mine, 1995). In addition, ovalbumin has been found to have antioxidant (protection of linoleic acid and docosahexaenoic acid), antimutagenic, and anticarcinogenic immunomodulatory properties (Mine *et al.*, 1991).

This work focuses on expanding the shape stability, printability, and nutritional profile of formulated purees that can provide new opportunities to leverage 3D food printing for consumer needs and health. The protein-rich formula is an encouraging food material in the 3D printing sector. Although still there is limited accessible information on food proteins for 3D printing. In the present work, protein-rich formulations have been prepared and different geometries produced by 3D food printing have been studied. The main aims of this study were to 1) investigate the rheological properties and 3D printability of different protein mixtures 2) evaluate the texture characteristics with new methods 3) understand the composition of different proteins on printability and 4) persuade people to have a personalized diet.

4.2. Materials and methods

4.2.1. Materials

Potato puree powder (Maggi) was purchased in a local supermarket with the nutritional values presented in Table 4.1.

Table 4. 1. Puree potato's nutritional value per 100 g of product.

Energy	1475 KJ / 348 Kcal
Fat	0.8 g
Carbohydrates	75 g
Fiber	6.8 g
Proteins	7.4 g
Salt	0.06 g
Moisture	16.7 g

Soy Protein acid hydrolysate (SPAH) was procured from Sigma-Aldrich (Nitrogen analysis ≥12% total= 75% protein, calculated applying a factor of 6.25 g protein/g N. Cricket protein powder 70% protein (*Acheta domesticus*) from Origen farms Albacete, Spain. Egg albumin 75% protein was obtained from Avantor®. According to the regulation (EC) N.1924/2006 all the formulations were high in protein (Zicari *et al.,* 2007) (Table 4.2).

Table 4. 2. Protein information.

Protein	Soy protein (S)		Cricket protein (C)		Egg albumin (A)	
Protein % in recipe	3	5	3	5	3	5
Protein / 100 g product	2.9	4.6	2.7	4.4	2.9	4.6
Energy Kcal / 100 g product	59.3	67.6	61.3	71.5	58.9	66.8

4.2.2. Sample preparation

The same base was applied for all the formulations: the different proteins (soy (SPAH), cricket, and egg albumin) were dissolved or dispersed in 100 mL of distilled water at 2 different levels (3 and 5%) with the addition of 17 g potato powder. The formulations were prepared by mixing 100 mL of distilled water and protein (albumin or cricket) and keeping the mixture in an oven at 40 °C for 30 min to denature the protein. For soy protein (SPAH), the conditions to denature the protein were 90 °C for 30 min. All the samples were kept in an oven with a lid to avoid evaporation. Finally, the dehydrated potato powder was added. Formulations were coded with a letter indicating the protein source (S, C, or A for soy, cricket, or egg albumin, respectively) and a number expressing the amount of protein added (3 or 5%). CS is the code for the control sample, which shares the same formulation but with no protein added, and the preparation condition was in an oven at 40 °C for 30 min.

Samples were kept overnight (22–24 h) in a fridge set at 2 °C and all samples were placed in an incubator at 20 °C for two hours before starting the rheological measurements. The whole experiment was repeated 3 times.

4.2.3. Frequency sweep test

The rheological measurements were performed with a rheometer (Rheostress RS1, version 127, Barcelona, Spain) and computer software (HAAKE RheoWin 3 Job and Data Manager Software, Thermo Fisher Scientific, Waltham, MA, USA). Samples were analyzed using a plate-plate geometry with a 1-mm gap between the 35-mm serrated plate (PP60 sensor) and the serrated base for their flow properties. The upper plate was placed into the measuring position, and the surplus sample was trimmed. Measuring rheological properties started after leaving samples to rest for 2 min. Two types of rheological tests were run: a rotational test and a dynamic oscillatory test. The temperature of the rheological tests was unvarying at 20.0 ± 0.1 °C for both measurements. The linear viscoelastic region was identified by a strain sweep test at a shear rate of 0.0025 s^{-1} before attempting the dynamic rheological measurement (Dankar *et al*., 2018a).

Oscillatory tests were performed to determine the strength and stability of the different formulations and explain the behavior of material from 0.1 to 10 Hz to understand how viscous or elastic properties dominated the behavior of the prepared formulations. Rheological properties can be evaluated through the storage modulus (G′), loss modulus (G″), and loss tangent (tan δ) which describes the ratio of the loss modulus to storage modulus (G″/G′). Samples were measured before the 3D printing test.

4.2.4. Rotational rheological measurements, viscosity, and yield stress

Shear rate was raised logarithmically from 0.1 to 10 s^{-1} during the first 30 s, after being preserved at 10 s^{-1} for 30 s, and finally was cut down logarithmically again to 0.1 s^{-1} over 30 s. For each sample, the shear stress (τ), viscosity (η), and yield stress were calculated following standard procedures (Tabilo-Munizaga & Barbosa-Cánovas, 2005).

4.2.5. TPA

3D printed hexagon shape made of 8 layers using a Foodini® 3D Food Printer machine (Natural Machines Iberia, S.L., Barcelona, Spain) was analyzed by TPA test to determine the textural properties of the printed formulations. Texture profile analyses of printed samples were performed at 20 °C using a Texture Analyser (TA. XT Plus, Stable Micro Systems, Godalming GU7 1YL, UK) with a compression plate (P/100). Double-cycle compression tests were performed at a test speed of 2 mm/s, a post-test speed of 2 mm/s, and compression strain was set at 35%, and with a time between compressions of 5 s. Each formulation was measured three times per sample. Hardness, chewiness, cohesiveness, adhesiveness, chewiness, and resilience were recorded in one replicate per sample.

4.2.6. Backward-extrusion test

The TA. XT Plus, Stable Micro Systems, UK, was used to perform a compression analysis with a 3.5 cm diameter plate probe. The purees were placed in a cylindrical container 5 cm in diameter and 6.5 cm high and filled to 75% of the volume of the container. For comparison, the method was adjusted to mimic the speed of the printing conditions of the Foodini® (speed of 2 mm/s for a distance of 30 mm).

4.2.7. Force of extrusion

To determine the firmness (N) and consistency (N·s) of the extrusion process with the Foodini®, the 304 stainless steel capsules of the printer (© 2021 Natural Machines), with a capacity of 100 mL and 4 mm mouthpiece have been used with a TA. XT (Stable Micro Systems, UK) was equipped with a 2.5 cm diameter cylindrical probe. The probe applied force to the plunger of the capsule (38 mm in diameter), pushing the puree out through the mouth placed at the bottom of the capsule. The maximum force needed to extrude the product was obtained by the analysis of the results of a test performed at a speed of 2 mm/s and for a distance of 30 mm (Figure 4.1).

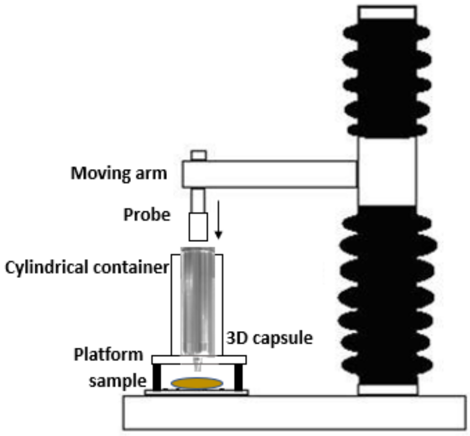


Figure 4. 1. Force of extrusion method.

4.2.8. 3D food printing conditions

For printing, Foodini®, an extrusion-based commercial system, was used. The formulations were produced using a nozzle size of 1.5 mm and a hexagon prism of 8 layers was created for printing and analyzing the samples. Before printing the samples all the formulations were passed through a sieve as a means to eliminate or reduce the presence of lumps that may disturb printing, with a nozzle of a small diameter (1.5 mm). Other more complex structures were printed, and if the printed sample's structures were able to keep their shape for at least 15 min, they were considered printable. The first ingredient holds at 4.2 mm, with the first layer nozzle height at 1.4 mm, and the print speed was set at 3500 mm/min.

4.2.9. Statistical analysis

Statistical analyses of the data were conducted on Minitab 18 (Minitab link. Coventry, UK). Data concerning Rheology and textural characteristics were tested for significant differences ($p < 0.05$) using analysis of variance, general linear model, and Tukey's comparison test.

4.3. Results

4.3.1 Viscosity and yield stress

For the formulation to have good printability, the rheological properties of extrusion-based products are critical (Sungsinchai *et al.*, 2019). Different mathematical models (e.g., Power Law, Herschel-Bulkley, Casson, and Bingham models) were tested to select the best performer. Bingham was determined to be the best model to fit the flow characteristics of the samples, with a high coefficient of determination (R > 0.98). The Bingham model is explained by the equation $\tau = \tau_0 + \eta_p \dot{\gamma}$, where τ (Pa) is the shear stress, τ_0 (Pa) is the yield stress, η_p (Pa·s) is the viscosity and $\dot{\gamma}$ (s−1) is the shear rate.

The addition of SPAH and cricket protein presented a positive correlation with the yield stress. These proteins could increase the mechanical strength in line with other studies (Cappelli *et al.*, 2020; Mirazimi *et al.,* 2022). The yield stress shows the mechanical strength of formulations. Yield stress is the ability of the formulations to keep their shape under gravity and point to the feasibility of extrusion. Also, they supported successive stacked layers after deposition. The larger the yield stress, the better self-supporting and easier smooth extrusion properties (Zhang *et al.*, 2015; Lille *et al.*, 2018). The formulation S5 and C5 presented the highest yield stress (535 Pa) and viscosity (451 Pa) among the tested formulations (Table 4.3) and the lower yield stress and viscosity belonged to the samples with egg albumin protein that they could not self-support for multilayer shapes so that the product collapsed after printing.

Table 4.3 shows that SPAH and cricket had a significant ($p < 0.05$) effect on increasing the viscosity. Increasing the concentration of SPAH had a significant effect on increasing viscosity but increasing the amount of cricket and egg albumin from 3% to 5% did not change the viscosity.

The high yield point and viscosity produced through the bonds between protein-starch and protein-protein internal microstructure, contribute to the elasticity of the network of the potato starch puree and consequently generate a starch internal microstructure that is more insistence to deformation. The formulations C5 (917 Pa), C3 (742 Pa), and S3 (636 Pa) showed high yield stress and viscosity (397 Pa), (302 Pa), and (254 Pa) respectively compared to the control sample which had good results for a multilayer shape.

Table 4. 3. The Bingham model $\eta = \eta_\rho + \tau_0/\dot{\gamma}$ parameters for the different proteins at different concentrations.

Samples	Yield point (Pa)	η_ϱ (Pa·s)	τ_0 (Pa)
CS	620^{bc}	105^{bc}	302^{b}
S3	636^{bc}	254^{b}	469^{ab}
S5	981^{a}	451^{a}	535^{a}
C3	742^{bc}	302^{ab}	486^{ab}
C5	917^{b}	397^{ab}	503^{a}
A3	530^{c}	87^{c}	290^{c}
A5	558^{c}	92^{c}	270^{c}

Note: CS (Control Sample), S3 (3 % Soy), S5 (5 % Soy), C3 (3 % Cricket), C5 (5 % Cricket), A3 (3 % Albumin), and A5 (5 % Albumin). Different superscript letters in the same column indicate a significant difference ($P < 0.05$).

4.3.2. Viscoelastic behavior

A frequency sweep test could be used to investigate the elastic modulus. Storage modulus (G′) and loss modulus (G″) of formulations were frequency-dependent revealing a gel-like structure with G′ higher than G″. Storage modulus (G′) indicates the elastic solid-like behavior and the mechanical strength. These parameters provided information about the mechanical properties that were directly connected to the self-supporting properties of 3D printing (Montoya *et al.*, 2021). G′ of formulations with SPAH and cricket protein were higher, indicating that can possess a better load-bearing capacity without deformation.

Food formulations with strong mechanical strength compared to control samples (S5 (995 Pa), C5 (827 Pa), C3 (816 Pa), and S5 (717 Pa)) determine excellent self-supporting performance after deposition and could withstand the complex printed shape.

A high tan δ means more viscous behavior and a low tan δ means more elastic behavior and a tan δ smaller than 1 signifies predominantly elastic behavior. All the formulations were significantly lower than 1 and show viscoelastic behavior.

Table 4. 4. Storage modulus (G'), loss modulus (G"), and tan δ at 10 Hz for different protein at different concentrations.

Samples	G´ (Pa)	G" (Pa)	TANGENT δ (-)
CS	686^{b}	106^{bc}	0.16^{ab}
S3	717^{b}	121^{b}	0.16^{ab}
S5	995^{a}	163^{a}	0.13^{b}
C3	816^{ab}	130^{ab}	0.15^{b}
C5	827^{ab}	131^{ab}	0.15^{b}
A3	661^{bc}	96^{c}	0.19^{a}
A5	404^{c}	88^{c}	0.19^{a}

Note: CS (Control Sample), S3 (3 % Soy), S5 (5 % Soy), C3 (3 % Cricket), C5 (5 % Cricket), A3 (3 % Albumin), and A5 (5 % Albumin). Different superscript letters in the same column indicate a significant difference ($P < 0.05$).

4.3.3. TPA

TPA is an important imitative test for textural characterization and is explained by mimics' human mastication behavior. It is performed as a two-bite compression test as it provides a link between mechanical properties and textural attributes during oral processing (Chen, 2009). The texture profile analysis (TPA) was done for the printed food samples. And the results are shown in Table 4.5.

The hardness, cohesiveness, springiness, gumminess, chewiness, and resilience of different proteins had no significant ($p < 0.05$) differences even with increasing the amount of protein concentration. The only parameter that changed in this test was adhesiveness where the lowest measurement belongs to the A5 (-3.80 N·s) and A3 (-3.73 N·s) respectively, and the highest measurements were for C5 (-5.75 N·s), S5 (-5.70 N·s) and S3 (-5.55 N·s). The other formulations (C3, and CS) showed the same behavior and had no significant differences ($p < 0.05$). The formulations prepared with egg albumin had a relatively low yield point and storage modulus which considering high adhesiveness can be translated into lower energy needed to make a swallow-able bolus and weak gel structure (Sharma *et al.*, 2017).

Table 4. 5. Textural properties of 3D printed samples for different protein at different concentrations.

Samples	Hardness (N)	Adhesiveness (N·s)	Cohesiveness (-)	Springiness (-)	Gumminess (N)	Chewiness (-)	Resilience (-)
CS	2.68[a]	-4.16[b]	0.005[a]	0.009[a]	2.21[a]	2.08[a]	0.0007[a]
S3	3.36[a]	-5.55[a]	0.006[a]	0.009[a]	2.30[a]	2.11[a]	0.0007[a]
S5	3.34[a]	-5.70[a]	0.006[a]	0.009[a]	2.39[a]	2.21[a]	0.0006[a]
C3	3.61[a]	-5.75[a]	0.006[a]	0.009[a]	2.52[a]	2.35[a]	0.0006[a]
C5	3.58[a]	-6.19[a]	0.006[a]	0.009[a]	2.55[a]	2.36[a]	0.0006[a]
A3	2.72[a]	-3.80[c]	0.005[a]	0.008[a]	2.12[a]	2.01[a]	0.0007[a]
A5	2.79[a]	-3.73[c]	0.005[a]	0.009[a]	2.32[a]	2.04[a]	0.0007[a]

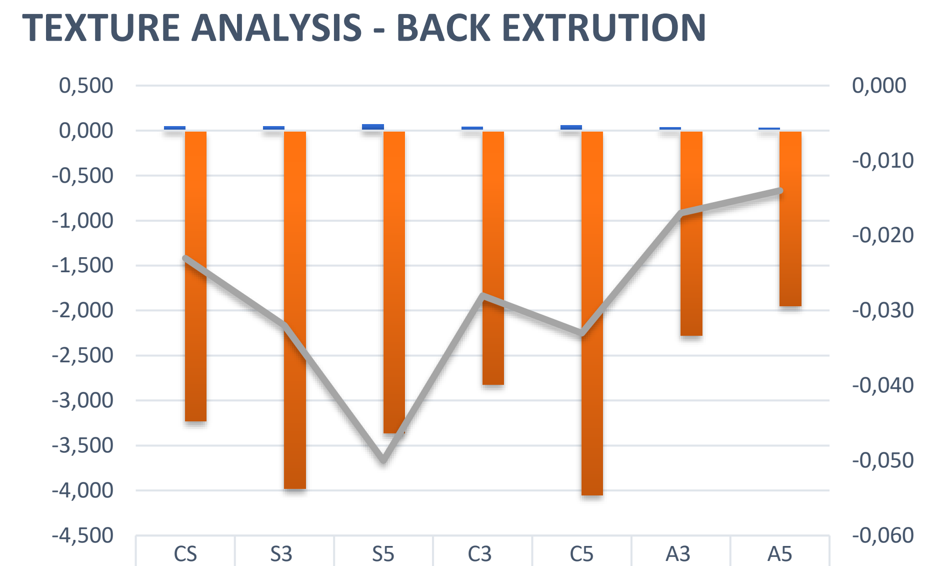
Note: CS (Control Sample), S3 (3 % Soy), S5 (5 % Soy), C3 (3 % Cricket), C5 (5 % Cricket), A3 (3 % Albumin), and A5 (5 % Albumin). Different superscript letters in the same column indicate a significant difference ($P < 0.05$).

4.3.4. Back extrusion

Back extrusion is a technique commonly used to assess the flow properties of Newtonian and non-Newtonian fluids in the industrial environment for various purposes, including quality control. Some studies show that the back-extrusion technique correlates well with the rheological tests (Chen *et al.*, 2021). Comparing the measurement of force applied by the annular space with the rheological properties of the samples, it can be observed that the force reflected the rheological properties of the samples. The consistency of samples with SPAH 5% and cricket 5% was higher than the rest of the formulations due to higher shear viscosity and therefore higher resistance to flow.

The index of viscosity is the extrusion energy or work of adhesion when it becomes higher; more resistance is required when pulling out the sample and it has been demonstrated that the index of viscosity is related to the consistency of the purees (Nasaruddin *et al.,* 2012). The formulations with SPAH had the highest index of viscosity followed by the samples with cricket protein which had a higher index of viscosity than the control sample. The formulations with albumin showed a low index of viscosity due to the less viscosity and yield stress referring to the rheological test (Table 4.3).

Cohesiveness means 'work of cohesion', and the more negative the value, the more 'cohesive' is the sample. The highest cohesiveness among the tested formulations is for sample C5 and significantly ($p< 0.05$) changed with increasing cricket protein from 3% to 5%. Samples with SPAH (S5 and S3) showed a different behaviour and did not change with increasing the amount of protein from 3% to 5% (Figure 4.2). The albumin had weak consistency and cohesiveness, which means less resistance to flow.



	CS	S3	S5	C3	C5	A3	A5
Consistency (N·s)	0,047 [ab]	0,047 [ab]	0,069 [a]	0,039 [bc]	0,058 [a]	0,037 [bc]	0,032 [c]
Cohesiveness (N)	-3,230 [ab]	-3,980 [b]	-3,360 [b]	-2,820 [ab]	-4,050 [c]	-2,280 [a]	-1,950 [a]
Index of Viscosity (N·s)	-0,023 [bc]	-0,032 [d]	-0,050 [e]	-0,028 [cd]	-0,033 [d]	-0,017 [ab]	-0,014 [a]

Figure 4. 2. Back extrusion measurements. The numbers represent the average of three replicates per sample.

Note: CS (Control Sample), S3 (3 % Soy), S5 (5 % Soy), C3 (3 % Cricket), C5 (5 % Cricket), A3 (3 % Albumin), and A5 (5 % Albumin). Different superscript letters in the same row indicate a significant difference ($P < 0.05$).

4.3.5. Force of extrusion

According to the force of extrusion method (Figure 4.3), the formulation S5 had the highest consistency and firmness ($p< 0.05$). With increasing the amount of SPAH from 3 to 5 % these values increased ($p< 0.05$). The formulations with cricket protein (C3 and C5) also increased the consistency (0.37 and 0.43 N. s) and firmness (0.028 and 0.035 N) respectively compared to the control sample (CS). However, the samples with SPAH and cricket all showed good printability for multilayer shapes. This good performance can be explained by the strong connections between starch granules and protein, being able to build a 3D network in the case of using soy and cricket protein. Huang *et al.,* (2007) showed that the mixtures of the protein and polysaccharides were able to increase the texture parameters that may be described similarly in the case of the mixture of proteins and starch in this work. The formulations with albumin A3 and A5 had poor consistency and firmness pointing to a difficulty to build a strong enough network with the potato starch, as opposed to the case of soy or cricket.

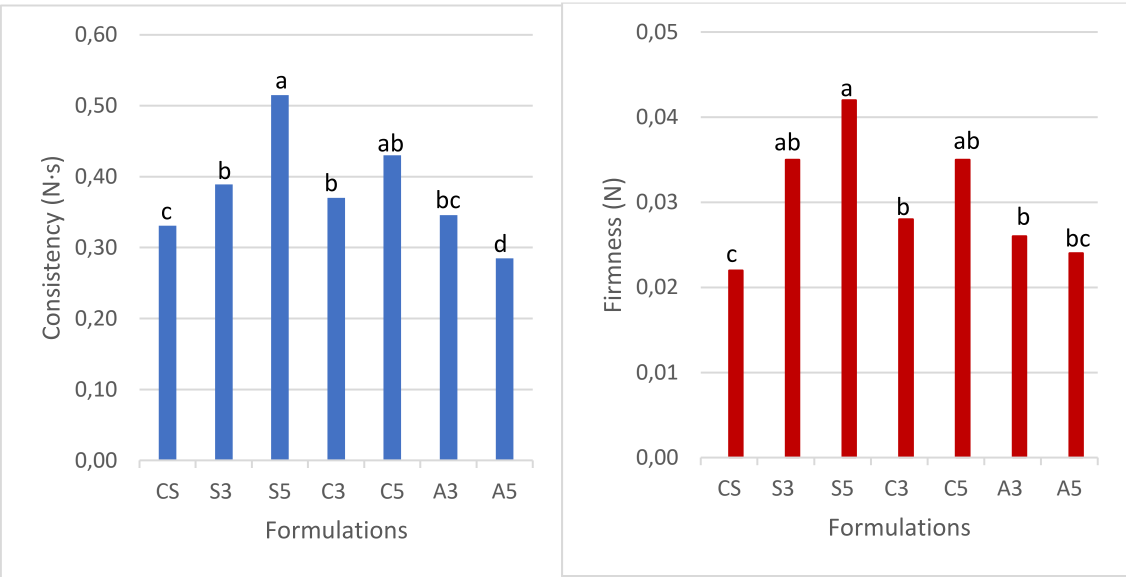


Figure 4. 3. Force of extrusion measurements. The numbers represent the average of three replicates per sample.

Note: CS (Control Sample), S3 (3% Soy), S5 (5% Soy), C3 (3% Cricket), C5 (5% Cricket), A3 (3% Albumin), and A5 (5% Albumin). Different superscript letters indicate a significant difference ($p < 0.05$).

4.3.6. 3D printing test and correlations

As observed in the printing experiments, all the formulations could be easily extruded out from the nozzle at 25 °C. Different shapes were applied to see the printability and stability of the formulations with different proteins. Figure 4.4 shows the result of 3D printing. The formulations with SPAH and cricket protein were able to support the extruded materials and stable self-supporting structures for all the shapes that have been designed even for the complex ones with 13- and 11-layers flower shapes. The hexagon shape for all food formulations as well as the control sample was able to keep the structure for more than 15 minutes, except for formulations with egg albumin that showed very poor resolution due to the low rheological and textural properties. The control formula, as well as those including SPAH or cricket, were able to form stable self-supporting structures when printing complex flower or mountain shapes (Figure 4.4 (G, H, I, and J)) of 9 printed layers. Conversely, the formulations fortified with albumin were not able to tolerate the load of the 9 layers of material and the 3D printed flower and mountain shapes collapsed under their own weight.

Nowadays many studies have shown that printed shape stability was closely related to rheological properties, especially the G′ and τ_0 of materials, and to develop ordinary structures through 3D printing and found that materials with higher G′ and τ_0 showed better shape retention. The τ_0 shows the minimum force necessary for material extrusion and G` reflect the mechanical strength of mixtures, which is critical for supporting sequentially deposited layers and maintaining printed shape. Liu *et al.*, (2018) determinate τ_0 of 312.16 (Pa), which had good extrudability and shape retention for the extrusion-based printer. In the present study, the samples with the best 3D shapes were produced from formulas fortified with SPAH and cricket protein, having high values for τ_0 (486 Pa - 535 Pa) and G′ (717 Pa - 995 Pa). The mixtures with either SPAH or cricket protein had higher τ_0 and G′ and were strong enough to support the deposited layers and hold the printed structures. The samples with different concentrations of egg albumin had low τ_0 (A3 290 Pa and A5 270 Pa) and G′ (A3 96 Pa and A5 88 Pa), lower even than the control sample, and does not possess enough mechanical strength to uphold complex shapes with many layers resulting in the deformation of the printed shape and the poor resolution supporting structure observed in formulations including egg albumin (Figure 4.4).

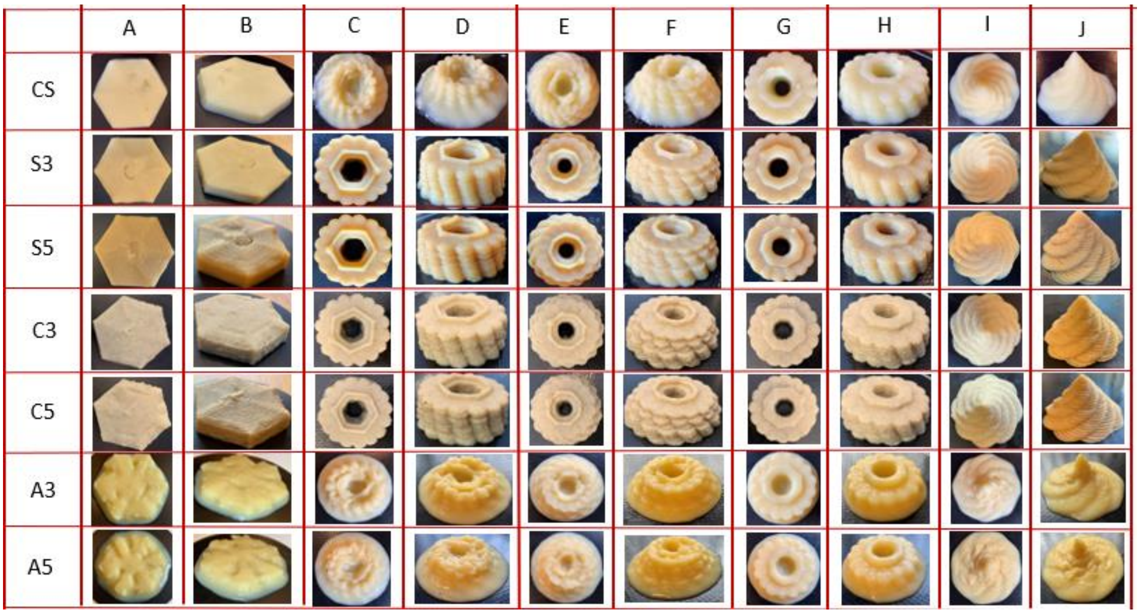


Figure 4. 4. 3D prints. Representative image of 3D printed shape with seven formulations.

A) 8 layers hexagon shape top B) 8 layers hexagon shape side C) 13 layers flower shape top D) 13 layers flower shape side E) 11 layers flower shape top F) 11 layers flower shape side G) 9 layers flower shape top H) 9 layers flower shape side I) 28 layers mountain shape top J) 28 layers mountain shape side.

Note: CS (Control Sample), S3 (3 % Soy), S5 (5 % Soy), C3 (3 % Cricket), C5 (5 % Cricket), A3 (3 % Albumin), and A5 (5 % Albumin).

4.4. Discussion

The 3D printing process can be divided into three stages including extrusion, recovery, and self-support, which are directly connected with the rheological properties including viscosity, yield stress and elastic modulus (G′), and viscous modulus (G″) (Liu et al., 2019). Extrusion correlates with yield stress and it is worth noting that although the smaller the yield stress, the easier for the material to be extruded, it also implied that the mechanical strength of the material may be unfavorable for the subsequent self-support and deformation resistance of the material extrusion (Qiu *et al*., 2022).

Adding SPAH and cricket protein improved the rheological characteristics, showed stronger mechanical properties, and furthered the printability. SPAH and cricket proteins were found to be ideal ingredients for 3D food printing.

Proteins and polysaccharides interact with each other and, accordingly, can change the structure and properties of the food formulations. Such interactions are attracting a lot of attention in the food industry as the structure and stability of many foods are dependent on the extent of the interaction between proteins and polysaccharides (Xiong *et al*., 2017).

Conventionally, when complexes of protein-polysaccharide are formed, their functional properties are potentially better than those of the proteins and polysaccharides alone. However, the characteristics depend on several parameters such as protein and polysaccharide type and concentrations, pH, temperature, and the concentration of cations present in the solution, which can form complexes or thermodynamic incompatibilities (Dickinson, 2003; Razi *et al.*, 2019).

Mixing two biopolymers in a solution, as a polysaccharide and a protein, can build interactions that may present two possibilities the interaction of the two biopolymers can be segregative (the biopolymers repel each other and are denoted as incompatible) or associative (the biopolymers attract one another) (De Kruif & Tuinier, 2001). Hence, in this work, with the addition of SPAH and cricket protein, the behavior of the polysaccharide-protein mixture was associative and could create a complexation mixture. In opposition, egg albumin protein behavior with polysaccharides in the condition of this work created a segregative mixture that causes incompatibility. Conditions for incompatibility are dependent on the structure and composition of the biopolymer pair (Polyakov *et al.*, 1997).

The samples with egg albumin showed low values on the rheological parameters compared to the control sample due to the segregation on the polysaccharide-protein mixture produced by their incompatibility. Grinberg and Tolstoguzov (1997) demonstrated incompatibility is strongly dependent upon the conformational state of the proteins and it is enhanced by protein denaturation. Egg albumin has a very low transition temperature and can be more susceptible to suffering incompatibility effects associated with its denaturation.

4.5. Conclusion

It is a new challenge to create a nutritional formulation that is customized, extrudable, and consistent for complex shapes. In conclusion, the printability and print stability of pureed food can be improved by adding protein. This study demonstrated the applicability of food ingredients (e.g., soy protein, cricket protein, and egg albumin protein) in 3D food printing, which is a starting point for the future development of healthy, nutritional, and customized foods. The addition of soy and cricket protein (in the doses tested) significantly increased all the rheological values (yield stress, viscosity, G′, and G″). The consistency and firmness according to the extrusion force test were increased significantly in the case of using soy and cricket protein which provided an accurate printing result and a stable geometry for multilayer complex shapes. Additionally, the rheological values and the consistency obtained from the extrusion force test increased with the amount of soy protein. Best printing results were achieved by using soy and cricket proteins which showed good fluidity with higher G′, G″, and τ_0. On the other hand, the addition of egg albumin could not support the designed structure during the 3D printing process and showed weaker rheological and textural properties because of protein incompatibility due to protein denaturation in the condition of this study.

Chapter 5. Study the correlation between the instrumental and sensory evaluation of fortified potato puree

This chapter is submitted to the Journal of **Food Quality and Preference**.

Mirazimi, F.; Saldo, J.; Sepulcre, F.; Pujolà, M. Study the correlation between the instrumental and sensory evaluation of fortified potato puree.

Abstract

The creation of 3D printed food with programmed texture has the ambitions of getting personalized properties through novel texture perceptions with many different ingredients and is helping to the swallowing or mastication problems of vulnerable people. This study is done to determine the correlation between the instrumental and sensory evaluation of 3D-printed fortified puree potato. At the moment there are not many studies about this correlation, and this information can be very helpful for food texture development. For people with swallowing difficulties, it is critical to have access to safe food with the desired texture. Therefore, understanding a correlation between texture-modified food will aid in the formulation of safe foods with desired sensory properties. Instrumental measurements of fortified puree were performed by a texture analyzer and the attributes obtained were firmness and consistency (from the force of extrusion test) and cohesiveness and index of viscosity (from the back extrusion test). Quantitative descriptive analysis with eight trained panelists was employed to characterize the texture of the 3D printed fortified puree based on six sensory attributes: firmness, thickness, smoothness, rate of breakdown, adhesive, and difficulty swallowing.

Three proteins (soy, cricket, and egg albumin with two different concentrations of 3% and 5%) were evaluated against puree potato without any protein as a control. The correlation results obtained from texture analysis and sensory evaluation were statistically significant ($P < 0.05$) and showed that a well-designed instrumental technique can be used to understand the impact of ingredients for textured modified puree.

Keywords: Sensory evaluation; soya protein; cricket protein; albumin protein; texture analysis; 3D printing

5.1. Introduction

Three-dimensional (3D) printing is a technology that is based on a layer-by-layer deposition technique to build computer-aided designed objects (Joshi *et al*., 2021). The application of 3D printing technology has been gaining interest in many industrial sectors, including the field of food and gastronomy (Lipson *et al.*, n.d.). In food manufacture, hydrocolloids or proteins are commonly used to improve quality attributes and sensory properties (Saha & Bhattacharya, 2010). 3D printing could also enable nutritional optimization of one's tailored diet. For instance, the prospect of personalized nutrition will benefit hospitalized patients with compromised eating ability or reduced appetite by providing appealing small portions adapted to their needs. Among this population, the provision of protein-enriched products will allow the fulfilment of nutrient requirements and facilitate recovery (Methven *et al.*, 2010). Several studies have successfully conducted research on 3D printed food with efforts made to elucidate the effect of printer parameters and textural properties of food inks on printability. However, investigation of the sensory and consumer aspect of 3D printed food is limited (Severini *et al.*, 2018).

Food is maintaining the energy supply necessary for survival in humans but has a very important role as a source of pleasure (Aguilera & Park, 2016). For creating the right textured food, characterization of food products instrumental and sensory evaluation can be done to achieve quantifiable textural attributes for pureed foods. In addition, the textural properties of novel food products are also of critical importance in the attempts of new product development for many food products (McGorrin, 2019).

The back extrusion method, based on compression and extrusion tests, has been used to measure the textural properties (cohesiveness and index of viscosity) of semisolid foods (Ilhamto, 2012). Whereas the new method of textural analysis such as the force of extrusion which is for 3D printing is developed for puree food textural measurements (cohesiveness and index of viscosity) (Mirazimi *et al.*, 2022).

Sensory properties have been used to characterize the perception in-mouth and associate the reasoning of acceptance or rejection of food (Sharma *et al.*, 2017). Quantitative descriptive analysis (QDA) is a comprehensive sensory technique that yields quantitative product description and can be used to correlate sensory characteristics to instrumental data. The main principle of QDA is to train panelists to measure specific quality attributes of a food product (Puri *et al*., 2016).

Both instrumental and sensory characterization of texture-modified food could be used to understand the qualitative and quantitative textural attributes of pureed foods, which ultimately seeks to improve the quality of life of people. However, few research papers have explored the correlation between instrumental and sensory measurements for puree foods (Zargaraan *et al*., 2013).

The aim of this study was to investigate the sensory properties of high protein puree potato products and to study the possible correlations with the textural attributes and 3D food printing extrusion. This correlation can be used as a quick and effective tool to measure relevant textural and sensorial attributes.

5.2. Materials and methods

5.2.1. Materials and sample preparation

Dehydrated potato puree (Maggi, purchased in a local supermarket) has the following nutritional values: energy 348 Kcal, fat 0.8 g, carbohydrates 75 g, fiber 6.8 g, and proteins 7.4 g. Soy Protein acid hydrolysate (SPAH) food-grade was procured from Sigma-Aldrich (Nitrogen analysis ≥12% total= 75% protein **(S)**, calculated applying a factor of 6.25 g prot/g N. Cricket protein powder70% protein (*Acheta domesticus*) **(C)** from Origen farms Albacete, Spain. Egg albumin 75% protein **(A)** was obtained from Avantor®. According to the regulation (EC) N.1924/2006 (Zicari *et al.,* 2007) all the formulations were high in protein 3S (2.9 g protein/100 g product), 5S (4.6 g protein/100 g product), 3C (2.7 g protein/100 g product), 5C (4.4 g protein/100 g product), 3A (2.9 g protein/100 g product), and 5A (4.6 g protein/100 g product).

Different proteins (soy (SPAH), cricket, and egg albumin) were dissolved in 100 mL of distilled water at 2 different levels (3 and 5%) and later 17 g potato powder was added. The formulations were prepared by mixing 100 mL of distilled water and protein (albumin or cricket) and keeping the mixture in an oven at 40 °C for 30 min (with a lid to avoid evaporation) to denature the protein. The control sample shares the same formulation but with no protein added, and the preparation condition was in an oven at 40 °C for 30 min. For soy protein (SPAH), the conditions to denature the protein were 90 °C (with a lid to avoid evaporation) for 30 min (Mirazimi *et al.,* 2022). All Formulations were coded with a number expressing the amount of protein added (3 or 5%) and a letter indicating the protein source (S, C, or A for soy, cricket, or egg albumin, respectively).

Samples were kept overnight (22–24 h) in a fridge set at 2 °C and before starting the textural measurements all samples were placed in an incubator at 20 °C for two hours. The whole experiment was repeated 3 times.

5.2.2. 3D food printing condition

For printing of all formulations Foodini®, an extrusion-based 3D printer, was used. Before printing the samples were passed through the sieve as a means to eliminate or reduce the presence of lumps that may avoid printing through the nozzle. The samples were produced using a nozzle size of 1.5 mm, and an 8 layers hexagon prism was created for printing. The print speed was set at 3500 mm/min, the first ingredient holds at 4.2 mm, and the first layer nozzle height at 1.4 mm.

5.2.3. Instrumental texture analysis

This part is according to the textural tests done by Mirazimi *et al.,* (2022). The back extrusion and force of extrusion measurements were analyzed by the TA. XT Plus, Stable Micro Systems, UK.

5.2.4. Backward-extrusion test

The different formulations of fortified puree were placed in a cylindrical container 5 cm in diameter and 6.5 cm high and filled to 75% of the volume of the container. A 3.5 cm diameter plate probe was plugged through the sample at a speed of 2 mm / s and for a distance of 30 mm. The method was adjusted to mimic the speed of the printing conditions of the Foodini® and the index of viscosity (N·s) and cohesiveness (N) were obtained from this method.

5.2.5. Force of extrusion

To determine the firmness (N) and consistency (N·s) the 304 stainless steel capsules of the printer (© 2021 Natural Machines), with a capacity of 100 mL and 4 mm mouthpiece have been filled with each of the formulae to be tested. The probe plunger of the capsule (38 mm in diameter) was forced into the capsule at a speed of 2 mm/s and for a distance of 30 mm, pushing the fortified puree out through the 4 mm mouth placed at the bottom of the capsule. The maximum force needed to extrude the product was obtained by the analysis of the results of a test performed.

5.2.6. Sensory evaluation

The textural attributes of the fortified puree potato samples were evaluated by trained panelists at BDF company using QDA (Quantitative Descriptive Analysis) and hedonic evaluation. Each sample of 10 ± 2 g was scooped into a small bowl and served at room temperature (25 °C) for sensory evaluation. Room temperature water was provided for the panelists to cleanse their palates after testing each sample. The control sample was considered as a central point and served to evaluate the other samples taking this one as a reference. Eight panelists (two males and six females, aged between 25 and 45 years) were recruited and trained for sensory evaluation. According to the definition (Table 1) following parameters were measured: firmness, Thickness, Smoothness, Rate of breakdown, Stickiness, and Difficulty to swallow (Peh *et al.,* 2022).

Table 5 .1. Sensory attributes and definitions.

Parameters	Anchor	Definitions
Firmness	Very High – Very low	The force that is required to compress the food in-between the tongue and upper palate.
Thickness	Very High – Very low	The sensation of how thick the product is and the degree of the mass of food and saliva to hold the food together.
Smoothness	Very High – Very low	The homogeneity of the food when rubbed between the tongue and the upper palate and considering bits or lumps.
Rate of breakdown	Very fast – Very slow	The rate the food takes to be broken down in the mouth without chewing action.
Stickiness	Very High – Very low	The stickiness of the food in the mouth.
Difficulty to swallow	Very High – Very low	The swallowing difficulty after deforming the food between the tongue and the upper plate.

5.2.7. Data analysis

Data obtained from both instrumental measurements and sensory evaluations were analyzed using Minitab 18. The ANOVA and general linear model at a 95 % confidence level were used to determine significant differences. A Tukey's honest significant difference test was used to identify the differences among the samples. In addition, Pearson's correlation was used to determine the correlation between instrumental and sensory parameters.

5.3. Results and discussion

5.3.1. Instrumental texture analysis

The average results of the different parameters derived from force of extrusion and back extrusion parameters for each formulation are listed in Table 5.2. The firmness and consistency values were obtained from the force of extrusion test while cohesiveness and index of viscosity values were obtained from the back-extrusion test.

The highest firmness and consistency belonged to formulation 5S. The formulations 5C and 3S showed high firmness respectively with significant differences (P<0.05). Formulation 3S for firmness had the same behaviour as formulation 5C. Regarding consistency, formulation 5S and 5C showed the highest measurement, followed by formulations 3S and 3C (P>0.05).

Additionally, the parameters achieved from the back-extrusion test showed the highest cohesiveness for formulation 5C and the highest index of viscosity for formulation 5S. Following these results, formulations 3S and 3C showed the same behaviour for cohesiveness and index of viscosity. Formulations with egg albumin protein had the lowest values referring to all parameters, even below those of the control sample.

Generally, with the addition of SPAH and cricket proteins from 3% to 5%, textural attributes measurements increased which can be explained by increasing the interactions that could be created between starch and protein that increase along with protein concentration. The formation of starch-protein mixed systems was weak in the case of egg albumin because the activity of ovalbumin depends more on protein concentration, temperature, and heating process (Mine *et al.*,1991). The condition of this study involved in the thermally-induced native proteins have become disrupted and thus, convert to denatured forms, cannot build a strong network between protein and starch (Clark *et al.,* 2001).

Table 5. 2. Textural attributes measurements.

Samples	Firmness (N)	Consistency (N·s)	Cohesiveness (N)	Index of viscosity (N·s)
Control	0.022^{c}	0.33^{c}	-3.230^{ab}	-0.023^{bc}
3S	0.035^{ab}	0.39^{b}	-3.980^{b}	-0.032^{d}
5S	0.042^{a}	0.52^{a}	-3.360^{b}	-0.050^{e}
3C	0.028^{b}	0.37^{b}	-2.820^{ab}	-0.028^{cd}
5C	0.035^{ab}	0.43^{ab}	-4.050^{c}	-0.033^{d}
3A	0.026^{b}	0.35^{bc}	-2.280^{a}	-0.017^{ab}
5A	0.024^{bc}	0.29^{d}	-1.950^{a}	-0.014^{a}

Note: 3S (3 % Soy), 5S (5 % Soy), 3C (3 % Cricket), 5C (5 % Cricket), 3A (3 % Albumin), and 5A (5 % Albumin). Different superscript letters indicate a significant difference ($p < 0.05$).

5.3.2. Sensory analysis

5.3.2.1. Hedonic evaluation

For a better interpretation of the results of sensory evaluation, they are represented in a spiderweb diagram (Figure 5.1) depicting the ratings for color, flavor, mouthfeel, aroma, and overall acceptability of all formulations. Regarding the aroma, flavor, and overall scores of formulations, the level of added protein (3% and 5%) had no significant differences. However, there was a noticeable and significant effect on the rating for the cited parameters between the different proteins following descending order: cricket > egg albumin > SPAH. The color rate score of all formulations was in the range 3.10 – 4.25 (3= acceptable color and 4= attractive color) for all the panelists for all the high protein puree potato products. However, the hedonic mouthfeel characteristics scores had significant differences and the highest liking score belonged to the cricket protein samples followed by the control sample, egg albumin, and SPAH in this order.

The SPAH protein likeability kept on declining in all the sensory attributes which shows that the addition of this protein was not acceptable for flavor and aroma attributes. Hence, it can be concluded that the cricket protein 3% and 5% are the best formulation in terms of hedonic characteristics.

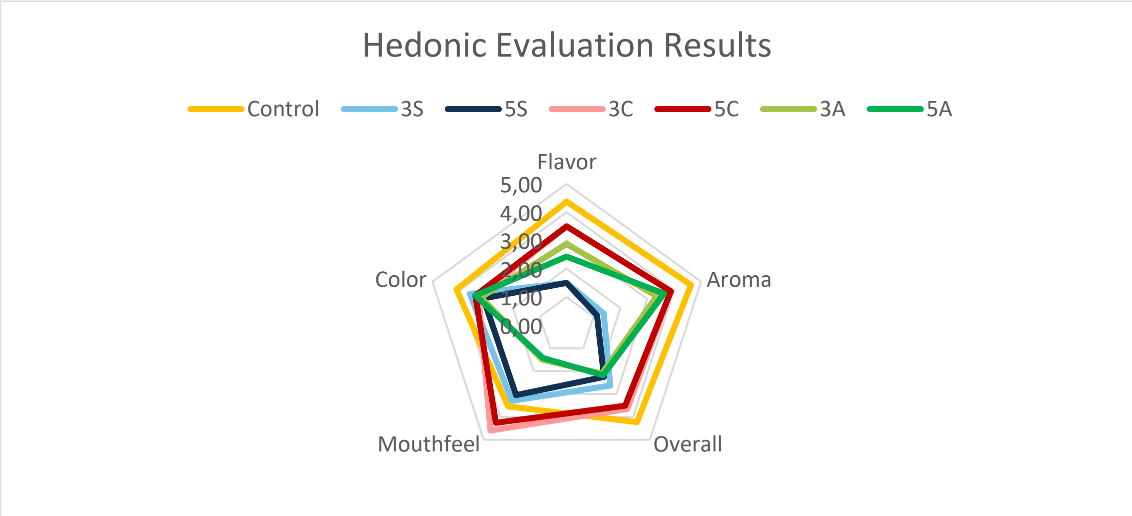


Figure 5. 1. Spider web chart of the hedonic evaluation.

Note: 3S (3 % Soy), 5S (5 % Soy), 3C (3 % Cricket), 5C (5 % Cricket), 3A (3 % Albumin), and 5A (5 % Albumin). Different superscript letters indicate a significant difference ($p < 0.05$).

5.3.2.2. Sensory evaluation

In this study, a trained QDA panel evaluated a similar set of fortified puree formulations that were also characterized by the texture analyzer as described earlier. The overall mean scores for each sample are shown in Figure 5.2.

Based on the plot, all samples with the sensory attributes: firmness, thickness, smoothness, stickiness, and difficulty to swallow, with the exception of the rate of breakdown, followed a descending order of 5C and 5S > 3C and 3S > control > 3A and 5A.

The 5C and 5S formulations had the highest intensity scores for firmness and thickness respectively with significant ($p < 0.05$) differences from the rest of the formulations. The formulations 3C and 3S showed a high score on these parameters as well. It has also been suggested (Stahlman *et al.*, 2000) that viscosity and firmness are interrelated as the more thick a product is, the more force will be needed to compress the pureed food. The force needed to compress the puree can be estimated from the measurement of firmness from the force of extrusion, as done in the present study.

The formulations with cricket and soy protein had not any significant differences in smoothness and stickiness to each other. However, they were different ($p < 0.05$) from the control sample and the samples with egg albumin. From our data, the degree of smoothness was observed in the order of 5S, 3C, 5C, 3S >control > 3A, and 5A with significant differences ($p < 0.05$). This could be contributed by cricket and SPAH interactions with starch granules, which provided a coating layer between the tongue and palate giving a smooth perception.

The formulations with cricket and SPAH with different concentrations had high stickiness and all formulations including these kinds of proteins (5C, 5S, 3C, and 3S) ($p > 0.05$). Control formulation, because not having any protein added, and the egg albumins formulations because of protein incompatibility that is discussed earlier, had the lowest rating for stickiness

Formulations with egg albumin protein were rated the highest in the rate of breakdown (p-value < 0.05). The rate of breakdown shows an opposing trend to the attribute difficult to swallow. This trend is conceivably logical for panelists with normal swallowing. This is because food that has a too low or too high rate of breakdown may not always be perceived to have the same degree on the difficult to swallow or easy to swallow parameter. The rate of breakdown from the highest to lowest are 5A and 3A > control > 3S, 5C, 5S, and 3C. The sample with egg albumin had the highest score for the rate of breakdown, which means that it could be deformed and disintegrated easily when subjected to an applied force between the tongue and the upper palate in the mouth. As the control did not have any protein added, it is expected that the puree sample was easily broken down in the mouth and can be explained by poor instrumental texture analysis (low measurements for different parameters) which can show these formulations can break very fast.

In the case of 3A and 5A samples containing egg albumin, the high rate of breakdown could be attributed to incompatibility between protein and starch due to limited protein denaturation of egg albumin and starch gelatinization, which rapidly reduces the binding ability and consistency of the samples.

The difficulty to swallow parameter had no significant difference among all samples.

Overall, the sensory evaluation results showed that the textural attributes of fortified potato puree formulations perceived during oral manipulation varied significantly with the different proteins and agree with the data obtained using back extrusion and force of extrusion by instrumental texture analysis.

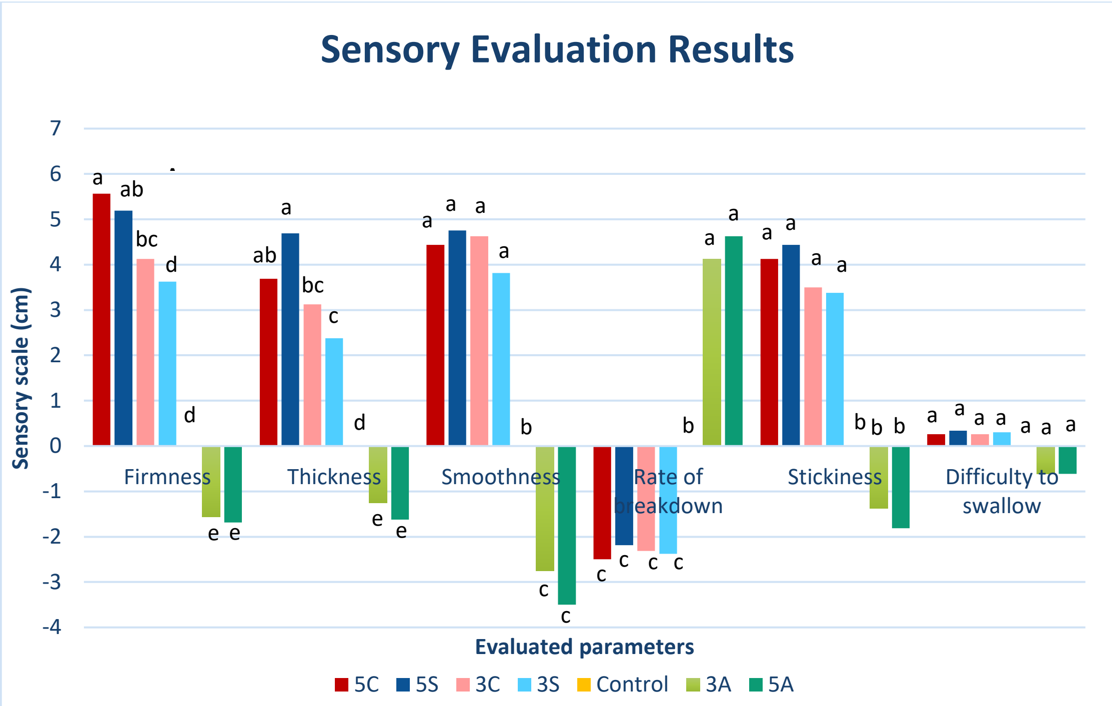


Figure 5. 2. Summary of sensory attributes evaluated by a trained panel (n= 8) for fortified formulated with five three proteins with the control sample as a central point.

Note: 3S (3 % Soy), 5S (5 % Soy), 3C (3 % Cricket), 5C (5 % Cricket), 3A (3 % Albumin), and 5A (5 % Albumin). Different superscript letters indicate a significant difference ($p < 0.05$).

5.3.3. 3D printing and correlation between textural analysis

All the formulations were extruded out from the nozzle at 25 °C. A hexagon shape was applied to see the printability and stability of the formulations. The hexagon shape for all formulations as well as the control sample was able to keep the structure for more than 15 minutes. Figure 5.3 shows the result of 3D printing. The formulations with SPAH and cricket protein were extruded very well in the printing process and the materials showed the ability to form self-supporting structures. Also, the control sample showed very good, extruded meantime printing and establish 3D printed shape. Egg albumin showed very poor resolution due to its low textural properties. In addition, during printing, the fortified formulations with albumin were not able to tolerate the load of layers.

In the present day, there are many studies that found that printed shape stability was closely related to textural properties (Huang *et al.,* 1947; Derossi *et al.,* 2021). Protein incompatibility is a reason that egg albumin during formation and functional properties of starch-protein mixed systems worked very weak which is on account that incompatibility is strongly dependent upon the conformational state of the proteins and it is enhanced by protein denaturation (Grinberg and Tolstoguzov, 1997).

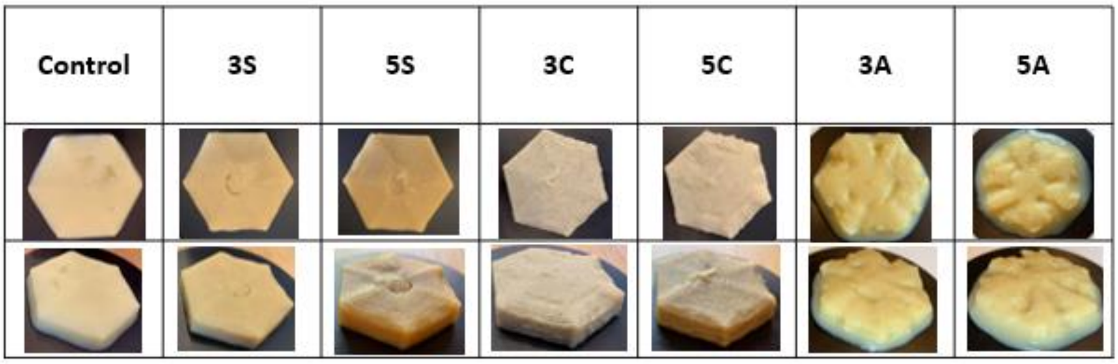


Figure 5. 3. 3D printed shape.

Note: 3S (3 % Soy), 5S (5 % Soy), 3C (3 % Cricket), 5C (5 % Cricket), 3A (3 % Albumin), and 5A (5 % Albumin). Different superscript letters indicate a significant difference ($p < 0.05$).

5.3.4. Correlation between sensory and textural analysis

For a better understanding of the behavior of different proteins in fortified puree potato, the Pearson correlation method was used. This method is used to examine the direction and strength of the linear relationship between two variables. In this study correlations of the attributes obtained by texture analysis and attributes from the sensory evaluation were calculated. The Pearson correlation method was a correlation coefficient with an absolute value of 1 point to a perfect linear relationship. Strong correlations (0.806–0.994) were obtained for certain attributes obtained by instrumental and sensory measurements as shown in Table 5.2. A p-value lower than 0.05 indicates that the attributes were significantly correlated to each other. From the analysis, the consistency (instrumental) was positively correlated to thickness (sensory) and smoothness (sensory) with coefficients of 0.994 and 0.893, respectively (Table 5.3). The cohesiveness attribute obtained by the texture analyzer was negatively correlated to the rate of breakdown from the sensory evaluation with a correlation coefficient value of -0.863 (p-value of 0.002) (Table 5.3). It is to be noted that the fortified puree potato samples tested in the sensory evaluation were subjected to the conditions in the mouth, especially where the samples were mixed and diluted with saliva. The mixing of saliva with the puree and the mechanical manipulation of the puree in the mouth resulted in the formation of a food bolus. The presence of saliva has been suggested to increase cohesion and viscoelastic properties of the bolus due to the human mucin components secreted in food-stimulated saliva (Sukkar *et al.,* 2018). Nevertheless, a higher cohesiveness value obtained from instrumental measurements means that an increase in the applied force is required for the puree processing in the mouth to facilitate the mixing of the puree with the saliva to form a bolus prior to swallowing. Hence, a fortified puree sample with a higher cohesiveness value would correspondingly result in a lower perceived rate of breakdown in the mouth. Although the results obtained from this study showed a strong correlation between these two attributes, factors such as dilution of foods by saliva in the mouth, differences in the applied shear forces in the mouths of dysphagia subjects as compared to healthy subjects, and so on are important factors to be considered in future studies.

For the adhesiveness attribute obtained by the texture analyzer, positive correlations were obtained with the sensory attributes of adhesive and difficulty to swallow.

The results from this work have shown that instrumental measurements could be used as a rapid and cost-effective tool to characterize texture-modified food.

The firmness attribute measured from either force of extrusion and sensory attributes were positively correlated. Consistency was positively correlated to thickness and smoothness. Cohesiveness was negatively correlated to the rate of breakdown. However, due to the effect of saliva on the food bolus and the different shear forces in the mouth further validation is needed to establish the correlation. The index of viscosity obtained from the texture analyzer was positively correlated to sensory attributes of stickiness and difficulty to swallow.

Table 5. 3. Correlation results between texture and sensory attributes.

Texture attributes	Sensory attributes	Pearson correlation coefficient	P-value
Firmness	Firmness	0.806	0.000
Consistency	Thickness	0.994	0.003
	Smoothness	0.893	0.000
Cohesiveness	Rate of breakdown	-0.863	0.002
Index of viscosity	Adhesive	0.968	0.000
	Difficulty to swallow	0.833	0.012

5.4. Conclusion

3D printing of food offers many possibilities for customized nutrition, including exceptional flexibility in geometries, textures, and flavors. Increasing the number of studies using 3D printing technology and increasing the number of people with swallowing difficulties is an opportunity to investigate more about sensory and textural attributes of 3D printed food and find the correlation that may exist between these attributes. In this study according to the hedonic test observed cricket and egg albumin protein had the best overall score compared to SPAH that is because the taste and odor of hydrolyzed protein were strong and not acceptable for panelists which has a solution with considering 3D printing technology is possible to mix this formulation with different flavor depends on each people taste.

The cricket and SPAH protein showed a very stable shape after 3D printing and self-supporting behavior, but egg albumin had no stable shape and could not tolerate the weight of shape layers. We found that there is a direct relevancy between 3D printing and textural measurements.

According to the sensory attributes measured using QDA were strongly correlated with instrumental measurements.

In general, the statistically significant correlations have demonstrated the usefulness of using an instrumental technique to characterize texture-modified food. This study also highlights the need for quantitative guidelines for formulators of different ingredients in selecting suitable substances and recommending appropriate concentrations for users preparing foods considering printability or material and people with special needs like dysphagia. The knowledge from this study is helpful to understand better the correlation between textural and sensorial attributes and showed the possibility of using textural analysis to predict the sensory attributes which require a lot of time for training panelists and are more cost-effective.

Chapter 6. General Discussion

Keywords: 3D food printing _ Nutrition _ Personalized meal _ elderly _ Dysphagia _ IDDSI

The number of elderly suffering from chewing/swallowing impairment is increasing rapidly with the coming aging society and the present investigations will allow understanding of the best ingredients and additives which can be used for creating personalized printable formulations (Giura *et al.*, 2021; Pattarapon *et al*., 2022). In this thesis, we created high protein formulations (3, 5, 7% SPAH and 0.2% agar) that can provide nutritional benefits from the nutritional point of view for the elderly and people that are in a risk group for malnutrition. In addition, all formulations were printable although the formulations with 0.2% agar showed more shape stability as Ying Wu *et al.,* (2009) results showed that agar and potato starch were compatible and the addition of agar improved the microstructure and mechanical properties of starch. Before 3D printing technology to be used for personalizing diets such as dysphagia and elderly, is possible to make sure about the safety of formulations by IDDSI tests (spoon tilt and fork pressure) which in this study all the tested formulations were acceptable for a dysphagia diet.

Keywords: 3D printing _ Extrusion based _ Rheology _ Material _ Rheology _ Yield stress_ Storage modulus_ Printability

There are many studies to prove to fabricate delicate and complex shapes during the soft-material extrusion process, it is necessary to use additional structural objects to support the product geometry (Rogers *et al.*, 2021).

Therefore, it is necessary to fully understand the material properties and relevant technologies thus, to be able to construct 3D structures. The printing precision and accuracy are critical in the production of an appealing object, and there are several factors that may be responsible for this: 1) extrusion mechanism 2) material properties, such as rheological properties, gelling, melting and glass transition temperature 3) processing factors, such as nozzle height, nozzle diameter and extrusion speed (Liu *et al.*, 2017). In extrusion-based printing, the properties of food material, such as the moisture content, rheological properties, specific crosslinking mechanisms, and thermal properties, are critical for a successful printing. We have investigated the impact of rheological parameters on 3D printing by the addition of different concentrations of different proteins and hydrocolloids on potato puree. We concluded that the highly desirable materials for 3D food printing would have suitable yield stress (τ_0) and elastic modulus (G′) to be capable of maintaining printed shapes. Additionally, is proved that by considering different rheological parameters such as consistency index (K) and flow behavior index (n) is possible to predict the material printability through a nozzle for using a extrusion-based type printer (Liu *et al.*, 2018). The addition of SPAH and cricket (3 and 5%) proteins created strong enough mechanical strength with yield stress (τ_0) of (465-535 Pa) and proper elastic modulus (G′) of (602-995 Pa), therefore the objects could withstand the shape over time and possessed smooth shape and resolution. On the opposite, the addition of egg albumin protein induced a drop in τ_0 (270-290 Pa) as compared with the control formulation thus printed objects deformed over time because of sagging. Nevertheless, with the addition of agar as a thickener at 0.2% to a formulation with SPAH protein produced a puree with excellent extrudability and printability due to proper τ0 (488-602 Pa) and G′ (974-1352 Pa). Cavin Tan et al., (2018) discussed how additives such as hydrocolloids may modify the rheological properties and texture of a pureed food for printing.

Keywords: 3D printing _ Rheology _ Dysphagia _ IDDSI _ Hydrocolloids _ Protein

The texture-modified food for people with dysphagia and its rheological properties has been studied., knowing the rheological properties of food are very important for the swallowing process (James *et al*., 2011). From all the experiments related above, it was observed that the samples with SPAH and agar had strong ability to enhance the viscosity and yield stress values of the potato puree at the concentrations used in the present thesis and its combination is favorable for food technological processing. According to IDDSI test, all samples were suitable for people with dysphagia but using agar as a thickener it is possible to achieve an improvement on all the mechanical properties such as yield stress, thixotropy, and viscoelastic properties and could increase the stability of printability for a multilayer shape. There are many studies (Dankar *et al.*, 2018; Dick *et al.*, 2020; Pant *et al.*, 2021) reporting the addition of hydrocolloids, such as agar, xanthan, or guar, increase the internal strength due to their relatively large molecular size, give them the ability to form conveyed network structure with the polysaccharide chains.

One of the most widely used interventions in the management of dysphagia is using thickening agents, as these normally present the greatest risk of aspiration *(Andersen et al.*, 2013; Newman *et al.*, 2016). As the thickener increases the viscosity, the flow rate of the bolus during swallowing is significantly reduced, increasing the chances of the airways being secured in time to prevent aspiration (Qazi *et al*., 2019). The viscosity-dependent therapeutic effect of this thickener has proven to be especially noticeable in patients with impaired swallowing physiology, such as elderly people, dysphagia disease patients, and post-stroke patients. Increasing viscosity through the use of this thickener allows these patients to swallow safely in up to 96% of cases, compared to just over 40% who are able to do so when swallowing low-viscosity liquids (Hyo Choi *et al.*, 2011).

Keywords: Printability _ Viscoelastic behavior _ Force of extrusion _ Back extrusion

On the other hand, in this study, there were included not only rheological properties such as G` and yield stress but also textural tests were done to evaluate such as the force of back extrusion. Back extrusion values correlated well with printability. As a result, the firmness and consistency values in the minimum range of (0.03 N) and (0.40 N·s) respectively measured from the force of extrusion test demonstrated its ability to assure a formulation favorable for 3D printing. Formulations with soy and cricket protein that had stability even for complex shapes and showed high firmness and consistency between the rage of (0.03– 0.04 N) and (0.38 – 0.52 N. s) respectively. It was observed that samples with egg albumin were not able to produce stable printed forms because of the very weak entanglement networks, recording the lowest values for firmness and cohesiveness (significantly different from all the other samples). The TPA test can present good information about the mechanical characteristics of formulations. But for the pureed kind of food, there are some difficulties to analyze correctly the results of this test as the samples tend to stick to the upper plate during the measurements and making it impossible to obtain good adhesivity measures.

As seen from our data, the formulations control and the samples with egg albumin could not support the complex designed shape when printed, which was attributed to their poor mechanical strength to be self-supporting and resist the gravity that compresses the printed shape causing deformation. The SPAH and cricket protein showed strong self-supporting for all the printed designs selected in this study. It is directly indicated by the higher and lower values of G′, yield stress from rheological measurements, and their firmness, consistency, cohesiveness, and index of viscosity obtained from the textural measurements. This was consistent with the previous studies, which reported that rheological and textural properties are related to the self-supporting capability of 3D printed structures (Ma *et al*., 2021; Vancauwenberghe *et al*., 2018). In addition, it was observed that when these proteins concentration was at 3% to 5%, then the 3D printed samples were stronger due to the ability to create more binds within the polysaccharide-protein mixture.

Keywords: Sensory evaluation _ Texture analysis _ Dysphagia _ 3D printing

Furthermore, the sensory evaluation of the texture-modified food products developed in this thesis has been considered of great importance. An interesting conclusion obtained was the detection of a relevant direct correlation between the back extrusion and force of extrusion tests with sensory evaluation. The sensorial parameters such as firmness, smoothness, thickness, stickiness, rate of breakdown and difficulty to swallow showed a correlation with instrumental measurement. All of them had a positive correlation except the rate of breakdown. Moreover, statistically significant correlations have demonstrated the usefulness of using an instrumental technique to characterize texture-modified formulations. The findings are in coincidence with some previous studies (Peh *et al*., 2021; Sharma *et al.*, 2021) and also can help scientists to develop and predict printable materials designed for dysphagic patients and general elderly and children with swallowing difficulties.

Samples with SPAH and cricket protein showed a high scoring on sensorial measurements, as the opposite of egg albumin. From these results, we can follow the 3D printability results of samples with higher firmness and consistency showed good self-supporting even for complicated printed designs. In general, the textural measurement results suggest that the instrumental data widely depends on protein type and concentration. This may occur because of the formation of continuous networks of starch-protein and protein-protein interactions. Differences in the type and concentration would likely change the binding strength (Kurotobi *et al.*, 2018). However, understanding these changes and evaluating its results through sensory analysis is very complicated and require some very well-trained panelist. The present findings that demonstrate a good correlation between textural and sensorial attributes have proved that is possible to consider a textural attribute to predict sensorial attributes and speed up the process of design and validation of novel formulation using instrumental evaluations.

Chapter 7. General Conclusion

From rheological scale:

- All fortified formulations with different ingredients at different concentrations possessed non-Newtonian, shear-thinning behavior, which is favorable for the flow behavior through extrusion 3D printing.
- For all the samples, storage modulus (elastic) G` was higher than loss modulus (viscous) G” without any intersecting or crossing over point.
- Agar demonstrated the capacity to moderately affect and stabilize potato puree because of its gelling ability and its capacity to increase the rheological parameters. Also, was an acceptable thickener according to IDDSI test and all samples were classified as level 4 puree dysphagia diet within the IDDSI.
- The sieving process that normally is done before the printing process to avoid the presence of air bubbles and clumps has a significant effect on the rheological properties of the formulations containing hydrocolloids.
- Mechanical characteristics such as yield stress and storage modulus are directly related to the shape stability and the self-supporting ability of the formulations.
- All formulations were printable. However, the **best printability** was accounted **for 6SA (6 g soy protein and 0.2 g agar) with Yield stress (1489 Pa) and thixotropy (15345 Pa·s),** capable to maintain complex shapes made of multiple layers. The second best formulations were those with cricket and SPAH protein with the dosage (3 and 5%) used in this study that also showed good printability for a complex shape with multiple layers.

From textural scale:

- The TPA test is not considered the best analysis due to the stickiness of pureed food to the texturometer probe.
- The method developed in this thesis including the force of back extrusion parameter can be used for predicting the formulations' printability for complicated designs (Firmness should be more than 0.03 N and consistency above 0.35 N·s).

- The back extrusion and force of extrusion parameters showed a significant positive correlation with sensorial analysis.
- Textural attributes correlated very well with firmness, thickness, smoothness, stickiness, and difficulty to swallow parameters from sensorial attributes.

From sensorial scale:

- A combination of textural and sensorial analysis was helpful to reveal more information regarding the influence of each protein and understanding the possible correlation between these attributes.
- Sensory characterization indicated that the texture of fortified 3D printed puree formulations varied with SPAH and cricket protein, representing high firmness, thickness, smoothness, and stickiness with significant differences $p<0.05$.
- Sensory attributes measured among proteins were strongly correlated with instrumental parameters and 3D printing, which helps in understanding oral processing and can be further used for developing diets for people with swallowing disorders by 3D printing technology.
- In addition, using cricket protein as the substrate of the printing ink not only provides a new printing material but may also provide us with an idea for the development of different food products. Inks with cricket protein showed good printability and good sensory parameters.
- Sensory evaluation showed a correlation with instrumental measurements which has an impact on the development of printable and safe products.

Common conclusions from different proteins:

With the results obtained from this thesis we can conclude:

- Cricket protein is a very good complement to enrich potato puree to be used for developing 3D food printing formulations with good taste and odor.
- SPAH protein enriched potato puree showed very good mechanical properties for printing and self-supporting, but it was not acceptable due to the flavor and odor induced by the acid hydrolysis process. Its use on 3D-food technology is possible if adding different flavors to the formulations to mask SPAH and making

it is possible to personalize the final product with a specific flavor depending on people's taste.

- Egg albumin protein was discarded according to the poor printability, rheological and textural properties presented due to the protein denaturation causing the incompatibility of the protein with the starch in the conditions of this study.

Future perspectives

Is very important to consider the nutritional and health status of patients with dysphagia and to use protein enriched formular to prepare 3D-printed preparation to make more appealing their meals. More study about how sieving and extruding may affect the nutrient content, digestibility, and health of the gut microbiome of the consumer is needed to be done.

Sensory evaluation analysis by real patients in elderly houses should also be incorporated to ensure consumer acceptability.

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