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3D printing of a high protein yoghurt-based gel: Effect of protein enrichment and gelatine on physical and sensory properties

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A B S T R A C T

The potential application of 3D printing technology in creating protein-rich desserts with multisensory design was investigated. Yoghurt-gel inks were formulated by varying the concentration of gelatine and whey protein isolates (WPI). Assessment of rheological and textural properties prior to printing, showed that an increase of gelatine concentration from 7.5 to 12.5% w/w increased the yield stress, storage modulus, loss modulus, firmness, and resilience of yoghurt gels. Addition of 12% WPI reduced these effects; creating softer gels with reduced resilience. However, these gels showed stable shape after printing, especially in formulations with higher gelatine concentrations. The changes in textural properties caused by the extrusion process need to be considered when designing yoghurt gels, as a significant reduction in firmness and resilience and an increase in adhesiveness were observed after 3D printing. The more stable and well-shaped 3D printed yoghurt gels were obtained by the combined effect of WPI and gelatine which provided a good balance of appearance, taste, flavour, and mouthfeel attributes evaluated by a trained sensory panel. A consumer study performed with thirty healthy adults showed the potential to improve sensory acceptance through the creation of multisensory layering design.

1. Introduction

Three-dimensional (3D) printing is a technology that adopts a layer-by-layer deposition technique to build computer-aided designed objects (Zhu, Stieger, van der Goot, & Schutyser, 2019). Application of 3D printing technology has been gaining interest in many industrial sectors, including the field of food and gastronomy (Lipson & Kurman, 2013). It offers a range of benefits such as flexibility to use alternative food ingredients, highly customisable, and efficient material use (Lille, Nurmela, Nordlund, Metsä-Kortelainen, & Sozer, 2018). Godoi (2019) described various studies that utilised 3D printing technology to create complex structures made of fruits and vegetables, confectionary, and cereals.

One of the most crucial aspects of 3D food printing is the rheological properties of the printing material, also called ‘food ink’. The printing material should demonstrate viscoelastic behaviour with appropriate ability to flow, rigidity which will allow smooth extrusion, and sufficient stability to retain its form after deposition (Kim, Bae, & Park, 2017). In food manufacture, hydrocolloids or proteins are commonly used to modulate rheological and sensory properties; these are polymers that can form gels or viscous solutions. Gelatine, along with pectin, carrageenan, agar, gellan, and alginate are commonly used due to their gelling ability (Saha & Bhattacharya, 2010). Whey proteins, in addition to its good protein bioavailability, has the ability to modulate gel properties (Cao & Mezzenga, 2020) and improve printability (Sager, Munk, Hansen, Bredie, & Ahrné, 2021).

In addition to the production of complex 3D design, the highly customisable aspect of 3D printing could also enable nutritional optimisation tailored to one’s diet (Lipson & Kurman, 2013). For instance, the prospect of personalised nutrition will benefit hospitalised patients with compromised eating ability or reduced appetite by providing small portions adapted to their needs. Among this population, provision of protein-enriched products will allow fulfilment of nutrient requirements and facilitate recovery. However, high-protein dairy beverages often elicit undesirable mouthfeel quality such as astringency or mouth-drying which impairs their consumer acceptability (Methven et al., 2010; Withers, Gosney, & Methven, 2013).

Several studies have successfully conducted research on 3D printed food. Efforts have been made to elucidate the effect of printer parameters and rheological properties of food inks on printability. However,
investigation on the sensory and consumer aspect of 3D printed food is limited (Keerthana, Anukritihuka, Moses, & Anandharamakrishnan, 2020; Severini, Derossi, Ricci, Caporizzi, & Fiore, 2018). Kouzani et al. (2017) aimed to use 3D food printing to produce visually attractive texture-modified food for patients with dysphagia. An approach to enhance hedonic and sensory perception is by creating an inhomogeneous distribution of tastants (Holm, Wendin, & Hermansson, 2009; Mosca, Bult, & Stieger, 2013). For example, Mosca et al. (2013) showed that layered agar-gelatin gels containing different concentrations of sucrose enhanced the perception of sweetness in comparison to gels with homogeneously distributed sucrose. A proof-of-concept of multisensory design approach has been demonstrated to stimulate desire to eat in a previous study (Chow, 2019).

In these studies the layered gels were manually created. 3D printing technology offers unique possibilities to design the food structure by combining food materials with different properties and their special distribution. Thus, we hypothesize that the consumer acceptance of high-protein 3D printed foods can be modulated. For instance, the astringency or mouth-drying commonly perceived in high protein formulations can be reduced by creating layered products where low and high concentration of protein are combined.

The present study aims to investigate the potential sensory modulation of a protein-enriched food through the creation of 3D printed yoghurt gels with multisensory layered design. To achieve this objective, the rheological and sensory properties of 3D printed yoghurt gels with different concentrations of whey protein isolate and gelatine were evaluated to assess the effect of formulation on printing and sensory profiles. Rheological measurements and texture profile analysis were conducted on gels before and after 3D printing and their sensory profiles were evaluated by a trained sensory panel. Subsequently, two formulations with satisfactory printability and sensory profiles were selected to formulate a multisensory layered design yoghurt gel and the hedonic response was compared with a homogenously layered yoghurt gel in a consumer study.

2. Materials and methods

A 2² full factorial design with three replicates in the central point was used to investigate the independent effects of whey protein isolate-enrichment (0% – 12% w/w) and gelatine concentration (7.5% – 12.5% w/w) on printability as well as physical and sensory properties of yoghurt gels. Given the results, yoghurt gel with a multisensory layered design was formulated and the hedonic response was assessed in a consumer study. The concentrations of gelatine and whey protein isolate were based on pre-trials and nutritional recommendations from the Kulinarium Aalborg University Hospital (Denmark).

2.1. Materials

Commercial yoghurt (Naturmelk natural flavour Greek yoghurt 10% fat) was used as the main ingredient in this study. Beef gelatine (Torsleif bovine gelatine leaf) and whey protein isolate (WPI) (Arla Foods Ingredients) Lacprodan) were used to modulate the texture and printability of the yoghurt gels. These two proteins were selected based on the fact that gelatine is commonly used to modulate rheological properties of gels and WPI has a good protein bioavailability. To ensure food safety, citric acid (Kryta) was added to keep the pH below 4.5 while sweetener (Hermetas liquid sweetener) were added to balance the taste.

2.2. Formulation of the yoghurt gels for 3D printing

Five different formulations were developed according to the 2² factorial design. First, gelatine stock (20% w/w) was prepared by soaking gelatine leaf in water for 5 min, removing excess water, then melting over a bain-marie. To prepare the yoghurt gels, citric acid (1% w/w) and sweetener (1.5% w/w) were incorporated into Greek yoghurt. Then, different concentrations of whey protein isolate (0% WPI -12% WPI) and cooled gelatine stock (7.5% Gel- 12.5% Gel) were added and whisked to form a homogenous paste. The yoghurt gel was then cooled in a 5 °C refrigerator to allow complete gelation. The five formulations were named: LL: 0% WPI-7.5% gelatine; LH: 0% WPI-12.5 %gelatine; MM: 6% WPI-10% gelatine; HL: 12% WPI-7.5% gelatine; HH:12% WPI-12.5% gelatine.

2.3. 3D printing process

The yoghurt gel was transferred to printing syringes immediately before printing. Printing experiments were conducted in a temperature-controlled 20 °C room. A cube shape was selected from the list of templates available in the built-in Foodini software. Samples were printed using Foodini extrusion-based 3D food printer (Natural Machines, Barcelona, Spain) to form a 2.5 x 2.5 x 2.5 cm cube. Nozzle diameter of 1.5 mm was used to print the samples consisting of 13 layers, with first-layer nozzle height of 1.4 mm, and print speed of 2500 mm/ min. These printing parameters were obtained through optimisation during the pre-trials. Samples were printed on a plastic lid and covered with a cup for storage. Printed yoghurt gels were kept under refrigeration at 5 °C until the execution of instrumental and sensory analysis.

2.4. Rheological measurement

The rheological behaviour of yoghurt gels before printing was characterised using a DHR-2 rheometer (TA Instruments, New Castle, USA) equipped with two serrated parallel plates (diameter of 25 mm) at 20 °C. Yoghurt gels were cut into slices with a height of 10 mm. After sample loading, the sample was rested for 30 s before measurements, and each sample was measured in triplicate.

2.4.1. Yield stress

The yield stress, storage modulus (G’), and loss modulus (G’’) of yoghurt pastes was determined by oscillation stress sweeps at a frequency of 1 Hz and a logarithmically increasing shear stress (0.1–1000 Pa). During the first part of the measurements (0.1–10 Pa), all samples showed constant G’ and G’’ values also called the linear viscoelastic region. The crossover point between G’ and G’’ was taken as the yield stress (Dinkgreve, Paredes, Denn, & Bonn, 2016; Liu, Bhandari, Prakash, Manithal, & Zhang, 2019).

2.4.2. Creep-recovery

The creep-recovery behaviour was determined by applying an instantaneous shear stress on the paste for 3 minutes (creep-time) whereupon the stress was released and the recovery was monitored (3 minutes). The creep-recovery behaviour was determined in the linear viscoelastic region of 1 Pa and measured in two successive cycles. The recovery strain was calculated as follows (Spotti, Tarhan, Schaffer, Corvalan, & Campanella, 2017):

\[
RS (%) = \frac{(initial strain - final strain)/initial strain) * 100}{(1)}
\]

2.5. Texture profile analysis

Texture analysis was performed on yoghurt gels before and after 3D printing using Texture Analyzer (Model TA-XT Plus, Stable Microsystems, Godalming, United Kingdom) equipped with a 5.0-kg load cell. The texture analysis was performed with double compression with a speed of 2 mm/second, 50% strain, and a trigger force of 5 g using a 0.5 mm sphere (SMS P/0.5 S). The before-printing samples were removed from refrigeration and measured directly in the plastic container in which they had been set while the after-printing samples were measured immediately after printing. The parameters firmness, adhesiveness, and
resilience were extracted using Exponent software (6.1.9.0 version). All samples were measured in triplicate.

2.6. Image analysis

Lateral-view of 3D printed samples were photographed in a light box to document the height while top-view images were taken using Vidoe-meterLab spectral imaging device (Videometer, Herlev, Denmark) immediately after printing in a room with controlled temperature at 20°C. ImageJ software (Schneider, Rasband, & Eliceiri, 2012) was used to measure the dimension (height and top area) of the samples. All samples were photographed at T₀ (immediately after printing) and T₃₀ (30 min after printing and storage at 5 °C) for comparison to simulate the storage condition at the hospital kitchen. Each sample was photographed and measured in three replicates for accuracy. Deformation rate, calculated as the percentage of change in height between T₀ and T₃₀, was used as an indicator of stability (modified from Kim et al., 2017).

2.7. Sensory evaluation of yoghurt gel

2.7.1. Sensory profiling of printed yoghurt gels

The sensory profiles of printed samples were evaluated using a descriptive sensory profiling. The evaluation was performed following ISO 13299 (2003) guidelines by a sensory panel consisting 7 assessors (5 females, 2 males) recruited from external trained panel of the Department of Food Science, University of Copenhagen. All assessors had received regular training in evaluating various sensory modalities of food and were compensated for their participation. Prior to the evaluation, the panel underwent two 2-h training sessions specific for the texture and taste of yoghurt gels where they generated comprehensive vocabularies to describe the sensory attributes. They were subsequently trained to rate the attributes using the provided scale and standard references were provided to assist the training. With clear sensory differences between the samples, a total of 20 sensory attributes were included in the vocabulary. The final set of attributes along with the definitions and references are specified in Table 1.

Assessors independently evaluated the yoghurt in terms of appearance, basic taste, flavour, texture, mouthfeel, and aftertaste. The intensity of attributes was rated on a 15 cm unstructured line scale using FIZZ software (Biosystemes, Couternon, France). Following a complete block design, samples were randomised and evaluated in duplicates over 2 h evaluation sessions. Five samples, coded with a 3-digit number, were served at 5°C in a monadic-sequential manner. Tap water and warm water were provided for palate cleansing and a 10-minutes break was enforced between samples.

2.7.2. Consumer study on multisensory layered yoghurt gel

Based on the physical and sensory characterisations, two formulations were selected to formulate a multisensory layered sample (hereafter referred to as ‘multilayer’). A homogenously layered sample (hereafter referred to as ‘monolayer’ was presented as a reference to evaluate the effect of multisensory layering on hedonic responses. Limited by the number of 3D printed products that could be produced, 30 healthy adults (21 f, 9 m; mean age: 35 years) were recruited from the consumer database of Department of Food Science, University of Copenhagen. Prior to evaluation, participants received an introduction to the study and written informed consent was obtained from each participant. Participants evaluated the hedonic attributes in terms of appearance, taste, texture, and overall liking on a 15-cm line scale. The scales were anchored with “dislike very much” and “like very much” at the two ends, with a description “neither like nor dislike” at the center of the scale. Additionally, desire to eat and degree of salivation were evaluated as indicators of appetitive response (Barkeling, Rossner, & Sjoberg, 1995; Keessman, Aarts, Vermeent, Häfner, & Papies, 2016). Desire to eat was anchored with “very weak” to “very strong” and degree of salivation was anchored with “not at all salivating” to “extremely salivating”. The two samples, coded with a 3-digit number, were randomized and served at 5°C in a monadic-sequential manner. The data was collected anonymously, i.e. the data processor had no personal identifiable information on the participants. The study was conducted in

| Table 1 |
| Description of sensory attributes and reference used for sensory profiling. |
|---|---|---|---|
| Modalities | Attributes | Definition (Reference) | Scale p-value |
| Appearance | Jelly-like | Springy when spooned (Redcurrant jelly fynbo) | Not at all → very | 0.394 |
| Initial shine | Amount of light reflected from surface (Yoghurt gel) | Not at all → very | 0.016 |
| Rough | Surface | Amount of irregularity/protrusion/bumps (Yoghurt gel) | Not at all → very | <0.001 |
| Basic taste | Sweet | Sweet taste sensation similar to sucrose (24 g/L sucrose) | Not at all → very | <0.001 |
| Sour | Sour taste characteristic for Greek yoghurt (Naturnælk, 10% fat) | A little → a lot | <0.001 |
| Flavour | Buttermilk | Buttermilk flavour (Arla, 0.5% fat) | Not at all → very | 0.003 |
| Milky | Milky flavour characteristic for Whole fat milk (Arla, 3.5% fat) | Not at all → very | 0.157 |
| Cream | Cream flavour characteristic for combination of Whipping cream (Coop Anglamark, 38% fat) and whole fat milk (Arla, 3.5% fat) | Not at all → very | 0.199 |
| Texture | Shiny | Amount of light reflected from surface after sample has been sitting in room temperature (Yoghurt gel) | Not at all → very | 0.0013 |
| Firm | Hard, dense (Mozzarella cheese Galbani) | Not at all → very | 0.002 |
| Airy | Light, soft (Lemon mouse Dr. Oetker) | Not at all → very | 0.022 |
| Jelly-like | Springy, rubbery when manipulated in mouth (Redcurrant jelly Fynbo) | Not at all → very | 0.130 |
| Lumpy | Inhomogeneous/ uneven particle distribution (Rice milk dessert pudding Milbona) | Not at all → very | <0.001 |
| Mouthfeel | Coating | Amount of film left on tongue or mouth surface (Whipping cream Coop Anglamark, 38% fat + whole fat milk Arla, 3.5% fat) | Not at all → very | 0.325 |
| Smooth | Soft on tongue surface and low resistance to manipulation (Cream pudding Milbona, milk chocolate flavour) | Not at all → very | <0.001 |
| Melting | Melt-in-mouth, easy to swallow (Chocolate mousse Dr. Oetker) | Not at all → very | 0.046 |
| Drying | Casing dryness on the tongue (Natural cream cheese Boku) | Not at all → very | 0.316 |
| Astringent | Shrinking or puckering of tongue surface (Black tea Medov) | Not at all → very | 0.061 |
| Prickly | Irritation due to high sourness (Yoghurt gel) | Not at all → very | 0.001 |
| Aftertaste | Sour | Sour aftertaste similar to Greek yoghurt (Naturnælk, 10% fat) | A little → a lot | 0.002 |
accordance with the Declaration of Helsinki Ethical Principles and Good Clinical Practices. Based on the “Danish Act on Research Ethics Review of Health Research Projects; Section 2” the study could be initiated without approval from The Committees on Health Research Ethics for the Capital Region of Denmark.

2.8. Statistical analysis

In order to examine the effects of WPI and gelatine concentrations on the physical properties, data of rheological and textural measurements were analysed based on the $2^2$ factorial design. To compare the magnitude of effect between the two factors, effect sizes ($\eta^2$) were obtained from partial eta-squared. Data from sensory profiling was analysed using a mixed model ANOVA in SPSS 25 (IBM, USA). Sample was assigned as a fixed factor in the model, with assessor and replicate as random factors and interaction effects of sample*assessor and sample*replicate. Principal components analysis on sensory profile of 3D-printed yoghurt gels were performed in OriginPro 2020 (OriginLab, USA). Due to the unequal subject (consumer) variance causing uneven weight in the analysis, data from the consumer study was normalised in order to account for the individual variance (i.e. some consumers tend to use the whole range of scale whereas others tend to avoid using the extremes). This was done by calculating the standard score of each observation based on individual consumer variance (Eq.2). The normalised data was then analysed using paired sample t-test in SPSS 25 to compare the ratings of multilayer and monolayer samples. For all analyses, the significance level was set at $p < 0.05$.

$$Z = \frac{x - \mu}{\sigma}$$

$Z$: standard score; $x$: observed value; $\mu$: mean of the individual rating across all attributes; $\sigma$: standard deviation of the individual rating across all attributes.

3. Results and discussion

3.1. Rheological and textural characterisation of yoghurt formulations before 3D printing

Storage modulus ($G'$) describes the elastic part of the material and loss modulus ($G''$) describes the viscous part of the material. Both parameters were obtained in the linear viscoelastic region, where $G'$ and $G''$ are constant. The yield stress, determined at the crossover point between $G'$ and $G''$ representing the minimum force required for the sample start to flow (Dinkgreve et al., 2016; Liu et al., 2019), is an essential parameter to understand the behaviour of printed materials during extrusion. During 3D printing the yield stress of a material has to be overcome by the printer, therefore, the yield stress should be enough to allow smooth and continuous extrusion (Liu et al., 2019). All formulations exhibited yield stresses suitable for extrusion (<1400 Pa) and $G'$ exceeded $G''$ which describes a material with viscoelastic properties (Fig. 1). Both gelatine concentration and WPI influenced yield stress, $G'$ and $G''$, with gelatine causing a more significant effect for all these substances.

![Fig. 1. Mean of a) yield stress, b) storage modulus, and c) loss modulus of yoghurt gels before printing, error bars represent standard deviation. Means with the same letter are not significantly different from each other ($p > 0.05$ ANOVA followed by Tukey LSD test). From left to right: LL: 0% WPI-7.5% gelatine; LH: 0% WPI-12.5% gelatine; MM: 6% WPI-10% gelatine; HL: 12% WPI-7.5% gelatine; HH:12% WPI-12.5% gelatine.](image-url)
parameters.

Increasing gelatine concentration from 7.5 to 12.5% strengthened the gel network structure and caused a significant increase in yield stress (up to 65%), storage modulus (up to 104%), and loss modulus (up to 64%). Similarly, Ares et al. (2007) also observed the same effect of gelatine on yield stress of stirred yoghurt. Gelatine influenced the rheological properties by entrapping caseins from the yoghurt in a three-dimensional compartmentalised network which has shown to significantly increase G’ (Fiszman, Lluch, & Salvador, 1999).

It was observed that incorporation of 12% WPI led to a decrease in yield stress up to 23% and storage modulus up to 46% (p = 0.02, p < 0.001 respectively). These findings are in accordance with earlier observations by Morell, Tarrrega, Foegeding, and Fiszman (2018) and Patocka, Cervenková, Narine, and Jelen (2006). Addition of 12% WPI also reduced the loss modulus by up to 25% (p < 0.001) although the effect was only observed on the high-gelatine sample (12.5%) (Fig. 1). Fortifying yoghurt with whey protein showed to be advantageous not only due to its nutritional properties but also its functional properties such as long-term stability, emulsification, water-holding, foaming, thickening, and gelling characteristics (Karam, Gaiani, Hosri, Burgain, & Seher, 2013). These characteristics were obtained due to the extensive formation of crosslinks within the gel network upon treatment using either heat, high pressure, enzyme, salt-induced gelation or acidification (Spotti et al., 2017), however in the present study the WPI powder was added to the yoghurt without involving any of these processes. Absence of the aforementioned treatments limited the cross-linking of whey protein, hence WPI acted as “inert filler” unable to form a cohesive network with the yoghurt matrix which consequently weakened the existing yoghurt gel network (Patocka et al., 2006). In the context of producing printable food gels, the addition of inert WPI can be considered as an advantage which enables smooth extrusion during printing. Similar findings have been reported by Kristiansen (2019) and Sager, Munk, Hansen, Bredie, & Ahnér (2020) where the addition of microparticulated whey protein, acting as an “inert filler”, significantly improved the printability and shape retention of heat-induced whey protein gels.

Creep-recovery test was performed to understand the viscoelastic behaviour of the different formulations. Fig. 2 visualised the behaviour during two cycles of stress application (3 min) and recovery (3 min). All samples showed typical viscoelastic creep curves with an initial steep increase in strain and a non-recoverable strain after the recovery cycle (de Faria, Minim, & Minim, 2013). It was observed that WPI increased the maximum strain, whereas gelatine decreased it. The highest strain was observed in WPI-enriched sample with 7.5% gelatine content (HL) which is indicative for gels that strongly deform during stress application. Contrary, samples with 12.5% gelatine (HH and LH) deformed less, indicated by a lower strain which is a characteristic for firmer and more elastic gels.

Recovery strain (RS) was calculated to understand the creep-recovery behaviour and is expressed in Fig. 3. An optimum recovery behaviour is necessary to support the subsequent layers upon deposition (Liu et al., 2019). In all samples, the RS varied between 59 and 94% and decreased in the second cycle. This was also observed in earlier studies by Spotti et al. (2017) and Sager et al. (2021) and is a characteristic for viscoelastic gels which lose part of their elasticity after the first creep. No significant effect of WPI and gelatine concentrations were observed on the recovery strain of gels before printing (p > 0.05). Samples that deformed the most also had the lowest RS indicating that the structure was less elastic. These samples were enriched with WPI and the reduced elasticity can be explained by the minimal interaction between WPI and the yoghurt structure (Patocka et al., 2006). Incorporation of inert components such as microparticulate whey protein and micellar casein isolate has earlier shown to increase the maximum strain and decrease the RS (Kristiansen, 2019; Sager et al., 2021). On the other hand, there was a tendency of gelatine to increase elasticity which can be attributed to a highly structured and elastic matrix formed by gelatine (Fiszman et al., 1999).

Firmness is the force needed for a predetermined deformation (Szczesniak, 1963) and is an important textural property in relation to printability as it will influence the shape retention and the extrusion characteristics of the printed material (Pérez, Nykvist, Brogger, Larsen, & Falkeborg, 2019). Fig. 4a demonstrates that addition of 12% WPI reduced the firmness (p < 0.001), while increasing the concentration of gelatine from 7.5 to 12.5% increased the firmness (p < 0.001) of the gels before printing by 100 to 115%. The result follows a consistent trend with the rheological measurements in which samples with firmer texture also had higher yield stress. Further, it was revealed that the effect size of the two factors were similar (η² = 0.96 for WPI and η² = 0.95 for gelatine).

Adhesiveness is one of the mechanical characteristics of food texture,
defined as “the work necessary to overcome the attractive forces between the surface of the food and the surface of other materials with which the food comes in contact” (Szczesniak, 1963). Fig. 4b indicates that increase in gelatine concentration from 7.5 to 12.5% resulted in a decrease in adhesiveness by 55–67% (p < 0.003), whereas addition of 12% WPI did not induce significant effects on the adhesiveness (p = 0.796). Gelatine is known to have a high water-retention capacity (Fiszman et al., 1999) therefore at a higher concentration, more water molecules are entrapped in the matrix resulting in a more compact and dry gel.

Resilience indicates a product’s ability to recover from the structural deformation during the printing process and retain its shape; it is related to elasticity of printed product (Keerthana et al., 2020). Fig. 4c shows that addition of 12% WPI reduced resilience 4–17% (p = 0.03), while increasing gelatine concentration from 7.5 to 12.5% increased resilience 19 to 37% (p < 0.001). This result is consistent with the creep-recovery behaviour in which samples with higher gelatine concentration deformed less and tended to recover more. Gelatine concentration played a more significant role in resilience compared to WPI addition as shown by the effect size ($\eta^2 = 0.59$ and $\eta^2 = 0.25$, respectively).

### 3.2. Printability of the yoghurt formulations

Printability of the yoghurt formulations was evaluated through qualitative assessment of the 3D printed yoghurt gels, textural changes caused by 3D printing, and evaluation of stability of the 3D printed object after 30 min of storage. The qualitative assessment of printability was evaluated based on the ability to support the following deposited layers, ability to retain the printed shape, and ease of extrusion during printing (Godoi, Prakash, & Bhandari, 2016).

Fig. 5 shows that three samples, namely low WPI-low gelatine (LL), medium WPI-medium gelatine (MM), and high WPI-high gelatine (HH) displayed satisfactory printing resolutions, moreover these samples were easily extruded, could retain their forms, and support subsequent layers. Low WPI-low gelatine (LL) could withstand the shape and support subsequent layers but displayed a distorted surface and fragmented extrudate thread. On the other hand, high WPI-low gelatine (HL) could be smoothly extruded but looked deformed and sagging, the layers were not well-defined, and could be seen melting on top of each other.

These observations can be associated with the textural and rheological properties of the yoghurt gels. LH exhibited the highest firmness and resilience due to the gel-strengthening effect of gelatine and the absence of the gel-softening effect of WPI, as opposed to HL which displayed the lowest firmness and resilience (Fig. 4). In relation to the rheological behaviour, a combination of optimum yield stress and recovery behaviour are respectively crucial for extrusion stage and recovery stage during 3D printing (Liu et al., 2019). A high elasticity, indicated by the high recovery strain (Fig. 3), coupled with a high yield stress lead to rupturing during extrusion as it was observed for LH with its fragmented extrudate. On the other hand, materials exhibiting a lower than optimum yield stress allowed smooth extrusion, but along with the low elasticity of HL resulted to a lack of mechanical strength to support the following deposited layers. This suggests that optimum properties for both the textural and rheological parameters are needed for the materials to be well-printed as displayed by the three formulations (i.e. LL, MM, and HH).

A side-by-side comparison on the textural properties of the yoghurt gels before and after 3D-printing is presented in Fig. 4. The mechanical stress application during the 3D printing has significantly reconstructed the physical texture of yoghurt samples from a firm gel into a soft gel as they were extruded layer by layer through a narrow nozzle. In comparison to samples before printing, the mechanical stress of 3D printing caused a decrease in firmness (p < 0.001) and resilience (p < 0.001), and an increase in adhesiveness (p < 0.001). Similarly, Le Tohic et al. (2018) also demonstrated a significantly lower (45–49%) firmness value of 3D-printed cheese compared to untreated cheese although no significant difference on resilience was observed. High-gelatine samples (LH and HH) were more affected by printing compared to low-gelatine samples.

After 3D-printing (Fig. 4, right), there was no clear effect of gelatine and WPI on adhesiveness. Further, gelatine also had no effect on firmness and resilience (p = 0.39 and p = 0.40), whereas addition of WPI reduced firmness and resilience (p < 0.001 and p = 0.005). The 3D-printing process eliminated the initial significance of gelatine on firmness and resilience that was previously observed on samples before printing, whereas the effect of WPI remained significant. This implies that the printing process mainly affected the structure created by the gelatine network, as confirmed by the profound textural change in high-gelatine samples, compared to low-gelatine samples.

Stability of 3D printed yoghurts gels was assessed by the deformation rate, comparing the lateral height immediately after printing ($T_0$) and after 30 min of storage ($T_{30}$). Fig. 6 illustrates the deformation rate with mean values ranging from −1.9 to −13.2%, suggesting that the samples begin to lose its ability to support the structure after 30 min. There was no significant effect of WPI and gelatine (p = 0.30 and p = 0.43, respectively) on the deformation rate due to the large standard deviations.
3.3. Sensory characterisation of the 3D printed yoghurt gels

All samples were described as having jelly-like appearance and texture, creamy flavour, and coating mouthfeel. Milky flavour and drying mouthfeel were rated low in most samples. However, these attributes did not significantly differ between samples and were thereby excluded from further analysis. Principal component analysis (PCA) was performed to summarise the sensory characteristics of the printed yoghurt gels. The sensory variation between samples could be projected into the first two principal components. The PCA explained 96.5% of the variations in the sensory attributes of yoghurt gels with the first two principal components accounting for 72.0% and 24.5%, respectively, of the total variance.

A biplot for scores and loadings (Fig. 7) was generated to visualise the difference between samples. The sensory attributes were separated into two clusters by the first principal component (PC1) primarily due to the differences caused by WPI-enrichment. The second principal component (PC2) was defined by lumpy texture on its negative axis and smooth mouthfeel on its positive axis; PC2 subdivided the low and high-gelatine samples. The five samples were distinctive in terms of their sensory profiles, as they were projected into different quadrants of the plot. The attributes sour (taste and aftertaste), buttermilk flavour, astringent, and prickly sensations were highly correlated as they were...
closely positioned. Together with firm texture and rough appearance, these attributes were shown to have large positive loadings on component 1, generally characterising the low-WPI samples (i.e. LL and LH). These samples were not enriched with WPI powder therefore the sensory profiles were dominated by the sensory attributes of natural Greek yoghurt (buttermilk, sour) and citric acid (astringent, prickly). LH was particularly associated with rough appearance and firm texture which can be explained by the high concentration of gelatine.

In contrast, WPI-enriched samples (i.e. HL and HH) were associated with sweetness, indicating that the WPI powder used in this study has a dominant sweet taste which moderated the sourness of yoghurt. These samples were also characterised with airy texture which may be related to the gel-softening property of WPI. Furthermore, HL was also characterised by its shiny surface, smooth, and melting mouthfeel which may be associated with its low gelatine concentration. MM displayed intermediate intensities for most of the sensory attributes, confirmed by its location close to the centre of the plot.

A potential concern in enriching the yoghurt gels with whey protein is its tendency to elicit astringent sensations as previously reported in dairy-based high-protein beverages (Methven et al., 2010; Withers et al., 2013). Astringency is described as a sensation that causes puckering, drying, or roughing of tongue and oral cavity (Bull et al., 2017). The two potential astringent or mouth-drying sources in dairy products are whey protein and casein (Withers, Lewis, Gosney & Methven, 2014). Contrary to expectations, WPI-enriched samples in this study were not associated with mouth-drying or astringent sensations. Drying mouthfeel was minimally perceived in most samples (average 6.6 out of 15.0). Moreover, astringent and prickly sensations were higher in low-WPI samples (average 10.9 and 8.8) compared to high-WPI samples (average 6.6 and 2.0). This suggests that the astringent and prickly sensations were rather associated with the sourness caused by the yoghurt and citric acid whereas the addition of WPI masked this sourness.

Kelly et al. (2010) described the mechanism of whey protein-induced astringency is due to interaction of saliva with whey protein aggregate, resulting in a reduced oral lubrication. At present, addition of WPI powder without heat treatment did not contribute to the formation of protein aggregate. This notion is supported by Bull et al. (2017) which showed that increased heating time resulted in a higher astringent-related sensations, suggesting that astringency is influenced by thermal denaturation. An alternative explanation may be due to the method of the sensory evaluation used in this study in which samples were evaluated at one time-point whereas previous studies used a temporal approach to demonstrate intensified astringent sensations over sequential consumption.

In relation to the physical characteristics, after printing-firmness from the instrumental texture analysis (Fig. 4a-right panel) showed similar trend with results obtained from the sensory analysis. The sensory analysis showed that samples high in firmness (i.e. LH and LL) were associated with firm attribute. In contrast, samples that were low in firmness (i.e. MM, HL, and HH) were described to be airy.

### 3.4. Consumer acceptance of multisensory yoghurt gels

Multilayer sample (Fig. 8) was formulated upon evaluation of printability along with the physical and sensory characteristics of the formulations. In addition to the satisfactory printability, HH and LL demonstrated contrasting yet complementary sensory profiles (dominantly sweet and dominantly sour, respectively, Fig. 7) suitable to create
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a multisensory perception of flavour for the multilayer system. The ingredient composition of MM corresponded to the overall composition of the multilayer sample, thus representing a monolayer reference. The configuration of layer placement was decided based on pre-trials. The multilayer system demonstrated significantly improved taste liking ($p = 0.022$) and a significant reduction on appearance liking ($p = 0.045$). No significant differences were observed for degree of salivation, desire to eat, and overall liking ($p = 0.898$, $p = 0.065$, and $p = 0.203$, respectively). Compared to the monolayer system, the multilayer system displayed a lower printing appearance and stability due to the textural differences between each layer, but this did not affect the texture liking ($p = 0.405$). The improved taste may be attributed to the perceived fluctuation of flavour caused by the alternating sweet and sour layers (Mosca et al., 2013). This work gives a first indication about the potential improvement on sensory acceptance by layering products with different sensory characteristics. However, it is important to take in account that a multilayer system requires good compatibility between the material properties of the layers in order to keep a good appearance and stability. In the current study, the lower stability of the multilayer system may have compromised the results.

4. Conclusions

Whey protein isolate and gelatine were used as ingredients to formulate protein-rich yoghurt gels for 3D printing. An increase of gelatine concentration significantly increased the yield stress, storage modulus, and loss modulus as consequence of the formation of a highly structured gelatine gel network. Contrary, the addition of WPI, acting as an inert filler, caused a gel-softening effect on yoghurt gel due to its inability to form a cohesive network. In the context of 3D printing this effect is an advantage, allowing smooth extrusion of the gel through a narrow printer nozzle. In order to design gels for 3D printing, the changes in gel texture caused by the 3D printing process needs to be accounted, as significant reduction in firmness and resilience as well as increased adhesiveness were caused by the extrusion.

WPI and gelatine also influenced sensory properties of yoghurt gel. WPI primarily influenced taste, flavour, and mouthfeel attributes whereas gelatine influenced appearance and texture attributes. A consumer study with a multisensory-layered design of 3D printed yoghurt gel provided a positive indication of improved taste liking, however a significant reduction on appearance liking was observed due to instability of the 3D printed object. Future studies can be directed into creating multilayer products with better stability. In addition, more studies are needed to investigate hedonic and appetitive responses of various multilayer designs to support the development of innovative personalised nutrition concepts.

CRediT authorship contribution statement

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Fig. 7. Biplot for covariance matrix of sensory profile data, texts in blue (■) display the yoghurt samples (score) and texts in black (●) display the sensory attributes (loading). From left to the right: LL: 0% WPI-7.5% gelatine; LH: 0% WPI-12.5 %gelatine; MM: 6% WPI-10% gelatine; HL: 12% WPI-7.5% gelatine; HH:12% WPI-12.5% gelatin. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 8. Illustrative representation of the monolayer (left) and multilayer (right) sample.
References


