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Printability, stability and sensory properties of protein-enriched 3D-printed lemon mousse for personalised in-between meals

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Abstract

3D printing of foods is an emerging technology with the potential to develop nutritious and appetising foods to accommodate needs of special consumer groups. For the technology to succeed, studies need to address the composition of printable foods and the stability of printed matrices delivering acceptable sensory properties. This study investigated the effect of gelatine (1–2% w/w), citric acid (0.9–1.5% w/w) and whey protein isolate (WPI) (8–18% w/w) concentration in lemon mousse formulations on printability, physical and sensory properties. The textural properties of the mousses before printing were highly influenced by the concentration of gelatine and WPI but less by citric acid. Gelatine had a gel firming effect, giving a higher firmness and yield stress in mousses, while WPI softened the gel structure. The gel firming effect of gelatine was beneficial to produce 3D-printed mousses with good storage stability after printing, while the addition of WPI gave better-defined layers and a glossier surface of the 3D-printed mousses. The extrusion process disrupted the foam structure, creating a more uniform air bubble distribution, but decreasing the firmness and resilience of the mousses, nevertheless, sensorially attractive and stable 3D-printed mousses were obtained. Increasing WPI concentration in mousses enhanced the shiny appearance, smooth texture and melting mouthfeel. It reduced the rough surface, lumpy and compact texture in mousses, which were associated with the gel firming effect of gelatine. This work shows that protein-enriched 3D-printed lemon mousses with good printability, stability and high consumer acceptance can be produced by formulation design.

1. Introduction

Three-dimensional (3D) printing, also known as additive manufacturing, was originally developed for material-based printing of metal and ceramics to build up 3D structures layer-by-layer. In recent years the technologies have been applied to the food sector (Dankar et al., 2018; Godoi et al., 2016). The technologies offer a culinary potential to create foods with complex geometric designs (Lipson & Kurman, 2013). It also makes it possible to customise foods to accommodate the needs of special consumer groups such as children, elderly or hospitalised patients (Dankar et al., 2018). This includes tailoring the shape, taste, texture and nutrient content of printed foods to suit the preferences and nutritional requirements of specific individuals. Previous research has shown that protein-enrich foods and in-between meals are effective strategies to promote food intake in patients at nutritional risk (Gall et al., 1998; Okkels et al., 2016). Applying 3D printing to develop personalised foods e.g. desserts can be a novel initiative to stimulate the desire to eat.

Extrusion-based printing is commonly used in food printing, along with three other methods that are currently available: inkjet printing, binder jetting and selective sintering printing (Liu et al., 2017). During printing, the food material is extruded out of a narrow nozzle into a pre-set 3D shape by the force of a hydraulic piston (Godoi et al., 2016). The extrusion method with temperature control is suitable for semisolid materials, especially for heat-sensitive food ingredients such as eggs and dairy products that are widely used in desserts (Lee et al., 2020).

High compositional complexity and large variations in physico-chemical properties of different foods make 3D printing of foods a challenging technology. The food material used for printing is also called food ink. When the material is printed without phase transition occurring, the rheological properties have to be balanced between a sufficient viscosity and shear-thinning behaviour (Godoi et al., 2016; Liu...
This allows the food ink to withstand extrusion forces, and at the same time be able to maintain the structural integrity after passing the narrow nozzle. Printability is the properties of the food ink that allow handling and deposition of the material by a 3D printer and the material to be self-supporting post-deposition (Godoi et al., 2016; Kim et al., 2017). It is affected not only by the food ink rheology but also intrinsic printing factors, such as printing speed, nozzle size, layer height and temperature (Derossi et al., 2018; Hao et al., 2010; Severini et al., 2016). To improve printing, hydrocolloids are commonly added to food ink formulations to modify their rheological properties. Studies on rheological, textural properties and printability have largely focused on using fruit gels and pastes as food inks. These include lemon juice gel (Yang et al., 2018), fish surimi gel (Wang et al., 2018), processed cheese (Le Tohic et al., 2018), fruit and vegetables (Derossi et al., 2018; Kim et al., 2018), and various protein and fibre-rich materials (Lille et al., 2018).

While studies on 3D food printing focused on the food ink rheology and its printability, investigation on the sensory aspects of printed foods is limited but essential. For instance, printed foods have a layer structure and unique appearance that can influence the sensory perception of foods (Severini et al., 2018; Sun et al., 2018). Stabilising the food ink by adding hydrocolloids affects the perceived texture and mouthfeel of printed foods (Cohen et al., 2009). Characterising the sensory properties of printed foods may provide indications for multi-sensory design, for instance, to create foods with layers differing in composition and sensory properties to improve palatability (Chow, 2019; Hyde & Witherly, 1993).

Foams contain a continuous and a gas phase stabilised by various food components such as proteins, emulsifiers and fat crystals (Zúñiga & Aguilera, 2008). Mousse is an example of an aerated gel, a form of viscoelastic foam, in which the continuous phase is solidified by gelatine (Campbell & Mouget, 1999; Zúñiga & Aguilera, 2008).

The present study aims to investigate the effect of varying gelatine, citric acid and whey protein isolate concentration in lemon mousse formulations on printability and sensory properties. This was achieved by characterising the properties of the not-printed and 3D-printed lemon mousse by textural, rheological and image analysis. The sensory properties and acceptance of selected 3D-printed lemon mousse were then evaluated by a trained sensory panel and a consumer panel respectively.

2. Materials and methods

A two-level three-factor design with a central point was used to determine the effect of gelatine, citric acid and whey protein isolate (WPI) concentration on the printability of lemon mousse. To assess the variability of the 3D printing process, the lemon mousse formulation representing the centre point was repeated three times in three independent experiments.

2.1. Preparation of lemon mousse

Eleven formulations were developed based on the lemon mousse recipe provided by Kuliunarian Aalborg University Hospital (Denmark). These formulations varied in gelatine, citric acid and WPI concentration (Table 1). Samples were prepared in the following way: whipping cream 38% fat (Arla Foods; protein content: 2.1% (w/w)) was first refrigerated (Table 1). Samples were prepared in the following way: whipping cream at 5°C and porcine gelatine (Dr. Oetker; protein content: 84% (w/w)) was softened in cold water. Whole pasteurized eggs (Danaeg; protein content: 11.4% (w/w)), sugar (Dan Sukker), fresh lemon zest and WPI were mixed with a fixed electric hand mixer (Bosch MFO4020) until the sugar dissolved (approx. 1 minute). The citric acid solution was prepared by dissolving the weighted food grade citric acid granules (Kryta) in tap water, mixed and melted with the softened gelatine in a water bath at approximately 30°C. The refrigerated cream was whipped in a 500 mL measuring cup with a fixed electric hand mixer for approximately 2 minutes until the volume was doubled (100% overrun). The cooled gelatine-citric acid solution was then promptly mixed into the egg mixture and gently folded with the whipped cream. After being properly mixed, the mousse was transferred to a piping bag.

Table 1

<table>
<thead>
<tr>
<th>Formulation</th>
<th>% w/w</th>
<th>pH</th>
<th>% dry matter</th>
<th>w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMM1</td>
<td>1.5</td>
<td>1.2</td>
<td>13</td>
<td>46</td>
</tr>
<tr>
<td>MMM2</td>
<td>1.5</td>
<td>1.2</td>
<td>13</td>
<td>46</td>
</tr>
<tr>
<td>MMM3</td>
<td>1.5</td>
<td>1.2</td>
<td>13</td>
<td>46</td>
</tr>
</tbody>
</table>

*The 3-letter code of the lemon mousse formulations refers the first letter to gelatine, second letter to citric acid and third letter to WPI concentration at H: high, M: medium, or L: low concentrations.

Lemon mousses that were not-printed were prepared by piping the freshly made mousse into 100 mL disposable portion cups with lids and refrigerated overnight at 5°C. Formulations to be 3D-printed were first refrigerated overnight in the piping bag and transferred to the printing syringes right before printing. The pH of each formulation was measured (pH meter HQ411d, HACH Company, Loveland, USA) and the % dry matter was calculated based on the dry matter of the ingredients (Table 1). The batch size of each formulation ranged from 432 to 480 g, depending on the gelatine, citric acid and WPI concentration.

2.2. Printing process

Lemon mousses were 3D printed using the Foodini cold extrusion-based printer (Natural Machines, Barcelona, Spain). Based on pre-trials, the print speed was set to 250 mm/min and line thickness to 3.5 mm for optimal printing condition. Cylinders of samples (≤25 mm × 21 mm; 6 layers) were extruded using a 4 mm circular nozzle. Excluding the time spent loading and unloading the mousses in the printer, it took approximately 50 s for printing a 6-layer cylinder of a sample (8 s for each layer). The printing process was conducted at 20°C.

2.3. Characterisation of material properties

2.3.1. Texture profile analysis

The instrumental texture properties of each formulation in the not-printed and 3D-printed samples were characterised using a texture analyser (Model TA-XT Plus, Stable Microsystem, Godalming, United Kingdom). The texture analysis was performed with double compression using a 0.5 mm sphere (SMS P/0.5 S) with a speed of 2 mm/s, 50% strain and a trigger force of 5 g. The time between the two compressions was 30 s.

The parameters firmness, adhesiveness and resilience were selected to describe the texture properties of the mousse. Firmness refers to the force required for the mousse to attain a given deformation. The firmness value was obtained from the peak force of the first compression. Adhesiveness represents the work necessary to overcome the attractive forces between the surface of the mousse and the probe, which was calculated using the area of the negative force-time curve between the two compressions. Resilience is measured as how the mousse recovers after deformation. The value was defined as the ratio of the area under
the upstroke energy and the downstroke energy of the first compression (Civille & Szczesniak, 1973; Hurler et al., 2012). The not-printed samples were measured directly in the disposable cups (60 mL; ø55 mm) which the mousse had been set and refrigerated. The 3D-printed samples were analysed right after the printing process. Each formulation was measured in triplicate for not-printed samples and 3D-printed samples. Measurements were conducted in a control room at 20 °C.

2.3.2. Rheological characterisation of the not-printed mousses

The rheological tests were performed on the not-printed samples to imitate the impact of printing and how it would affect the properties. Rheological measurements were performed on a rheometer (DHR-2, TA Instruments, New Castle, DE, USA), using a 25 mm serrated parallel plate geometry, a temperature of 20 °C and a gap of 10 mm. The sample was cut to a height of 10 mm and transferred to the plate using a spatula. Then, the probe was lowered to a height of 10 mm. There was a visual inspection before each measurement to ensure that the sample was placed correctly, which the probe was touching but not squeezing the sample.

After 30 s of equilibration, oscillation stress sweep tests with logarithmically increasing shear stresses (0.1–1000 Pa) and a constant frequency of 1 Hz were performed to determine yield stress, storage modulus (G’), and loss modulus (G”). The G’ and G” values were obtained from the plateau region i.e. linear viscoelastic region (LVE); the yield stress was determined as the crossover point between G’ and G” (Liu et al., 2019; Wilson et al., 2017). For each formulation, measurements were conducted in triplicate.

2.3.3. Bubble size and image analysis

Bubble size analysis was performed with a microscope (Leica Microsystems, Wetzlar, Germany) for the not-printed and 3D-printed lemon mousses. Microscopy samples were taken by extracting a thin layer of mousse from the centre of the samples using a spatula (approx. 1 g), then placed at the centre of the microscope slide and measured with an × 40 magnification. ImageJ (U.S. National Institutes of Health, Bethesda, Maryland, USA) was used to settle the scale bar and resize images for comparison between samples. Three images were taken per formulations for the not-printed and 3D-printed samples.

Pictures of the lateral view of 3D-printed samples were taken to compare the printability and stability of formulations at two time points: immediately after printing (T₀) and 30 min after printing (T₃₀). For each formulation, three images were taken in a lightbox. Samples were refrigerated at 5 °C between the two time points.

2.4. Sensory evaluation of 3D-printed lemon mousses

2.4.1. Sensory profiling

The sensory characteristics of 3D-printed lemon mousse were evaluated by descriptive analysis using a trained panel. Five samples with the best printing stability were selected. They were printed on a plastic lid, covered with a cup and refrigerated at 5 °C overnight before serving.

The sensory evaluation was performed according to the guidelines in ISO 13299 (2016). Ten panellists (9 females, 1 male) were recruited from the trained external sensory panel of the Department of Food Science, University of Copenhagen, Denmark. All these panellists received regular training for descriptive analysis and were familiar with evaluating different types of dairy products.

The panel first developed a consensus vocabulary to describe the variation between the samples and trained for scale use in two training sessions. Reference materials were used to support the training and a total of 23 sensory attributes were included in the final vocabulary (Table 2). Then, the panel evaluated attributes for each sample in two profiling sessions. Each session consisted of 2 hours. Following a complete balanced block design, the panel evaluated each sample in triplicate during the two profiling sessions. Within each block, samples were blinded with random 3-digit numbers and presented in a randomised order. The panel scored the intensities of attributes on a 15-cm line scale with anchors described in Table 2 at each end. Tap and lukewarm water were provided for palate cleansing. Data were collected using the Fizz software 2.50B (Bioysstèmes, France).

2.4.2. Consumer test

Thirty healthy adults (21F, 9M; mean age 35 years) were recruited for the consumer study. Participants performed product evaluation individually in sensory booths at the University of Copenhagen. They received six 3D-printed desserts, including two samples of the 3D-printed lemon mousse. Using a 15-cm line scale, the hedonic attributes in terms of liking of appearance, -taste and -texture, desire to eat, degree of salivation and overall liking were evaluated. The anchors at two ends of the liking scale were “dislike very much” and “like very much.”

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Definition (Reference material)</th>
<th>Anchors (15-cm line scale)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Appearance</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shiny-A</td>
<td>Amount of light reflected from surface (Milbbona cream pudding)</td>
<td>not at all → very</td>
</tr>
<tr>
<td>Rough surface-A</td>
<td>Amount of irregularity, protrusion or bumps</td>
<td>not at all → very</td>
</tr>
<tr>
<td>Jelly-A</td>
<td>Springy when shaking the cup of the sample and elasticity when spooning the sample (COOP currant jelly)</td>
<td>not at all → very</td>
</tr>
<tr>
<td><strong>Taste</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sour-BT</td>
<td>Sour taste sensation reminiscent to citric acid solution (2.4 g/L)</td>
<td>a little → very</td>
</tr>
<tr>
<td>Sweet-BT</td>
<td>Sweet taste sensation reminiscent to source solution (24 g/L)</td>
<td>a little → very</td>
</tr>
<tr>
<td>Salty-BT</td>
<td>Salt taste sensation reminiscent to sodium chloride solution (2 g/L)</td>
<td>not at all → very</td>
</tr>
<tr>
<td>Bitter-BT</td>
<td>Bitter taste sensation reminiscent to caffeine solution (0.54 g/L)</td>
<td>not at all → very</td>
</tr>
<tr>
<td><strong>Flavour</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lemon-F</td>
<td>Lemon flavour reminiscent to fresh lemon juice with zest</td>
<td>a little → very</td>
</tr>
<tr>
<td>Cream-F</td>
<td>Cream flavour reminiscent to a solution of 50:50 whole milk and whipping cream 38% fat</td>
<td>not at all → very</td>
</tr>
<tr>
<td>Synthetic-F</td>
<td>Unnatural experience of sourness</td>
<td>not at all → very</td>
</tr>
<tr>
<td><strong>Texture</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compact-T</td>
<td>Hard, firm and dense (BUKO cream cheese)</td>
<td>not at all → very</td>
</tr>
<tr>
<td>Foamy-T</td>
<td>Light, bubbles and soft (Dr. Oetker lemon mousse)</td>
<td>not at all → very</td>
</tr>
<tr>
<td>Smooth-T</td>
<td>Soft on the surface of tongue and low resistance when swallowing (Milbbona milk chocolate cream pudding)</td>
<td>not at all → very</td>
</tr>
<tr>
<td>Lumpy fracture-T</td>
<td>Lumpy and fracture of the sample texture but when first manipulate in mouth then it soon disappears</td>
<td>not at all → very</td>
</tr>
<tr>
<td>Stickiness-T</td>
<td>Thickening of saliva to become slimy (Matilde® milkshake)</td>
<td>not at all → very</td>
</tr>
<tr>
<td><strong>Mouthfeel</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coating-MF</td>
<td>Mouth coating by fat or cream (50:50 whole milk &amp; whipping cream 38% fat)</td>
<td>not at all → very</td>
</tr>
<tr>
<td>Melting-MF</td>
<td>Melt-in-mouth, easy to swallow (Dr. Oetker chocolate mousse)</td>
<td>not at all → very</td>
</tr>
<tr>
<td>Salivating-MF</td>
<td>Amount of saliva produced after swallowing the sample as compared to before eating the sample</td>
<td>not at all → very</td>
</tr>
<tr>
<td>Astringent-MF</td>
<td>Dryness of tongue surface (Medova black tea)</td>
<td>a little → very</td>
</tr>
<tr>
<td>Irritating-MF</td>
<td>Irritation due to high sourness</td>
<td>not at all → very</td>
</tr>
<tr>
<td><strong>Aftertaste</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sour-AF</td>
<td>Sour aftershave sensation reminiscent to citric acid solution (2.4 g/L)</td>
<td>not at all → very</td>
</tr>
<tr>
<td>Bitter-AF</td>
<td>Bitter aftershave sensation reminiscent to caffeine solution (0.54 g/L)</td>
<td>not at all → very</td>
</tr>
</tbody>
</table>
much”, with a description of “neither like nor dislike” in the middle. The anchors at two ends of the scale for the desire to eat were “very weak” and “very strong” and the anchors at two ends of the scale for the degree of salivation were “not at all salivating” and “extremely salivating”. The 3D-printed lemon mousse samples were served to participants in random order. Only the data on 3D-printed lemon mousse were reported in this paper. The subjects participated by written informed consent and received a gift bag at the end of the study.

2.5. Statistical analysis

A 2²3 factorial ANOVA was used to analyse the effect of gelatine, citric acid and WPI concentration on the instrumental measures. This included the textural and rheological properties of the not-printed lemon mousses. For each formulation, the percentage of change on texture parameters after 3D printing was calculated.

Principal Components Analysis (PCA) of the mean rating of sensory data was used to illustrate the relationship among variables and 3D-printed lemon mousse samples. The relationship between texture-related sensory attributes and instrumental measures in the not-printed lemon mousses (i.e. texture profile analysis and rheological measurements) was assessed using Spearman’s rank correlation ρ. To account for the different batches of sample used in the instrumental measures and sensory profiling, a cross-product matrix with 9 combinations of the triplicate measurement of the two tests was created for each formulation for correlation analysis (i.e. $T = \{T_{R1} T_{R2} T_{R3}\}$, $S = \{S_{R1} S_{R2} S_{R3}\}$, where $T$: instrumental measures e.g. firmness; $S$: texture-related sensory attributes; $R$: replicate).

For the consumer test, data were first normalised to take into account the unequal subject (consumer) variance. This was achieved by calculating z-scores for each hedonic rating per subject based on the individual’s variance (Equation (1)). A paired sample t-test was used on these normalised data to compare the hedonic ratings between samples.

$$Z = (x - \mu) / \sigma$$  
(Equation 1)

where: $Z$: standardised score; $x$: hedonic rating (observed value); $\mu$: mean of the individuals’ ratings across all attributes; $\sigma$: standard deviation of the individuals’ ratings across all attributes.

Statistical analyses were performed using SPSS 27 (IBM, USA). Significance was set at $p < 0.05$ for all analyses. PCA and all the graphs were generated using OriginPro 2020 (OriginLab, USA).

3. Results and discussion

3.1. Characterisation of textural and rheological properties of lemon mousses

The main effects and interactions of gelatine, citric acid and WPI concentration on the texture of the not-printed mousses are reported in Table 3. The textural properties were highly influenced by the concentration of gelatine and WPI but were less affected by citric acid. Gelatine concentration had a positive effect ($p < 0.001$) on the firmness of mousses whereas WPI concentration had a negative effect ($p < 0.001$, Fig. 1a). There was a significant gelatine*WPI interaction ($p < 0.001$). At high gelatine concentration, the firmness of mousses decreased more significantly when adding WPI than at low gelatine concentration. There was also an interaction between WPI and citric acid concentration ($p < 0.001$). The difference in the firmness of mousses between high and low WPI samples was more significant at high citric acid concentration.

Increasing the gelatine and WPI concentration significantly increased the adhesiveness of mousses (both $p < 0.001$, Fig. 2a). The significant gelatine*WPI interaction ($p < 0.001$) indicates a more profound effect of WPI at a high gelatine concentration. Increasing WPI concentration had a negative effect on the resilience of mousses ($p < 0.001$, Fig. 3a). The significant interaction effect between citric acid and WPI concentration ($p = 0.001$) showed the effect of WPI in reducing the resilience of mousses was mainly observed at high citric acid concentration. When the citric acid concentration was low, the resilience of mousses was similar for both low and high WPI samples.

Gelatine can create firm gels by forming a three-dimensional network made up of small laminas entrapping proteins and air bubbles (Zúñiga & Aguillera, 2008). This contributes to improved stability by increasing the firmness of mousses, which is an important property for a food ink as it influences the shape retention and the extrusion characteristics of the materials after printing (Chuanxing et al., 2018). Whey proteins can form strong gels after heat or enzyme treatments, however, the addition of whey ingredients without heating can lead to softening of the gel matrix as the protein acts more as an inert filler than as an active ingredient (Patocka et al., 2006). A similar effect was observed in our samples since they were not heated or enzymatically treated after adding the WPI into the formulations. An increase in WPI concentration decreased both firmness and resilience but increased adhesiveness of mousses, and the negative effect of WPI on firmness and resilience was more pronounced at high citric acid concentration. It is unclear the reason for the pronounced effect observed for citric acid at higher WPI concentration. The pH of the mousses ranged from 4.2 to 5.1 and taking into account the complex protein formulation of the mousse composed of gelatine, egg and whey proteins, more systematic studies are needed with model systems to understand this effect.

The effect of gelatine, citric acid and WPI concentration on the rheological behaviour of not-printed mousses was determined by oscillation stress sweeps and reported in Table 4. Yield stress, storage modulus ($G'$) and loss modulus ($G''$) at different gelatine, citric acid and WPI concentration are presented in Fig. 4. These parameters have earlier shown to be important properties in relation to printability. For instance, the yield stress is closely related to the force that has to be applied to materials before it starts to flow out of the nozzle (Liu et al., 2019) and storage modulus affects the extrudability of materials (Lille et al., 2018). Both parameters are critical for shape stability after printing (Lille et al., 2018; Liu et al., 2018, 2019).

Depending on the concentration, gelatine forms a strong gel network which increases yield stress (Ares et al., 2007). Increasing gelatine and WPI concentration increased and decreased the yield stress of mousses, respectively (both $p < 0.001$). There was a significant gelatine*WPI interaction ($p < 0.001$). At high gelatine concentration, yield stress decreases more significantly when adding WPI than at low gelatine concentration. There was no significant effect of citric acid concentration on yield stress.

Storage modulus ($G'$) describes the elastic response of a material and loss modulus ($G''$) describes the viscous response of a material. All formulations exhibited $G' > G''$ (phase angle $< 45°$) in the linear viscoelastic region, indicating a characteristic of viscoelastic gels (Mezger, 2012). Both gelatine and citric acid concentration had a positive effect on the storage modulus of mousses (gelatine: $p < 0.001$ and citric acid: $p < 0.001$).
As a higher storage modulus indicates a greater structural strength of a material at rest (Mezger, 2012), thus up to a given point, increasing the gelatine or citric acid concentration could be desirable for 3D printing. The loss modulus of mousses increased significantly with increasing concentration of gelatine, WPI and citric acid (gelatine: \( p < 0.001 \), WPI: \( p = 0.001 \), citric acid: \( p = 0.005 \)).

Fig. 5 (left) shows differences in air bubble size between the not-printed lemon mousse samples. High gelatine samples showed fewer large size bubbles (~0.1 mm) than low gelatine samples. Gelatine created a denser and more homogeneous network when presented in high concentration, however, at low gelatine concentration a softer network was created, which allowed the coalescence and formation of
large air bubbles. Interestingly, the bubble size of the high gelatine-high acid-high WPI sample (HHH) was heterogeneously distributed, whereas a finer, more homogeneous distribution was seen in the high gelatine-low acid-high WPI sample (HLH). Further, low WPI samples tended to have smaller size bubbles. These samples were earlier characterised as firmer and had higher yield stress than their high WPI counterparts. This may be due to the WPI stabilising the foam when presented at a low concentration (Sarkar & Singh, 2016; Singh, 2011).

### 3.2. Printability and stability of lemon mousse

Pictures of the lateral view of 3D-printed lemon mousses at $T_0$ (directly after printing) and $T_{30}$ (30 minutes after printing) are presented in Fig. 6. The printability of lemon mousses was evaluated based on the sample’s ability to hold the weight of the accumulative layers without compromising the shape and height of the print.

At $T_0$, gelatine concentration clearly affected the printability of mousses. High gelatine samples ($T_0$, left column) maintained most of their heights; their structures had less tendency to collapse or lose their shapes than low gelatine samples ($T_{30}$, right column). This effect can be further observed at $T_{30}$, where low gelatine samples deformed and collapsed significantly after 30 minutes of storage, for instance, the low gelatine-high WPI samples (LLH and LHH). Gelatine gave higher firmness, yield stress and storage modulus in mousses (see Section 3.1), which was beneficial to shape stability after printing. Similar findings were reported by Lille et al. (2018) and Liu et al. (2018, 2019). Higher structural strength in materials can better support the stress generated by subsequently deposited layers and therefore maintain the printed shape.

The appearance of the printed lemon mousses was affected by WPI concentration. High WPI samples had a “glossier” surface and low WPI samples had a drier and crumblier surface. The limited interaction between WPI and the gel matrix may cause syneresis, which could explain the glossier surfaces observed. The layers in high WPI samples were more fused together than in low WPI samples, especially when the concentration of gelatine was high. Interestingly, the high gelatine-high acid-high WPI sample (HHH) was characterised by a tip formed on the

![Fig. 4. Rheological behaviours of not-printed lemon mousses at different gelatine, citric acid and WPI concentrations: (a) yield stress; (b) storage modulus $G'$ and (c) loss modulus $G''$. Each formulation (Table 1) was measured in triplicate for not-printed and 3D-printed samples.](image-url)
top of the sample. This was created at the end of the printing process when the nozzle retracted from the printed mousse. At high gelatine concentration, adhesiveness of mousses greatly increased when adding more WPI (Fig. 2b). Notably, the middle point formulation (MMM) created a 3D structure that looked very similar to the high gelatine-high WPI samples but collapsed less after 30 minutes of storage. This may be due to the effect of WPI in stabilising the foam (Sarkar & Singh, 2016; Singh, 2011), as previously observed in the bubble structure.

Samples with high citric acid concentration appear to be less crumbly than their low citric acid concentration counterparts, especially when the gelatine concentration was high. This indicates an improved printability of mousse when the acidity of the formulation increased. Adding WPI into the formulations increased the buffering capacity of the mousses (Salaün et al., 2005). However, further studies are needed to understand the interplay between pH and the complex WPI formulation (gelatine, egg and whey proteins) on the appearance of the 3D-printed mousses.

During the 3D printing process, the lemon mouse was extruded from the printing syringe through a narrow nozzle. It was expected that this process would lead to a change in the physical properties of the aerated gel. Bubble size analysis showed a formation of more uniform, medium-sized air bubbles (< 0.1 mm) in nearly all 3D-printed samples (Fig. 3 right). This indicates that the printing process led to a more uniform aerated gel network in foams, in which large bubbles may collapse and form smaller bubbles and at the same time, small bubbles coalesce to form larger bubbles.

The percentage of change on texture profile analysis parameters measured in samples after printing was calculated to assess the effect of 3D printing on mousses in each formulation. At low gelatine concentration, the firmness of mousses decreased by approximately 70% independent of citric acid or WPI concentration. At high gelatine concentration, the firmness of mousses decreased ranging from 30% to 70% (average 50%) and the percentage change was affected by both citric acid and WPI concentration. This value is comparable to Le Tohic et al. (2018), which showed a decrease in firmness (45–49%) when comparing the 3D-printed cheeses to the untreated cheeses. High gelatine samples had a higher firmness. They were more resistant to deformations during printing, resulting in a firmer mousse after printing (Fig. 7a). Among the four high gelatine formulations, low WPI samples (HHL and HLL) had a greater decrease in firmness than high WPI samples (HHH and HLH) after printing (average 37% and 64%, respectively). The initial differences in firmness observed in samples due to the low or high WPI concentration were eliminated by printing. Moreover, high acid samples (HHH and HHL) were less affected by printing than low acid samples (HLH and HLL) with a reduction of firmness in an average of 40% and 60%, respectively.

The adhesiveness of mousses tended to decrease after printing for low gelatine samples but increase for high gelatine samples (Fig. 7b). The marked increase of adhesiveness in high gelatine samples, especially when the concentration of WPI was high (HLH and HHH), was also reflected in the tip formed on the top of the printed mousse earlier shown in Fig. 6.

WPI concentration played a major role in the percentage of change in resilience after printing. The percentage decrease of resilience in low WPI samples was approximately double that of high WPI samples (average 38% and 20%, respectively; Fig. 7c). In the non-printed high WPI samples, whey protein ingredients softened the gel matrix (Patocka et al., 2006), resulting in a lower resilience of mousse. It appears that such initial differences between samples were eliminated by the printing process, thus the decrease in resilience was more drastic for low WPI
3.3. Sensory profile of 3D-printed lemon mousses and their correlations with instrumental measurements

Based on the physical characterisation, the high gelatine formulations (HHH, HLL, HLH and HHL) showed high stability after 3D printing and were selected for sensory profiling. A new high gelatine formulation (HMM) was further developed and included in the test, giving a total of five 3D-printed lemon mousses. The HMM formulation represented the middle-point of the four high gelatine formulations (i.e. 2% gelatine concentration, 1.2% citric acid concentration and 13% WPI concentration).

PCA was performed to present the relationship between the 3D-printed lemon mousses and the sensory attributes. The high gelatine formulations with different combinations of citric acid and WPI concentration gave sensory profiles of printed lemon mousses that were distinctly different (Fig. 8). The sensory variation between samples could be projected into the first two principal components.

PC1 explained mostly the texture properties of the mousses, showing the differences in rough surface appearance and lumpy fracture texture versus shiny appearance and smooth texture. Samples with high and middle WPI concentration (HHH, HLH or HMM) were characterised with a shiny appearance, a smooth and stickiness texture and melting mouthfeel, whereas samples with low WPI concentration (HHL and HLL) had a rough surface and jelly appearance, and a lumpy and compact texture. When comparing between samples with the same WPI concentration (high protein: HHH vs. HLH or low protein: HHL vs. HLL), a low citric acid concentration in formulations gave less shiny and rougher appearances. The results resemble the observations from the lateral view pictures described in Section 3.2.

PC2 explained the taste and flavour differences of the samples. Sour taste and its relating attributes, e.g. synthetic flavour, sour aftertaste and salivating were negatively correlated with a sweet taste and cream flavour. Increasing citric acid concentration in formulations increased the intensity levels for the sour taste and relating attributes. Moreover, adding more WPI increased the pH and buffering capacity in mousses (Salaün et al., 2005), therefore counteracting the acidity generated by citric acid. Samples with high WPI concentration (HHH and HLH) were more characterised by a sweet taste and cream flavour than the low WPI ones.

The middle-point formulation (HMM) was characterised by a moderate smooth and stickiness texture and melting mouthfeel, and a sour and sweet taste comparable to the high WPI-high acid formulation (HHH).

The relationship between the texture-related sensory attributes and instrumental measurements for four high gelatine formulations (HHH, HLL, HLH and HHL) were assessed by Spearman’s correlation. TPA parameters obtained in the not-printed lemon mousses showed correlations with two groups of texture attributes that could be distinguished on the first PC (Table 5 and Fig. 8). First, firmness and resilience were strongly correlated to the lumpy fracture and compact texture and moderately correlated with the foamy texture of mousses after printing. Similar to firmness and resilience, the yield stress of the not-printed lemon mousses were related to this group of texture attributes. These indicate that a stronger gel structure in the mousse, despite giving good storage stability after printing, was more subjected to disruption by extrusion during the printing process and eventually gave poor textures in the 3D-printed mousses (HHH and HLL). Second, adhesiveness was positively correlated to smooth texture, stickiness, coating and melting.
mouthfeel (Nishinari et al., 2019; Szczesniak et al., 1963). Increasing the WPI concentration in formulations softened the gel structure, reduced firmness and increased adhesiveness. However, it helped improve the texture perception of the 3D-printed lemon mousses (HMM, HHH and HLH).

Fig. 7. Percentage change of mean (a) firmness, (b) adhesiveness and (c) resilience after 3D printing in relation to the properties of the lemon mousse before 3D printing (Table 1). The error bar in the MMM formulation indicates the standard deviations of three 3D independent experiments.

Fig. 8. PCA plot showing the variation between the (a) 3D-printed lemon mousse samples in red dots (scores) and (b) the sensory attributes in black squares (loadings). The circled samples represent formulations selected for multisensory design (HLH and HHL) and compared with (HMM) in the consumer test.
In the consumer test, the HLH and HHL formulations were used to formulate the multisensory layered sample (hereafter referred to as “multilayer”) to evaluate the hedonic effects of multisensory design in printed foods. The multilayer sample was compared with a homogeneously layered sample (hereafter referred to as “monolayer”) using the HMM formulation. The three formulations were selected based on satisfactory printability and sensory characterisation. The HLH and HHL formulations were characterised by low and high sourness respectively, which their complementary sensory profiles could be suitable for applying multisensory design in printed foods. The HMM formulation was close to the average of the sensory dimensions that could be set as a reference formulation. The multilayer samples consisted of 50:50% HLH and HHL formulations distributed across six layers of a mousse (i.e. from top to bottom: 1 layer of HHL – 3 layers of HLH – 2 layers of HHL). This arrangement can cause a shorter period of maximum sour intensity and a more rapid decline in perception than in the monolayer sample (i.e. 6 layers of HHL formulation. The three formulations were selected based on satisfactory printability and sensory characterisation. The HLH and HHL formulations were characterised by low and high sourness respectively, which their complementary sensory profiles could be suitable for applying multisensory design in printed foods. The HMM formulation was close to the average of the sensory dimensions that could be set as a reference formulation. The multilayer samples consisted of 50:50% HLH and HHL formulations distributed across six layers of a mousse (i.e. from top to bottom: 1 layer of HHL – 3 layers of HLH – 2 layers of HHL). This arrangement can cause a shorter period of maximum sour intensity and a more rapid decline in perception than in the monolayer sample (i.e. 6 layers of HMM) with an identical overall composition (Chow, 2019). Both samples were produced using the 3D printing parameters as described in Section 2.2.

The hedonic attributes of the multilayer and monolayer sample were evaluated by a consumer panel and the results of paired t-test on normalised data are reported in Table 6. Both samples scored high for overall liking (monolayer: 9.8 and multilayer: 10.6 on the 15-cm line scale). This indicates that 3D-printed lemon mousses were well-accepted by consumers, regardless of the multisensory layer arrangement. There was no significant difference between the two samples for most hedonic attributes except for texture liking, in which the multilayer sample demonstrated a significant slight reduction in texture liking (p = 0.044).

As reflected in the high scores of appearance and texture liking of the monolayer sample (9.1 and 10.6 on the 15-cm line scale, respectively), a good printing appearance and stability was achieved when the formulation was printed homogeneously (see also Section 3.2 and 3.3). However, in the multisensory system, when formulations differing in texture properties were printed on top of the deposited layers, the overall print stability was lowered. Further research can focus on improving the print stability of lemon mousses with a multisensory layered design, which has been demonstrated as a potential tool for promoting food intake (Chow, 2019).

4. Conclusion

Lemon mousse was successfully printed using an extrusion-based printer. Although extrusion through a nozzle during the printing process disrupted the gel structure, leading to a substantial change in the textural properties which in turn affecting the sensory properties, sensorial attractive and stable 3D-printed mousses were obtained. Texture profile and rheological analysis performed in not-printed samples showed that the concentration of gelatine and WPI highly changed the properties of the mousses, and citric acid had some effect but to a lesser extent. These changes had consequences for the printability and stability of the 3D-printed mousses. The gel firming effects of gelatine, giving a higher storage modulus and yield stress, were contra-balanced by the softening effect of WPI that although acting as inner filler, improved the adhesiveness of the mousses. Using a standard lemon mousse formulation, 2% gelatine was required to produce 3D-printed mousses with acceptable shape and stability after printing. The addition of WPI up to 18%, apart from increasing the nutritional value of the product, improved the properties of the 3D-printed mousses, giving better-defined layers that fused together to form a more glossy and smooth mousse surface. An increased concentration of citric acid, although had a minor effect on the textural attributes measured, clearly improved the printability quality and stability of the mousses, which may be due to the strengthening of the mousse network as observed by the higher storage modulus.

The sensory profiling of these formulations showed that a high WPI concentration was associated with a shiny appearance and a smooth and sticky texture, whereas increasing citric acid concentration increased the intensity levels for the sour taste. Lemon mousse formulations with a high gelatine concentration and a medium to high WPI concentration were stable after printing and had attractive sensory profiles. The softening effect of WPI improved smoothness and reduced the lumpy fracture texture perception, which was associated with the gel firming effect of high gelatine concentration in 3D-printed mousses. This may contribute to the consumer acceptance of 3D-printed mousses. Further research can focus on improving the multisensory layered design in 3D-printed mousses, which have the potential to be served as appetising and protein-enrich snacks for specific consumer groups with different needs.

### Author contributions

Ching Yue Chow: Methodology, Investigation, Visualisation, Formal analysis, Writing – original draft. Camilla Doris Thybo: Methodology, Investigation, Formal analysis, Writing – original draft. Vale ska Farah Sager: Methodology, Investigation, Formal analysis, Writing – original draft. Reisy Rizki Riantiningtyas: Methodology,
Investigation. Wender Bredie and Lilia Ahrnè: Conceptualisation, Methodology, Supervision, Writing – review and editing, Project administration, Funding acquisition.

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.

Appendix

Table 1

<table>
<thead>
<tr>
<th>Food ingredient</th>
<th>Supplier</th>
<th>Address</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whipping cream 38% fat</td>
<td>Arla Foods</td>
<td>Sønderhøj 14, 8260 Viby J, Denmark</td>
</tr>
<tr>
<td>Gelatine</td>
<td>Dr. Oetker</td>
<td>Sødvestvej 15, S. sal 2600 Glostrup, Denmark</td>
</tr>
<tr>
<td>Whey protein isolate (Lacprodan® SP-9224)</td>
<td>Arla Foods Ingredients</td>
<td>Sønderhøj 10–12, 8260 Viby J, Denmark</td>
</tr>
<tr>
<td>Whole pasteurized eggs</td>
<td>Danèrg</td>
<td>Danåvej 1, 6070 Christiansfeld, Denmark</td>
</tr>
<tr>
<td>Sugar</td>
<td>Dan Sukker</td>
<td>Edvard Thomssens Vej 10, 7.sal 2300 København S, Denmark</td>
</tr>
<tr>
<td>Citric acid granules</td>
<td>Kryta</td>
<td>Navevej 32 4000 Roskilde, Denmark</td>
</tr>
</tbody>
</table>

References


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