Pectin based food-ink formulations for 3-D printing of customizable porous food simulants

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ABSTRACT

Additive manufacturing is revolutionizing processing in many applications including 3-D food printing. So far the number of suitable printable materials for food is limited and the microstructure and texture properties of 3-D printed food have been characterized poorly. This study introduces the novel concept of customizable water-based porous food simulants by 3-D printing using low methoxylated pectin gel as food-ink. A series of pectin gels was successfully 3-D printed by changing the formulation parameters including stirring rate and pectin, $CaCl_2$, bovine serum albumin and sugar syrup concentrations. It was shown that food objects with variable microstructure and texture properties can be 3-D printed by using different pectin based food-inks. The pectin concentration was the main determinant of firmness and strength of the printed object. Sugar and pectin concentrations increased viscosity and affected the build quality. BSA stabilized and promoted the aeration of the food-ink. This resulted in a pore size distribution after printing that was influenced by the viscosity of the food-ink. A predictive model was established for designing the food-ink composition to obtain 3-D printed food simulants with a priori defined microstructure and texture.

1. Introduction

Almost 30 years since its introduction, additive manufacturing (AM), commonly referred to as "3-D printing", is revolutionizing processing in many industrial applications ranging from the aerospace and automotive to the medical industries (Berman, 2012; Getschmann, 2013). The success of 3-D printing is owing to (i) the ability to produce complex and customized objects while causing little material loss, (ii) the possibility to rapidly exchange manufacturing data in digital format, and (iii) the combination with 3-D scanners that can reduce the development cycle time of products. The 3-D object is generally manufactured from the bottom up by assembling successive layers of powder, liquid, or sheets of material. Each layer corresponds to a cross-section of the 3-D object that requires additional computing treatments to define the slice resolution and give command instructions to the machine (Getschmann, 2013; Gibson, Rosen, & Stucker, 2010; Rengier et al., 2010).

Recently, interest in 3-D printing for food manufacturing has

increased (Godoi, Prakash, & Bhandari, 2016; Lipton, Cutler, Nigl, Cohen, & Lipson, 2015). 3-D food printing (3DFP) fits the concept of digital gastronomy that aims at combining traditional food cooking with 3-D printing in order to create food having new textures and flavors taking into account individual nutritional needs (Food Factory of the Future, 2013; Sun et al., 2015; van Bommel, 2012; Zoran & Coelho, 2011). Deposition methods based on material extrusion have been used to print liquid or mashed food that could easily solidify through the addition of a gelling agent such as an edible biopolymer (Cohen et al., 2009; Wegrzyn, Golding, & Archer, 2012). Prototypes of 3-D food printers and applications are now emerging. An extrusion printer was developed to create complex geometries with several food products including chocolate, cookie dough or mashed meat and vegetable with gelatin and xanthan gum as gelling agents (Cohen et al., 2009; Hao et al., 2010; Lipton et al., 2010). Different printing technologies including selective laser sintering, inkJet printing and fused deposit modelling have been explored (van Bommel, 2012). While these techniques were introduced as a means to produce foods, it remains a

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challenge to enable the technology to produce the desired food properties including shape, taste and texture.

Biocompatible hydrogels are often used as gelling agent for food printing and bioprinting which uses AM to produce biological tissues and organs (Mironov et al., 2006). Appropriate biopolymers have to be selected based on several, sometimes conflicting criteria, including edibility, final rigidity (stability of the 3-D structure) and flowability (in order to be easily manipulated and extruded). Typically, gellification is initiated either by changing pH, temperature, UV radiation or addition of crosslinkers like multivalent ions or enzymes (Malda et al., 2013). Gelatin, xanthan gum, starch and alginate are the most used polymers in 3DFP reported so far (J. Lipton et al., 2010; Sun et al., 2015; Wegrzyn, Golding, & Archer, 2012).

This study explores a novel use of pectin as a suitable model system for 3DFP of customizable water-based food simulants in which different additives such as sugar can be added and printed using the same printing setting. Examples of such foods are pectin based candies. Pectin represents a wide family of heterogeneous polysaccharides generally constituted of rhamnogalacturonan and homogalactruronan backbones which can be methyl esterified on a proportion of galacturonic acid residues (Duvetter et al., 2009; Fraeye, Duvetter, Doungla, Van Loey, & Hendrickx, 2010; Sila et al., 2008; Van Buren, 1991). Depending on its degree of methoxylation (DM), pectin is classified as low methoxylated (LM) or high methoxylated (HM), (Willats, Knox, & Mikkelsen, 2006). The gelation mechanisms vary according to the degree of methoxylation. HM pectin gels (DM > 50%) are formed by hydrophobic forces and hydrogen bonds at low pH and high sugar concentrations. In comparison, LM pectin gels (DM < 50%) are generated through the formation of calcium crosslinks between free carboxyl groups (Fraeye, Colle, et al., 2010; Willats et al., 2006). The gelation mechanism of LM pectin is similar to the gelation model of alginate with calcium, also called the "egg-box" model (Fang et al., 2008). Pectin gelling properties thus depend on many factors and can be tuned by changing the chemical structure of the backbone, temperature, pH, source of pectin and the concentration of calcium (El-Nawawi & Heikal, 1995; Fraeye, Colle, et al., 2010). In addition, cosolutes can also affect pectin gelling properties, depending on their ionic strength (Garnier, Axelos, & Thibault, 1993; Wang et al., 2007; Thirawong et al., 2008).

The objective of this work was to 3-D print pectin-based porous food simulants with variable properties by extrusion printing and characterize them. First, a rheology study of Ca^{2+}/pectin food-ink was performed to highlight the importance of calcium addition to obtain printable pectin gels. Then, new food-inks based on LM pectin gel were formulated incorporating sugar syrup added for sweetness. Air pores were trapped using bovine serum albumin (BSA) as a surfactant protein to provide a specific porosity. Second, pectin inks were 3-D printed using a simple extrusion printer at room temperature. The effect of chemical composition of the food-ink on the object build quality and mechanical and microstructural properties was investigated. Finally, from these measurements, a model was developed for correlating the chemical composition of the food ink to the properties of the 3-D printed food simulant.

2. Materials & methods

2.1. Materials and food-ink preparation

Low methoxylated pectin was produced by alkaline saponification of high methoxylated pectin from citrus peel (≥ 74% Galacturonic acid) (Sigma-Aldrich P9135) (Fraeye et al., 2009). A solution of 25 g/L HM pectin was set at pH of 11 with NaOH. The saponification of methyl esters by OH^- ions resulted in a pH decrease. This decrease was titrated with 2 M NaOH for 1 h at 20 °C. Then, the pH was reduced to a value of 4.5 with HCl. After 2 days of dialysis, the solution was set at pH 6 and freeze dried at −80 °C. The DM was determined by infrared

Table 1
Food-ink parameters and levels that were used in the central composite design (see text).

Parameters	Min	Central	Max	Units	Role
LM pectin concentration	15	35	55	g/L	Maintain 3-D structure
CaCl₂ concentration	12.5	15	17.5	mM	Increase the food-ink viscosity
BSA concentration	0	2.5	5	g/L	Foaming agent
Sugar syrup volume	0	25	50	% (v/v)	Provide sweet taste
Stirring rate	3200	6700	10,200	rpm	Aerate gel

spectrometry following the protocol of Kyomugasho et al. (Kyomugasho, Christiaens, Shpigelman, Van Loey, & Hendrickx, 2015). Several batches of LM pectin were produced resulting in a LM pectin mixture with a DM varying from 12 to 25%.

The food-inks were formulated as water solutions composed of different concentrations of LM pectin, CaCl₂ (Sigma-Aldrich C3881), bovine serum albumin (BSA) (Sigma-Aldrich A3059) and sugar syrup (63% w/w of cane sugar) (local supermarket). Stock solutions of appropriate CaCl₂ concentrations were prepared. Table 1 summarizes the levels of the food-ink constituents and operational parameters used in the experimental design. To prepare 60 mL of solution, LM pectin was first dissolved in 45 mL of a mix of distilled water and sugar syrup of appropriate proportion under magnetic stirring at room temperature (23 °C) for 3 h at least. Then, 15 mL CaCl₂ stock solution was added drop-by-drop to the pectin solutions in order to reach the desired concentrations with the values given in Table 1. The food-ink was stirred under magnetic agitation for 1 h and used for the rheological characterization and 3-D printing within 24 h after their preparation. Just before printing, the appropriate amount of BSA was added and the food-ink was mixed for 10 min under vigorous stirring (IKA® T25 Digital Ultra-Turrax, head size 18 mm) resulting in the homogenization of the food-ink and the formation of air bubbles ('pores'). The pH of the food-inks remained at 6 ± 0.5 and did not require any pH adjustment. The food-ink was transferred to the syringe using a spatula to a volume of about 45 mL.

After the printing, the pectin objects were incubated into a water bath with 300 mM CaCl₂ for 10 min. Afterwards they were manually sprayed every 10 min with the 300 mM calcium solution for 90 min. This post treatment was required to solidify the pectin gel and let the Ca^{2+} ions diffuse through the gel. The incubation and spraying time was set from pre-experimental trials for a cubic sample of $15 \times 15 \times 15$ cm³. For larger samples a longer spraying time may be required.

All components used to prepare the food-inks were food-grade.

2.2. Rheology

The dynamic viscoelastic properties of LM pectin/CaCl₂ food-inks before printing were measured with a stress-controlled MCR501 rheometer with Direct Strain option (Anton Paar, Graz, Austria), using a cone-plate system with a diameter of 25 mm and a cone angle of 2°. The evolution of the storage modulus (G') and the loss modulus (G'') with increasing time, strain amplitude γ_0 and angular frequency ω was determined for six gels of LM pectin (15, 35 and 55 g/L) and CaCl₂ (12.5 and 17.5 mM). All measurements were carried out in triplicate. The temperature was kept constant at 25 °C by means of a Peltier heating system, and a low viscous oil was used to prevent water evaporation (Rhodorsil 47v5). Strain sweeps were performed in the strain range from 0.01 to 100% at $\omega = 1$ rad/s to determine the linearity limit γ_c, we defined as the strain amplitude at which the value of $G'(\gamma_0)$ has dropped to 95% of its plateau value. Frequency sweeps were carried out in the linear region to determine the frequency dependency of the dynamic moduli and the phase angle δ (tan(δ)) for ω ranging from 0.1 to 100 rad/s. The tan(δ) can directly be obtained from the dynamic