Effect of Hydrocolloids on Rheological Properties and Printability of Vegetable Inks for 3D Food Printing

Hyun Woo Kim, Jang Ho Lee, Sae Mi Park, Min Hyeock Lee, Il Woo Lee, Han Sol Doh, and Hyun Jin Park

Abstract: In food ink systems in which the particles are dispersed in a hydrocolloid matrix, the source of the particles and the particle content are the main factors affecting the printability and rheological properties of the system. In this study, different contents (10% and 30% w/w) of vegetable (broccoli, spinach, or carrot) powders were added to hydrocolloid matrices with different hydration properties, and their influence on the printability and rheological properties was investigated. At low powder contents (10%), slight differences in the printability and rheological values were observed between the different vegetable sources in all hydrocolloids. When the powder content was increased to 30%, the hydrocolloid with the lowest water hydration capacity, hydroxypropyl methylcellulose, showed the greatest differences in rheology and printability when different vegetable sources were used. Xanthan gum, with its higher water hydration capacity, inhibited the swelling of the particles, thus minimizing the increase in the rheological values at high volume fractions of powder and reducing the differences in printability between different vegetable sources. Confocal laser scanning microscopy analysis of the vegetable inks showed that xanthan gum inhibited swelling of the particles regardless of the vegetable powder source. The mixtures using xanthan gum could be smoothly extruded from the nozzle due to their low extruded hardness (2.96 ± 0.23 to 3.46 ± 0.16 kg), and the resulting objects showed high resolution without collapse over time.

Keywords: additive manufacturing, food 3D printing, food ink, printability, printing parameters

Practical Application: The powder-based texturization technology introduced in this study provides a standardized method of preparing food ink that can be universally applied to all food materials that can be powdered. In addition, the present invention can be applied to a 3D printing technique in which a powder and a hydrocolloid matrix are independently stored and mixed immediately before printing. This technique can minimize the inherent rheological differences between formulations with different food sources and compositions.

Introduction

3D food printing is expected to generate a paradigm shift in food technology. This technique allows free design of the shape and texture of food, and makes it possible to manufacture completely novel foods with any desired composition, taste, or flavoring. In 3D food printing, food materials are thoroughly ground and reconstituted immediately before printing. This processing enables the addition of functional ingredients or the removal of specific materials from the final food product. For instance, this technology can be used to provide personalized food products for people who are sensitive to specific ingredients, such as those with lactose intolerance or gluten digestion problems. In addition, nontraditional natural resources, such as microalgae and insects, can be used as food ingredients. However, a number of unsolved challenges remains in the use of 3D printing technology in the food industry. Few natural food materials are printable through a nozzle to form a defined structure without significant deformation. Additionally, in 3D food printing, both the integrity of the 3D structure and its nutritional content are important. Therefore, the development of processed food materials from various sources, such as cereals, meat, vegetables, and fruits, is required.

Vegetables contain significant amounts of bioactive substances that provide health benefits and play a critical role in the prevention of chronic diseases (Liu, 2003; Van Duyn & Pivonka, 2000). However, vegetables have traditionally been considered nonprintable because of their high water content and their low contents of carbohydrates, fats, and proteins, which provide printability through mechanisms such as gelation, agglomeration, and solidification (Godoi, Prakash, & Bhandari, 2016; Sun et al., 2015). Few studies of processes to create vegetable-based materials for 3D printing have been reported. Lipton et al. (2010) attempted to create a printable vegetable (celery) formulation using agar as an additive to provide printability. However, a scaffold structure was required to form the product due to the long gelation time needed to achieve sufficient rigidity after deposition.

As the water content of food materials is a major factor affecting their printability, moisture control methods may facilitate the achievement of suitable rheological properties for 3D printing. The use of powdered materials, in which the moisture is minimized through drying methods, in hydrocolloid-based food ink preparations could enhance the printability of the product. The nutrient content of 3D printed foods can also be increased by increasing the amount of food powder used. The rheological

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properties of mixtures consisting of dispersed particles in a hydrocolloid matrix are affected by factors including the powder source, powder volume, particle swelling, and particle size distribution. In particular, the rheological properties are strongly affected by the powder volume fraction, which in turn is related to particle swelling, and can be controlled by the usage of hydrocolloids to inhibit swelling (Silva, Birkenhake, Scholten, Sagis, & Van der Linden, 2013). However, to the best of our knowledge, no studies have been conducted to determine whether this approach can eliminate the inherent rheological differences between formulations with different food sources and compositions (especially in terms of carbohydrate, protein, fat, and fiber content).

The current study was designed to investigate the effect of the hydrocolloid and the food powder source on the shear rheology and printability of the resulting materials, and to determine the optimal hydrocolloids for food ink preparation. Three different vegetable powders: (1) broccoli, (2) spinach leaves, and (3) carrots were added to hydrocolloid matrices and used in 3D printing experiments. Four hydrocolloids were characterized by complex viscosity and water hydration capacity (WHC) measurements to correlate these properties with the rheological properties and printability of the resulting vegetable mixtures. Furthermore, we studied the relationship between the 3D printing behavior and the rheological, printability, and microstructural properties of vegetable mixtures with different powder contents.

Materials and Methods

Materials

Hydroxypropyl methylcellulose (HPMC) and locust bean gum (LBG) were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPMC (CAS: 9004-65-3) had a molecular weight of approximately 86 kDa. Xanthan gum (XG) was obtained from Daejung Chemicals & Metals (Gyeonggi-do, Korea). Guar gum (GG), sucrose, NaCl, and acetic acid were purchased from Duksan Pure Chemicals (Gyeonggi-do, Korea). All freeze-dried vegetable powders were acquired from Sanmaeul Co., Ltd. (Gyeongsangnam-do, Korea).

Preparation of vegetable inks

The particle size of the vegetable powder was adjusted from 100 to 150 μm by passing the powder through standard sieves with mesh sizes of 100 μm and 150 μm. All hydrocolloid mixtures were prepared at a concentration of 10% and stored in a refrigerator at 4 °C for 1 day to allow hydration. For ink formulation, the freeze-dried vegetable powders were added to the hydrocolloid mixtures and stirred continuously (30 min) until a homogeneous mixture was obtained. To characterize of the effects of different powder contents and the use of different hydrocolloids, the vegetable powders were added at concentrations from 10 to 30% (w/w) in 10% hydrocolloid mixtures.

Complex viscosity measurement

The effect of various food ingredients, such as salt, sucrose, and organic acids, on complex viscosity (η^*) of the hydrocolloids was observed using a Paar Physica MCR 302 (Anton Paar, Graz, Austria) stress-controlled rheometer. All hydrocolloid solutions were prepared at 2% (w/w) concentration and maintained at room temperature for 6 hr to allow prehydration. Sucrose, the salt NaCl, and acetic acid were dispersed (2% w/w) in distilled water by thorough mixing and added in a 1:1 (v/v) ratio to the hydrocolloid solution. The measurements were conducted using parallel plates with a diameter of 50 mm (PP50) and a gap of 1 mm. The angular frequency (ω) was swept from 1 to 100 rad/s at a strain of 0.1% at 25 °C, and the complex viscosity (η^*) was recorded using Rheocompass software (Anton Paar).

Water hydration capacity

The WHC of the hydrocolloids and vegetable powders was determined using a slight modified method reported by Chen et al. (2015). Accurately weighed samples (0.2 g) were dispersed in 30 mL of deionized water in centrifuge tubes. The dispersions were hydrated for 1 hr in a water bath at 60 °C, and then placed in cold water for 10 minutes. The samples were centrifuged at 10000 *g* for 20 min using a high-speed refrigerated centrifuge (CR21G, Hitachi, Ltd., Tokyo, Japan). The supernatant was decanted, and the centrifuge tubes containing the residue were weighed. Each sample was measured in triplicate in each repetition, and the average value from these tests was reported. The following formula was used for the calculation:

$$\text{WHC (g/g)} = \frac{W_{T+R} - W_T - W_P}{W_P}$$

where W_P is the weight of the sample, W_{T+R} is the weight of the centrifuge tubes and the residue after the supernatant was removed, and W_T is the weight of the empty centrifuge tube.

Viscoelastic properties of vegetable inks

Oscillatory measurements were conducted using an MCR 302 controlled stress rheometer (Anton Paar) with a sandblasted parallel plate system (gap of 1.0 mm) with a diameter of 25 mm (PP25/S) at 25 °C. Prior to the measurement, a strain sweep was performed at 10 rad/s to determine the linear viscoelastic (LVE) interval between the shear stress and shear strain. A strain value of 0.1% was determined within the identified LVE region. All samples loaded in the rheometer were allowed to rest for 10 min before starting the measurement. To prevent the sample from drying during the long measuring time, the exposed surfaces around the samples were coated with corn oil. The dynamic viscoelasticity measurements were carried out using an angular frequency sweep test from 1 to 100 rad/s, and the storage modulus (G') and loss modulus (G'') were measured.

Printability assessment

The printability assessment and classification were conducted according to the methods described in our previous work (Kim, Bae, & Park, 2017). Briefly, the printability of a vegetable ink was determined by measuring its shear modulus and extrusion force. For the shear modulus assessment, the samples were sheared in a Paar Physica MCR 302 (Anton Paar) rheometer using a sandblasted measuring system with a diameter of 25 mm (PP25/S) and with a gap of 1.0 mm. A time sweep of 200 s over a variable range of strains (from 0.001% to 10%) at 25 °C was performed, and the shear modulus (G) data were obtained in the linear elastic region of the stress/strain curve. For the extrusion force measurement, a 30 mL sample was placed into an extrusion cell with a reservoir diameter of 25 mm and a nozzle aperture size of 3 mm. The samples were extruded using a texture analyzer (TAXT plus 50, Stable Micro Systems Ltd., Vienna, UK) with a test speed of 1.0 mm/s and target distance of 20.0 mm in compression test mode. For both analyses, three measurements were performed and the average value was taken.

Confocal laser scanning microscopy (CLSM)

The vegetable ink specimens were visualized using a Zeiss LSM 700 confocal microscope (Carl Zeiss Inc., Oberkochen, Germany). A mixture of a fluorescent dye (Rhodamine B, 0.0025% w/v) in distilled water was maintained in the dark until sample labeling. A sample of the vegetable powder and hydrocolloid mixture was spread over a glass microscope slide and stained with the fluorescent dye solution. Rhodamine B will preferentially label the protein in vegetable powder. The sample was inspected under a 10× objective lens (EC Plan-NEOFLUAR 10x/0.3 M27) and focused on the 555 nm emission wavelength. The confocal laser scanning microscopy (CLSM) images, which were acquired with a size of 1277.8 μm × 1277.8 μm and a pixel size of 2.50 μm, were recorded and further processed using Zen 2012 light edition software (Carl Zeiss MicroImaging, Inc., Thornwood, NY, USA).

3D printing process

The A YL-CUBE 3D food printer (YOLILO Co., Ltd., Seoul, Korea) was used in this study. Samples with 10, 20, and 30% vegetable powder concentrations were prepared as described in the section "Preparation of vegetable inks" above, and approximately 25 mL of the sample was loaded in a Luer lock syringe (30 mL volume) equipped with a 1 mm nozzle. Before printing the object, a 3D design (.stl file format) with a leaf shape was created using 3D printing slicing software (Cura 2.4, Ultimaker B.V., Geldermalsen, Netherlands). The printing process was conducted using a nozzle diameter of 1.0 mm, a first layer height of 0.9 mm, a nozzle height between layers of 0.95 mm, an infill level of 100%, and an extruder head moving speed of 20 mm/s at room temperature (25 °C). The 3D printed products were deposited to a height of 20 mm on a plastic (polyvinyl chloride) bed and analyzed after a 1 hour retention time.

Statistical analysis

All data are presented as mean ± SD, and were analyzed using the available SPSS software package (SPSS 20.0, IBM, Chicago, IL, USA). One-way analysis of variance (ANOVA) and a Duncan's multiple comparison test were performed to statistically analyze of the data. In all analyses, $P < 0.05$ was defined as statistical significance.

Results and Discussion

Influence of food ingredients on complex viscosity of hydrocolloid mixtures

The effect on the values of (η^*) of hydrocolloid mixtures by addition of food ingredients is shown in Figure 1. For all the studied hydrocolloids, the values of (η^*) decreased as the angular frequency (ω) increased, indicating that these hydrocolloids were pseudoplastic materials. Pseudoplastic material behavior is ideal for extruder-type 3D printing, as these materials flow well through narrow nozzles at high speed during 3D printing due to the shear thinning that occurs when the applied stress exceeds the yield stress, and also exhibit enhanced structure holding ability (Costakis, Rueschhoff, Diaz-Cano, Youngblood, & Trice, 2016; Liu, Zhang, Bhandari, & Yang, 2017).

Figures 1A and B show the effect of the addition of food ingredients on the complex viscosity of XG and GG, respectively. Neither hydrocolloid exhibited significantly different rheological values after the addition of food ingredients. This result indicated that these hydrocolloids were very stable to organic acids, sugars, and ion sources, which may be naturally present in foods or may be added for seasoning purposes during the food ink manufacturing process. In fact, it has been reported that once XG is hydrated, its mixture is independent of pH ($2 > pH > 10$), and that 20 to 30% salt can be tolerated without rheological properties change (Wüstenberg, 2015). Also, since GG is a neutral polysaccharide, its solution is hardly affected by ion or pH (Arendt & Dal Bello, 2011).

On the other hand, in the LBG samples (Figure 1C), the addition of food ingredients caused a decrease in the complex viscosity compared to the blank solution. In particular, the addition of sucrose greatly reduced the viscosity of LBG. It is inferred that the addition of food ingredients reduced the solubility of the polysaccharides, resulting in relatively low interaction between the polymer chains and water molecules (Elfak, Pass, & Morley, 1977; Higiro, Herald, Alavi, & Bean, 2007; Shiroodi & Lo, 2015). Thus, food ingredients competed with the polysaccharide for water molecules, resulting in the complex viscosity reduction. In the samples containing HPMC, a trend similar to that of the LBG solutions was observed. When NaCl and acetic acid were added to HPMC solutions, decreased viscosity values were observed. Most hydrocolloids including HPMC and LBG are polyelectrolytes and the charged groups per molecule of hydrocolloids ensure hydration strongly. Thus, the presence of an ionic agent causes the complex behavior of charged groups, and leads to changes in the rheological properties of the hydrocolloid (Vlachy, 2008). In addition, the hydrodynamic volume that is the length scale associated directly with changes in viscosity of hydrocolloid mixtures depends on variation of pH and ionic strength, resulting in loss of complex viscosity (Scheraga & Mandelkern, 1953). On the other hand, the addition of sucrose resulted in increased viscosity of HPMC. The addition of sugar may have led to symmetric bending of the methoxy group in the HPMC matrix, increasing the hydrophobic interactions between their contact surfaces (Joshi, 2011). This tendency was observed in a previous study by Punitha, Uvarani, Panneerselvam, and Nithiyanantham (2014), in which the viscosity of a HPMC solution increased from 0.908 mPa·s to 0.949 mPa·s when 1% sucrose was added. Based on these results, the rheological properties of inks will depend on the hydrocolloid type and composition of the vegetable powder.

Water hydration capacity

The results of the WHC measurements of hydrocolloids and vegetable samples are shown in Figure 2. Due to the very high WHC values of the hydrocolloids, no significant differences in WHC between the vegetable samples were observed. However, the broccoli powder (BP) showed relatively higher WHC values (9.56 g/g) than carrot powder (CP) (7.62 g/g) and spinach powder (SP) samples (6.32 g/g). In the system that consists of a gel-like matrix with dispersed powder, the rheological properties were mostly affected by powder volume fraction. Silva, Scholten, van der Linden, and Sagis (2012) reported that when BP incorporated in starch matrix, the volume fraction was significantly increased by powder swelling and this led to an increase in shear modulus. The increase in viscosity of the dispersion with apple and pear powder proportionally to WHC (7.5 g/g and 4.9 g/g, respectively) was also observed by Bchir, Rabetafika, Paquot, and Blecker (2014). Therefore, it is inferred that the incorporation of BP could influence the rheological properties of the vegetable inks, due to the rather large swelling of the particles caused by its relatively high hydration capacity.

The WHC values of the hydrocolloids were much higher than those of the vegetable samples. In particular, XG (51.18 g/g) had

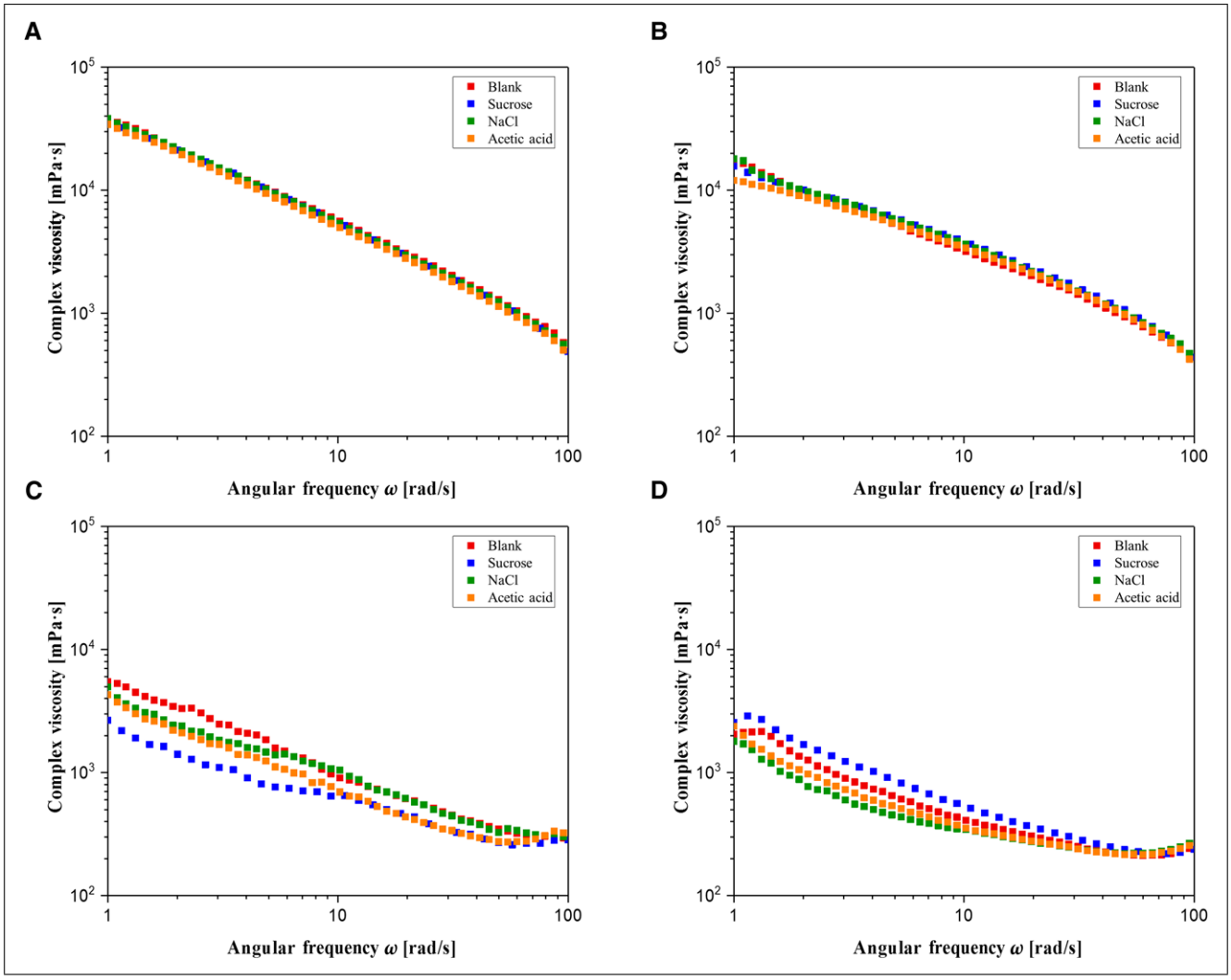

Figure 1–Effect of food ingredients on the complex viscosity (η^*) of XG (A), GG (B), LBG (C), and HPMC (D).

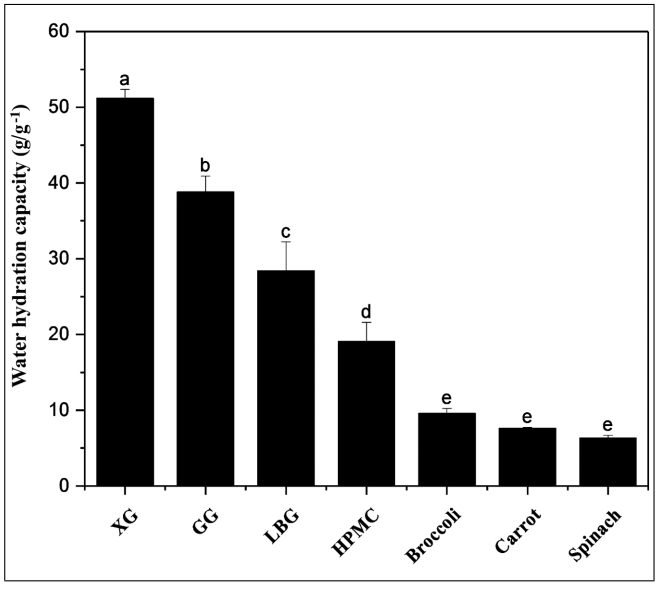

Figure 2–WHC of the hydrocolloids and vegetable samples. The letters above the bar indicate a significantly different WHP at $P < 0.05$ by Duncan's multiple range test.

the highest WHC of the tested hydrocolloids. This indicated that when XG is applied as a matrix in a powder-based ink system, it can competitively inhibit the hydration of the powder. Its high water hydration ability may also greatly reduce powder swelling (Silva et al., 2013), minimizing the rheological differences between inks based on different powders. In contrast, HPMC showed the lowest hydration capacity, suggesting that the use of HPMC could result in an increase in mechanical strength as the increases in powder volume fraction of the food ink matrix due to its low inhibition effects of powder swelling, and lead to brittleness in the layers passed through the thin nozzle.

Viscoelastic properties of vegetable inks

The vegetable mixtures can be regarded as composite materials composed of vegetable particles dispersed in a continuous hydrocolloid matrix. Therefore, the rheological properties of the vegetable mixture are determined by the dispersed phase (expanding particles), the continuous phase (network structure), and the interaction between these two phases. In the oscillation frequency sweep test, two dynamic moduli (storage modulus G' and loss modulus G'') are obtained as a function of frequency at a constant amplitude. The storage (elastic) modulus G' is a measure of elasticity, and the loss (viscous) modulus G'' indicates the

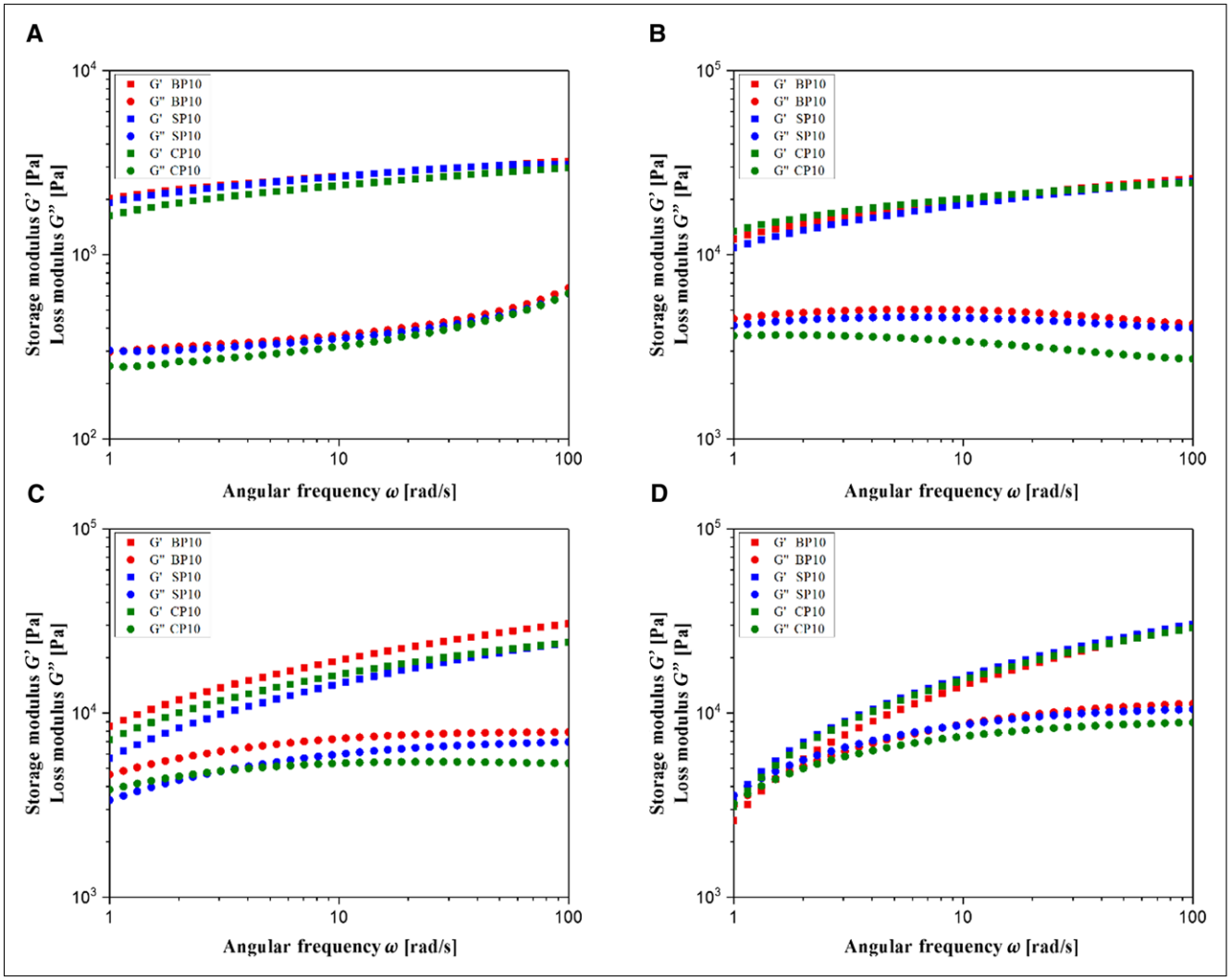

Figure 3–Storage modulus (G') and loss modulus (G'') compared to angular frequency (ω) for 10% vegetable mixtures with XG (A), GG (B), LBG (C), and HPMC (D).

Table 1–Printability of the vegetable inks produced using various hydrocolloids (XG, GG, LBG, and HPMC), vegetable sources (broccoli, spinach, and carrot), and powder contents (10% and 30%).[a]

		Shear modulus (kPa)			Extruded hardness (kg)		
Hydrocolloid	Powder content (%)	Broccoli	Spinach	Carrot	Broccoli	Spinach	Carrot
XG	10	1.90 ± 0.08b	1.81 ± 0.07^{b}	1.28 ± 0.05^{b}	0.35 ± 0.05^{a}	0.39 ± 0.04^{a}	0.42 ± 0.06^{a}
	30	7.73 ± 0.42a·b	9.73 ± 0.52^{b}	5.68 ± 0.31^{a}	2.96 ± 0.23^{a}	3.46 ± 0.16^{a}	3.00 ± 0.18^{a}
GG	10	6.39 ± 0.02^{e}	5.96 ± 0.44^{de}	7.84 ± 1.16^{f}	3.38 ± 0.44^{d}	4.24 ± 0.30^{e}	4.60 ± 0.06^{f}
	30	52.15 ± 0.66^{f}	39.07 ± 2.57^{e}	23.5 ± 1.28^{c}	10.39 ± 0.43^{c}	8.37 ± 0.17^{bc}	8.34 ± 0.37^{bc}
LBG	10	5.53 ± 0.22^{d}	3.81 ± 0.04^{c}	3.77 ± 0.24^{c}	2.53 ± 0.08^{c}	2.54 ± 0.22^{c}	2.12 ± 0.14^{b}
	30	68.78 ± 1.44^{h}	77.68 ± 3.66^{i}	32.94 ± 1.51^{d}	10.36 ± 0.43^{c}	9.18 ± 0.12^{c}	4.71 ± 0.25^{a}
HPMC	10	0.46 ± 0.02^{a}	0.49 ± 0.03^{a}	0.45 ± 0.03^{a}	4.63 ± 0.13^{f}	5.52 ± 0.23^{g}	5.51 ± 0.19^{g}
	30	58.12 ± 1.85^{g}	39.49 ± 1.25^{e}	24.09 ± 2.17^{c}	23.37 ± 3.27^{e}	19.69 ± 2.26^{d}	6.92 ± 0.27^{b}

[a]Data are mean values (± standard deviations).
The different superscripted letters indicate that a value is significantly different ($P < 0.05$) from others in the same column with the same powder content (10% or 30%) according to the Duncan's multiple range test.
XG, xanthan gum; GG, guar gum; LBG, locust bean gum; and HPMC, hydroxypropyl methylcellulose.

viscous properties of the material at a given frequency of oscillation (Doublier, Launay, & Cuvelier, 1992; Ross-Murphy, 1994; Wong & Lelievre, 1981). Thus, a gel or gel-like materials exhibit a mechanical spectrum, that is, the values of G' higher than G'' in the experimentally accessible frequency scale and the moduli show little frequency dependence (Almdal, Dyre, Hvidt, & Kramer, 1993; Clark & Ross-Murphy, 1987). However, in the case of the mixtures with entangled network between polymer chains, it could be challenging to determine whether the mixture is a liquid or a gel based on the mechanical spectrum. The dynamic response

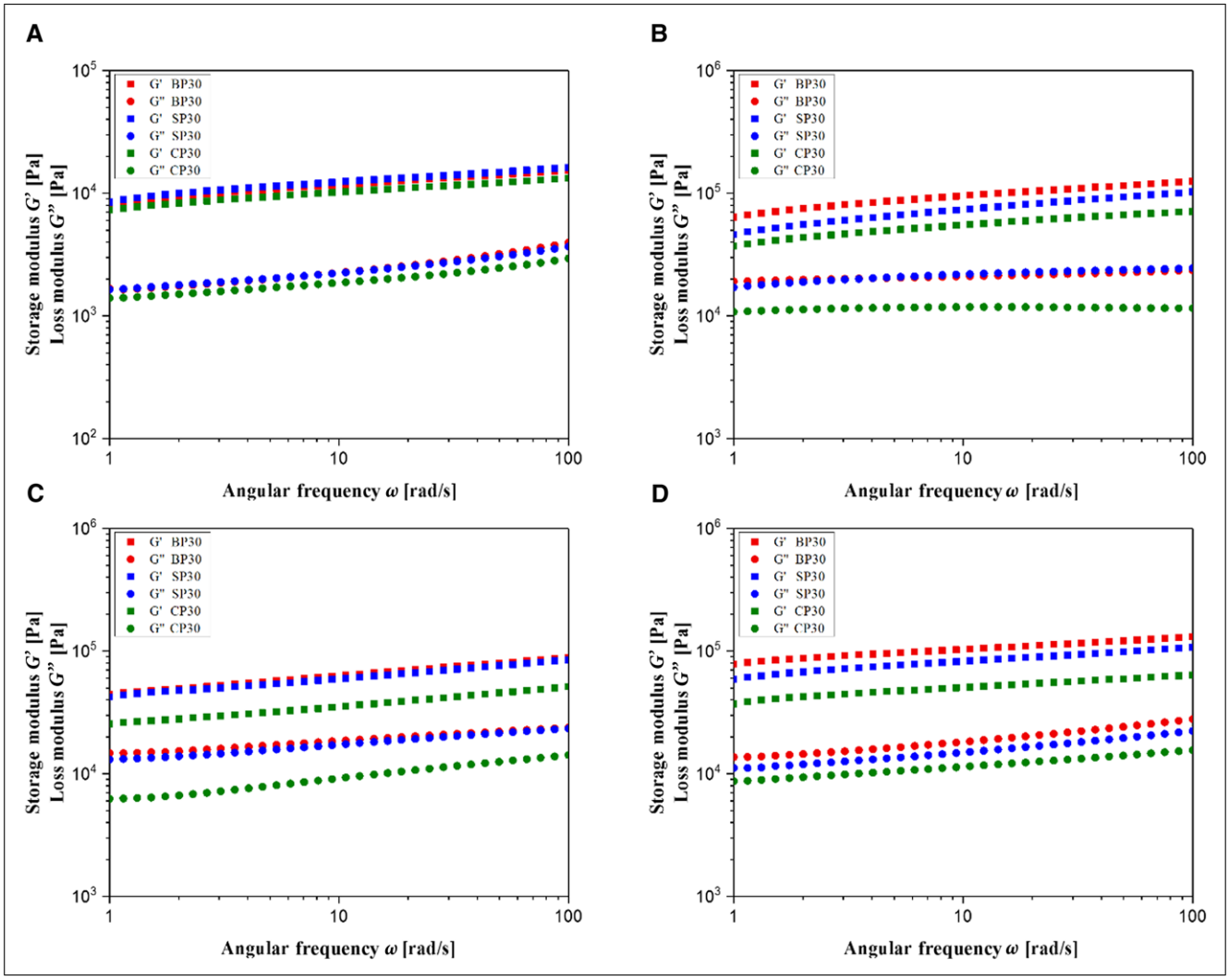

Figure 4–Storage modulus (G') and loss modulus (G'') compared to angular frequency (ω) for 30% vegetable mixtures with XG (A), GG (B), LBG (C), and HPMC (D).

for such a system has sufficient time for the polymer networks to come apart and flow at low frequencies. On the other hand, the system responds as a gel-like behavior at high frequencies because there is no sufficient time to disentangle within the single oscillation period (Ross-Murphy, 1994). Therefore, the dynamic response could be crossover depending on the frequency and affect the 3D printing performance of the materials.

Figures 3 and 4 show the plots of G' and G'' compared to angular frequency (ω) for the hydrocolloid mixtures containing 10% and 30% vegetable powder, respectively. When 10% vegetable powder was incorporated in the hydrocolloids (Figure 3), slight differences in the rheological values were observed among the mixtures containing different vegetable sources. The variation of the volume fraction due to the swelling characteristics of each vegetable was assumed to be negligible due to the relatively small amount of powder incorporated. In the samples containing XG (Figure 3A), GG (Figure 3B), and LBG (Figure 3C), the G' values (1.64 to 30.66 kPa) were higher than the G'' values (0.25 to 7.88 kPa), indicating that these materials exhibited gel-like properties. In contrast, in the samples containing HPMC (Figure 3D) the G' (2.61 to 3.44 kPa) values were lower than G'' values (3.15 to 3.57 kPa) in the lower frequency range (1 rad/s), meaning that the HPMC mixtures showed liquid-like properties at low shear rates. A crossover of G' and G'' indicates that it is an entangled network exhibiting stress relaxation causing plastic deformation by maintaining the structure-deformed condition for finite time interval and not a gel-like structure (Dooling, Buck, Zhang, & Tirrell, 2016). Therefore, it was inferred that the HPMC mixtures with 10% vegetable content would be insufficient to support the deposition of multiple layers.

In comparison to the 10% vegetable powder mixtures, all the hydrocolloid mixtures with the highest vegetable content of 30% were found to have significantly higher G' and G'' values (Figure 4). In all the hydrocolloid mixtures except XG, the BP containing mixtures showed the greatest increase in their viscoelastic values. The higher rheological values were postulated to result from the increase in the BP volume fraction by swelling due to its relatively high WHC. On the other hand, the CP containing systems showed the least increase in their viscoelastic values. CP contains a relatively large amount of water-soluble components, and thus may not experience a significant increase of total volume fraction because these components are dissolved in the hydrocolloid matrix. In fact, the carrot contains about 7.03% carbohydrates having high value of soluble sugar content of 6.23%, whereas

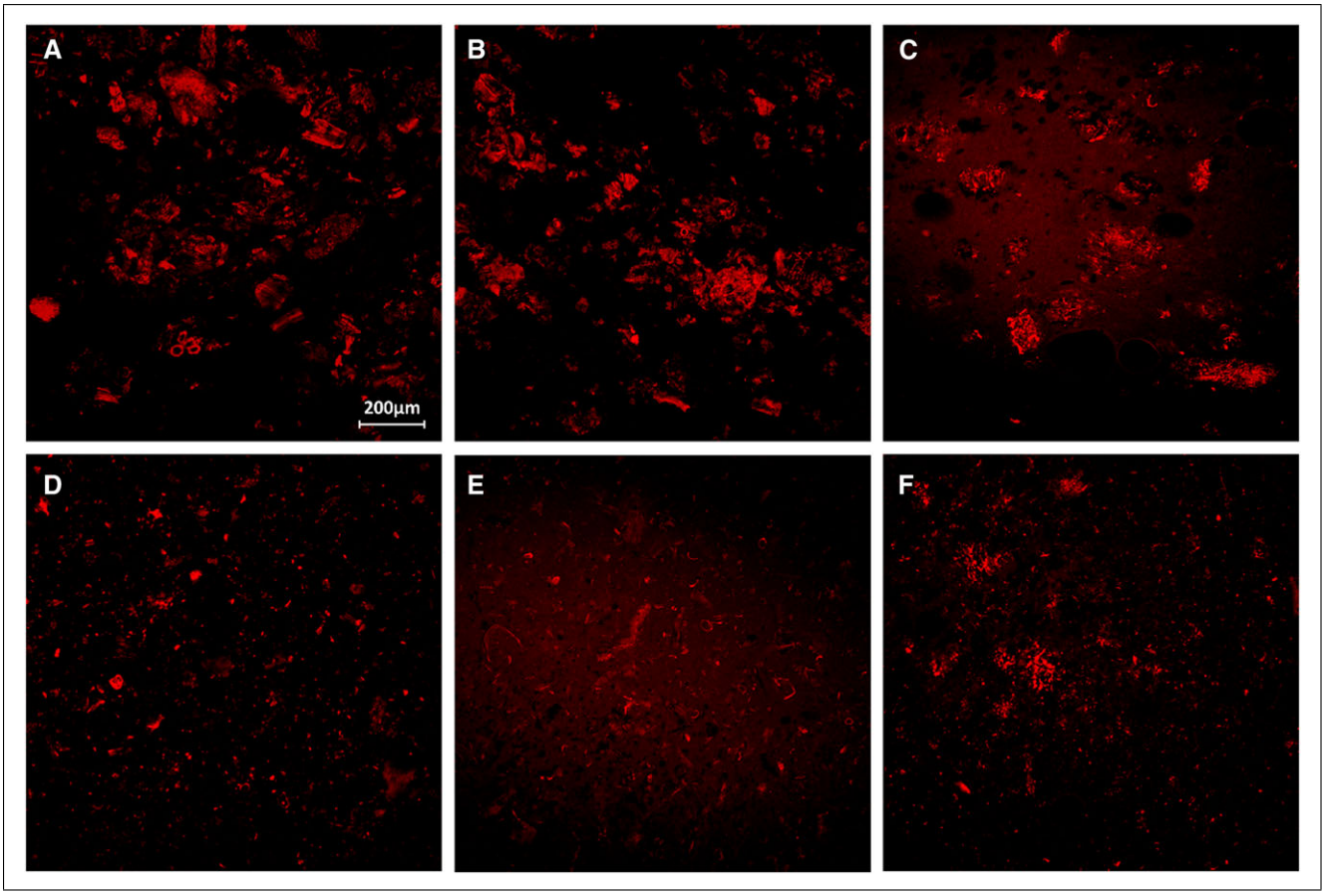

Figure 5–Microstructure of the vegetable inks with 10% powder content. Hydrocolloids: (A–C) 10% XG-based matrix; (D–F) 10% HPMC-based matrix. Vegetable: (A, D) broccoli; (B, E) spinach; (C, F) carrot.

the broccoli contains about 6.32% carbohydrates having 0.79% sugar (National Institute of Agricultural Sciences, 2016). The BP- and CP-based HPMC mixtures (Figure 4D) showed the greatest difference in rheological properties among the hydrocolloids tested, with the G' values of the sample containing CP (37.20 to 64.02 kPa) being less than half than those of the BP-based mixture (78.17 to 131.24 kPa). The reason for this large difference may be that the relatively low WHC of HPMC was insufficient to inhibit the swelling of the vegetable powder. It is also possible that the reduced viscosity of the HPMC matrix resulted from a decrease in the interactions between the polysaccharide chains due to the binding of the water-soluble component. As described above, HPMC also showed notable differences of (η^*) values in the presence of all food ingredients (Figure 1D). On the other hand, the XG-based mixtures showed the least difference between the viscoelastic values of the different vegetables. This suggested that XG, which has a very high WHC, could inhibit the swelling of the incorporated vegetable powders to an extreme degree in the hydrocolloid system, minimizing the rheological differences between mixtures with different vegetable powder sources.

Printability assessment

The printability of the vegetable mixtures was investigated by measuring their shear modulus and extruded hardness. In our previous work, we verified that the shear modulus can be used to predict and quantify the deformation behavior of samples after the 3D printing process. Table 1 shows the effects of the addition of vegetable powders to the hydrocolloids on the printability of the samples. The shear modulus values of the GG mixtures containing 10% vegetable powder were the highest among all the hydrocolloids tested, and ranged from 5.96 ± 0.44 kPa for SP to 7.84 ± 1.16 kPa. On the other hand, the mixtures based on HPMC showed the lowest shear modulus values, ranging from 0.45 ± 0.03 for CP to 0.49 ± 0.03 for SP. These low values indicated that HPMC has poor dimensional stability and could easily collapse under its own weight after deposition. In all samples, the shear modulus values increased significantly when a higher vegetable powder content (30%) was used. The incorporation of CP resulted in the lowest shear modulus increase for all samples, which was in agreement with the results of the rheology measurements (Figure 4). Compared to their low values at 10% powder content, the HPMC mixtures showed a dramatic increase in shear modulus (ranging from 24.09 ± 2.17 kPa for CP to 58.12 ± 1.85 kPa for BP) when the powder content was increased to 30%. This indicated that increasing the volume fraction of the particles dispersed in the hydrocolloid matrix could provide sufficient mechanical strength for the mixtures to retain a printed shape.

The extruded hardness, that is, the force required to extrude the material, was measured to assess the handling properties of the samples. At 10% powder incorporation, the extruded hardness of the samples ranged from 0.35 ± 0.05 kg for XG with BP to 5.52 ± 0.23 kg for HPMC with SP. The XG mixtures showed the lowest extruded hardness values among the tested hydrocolloids. The HPMC mixtures showed the highest values, even though their shear modulus values were the lowest. Increasing the vegetable powder content to 30% increased the extruded hardness of

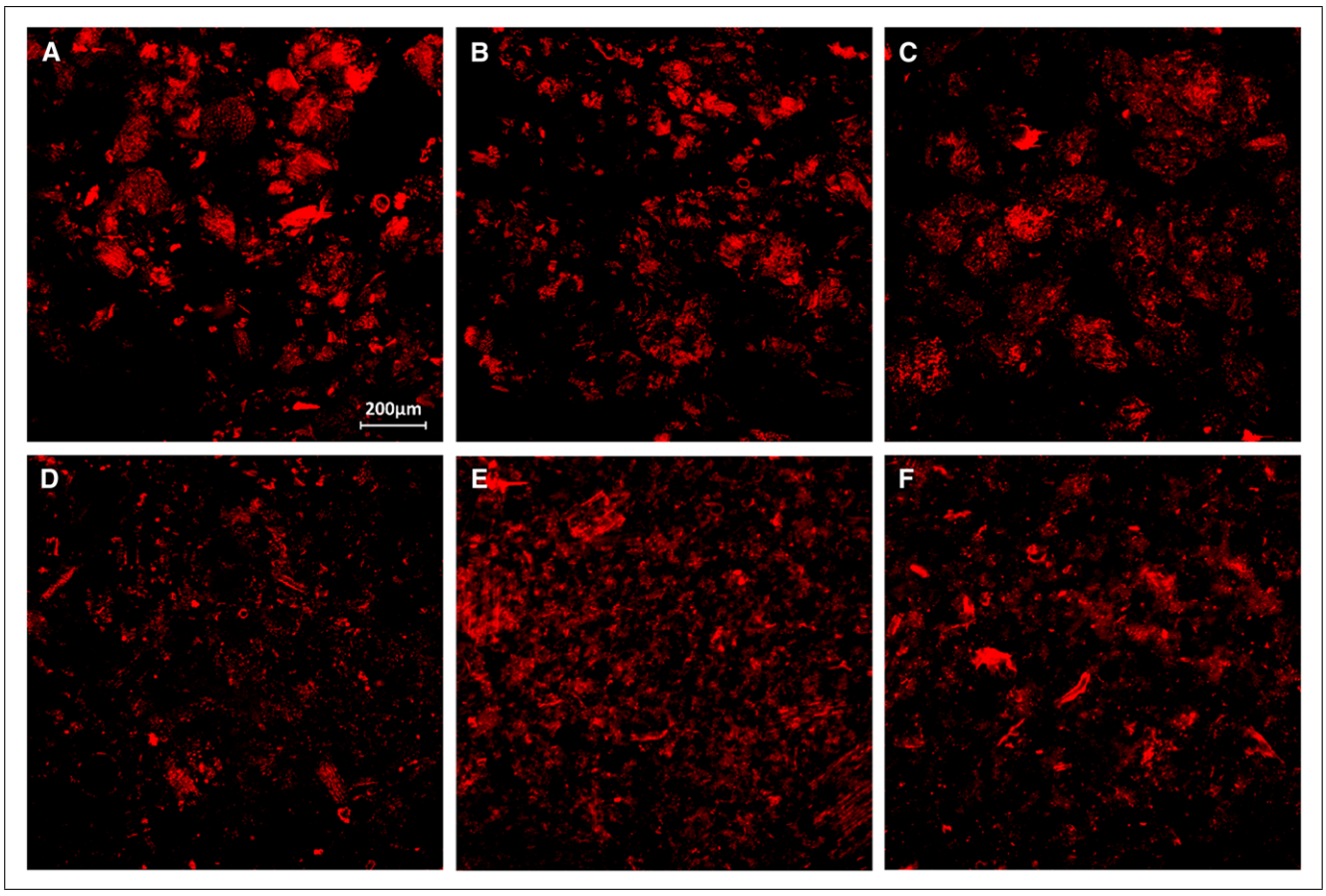

Figure 6–Microstructure of the vegetable inks with 30% powder content. Hydrocolloids: (A–C) 10% XG-based matrix; (D–F) 10% HPMC-based matrix. Vegetable: (A, D) broccoli; (B, E) spinach; (C, F) carrot.

all the hydrocolloids. In the samples containing XG, the extruded hardness ranged from 2.96 ± 0.23 kg for BP to 3.46 ± 0.16 kg for SP, and, unlike the other hydrocolloids, no significant differences were observed among the vegetable powders used. The extruded hardness of GG and LBG ranged from 8.34 ± 0.37 kg for CP to 10.39 ± 0.43 kg for BP, and 4.71 ± 0.25 kg for CP to 10.36 ± 0.43 for BP, respectively. HPMC exhibited the greatest increase in extruded hardness due to increased vegetable powder content. In addition, its hardness values varied remarkably depending on the source of the incorporated vegetable powder. The extruded hardness value resulting from BP incorporation (23.37 ± 3.27 kg) was more than three times greater than that from CP (6.92 ± 0.27 kg).

From these results, we can conclude that the use of hydrocolloids with high WHC, such as XG, in the preparation of vegetable inks with a high powder volume can improve printability. In addition, since XG can minimize the differences in printability between mixtures incorporating different powder sources, a standardized ink formulation for 3D printing can be prepared.

Confocal laser scanning microscopy

The microstructures of the systems containing vegetable particles dispersed in a hydrocolloid were visualized using CLSM. The results for the 10% and 30% vegetable powder content samples are shown in Figures 5 and 6, respectively. Only the samples containing XG and HPMC were observed, because these hydrocolloids showed the greatest influence on the rheological properties and printability of the systems. Rhodamine B preferentially binds to the hydrophobic zones of proteins in vegetable powder; thus, the red-labeled areas indicate the vegetable particles (Rodriguez, Torrez Irigoyen, Navarro, & Yamul, 2017). Figures 5A, B, and C show the XG-based matrices with 10% (w/w) of BP, SP, and CP, respectively. The results indicated that all three types of vegetable particles remained intact in this matrix. On the other hand, the vegetable particles in the HPMC-based mixtures were completely swollen (Figures 5D, E, and F).

The effect of the powder content was further investigated (Figure 6) by adding a higher powder content (30% w/w) than that of a normal vegetable powder ink mixture (10% w/w). The XG-based dispersions containing the three vegetable powders (Figure 6A, B, and C) suppressed the powder swelling at this high powder content effectively enough for particles to be observed. In the images of the HPMC-based dispersions (Figures 6D, E, and F), the vegetable powders were completely swollen. The results of the present study are in close agreement with previous reports in which hydrocolloids with high WHCs prevented the particle swelling of starch granules (Achayuthakan & Suphantharika, 2008; Chaisawang & Suphantharika, 2006) and BP (Silva et al., 2013) in gel-like matrices containing dispersed solid particles.

Printing behavior of vegetable Ink

The 3D printed objects based on the XG and HPMC formulations are shown in Figure 7. To investigate the effects of the powder content and the hydrocolloid type on the flexibility of the layers and the resolution of the products, a leaf-shaped model with many edges, requiring the layers to bend sufficiently, was

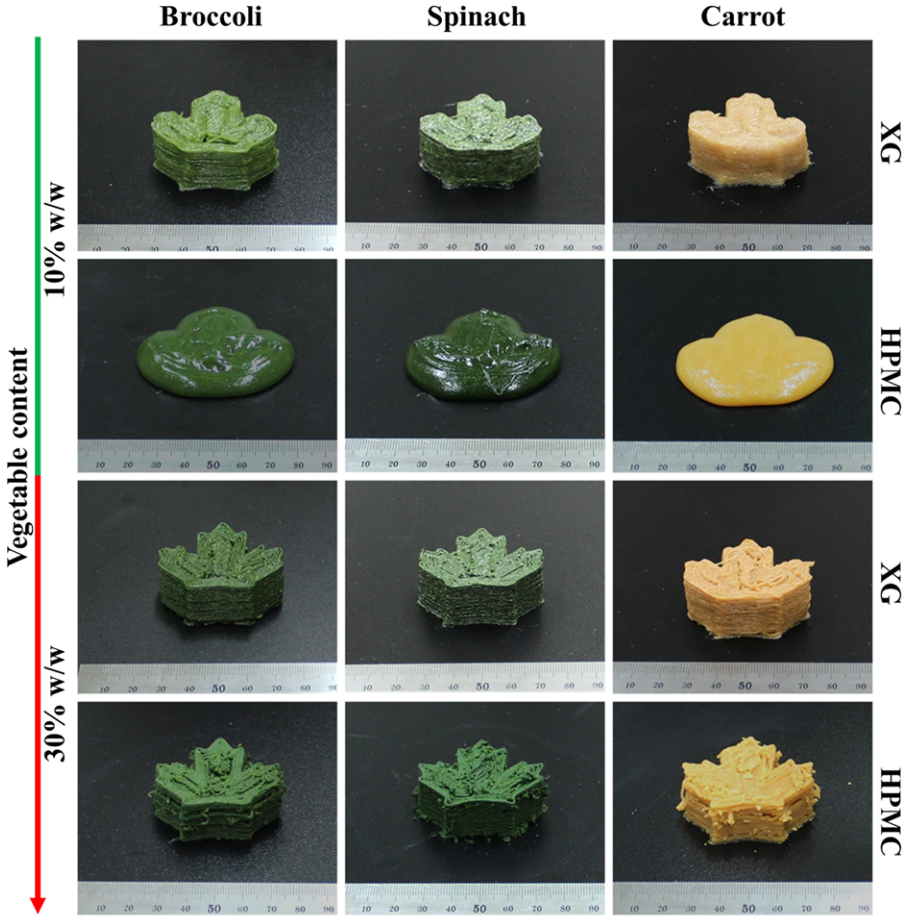

Figure 7–Images of XG and HPMC-based products containing 10% and 30% vegetable powder.

chosen. At 10% powder content, the XG-based products showed high resolution in the printing process, whereas the HPMC-based products completely collapsed during deposition. On the other hand, when the powder content was further increased to 30%, although the shear modulus and G' values of HPMC were evaluated as being highly suitable, the printed objects showed poor resolution. This could indicate that increasing the particle volume fraction in the system too far led to an excessive increase in its mechanical strength, resulting in poor layer flexibility and extrusion properties. Thus, layer breakage and surface cracking occurred in the printed object during the deposition process.

In contrast, as observed in the handling properties study, mixtures of XG with increased vegetable powder content could be smoothly extruded from the nozzle due to their low extruded hardness (2.96 to 3.46 kg). In addition, the resulting objects displayed fine resolution and a smooth surface, and did not collapse over time. These results confirmed that the maximum vegetable powder content at which objects with fine resolution can be obtained depends on the dispersion medium used. Furthermore, materials used for 3D printing should not only have suitable shape retention properties, but also proper handling properties to allow them to extrude smoothly through the nozzle during the extrusion process. Therefore, we suggest that the use of hydrocolloids with high WHC, such as XG, could improve the extrudability of systems by suppressing the swelling of incorporated particles while exhibiting sufficient shape retention.

Conclusions

Influence of food ingredients on (η^*) of various hydrocolloids was investigated, and confirmed that the shear rheology of XG and GG were not affected by the composition of the incorporated particles (sugar, acid, and ionic components). XG showed the highest WHC (51.18 g/g) of all the tested hydrocolloids. In all the vegetable powder mixtures except those based on XG, the rheology and printability of the mixtures varied depending on the vegetable powder source, with the variation becoming more apparent as the powder content was increased. Only the mixtures based on XG showed no significant differences in shear rheology and printability properties at 10% and 30% powder content. CLSM analysis confirmed that the variations of the shear rheology and printability at different powder contents were affected by the rapid increase in the total volume fraction of the system due to particle swelling, and demonstrated that XG strongly inhibited swelling. Even when a high vegetable powder content (30%) was incorporated in the XG mixtures, the resulting printed objects showed good extrudability and high resolution, with no cracks in the layers. Our results determined that XG was the optimum hydrocolloid for the preparation of food ink systems that contains a high volume fraction of powder, allowing increased nutritional value as well as 3D printing with good extrudability and resolution. Furthermore, we suggest that XG can be used to develop a standardized food ink preparation method because of its ability to minimize the rheological differences between systems containing different powders.

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Author Contributions

Hyun Woo Kim designed the experiment and prepared and experimented all the samples and analyzed the results. Jang Ho Lee participated in the interpretation of the influence of food ingredients on complex viscosity of hydrocolloid mixtures. Sae Mi Park participated in the rheological analysis and interpretation of the result. Min Hyeock Lee and Il Woo Lee conducted the water hydration capacity and printability experiment. Han Sol Doh participated in the 3D printing test and interpretation of the result. Hyun Jin Park participated in the design of all experiments and interpretation of the experimental results as corresponding authors.

References

Achayuthakan, P., & Suphantharika, M. (2008). Pasting and rheological properties of waxy corn starch as affected by guar gum and xanthan gum. *Carbohydrate Polymers*, *71*(1), 9–17.

Almdal, K., Dyre, J., Hvidt, S., & Kramer, O. (1993). Towards a phenomenological definition of the term 'gel'. *Polymer Gels and Networks*, *1*(1), 5–17.

Arendt, E., & Dal Bello, F. (2011). *Gluten-free cereal products and beverages*. Amsterdam, Netherlands: Elsevier.

Bchir, B., Rabetafika, H. N., Paquot, M., & Blecker, C. (2014). Effect of pear, apple and date fibres from cooked fruit by-products on dough performance and bread quality. *Food and Bioprocess Technology*, 7(4), 1114–1127.

Chaisawang, M., & Suphantharika, M. (2006). Pasting and rheological properties of native and anionic tapioca starches as modified by guar gum and xanthan gum. *Food Hydrocolloids*, *20*(5), 641–649.

Chen, Y., Zhang, B. C., Sun, Y. H., Zhang, J. G., Sun, H. J., & Wei, Z. J. (2015). Physicochemical properties and adsorption of cholesterol by okra (*Abelmoschus esculentus*) powder. *Food and Function*, *6*(12), 3728–3736.

Clark, A. H., & Ross-Murphy, S. B. (1987). Structural and mechanical properties of biopolymer gels. *Biopolymers*, 57–192. https://doi.org/10.1007/BFb0023332

Costakis, W. J., Rueschhoff, L. M., Diaz-Cano, A. I., Youngblood, J. P., & Trice, R. W. (2016). Additive manufacturing of boron carbide via continuous filament direct ink writing of aqueous ceramic suspensions. *Journal of the European Ceramic Society*, *36*(14), 3249–3256.

Doublier, J. L., Launay, B., & Cuvelier, G. (1992). Viscoelastic properties of food gels. In *Viscoelastic properties of foods*, 371–434. Amsterdam, Netherlands: Elsevier.

Dooling, L. J., Buck, M. E., Zhang, W. B., & Tirrell, D. A. (2016). Programming molecular association and viscoelastic behavior in protein networks. *Advanced Materials*, *28*(23), 4651–4657.

Elfak, A. M., Pass, G., & Morley, R. G. (1977). The viscosity of dilute solutions of guar gum and locust bean gum with and without added sugars. *Journal of the Science of Food and Agriculture*, *28*(10), 895–899.

Godoi, F. C., Prakash, S., & Bhandari, B. R. (2016). 3D printing technologies applied for food design: Status and prospects. *Journal of Food Engineering*, *179*, 44–54.

Higiro, J., Herald, T. J., Alavi, S., & Bean, S. (2007). Rheological study of xanthan and locust bean gum interaction in dilute solution: Effect of salt. *Food Research International*, *40*(4), 435–447.

Joshi, S. C. (2011). Sol-gel behavior of hydroxypropyl methylcellulose (HPMC) in ionic media including drug release. *Materials*, *4*(10), 1861–1905.

Kim, H. W., Bae, H., & Park, H. J. (2017). Classification of the printability of selected food for 3D printing: Development of an assessment method using hydrocolloids as reference material. *Journal of Food Engineering*, *215*, 23–32.

Lipton, J., Arnold, D., Nigl, F., Lopez, N., Cohen, D. L., Norén, N., & Lipson, H. (2010). Multi-material food printing with complex internal structure suitable for conventional post-processing. Paper presented at the Solid Freeform Fabrication Symposium.

Liu, R. H. (2003). Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *American Journal of Clinical Nutrition*, *78*(3), 517S–520S.

Liu, Z., Zhang, M., Bhandari, B., & Yang, C. (2018). Impact of rheological properties of mashed potatoes on 3D printing. *Journal of Food Engineering*, *220*, 76–82.

National Institute of Agricultural Sciences. (2016). Food composition table (9th revision). *Suwon: National Institute of Agricultural Sciences*, *1*, 1–520.

Punitha, S., Uvarani, R., Panneerselvam, A., & Nithiyanantham, S. (2014). Physico-chemical studies on some saccharides in aqueous cellulose solutions at different temperatures–Acoustical and FTIR analysis. *Journal of Saudi Chemical Society*, *18*(5), 657–665.

Rodriguez, A. C., Torrez Irigoyen, M. R., Navarro, A. S., & Yamul, D. K. (2017). Obtention and characterization of dried gels prepared with whey proteins, honey and hydrocolloids mixture. *Journal of the Science of Food and Agriculture*, *97*(14), 4969–4977.

Ross-Murphy, S. B. (1994). *Physical techniques for the study of food biopolymers* (pp. 343–392). Berlin, Germany: Springer.

Scheraga, H. A., & Mandelkern, L. (1953). Consideration of the hydrodynamic properties of proteins1, 2. *Journal of the American Chemical Society*, *75*(1), 179–184.

Shiroodi, S. G., & Lo, Y. M. (2015). The effect of pH on the rheology of mixed gels containing whey protein isolate and xanthan-curdlan hydrogel. *Journal of Dairy Research*, *82*(4), 506–512.

Silva, E., Birkenhake, M., Scholten, E., Sagis, L. M. C., & Van der Linden, E. (2013). Controlling rheology and structure of sweet potato starch noodles with high broccoli powder content by hydrocolloids. *Food Hydrocolloids*, *30*(1), 42–52.

Silva, E., Scholten, E., van der Linden, E., & Sagis, L. M. C. (2012). Influence of swelling of vegetable particles on structure and rheology of starch matrices. *Journal of Food Engineering*, *112*(3), 168–174.

Sun, J., Peng, Z., Zhou, W., Fuh, J. Y. H., Hong, G. S., & Chiu, A. (2015). A review on 3D printing for customized food fabrication. *Procedia Manufacturing*, *1*, 308–319.

Van Duyn, M. A. S., & Pivonka, E. (2000). Overview of the health benefits of fruit and vegetable consumption for the dietetics professional: Selected literature. *Journal of the American Dietetic Association*, *100*(12), 1511–1521.

Vlachy, V. (2008). Polyelectrolyte hydration: Theory and experiment. *Pure and Applied Chemistry*, *80*(6), 1253–1266.

Wüstenberg, T. (2015). General overview of food hydrocolloids. Cellulose and Cellulose Derivatives in the Food Industry: Fundamentals and Applications.

Wong, R. B. K., & Lelievre, J. (1981). Viscoelastic behaviour of wheat starch pastes. *Rheologica Acta*, *20*(3), 299–307.