Please use this form to clearly and concisely report on project progress. The information included should reflect quantifiable results that can be used to evaluate and measure project success. Comments should be limited to the designated boxes. Technical reports, no longer than 4 pages, may be attached to this summary report.

Project Number:	AWD-100936
Project Title:	Novel new functional edible protein films from soybean using innovative 3D printing technology
Organization:	Department of Food Science, Department of Biological and Agricultural Engineering, University of Arkansas Division of Agriculture
Project Lead Name:	Dr. Ali Ubeyitogullari
Report Date:	June 15, 2022 to September 15, 2022

National Soybean Checkoff Research Database https://www.soybeanresearchdata.com/ (public website funded by USB). Please include a non-technical project status along with your project status. The non-technical project status will be published to the website. If a non-technical project status is not provided, the contents of this entire report will be published.

Project Status: On-going (Year 2 of 2)

Objectives for the project:

1. Prepare soy protein isolate and hydrolysates from soybean seeds grown in Mid-South (lines R16-5065 from Arkansas and S17-17168 from Missouri).
2. Optimize conditions and prepare homogeneous film solutions with soy protein isolate, glycerol, malic acid and natural phenolic extract (antioxidant), and investigate flow properties.
3. Optimize conditions and extrude soy protein film using 3D printing technology, investigate the physical properties of the extruded films for color, tensile strength, and antioxidant activity of the extruded film.

Accomplishments this (June-September) quarter:

Objective 3: Optimize conditions and extrude soy protein film using 3D printing technology, investigate the physical properties of the extruded films for color, tensile strength, and antioxidant activity of the extruded film.

1. Methods

1.1. Preparation of 3D printed soy protein (SP) films

Aqueous suspension of 11% SP was homogenized in a glass container for 15 min. The pH of the solution was adjusted to 8.5 using 1M NaOH solution. Green tea (GT)/or grape seed (GS) extracts (1, 3, and 5%, w/w based on SP content) were added and stirred at room temperature (23 °C) for 15 min. The prepared solution was then sonicated (Probe sonicator Branson Ultrasonics, USA) for 5 min (duty cycle: 70%, output control: 9) in ice to prevent overheating. To this solution, glycerol (30%, w/w based on SP content) was added as a plasticizer and then degassed under the vacuum. The final solution was employed as the ink for printing. 3D food printing was carried out using an Allevi 2 Bioprinter (Allevi, Inc., Philadelphia, PA, USA) that was equipped with two 10 mL extruders. The developed SP solution was loaded into a 10 mL cartridge, which was then transferred to one of the extruders of the 3D printer previously warmed up to 85 °C, sustained at this temperature for 30 min. The temperature of the extruder was then reduced to room temperature (23 °C). The SP-based ink was then extruded through nozzles with different diameters (i.e., 23, 25, and 30 G corresponding to 0.330, 0.250, and 0.152 mm internal diameters, respectively). The extrusion pressure was optimized for each printing condition in the range between 3 and 9 psi. The printing speed was kept constant at 5 mm/s. The printed films were dried for 4 h in a controlled humidity chamber (Hot Pack, USA) at 50 °C and 45% RH. The dried films were then peeled and placed between wax-paper sheets in a chamber at 25 °C, 50% RH.

1.2. Viscosity measurement

The apparent viscosity was measured at 95 °C in shear rates ranging from 0.1 to 100 1/s using a controlled-stress rheometer equipped with a Peltier Plate system for temperature control (AR 2000 Rheometer, TA Instruments, DE, USA) and a parallel-plate geometry with a diameter of 40 mm.

1.3. FTIR analysis

The soy protein (SP), green tea (GT) extract, grape seed (GS) extract, and soy protein-based films were investigated for their structural properties using an FTIR spectrometer (IRAffinity-1S Fourier transform infrared spectrometer, SHIMADZU Corp., Japan) that was equipped with attenuated total reflectance (ATR) accessory (Specac Company, Orpington, UK). The spectra were recorded in the range of 4000 to 400 cm^{-1} with 64 scans.

2. Results

2.1 Printing performance

Fig. 1A, B depicts the 3D printing performance as a function of extract type and concentration, printing pressure, and nozzle diameter. The surface of the GS-containing films was smoother than the control and GT-containing films, which was due to the lower viscosity of the inks prepared with GS extract (Fig. 1A). The results showed that the extract affected the interactions of protein molecules, resulting in a change in printing quality. The GT extract used in this study, however, was discovered to contain a hydrocolloid, which could be the primary cause of the difference observed between the extracts.

As shown in Fig. 1B, printing pressure and nozzle diameter were also important factors in printing performance. The best printing pressure and nozzle diameter were determined to be 9 psi and 0.25 mm, respectively.

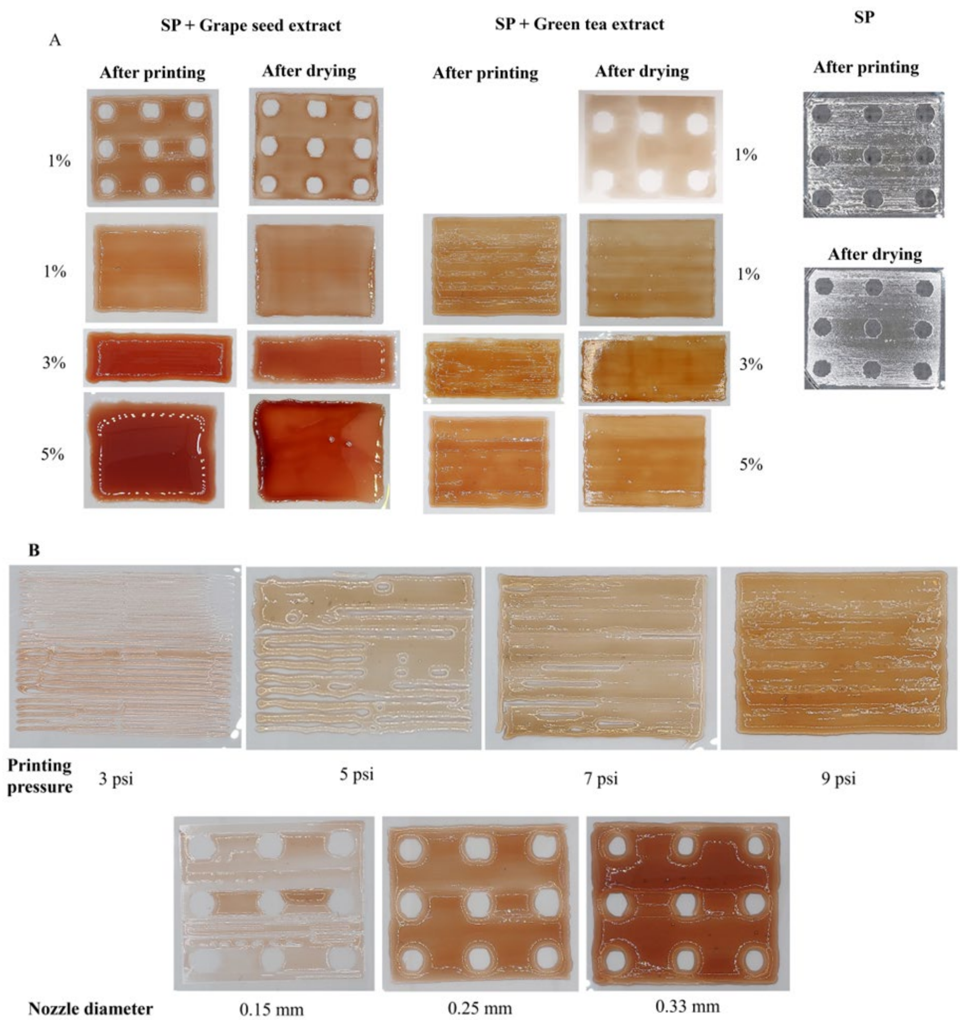

Figure 1. Effects of grape and green tea extracts on the printability of the films at different concentrations, pressures, and nozzle diameters.

2.2. Viscosity

The viscosity of protein-based ink as a function of extract type and concentration at 95 °C is depicted in Fig. 2, where the results revealed the shear-thinning properties of the inks. However, the addition of extracts reduced the viscosity of the protein solution, confirming the influence on weakening the interactions between protein chains. When compared to green tea extract, grape seed extract significantly reduced ink viscosity, which affected printing quality. However, as explained in the previous section, the hydrocolloid present in GT extract could be the main cause of the difference observed between the extracts. Overall, the apparent viscosity indicated that the addition of a high concentration of extracts prevented the degree of soy protein gelation upon thermal treatment.

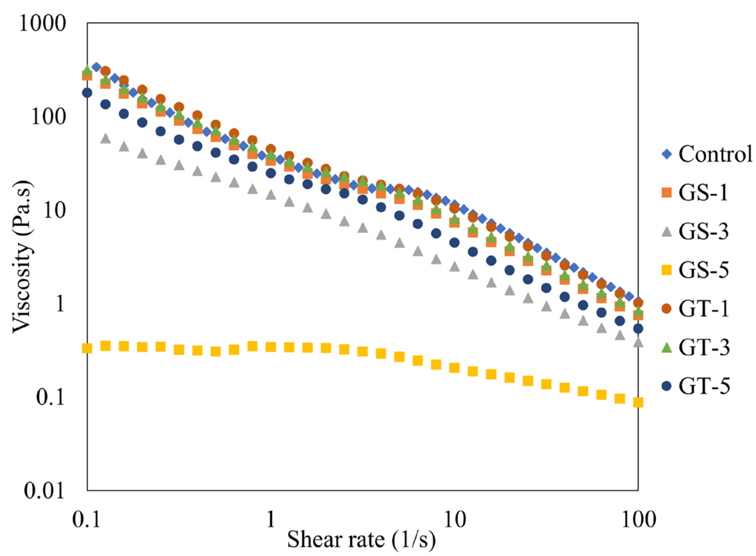

Figure 2. The viscosity of protein-based inks as a function of extract type and concentration, at 95 °C. GS: Grape Seed extract; GT: Green Tea extract.

2.3. FTIR spectra

As shown in Fig. 3, the FTIR spectrum of the SP film indicated an absorption band at 3270 cm^{-1}, associated mainly with the free O–H groups and amine N–H stretching. The bands that appeared at 1630 cm^{-1}, 1532 cm^{-1}, and 1235 cm^{-1} corresponded to C=O stretching (amide I), N–H bending (amide II), and C–N stretching (amide III), respectively. The asymmetric stretch vibrations of =C–H and $–NH3^{+}$ indicated a peak at around 2928 cm^{-1}. The peak at around 1039 cm^{-1} was associated with C–H and C–O–H deformations.

By inspecting the frequencies of amide bonds, FT-IR spectroscopy can determine the effect of phenolic compounds on the structure of proteins. Amide band I is the most sensitive spectral region for protein structure. Therefore, in the next step of this study, the curve fitting method will be applied to resolve the amide band I region of soy protein–GT/GS films, helping us to estimate the effect of the extract on the structure of proteins.

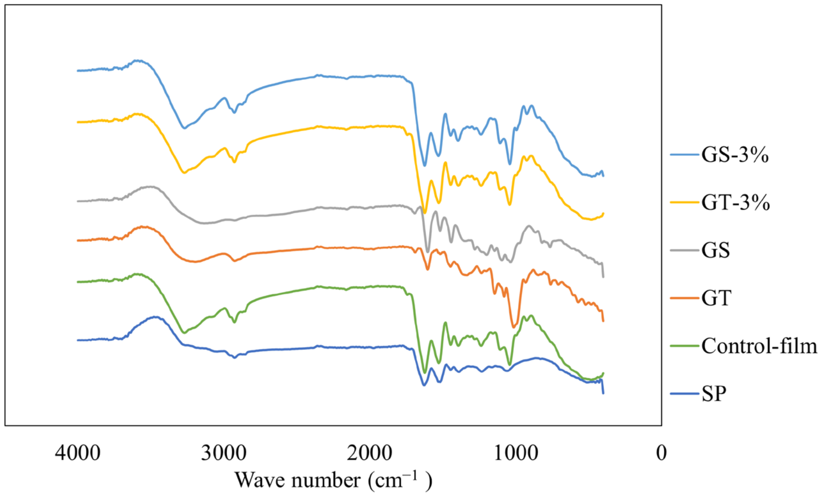

Figure 3. ATR-FTIR spectra of the soy protein, green tea and grape seed extracts, and the protein-based films incorporated with 3% of extracts.

Non-technical project status:

Edible films from soy proteins were successfully printed along with grape seed and green tea extracts via a 3D food printer. The effects of the concentration of grape seed and green tea extracts on printability, printing pressure, and nozzle size were investigated. All soy protein inks showed a shear-thinning behavior. However, their viscosity was reduced with the addition of the phenolic extracts, which also affected their printability. The chemical interactions between the soy proteins and phenolic extracts are currently being analyzed. Next, the antioxidant activities of the printed films will be determined. In addition, the printed films will be characterized for their mechanical strength, surface morphology, and color.