

Starch-based food inks in extrusion 3D printing: formulation, physico-chemical
properties and printing performance

by

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B.S., Kansas State University, 2020

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Grain Science and Industry
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2024

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Abstract

Food 3D printing offers the possibility of manufacturing novel products with intricate shapes, unique textures, and alternative methods for delivering nutrition. This research aims to understand the impact of key formulation attributes for 3D printing inks, such as starch source and plasticizers (sucrose and moisture), on their physico-chemical properties and rheology, printing performance, and end-product texture. Impact of 3D printing process parameters on end-product quality was also assessed. The focus of this thesis was to study the rheological and physico-chemical properties of starch-based food inks and how they impact performance for extrusion based printing, but the knowledge gained in this research can be transferred to other types of printing technology as well, including other types of protein and chocolate-based food 3D printing.

In the first part of the study, 3D printing inks were formulated based on a degermed corn flour or wheat base with varying level of sucrose (0, 4, 8 and 12%) while keeping the moisture constant at 50% wb in a 2x4 factorial design; and varying levels of water (50, 54, 58 and 62% wb) with no sucrose in the formulation in another 2x4 factorial design. Sucrose inclusion up till 12% significantly decreased the rapid visco analyzer (RVA) pasting viscosity of both corn-based (from 4655 to 1410 mPa.s) and wheat-based formulations (from 2591 to 703 mPa.s). Peak gelatinization temperature obtained using differential scanning calorimeter, increased from 73.6 to 77.2°C and from 65.5 to 69.0°C for corn and wheat-based inks, respectively, as sucrose level increased in the abovementioned range. Phase transition analyzer (PTA) data had conflicting results as sucrose level increased, with flow temperature decreasing from 130 to 91°C for corn-based inks, showing a clear plasticizing effect; but increasing from 101 to 147 °C for wheat-based inks, which indicated competition for water between sucrose and starch might have had an

important role, which was also affirmed from water absorption index data. Products based on corn-based inks had considerably poor 3D printing performance with increase in moisture content from 50-62% as ascertained by the dimensional distortion ratio (DDR) as compared to wheat-based inks (DDR absolute values of 0.032 to 1.110 for corn as compared 0.040 to 0.092 for wheat). Both corn and wheat-based 3D printed samples showed more resiliency in shape and dimensional distortion with increase in sucrose content, although wheat products exhibited an increase in DDR as sucrose level increased. 3D printed product was also tested using RVA and DSC, with results indicating that corn-based prints were only partial gelatinized, while wheat-based prints were completely gelatinized during the process.

In the second part of this study, rheology of corn and wheat-based food inks were evaluated using a dynamic rheometer. In general, frequency sweep storage modulus (G') and loss modulus (G'') values decreased ($p < 0.05$) for both corn and wheat-based inks with increase in sucrose inclusion levels from 0-12%, showing a plasticizing effect that was partly contradictory to the results obtained from PTA earlier. The order of magnitude of G' and G'' for corn ($\sim 10^4$ - 10^5 and $\sim 10^3$ - 10^4 Pa, respectively) were higher than that for wheat-based inks ($\sim 10^3$ - 10^4 and $\sim 10^3$ Pa, respectively). Impact of 3D printing infill density (20, 40, 60 and 80%) was also investigated. DDR for corn-based ink increased significantly from 0.064 to 0.701, as 3D printing infill density was varied from 20-80%; while the DDR for wheat-based ink was more resilient with no significant differences with change in infill density (DDR ranging from -0.026 to -0.052). High infill density significantly increased the structural integrity both corn and wheat-based 3D printed products. At infill density of 20%, an average peak force of 4.59 and 3.75 kg was observed for corn and wheat-based 3D printed products, respectively, as determined by a texture

analyzer in compression mode; while at 80% infill density corn and wheat-based products had average peak force of 21.16 and 24.70 kg, respectively.

In conclusion, key methodology was developed for evaluating physico-chemical properties and performance of food inks. The approach outlined in this study can help to design high performance materials for food 3D printing in the future.

Keywords: Food 3D Printing; Extrusion; Starch; Rheology; Printability

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Acknowledgements

I would like to thank the members of my committee, Dr. Sajid Alavi, Dr. Yong-Chen Shi, Dr. Martin Talavera, and Dr. Kaliramesh Siliveru for their input and expertise throughout this process. I would also like to thank the department as a whole for supporting my research and professional development as graduate student. And lastly, my lab mates, friends and family for helping to make all this possible.

Chapter 1 - Introduction

1.1 Background

Food 3D printing offers the possibility of manufacturing novel food products with intricate shapes, unique textures, and alternative methods for delivering nutritional value through the combinations of different food ingredients and printing methodologies. Many applications exist for 3D printing including plant-based meats, meals for those who have trouble chewing and swallowing, and personalized products, but further research is needed to scale-up for industry and improve sensory aspects of 3D printed food. 3D printing, also known as additive manufacturing is an automated manufacturing process that works through depositing layers of raw materials or binding raw materials to create physical 3D structures (Lipson et al., 2013). The application of 3D printing has been widely used in industry and research including in automotive, architecture, medical and fashion design fields. But in recent years, research and development has been conducted to use 3D printing within food manufacturing to create new products or innovate processing methods (Değerli et al., 2017).

Potential opportunities within the food industry sector for using 3D printing technology include: customized food design, personalized nutrition, efficient use of raw materials and expansion of food material sources (Fahmy et al., 2020; Ma et al., 2022). An advantage of Food 3D printing includes its application in creating complex products without the use of a mold, cutting tools, and (in certain cases) coolants. This review aims to explore current techniques and research being conducted for 3D printing of food, factors affecting printability, applications for use in industry and research, food material being used for printing, and areas for further research.

Current 3D food printing techniques can be classified into three general categories that include extrusion-based printing, binder jetting and inkjet printing. Post-printing treatments are

also used to manipulate the food products further to either make them edible or to set their structure. After a food item has been 3D printed, it can be frozen, baked or deep-fried, among other methods (Tan et al., 2018).

Extrusion-based printing builds food models by extruding food through a nozzle with constant pressure (Placone et al., 2018). The starting food material can either be a solid or paste with relatively low viscosity, which is generally homogenous in nature that is either heated or uses starch and hydrocolloids to stabilize the food ink (but some studies have produced multilayered materials for printing) (Dick et al., 2019). In the extrusion-based 3D printing process, food material can be loaded in an extruder cylinder/syringe before it is extruded through nozzle by ram pressure to create a shape layer – by – layer. The food material can also be partially cooked as they are being printed in the extruder (Sun et al., 2015). The temperature of the extruder depends on the type of material and desired printing result (Sun et al., 2015).

Many examples of food that have been produced using this technique include doughs, meat paste and cheeses (Kewuyemi et al., 2021). Results from those studies discussed by Kewuyemi in 2021 show that nozzle diameter, the nozzle movement speed, and the extrusion rate affect the quality of 3D food printing. Some food products that are produced from this method are typically gels/rigid structures such as meat analogues, confectionary products, and hydrogels (Pusch et al., 2018). Some advantages of this process include low cost for start up with entry level machines, a variety of raw materials that can be used, and compared to the other methods listed in this section its the easiest to customize (Piyush, et al., 2020).

3D printing of foods that utilize inkjet printing operate by using thermal heads to dispense a material stream of droplets to specified regions on a food surface to create a surface filling (Pallottino et al., 2016). Inkjet printing is typically used to decorate foods such as pizza,

cookies, and cake, or create surface fillings as in meat paste, cheese, jam and sugar icing. In a thermal inkjet printer, the print head is electrically heated to establish pulses of pressure that push droplets from the nozzle (Godoi et al., 2016).

Within inkjet printing, two general methods exist: a continuous jet printing and a drop-on-demand printing. For the continuous jet printing, an ink is ejected continuously through a piezoelectric crystal by vibrating with a constant frequency. For drop-on-demand printing, a valve is used as a controller to eject food material out under a designed pressure. The printing rates of drop-on-demand systems are generally slower than the continuous jet systems. Inkjet printing normally handles low viscosity materials and therefore does not find application in the construction of complex food structures that need strong formability/rigidity. Some advantages of this process include little to no waste of model material, high resolution and accuracy, multiple materials and multiple colors, and relatively fast fabrication.

The binder jetting, which is also classified as additive manufacturing technology, constructs food models by using a liquid binder to selectively bond powdered material layers. In this process, powder material is spread on a build platform using a roller machine, then small droplets of binder are deposited on to it. After deposition of the liquid binder, the entire surface of the powder bed is exposed to a fixed amount of heat to seal the models layers, then another layer of powder is then spread over the previous layer, and repeated (Mostafaei et al., 2021). The binder is usually in liquid form and the build material is in powder form. For binder jetting process, rheological properties of powdered material and binder are important to the successful fabrication of parts. The liquid binder has to be used at a low viscosity in which surface tension and ink density are suitable properties to prevent spreading from nozzles. Some disadvantages of

binder jetting process include models with a rough or grainy appearance and post-processing required to remove moisture or improve strength.

The 3D printing of food materials is reliant on their physical properties such as viscosity, water binding potential, pasting temperature, starch gelatinization characteristics, etc. (Li et al., 2021). The rheological properties of food material ink are critical to 3D printing precision and accuracy (Godoi et al., 2016). Generally, it is necessary that the food material inks possess low viscosity so that they can be easily ejected through the nozzle of the printhead to create accurate and firm prints (Godoi et al., 2016). Additionally, researchers have also highlighted that food 3D printing technology can construct some complex patterns internally besides developing a three-dimensional shape, referred to as infill density (Huang et al., 2019). Studies can alter the infill density of printed products to change internal structure; consequently affecting the texture properties of 3D printed objects.

Temperature is another important factor in 3D printing of foods, as it can be used to modify the rheological properties and surface energy of food material inks. For example, a low temperature may be applied to lower surface energy and reduce the spreading tendency of inks across a chocolate surface for a 3D print (Liu et al., 2017). Many studies currently researching 3D printing of food utilize room temperature extrusion-based technology, however increasing or altering the temperature of food material inks prior to printing has been found to help in printing flowability (Chen et al., 2022; Wang et al., 2022).

To achieve ideal rheological properties in food models that are capable of holding the 3D structures, rheological modifiers, such as hydrocolloids (polysaccharides such as starch), soluble protein, can be added. In addition, the crystallization state and T_g of material is also critical to make the deposited material able to support its own structure after printing (Godoi et al., 2016).

Additionally, hydrocolloids, such as xanthan gum, may be used within the printing process to improve the texture of a printed product. Controlling printing infill density and the spatial distribution of ingredients are common strategies to modify texture and flavor perceptions of printed foods. One study from 2022 utilized printing infill density and the spatial distribution to alter textural and stability of 3D printed meat for applications in frying after printing, but future research is needed for longitudinal studies in terms of consumer acceptance of 3D food printing and potential health impacts by consuming printed foods (Chen et al., 2022).

In preparation for food 3D printing, food materials must be in a usable form, for instance, a powder, liquid, dough or puree (Li et al., 2021). In recent years, research has been conducted to produce food material inks that can accommodate technical complexity and consumer needs. 3D printing can alter appearance and sensorial properties of foods, to potentially meet consumer preferences and impact consumer eating behaviors. 3D food printing technology is already redefining aspects of food processing because of its potential to deliver meals tailored to unique standards of taste, convenience, and cost, along with any dietary restrictions (Pitayachaval et al., 2018). Many studies that have researched 3D printed food utilized starch and protein based food materials that can form structures that maintain shape under stress and time (Cheng-Rong et al., 2022). Many of those studies were researching methods for using 3D printing in application for emulating baked or extruded products such as snacks, cookies, and other confectionary food products (Chen et al., 2021). Additionally, 3D printed food has been used in the culinary and wider food industry with products such as crystallized sugar cake, detailed chocolate designs, ready-to-bake pizzas and ravioli (Vukušić et al, 2021).

One study utilized 3D food printers to create meals that are soft and pureed in texture and mimic taste and texture of real food while adding nutritional value. The project is called

PERFORMANCE (Personalized Food for the Nutrition of the Elderly Consumers), which uses 3D-printing technology to create easy-to-swallow and nutritious 3D-printed meals for the elderly (Forstner et al., 2015). Another emerging application in 3D printing of food is the fabrication of customized meat products from non-meat sources (plant protein, lab grown meat, etc.). Methodologies applied to 3D printing of meat analogues are extrusion, inkjet printing, binding deposition, and bio-printing (Godoi et al., 2016). However, extrusion is the most commonly used due to its suitability to a wide variety of food rheological properties (Chen et al., 2021).

One aspect for future development in food 3D printing includes reducing food waste by only printing the amount of food that is needed and using food that is usually discarded, such as fruits and vegetables that are deemed to misshapen to be sold in grocery stores (Kewuyemi et al., 2021).

Consumer acceptance of this novel technology and its resulting products are also important and needed for future success of 3D printed food as well. While texture and physical modifications of 3D printed foods are often characterized by instrumental analysis (e.g. texture analysis) that are reviewed in this study, data from sensory studies are still limited in current literature.

3D printing of food has many implications for the future of the food industry and offers the possibility of manufacturing novel food products with intricate shapes through the combination of different food ingredients and printing methodologies. Another major challenge for this technology lies upon industry: scale-up. This technology has been developed in many labs, but still has yet to expand into consumers sectors and needs further development to make it accessible to the commercial food industry. One hurdle for growth of food 3D printing includes consumer perception, which is vital toward the acceptance and growth of this field (Mabotja et

al., 2021). While there are indeed potentials for scale-up, appropriate stakeholders and funding will require consumer education for growth in acceptance and consumption of 3D printed foods. This information should be used for future planning and development of food 3D printing projects.

1.2 Objectives for Chapter 2

Chapter 2 focuses on identifying raw materials for starch-based food ink for extrusion-based 3D printing and assessing physico-chemical properties and their 3D printing performance. Starch-based food materials for 3D printing need to be able to flow out of a nozzle head of the 3D Printer and set properly on the print deck or previous layer during the printing process. When starch is fully or partially gelatinized it is able to achieve both of these elements essential in food 3D printing. Formulations for starch-based food ink for 3D printing were developed, and analysis was performed on the dry ingredients within the formulations to identify differences in their composition that could affect viscosity, water absorption, starch gelatinization, and other physico-chemical properties. Additionally, starch-based food ink formulations were 3D printed and assessed for printing performance

1.3 Objectives for Chapter 3

Chapter 3 focused on expanding what was learned from the raw material analysis and extrusion-based food 3D printing tests in the previous chapter. The highest performing food inks for both wheat and corn (in terms of dimensional distortion ratio) were selected from the previous study and used to test various infill densities for 3D printed food models. Additionally,

further testing was conducted on finished models using simple compression texture analysis tests and on post-printed food inks with dynamic rheology oscillatory strain sweep tests.

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Chapter 2 - 3D printing of starch-based food inks: Impact of starch source, moisture and sucrose on physico-chemical properties and printing performance

Abstract:

Food 3D printing is an emerging technology used for the creation of innovative products. Extrusion-based 3D printing deposits ‘food ink’ through a nozzle to create shapes layer-by-layer. The performance of food inks for 3D printing and end-product quality rely on numerous factors including formulation of ingredients, processing conditions and ingredient functionality. Within this study, starch-based food ink formulations were evaluated on their physico-chemical properties and their 3D printing performance.

Food ink mixes were formulated with a raw degermed corn or wheat base to study the impact of starch source, how moisture as a processing agent effected the treatments (with moisture ranging from 50-62%) and sucrose (0-12% inclusion) as a plasticizer. Water absorption index (WAI), phase transition analysis (PTA), differential scanning calorimetry (DSC) and rapid visco analysis (RVA) were used to determine physico-chemical properties of the raw materials. DSC and RVA were again used to study the printed samples, which were also were evaluated for printing accuracy and textural properties. Sucrose significantly decreased the pasting viscosity of the raw materials (2591 and 703 mPa·s, respectively, for wheat base and 12% wheat sucrose inclusion, and 4655 and 1410 mPa·s, respectively, for corn base and 12% corn sucrose inclusion). Products based on corn-based inks had considerably poor 3D printing performance with increase in moisture content from 50-62% as ascertained by the dimensional distortion ratio (DDR) as compared to wheat-based inks (DDR absolute values of 0.032 to 1.110 for corn as

compared 0.040 to 0.092 for wheat). Both corn and wheat-based 3D printed samples showed more resiliency in shape and dimensional distortion with increase in sucrose content, although wheat products exhibited an increase in DDR as sucrose level increased. Sucrose was also observed to lower water absorption for the raw material samples (with an average decrease of 0.656 from the base formulation to 12% inclusion), which might have had an impact.

Post-printed material were collected as samples after extrusion-based 3D printing, and again tested with RVA and DSC. It is important to note that these samples were subjected to a pre-printing ‘cooking’ step in a water bath before being printed on a Food 3D Printer. Samples tested after 3D printing exhibited partial gelatinization (Corn) or complete gelatinization (Wheat) via DSC tests. This research acts as a gateway for a new innovative food processing method as food 3D printing can produce more intricate shapes and unique textures with the approach outlined in this study helping to design high performance food inks for 3D printing in the future.

2.1 Introduction

Food 3D printing offers the possibility of manufacturing novel food products with intricate shapes, unique textures, and alternative methods for delivering nutritional value through the combination of different food ingredients and printing methodologies (Fahmy et al., 2020; Ma et al., 2022). An advantage of Food 3D printing includes its application in creating complex products without the use of a mold, cutting tools, and (in certain cases) coolants. Many applications exist for 3D printing including plant-based meats, meals for those who have trouble chewing and swallowing, and personalized products, but further research is needed to scale-up for industry and improve sensory aspects of 3D printed food. It is also important to understand

that for food 3D printing, physico-chemical properties of the raw materials impact the printing performance (Li et al., 2021).

The rheological properties of food material ink are critical to 3D printing precision and accuracy (Godoi et al., 2016). Generally, it is necessary that the food material inks possess low viscosity so that they can be easily ejected through the nozzle of the printhead to create accurate and firm prints (Godoi et al., 2016).

Temperature is another important factor in 3D printing of foods, as it can be used to modify the rheological properties and surface energy of food material inks. For example, a low temperature may be applied to lower surface energy and reduce the spreading tendency of inks across a chocolate surface (Liu et al., 2017). Many studies currently researching 3D printing of food utilize room temperature extrusion-based technology, however increasing or altering the temperature of food material inks prior to printing has been found to help flowability during printing (Chen et al., 2022; Wang et al., 2022).

Additionally, ideal rheological properties in food 3D printing can be achieved when rheological modifiers, such as hydrocolloids (polysaccharides such as starch), soluble protein are added to hold rigidity in the final printed samples. In addition, the crystallization state and T_g of material is also critical to make the deposited material able to support its own structure after printing (Godoi et al., 2016).

After conducting a literature review on food 3D printers being used for peer review research, a printer was acquired for use in this study based on its capabilities to perform 3D printing on a wide variety of raw materials including plant-based meat, fortified snacks, rice-based intermediate products and buckwheat snacks (Chen et al., 2021, Huang et al., 2019, Guo et al., 2021).

Before the study, an extensive testing phase was carried out to understand and access the capabilities of the food 3D printer. Initially, combinations of starch sources (enriched wheat flour and degermed corn flour) and distilled water were mixed to create a dough-like pastes and printed. Ratios of flour as starch sources to water were based on previous literature for food 3D printing using starch-based sources in their formulations (Ma et al., 2022; Chen et al., 2021; Dick et al., 2020; Fahmy et al., 2020). After those tests were unable to produce a successful food 3D print, a second round of initial tests were done with dough-like or starch slurry inks being ‘cooked’ to test if partial or full gelatinization of the material would led to better printing performance. After those tests were unable to produce a successful food 3D print, a third round of initial tests were again conducted where food inks were subjected to a ‘cooking’ step using a “pre-heater machine set” heat exchanger provided by FoodBot. However, samples were not evenly cooked during this process and resulted in inconsistent printing performance for all samples that were tested with the heat exchanger. Afterward, a fourth round of initial tests were conducted where samples were again ‘cooked’ by being subjected to pre-printing ‘cooking’ step in a water bath before being printed on a Food 3D Printer. These tests provided more consistent models and better printing performance.

After more successful tests using the water bath pre-printing ‘cooking’ step, minor changes were made in the formulation to help improve printing performance including adding a lipid source (Vegetable Oil, Kroger, Cincinnati, OH) for lubrication to help prevent “plugging” of the nozzle head during the printing stage. Modified Starch (Tapioca Starch, Cargill, Wayzata, MN) was also added to help keep rigidity in the models and help the food ink flow consistently in a homogenous manner. Several of these initial tests were conducted using a combinations of different mixing, heating and formulation compositions before a successful print was made with

the material able to flow consistently in a homogenous manner out of the nozzle head and set properly on the print deck, while also allowing further layers to adhere to each other while printing.

2.2 Materials and Methods

2.2.1 Food 3D Printer

The printer that was used for the study was a Foodbot S2 multi-ingredient food 3D printer (Changxing Shiyin Technology Co., Ltd., Hangzhou, China). The Foodbot 3D printer possesses a motor-driven plunger with a maximum extrusion force of 3 Kg. The Foodbot is a multi-ingredient food 3D printer with a 150 x 150 x 73 mm max build volume (i.e. the maximum amount of volume that can be printed by the machine based on the xyz dimensions in prints on), and has a printing speed range 15-70 mm/s. Moreover, the nozzle heads the Foodbot food 3D printer can use range from 0.3 – 1.5 mm in diameter.

The printer uses Repetier Host 2.0.5 as its software (Ultimaker Cura was used for slicing files) and includes English, Chinese and Korean as its support languages.

Afterward final formulations were created for the studies to study the impact of corn or wheat base as a starch source, how moisture as a processing agent effected the treatments (with moisture ranging from 50-62%) and sucrose as a plasticizer. The process for food 3D printing in the study was informed by the initial 3D printing tests.

2.2.2 Ingredient Selection and Formulation

After an extensive initial testing phase with various ratios of ingredients for formulations, 14 formulations were determined for testing in the study. Each formulation was targeted to test how moisture, starch source and sucrose inclusion levels impact food 3D printing performance

and physicochemical properties of the raw formulation. For testing sucrose inclusion levels, four mixes were formulated with a base mix control and three inclusion levels of sucrose. The base mix and sucrose were combined to obtain ratios of 100:0, 96:4, 92:8 and 88:12 (base mix: sucrose). These ratios were referred to as Wheat or Corn Flour Base, 4%, 8% and 12% sucrose inclusion level, respectively and outlined in Table 2.1.

The first formulation, a control base mix, was processed with a 50/50 ratio of raw material ingredients to distilled water. The raw material composition included 74% flour from a starch source (i.e. degermed corn flour or enriched wheat flour) as the main structure forming component for the formulation, with that percentage being based on previous literatures formulations (Ma et al., 2022; Chen et al., 2021; Dick et al., 2020; Fahmy et al., 2020). Additionally, 22% of the control base mix formulation included a lipid source (Vegetable Oil, Kroger, Cincinnati, OH), 3% modified starch (Tapioca Starch, Cargill, Wayzata, MN), and 1% iodized salt source (which was used in the formulation as a minor flow agent to prevent the nozzle head from plugging during the 3D printing process) (Kroger, Cincinnati, OH). The second formulation for the sucrose treatments contained 4% sucrose, denoted as “4% sugar level” in the study, contained a 4% inclusion of sucrose while keeping the ratios of the dry ingredients from the base mix formulation the same to only study the effect of the presence of sucrose in the formulation. Similarly, formulations denoted as 8% Sugar Level and 12% Sugar Level were formulated the same way with 8% and 12% sucrose inclusion.

Additionally, other treatments were included for evaluating the effect of moisture as a processing agent in 3D printing performance. The first formulation for the treatments, a control base mix, was processed exactly the same as the control base mix for the sucrose treatments with a 50/50 ratio of raw material ingredients to distilled water. The other treatments were combined

to obtain ratios of 46/54, 42/58 and 38/62 of raw material ingredients to distilled water, with distilled water being used for the 3D printing process as a processing aid, much in the way of traditional food processes. These formulations are outlined in Table 2.2 below:

These formulations were specially developed with previous preliminary tests in consideration so that they would be able to at least flow out of the nozzle head of the 3D printer and set on the print deck. They would also serve as benchmarks for the future studies.

The two starch sources (degermed corn flour and enriched wheat flour) that were studied constituted the majority (56.24-74%) of the raw material ingredients in the formulation for the treatments.

2.2.3 Food 3D Printing Procedure

The following print settings were attuned during preliminary experiments: 1.2 mm nozzle diameter, 1 mm layer height, 2 perimeters, 2 solid top and bottom layers, 30% infill density, rectilinear infill pattern, 15 mm/s speed, and 100% flow rate. These settings are outlined in Figure 2.1. 3D printing was done on a Foodbot S2 multi-ingredient food 3D printer (Changxing Shiyin Technology Co., Ltd., Hangzhou, China). The model was designed in TinkerCAD (TinkerCAD, San Francisco, CA) and exported as an .stl file to Ultimaker Cura (Zaltbommel, the Netherlands) for slicing, afterward the file was transferred to a FoodBot 3D Printer which utilizes Repetier Host 2.0.5 as its software (Repetier, Hot-World GmbH & Co. KG, Willich, Germany).

The formulations were measured and combined in a lab scale food blender (Waring Laboratory Services, McConnellsburg, PA) and mixed under high shear for 30 seconds. Then, the raw material formulation was deposited into a specialty plastic syringe for the food 3D printer. Afterward, the filled syringe with the raw material formulation and a thermocouple

temperature probe were placed in a water bath at 90 °C until the geometric center of the material in the syringe was heated to a temperature range of 65-70 °C (material was not agitated or stirred during the heating process to promote viscosity build-up) and the heat exchange would typically last 5-10 minutes depending on formulation. The heated formulation in the syringe was then allowed to cool to a temperature range of 33-38 °C at ambient temperature before 3D printing and then loaded into the printer (typically 30 minutes of cooling time would occur). Finally, the syringe with the material was loaded into the food 3D printer and printed at a printing speed of 15 mm/s until completion of the print.

Printed samples were placed directly into a freezer at -9°C for 2 hours. Afterward they were carefully measured for Dimensional Distortion Ratio values, and returned to the freezer for an additional 46 hours. Samples were then removed from the freezer and dried at 100°C for 3 hours and stored in a sealed container at room temperature to be later used for pictures and subjective analysis of the models.

2.2.3 Raw Material Analysis:

2.2.3.1 Water Absorption Index:

(2.5 g in 30 ml distilled water) would interact with water. In previous literature, the ability of water to be absorbed by starch granules has been referred to with several names (water holding capacity, water binding capacity, water absorption capacity). The method used was adapted from the method utilized by Webb (2021) and García-Tejeda (2018).

For testing of raw materials, 2.5 ± 0.05 g of the raw material ingredients for the 3D printing food ink formulations were immersed in 30 mL of distilled water at room temperature. The sample was mixed with a metal spatula to ensure no clumps remained and vortexed with a test tube mixer (Type 37600 mixer, Thermolyne, Dubuque, Iowa, USA) for 15 seconds. The

samples were then continuously agitated using an Innova 44 shaker table (New Brunswick Scientific, Edison, New Jersey, USA) at 100 rpm for 30 minutes. After agitation the samples were centrifuged at 3000G for 15 minutes in a centrifuge (Eppendorf 5810R 15 Amp).

The supernatant was discarded, and the remaining gel was weighed, and WAI was calculated using the equation below.

$$WAI = W_{gel} / W_{sample}$$

Where W_{gel} is the weight of the gel and W_{sample} is the weight of the dry sample. Each sample tested was done in triplicate.

2.2.3.2 Rapid-Visco Analysis:

RVA measures the viscosity of slurries, and was used to infer properties of the raw materials used in food 3D printing. The measured viscosity response in an RVA test is used to infer elements for raw material processing such as intactness and/or degree of degradation of starch granules and their ability to build viscosity. However, it should be noted in RVA tests, the slurries being tested have a much higher water content when viscosity is measured, so accounting for potential difference in gelatinization level, tendency of starch granules to break down and ability of the starch molecules to come together or retrograde during cooling is important to keep in mind for other tests and processing.

During the 3D printing process, the starch-based formulation food inks are subjected to heating during the cooking step, and under pressure from the Foodbot 3D printer when printed. The heat and shear applied in rapid visco-analysis tests are used to inform how starch undergoes transformations. Pasting properties of the starch-based formulations for 3D printing food inks was quantified according to the standard AACC method 76-21 STD 1, using rapid visco analyzer (RVA) 4800 (Perten Instruments, Perkin Elmer, NSW, Australia).

Approximately $3.5 \text{ g} \pm 0.05$ of raw material ingredients from the formulations were combined with 25 mL of distilled water to create a slurry. This slurry contained a 14% (w/v), dry matter basis; the moisture content of the final test was adjusted to the same level across all treatments accordingly to correct for inherent moisture differences between the formulations (Crosbie et al., 2007). Sample slurries were heated to 50 °C and held for 1 min, with initial stirring at 960 rpm for 10 seconds. Then, during the remainder of the test, slurries were stirred at 160 rpm. Slurries were heated to 95 °C at 12 °C/min, then held for 2.5 min and cooled again to 50 °C. Peak viscosity, time and temperature of peak viscosity, setback, breakdown, pasting temperature and end viscosity were measured and recorded. Testing was done in triplicate.

2.2.3.3 Phase Transition Analysis:

Phase transition analysis was used to predict and assess pressure induced thermodynamic flow behaviors of the formulations for 3D printing. The phase transition analyzer (PTA™) (Wenger Manufacturing, Sabetha, KS) is a modified capillary rheometer that uses a combination of time, temperature and pressure to measure the softening (T_s) and flow (T_f) temperatures of a material by compressing it with a piston under a constant pressure within a barrel that is heated to temperatures up to 160 °C. Typically PTA is a test used for studies pertaining to extrusion, as the test emulates the conditions in thermal processing with similar temperatures and pressures used in the process, and allow a meaningful and practical measure of material deformation and flow behavior (Karkle et al., 2012; Plattner et al., 2001). (Webb et al., 2020).

Starch-based formulations of food inks for 3D Printing were hydrated to a moisture content of 20% wt basis (with inherent moisture of raw material taken into account, with all treatments at the same moisture level). During initial tests, wheat-based formulations were having issues being analyzed on the PTA at this moisture level, due to the naturally high water

absorption capacity of wheat flour. The starch content of the food ink dry ingredients, particularly the flour plays a major role in water absorbance. Wheat is known to have different water absorption and rheology behavior compared to corn flour (Li et al., 2020). Raw samples being analyzed on the PTA must still have a powder-like consistency before being subjected to the test, but if the material going into the test initially have a dough-like consistency it results in failure of the test altogether. Hydrating above 20% wb moisture turned the dry ingredients of the starch-based formulation into a dough-like consistency, which could not flow through the PTA die orifice properly for the test. Higher than 20% wb moisture was desired for the test to emulate the conditions later used during the actual 3D printing. During that process, the moisture level ranged from 50-62% as a processing aid for the process. And typically a granular or free flowing powder form for the sample is needed for an accurate PTA test.

Analysis was conducted on hydrated samples following the procedures outlined by Webb et al. (2020) and Roberts (2013). A blank die was inserted into the PTA and $2.0 \text{ g} \pm 0.5$ of raw material were weighed out and placed into the PTA chamber.

The dry ingredients of the starch-based formulation were compressed in the chamber with a blank die to 120 bars for 15 s, then compressed to 100 bars while the sample was heated at a rate of $8 \text{ }^{\circ}\text{C}/\text{min}$, with a starting temperature between $5\text{-}7 \text{ }^{\circ}\text{C}$. After the material softened, the closed die was replaced by a 2-mm capillary die and heating continued at the same heating rate and operating pressure. The flow temperature was obtained from the temperature at which the material started to flow through the capillary (identified by a steep increase in displacement). Flow point (T_f), is defined as the temperature at which the material began to flow through the capillary and was identified by a steep increase in displacement (Karkle et al, 2012; Webb et al., 2020). All samples were tested in triplicate.

2.2.3.4 Differential Scanning Calorimetry:

Differential Scanning Calorimetry (DSC) is a thermal analysis test, which determines properties of biopolymers such as starch gelatinization through heat flow changes. Results of DSC are highly dependent on the rate of heating and the hydration level of samples. Most of the reviewed literature for other 3D food printing studies that used DSC as a method for testing enthalpy and gelatinization used a heating rate of 5-10 °C/min with samples generally being heated from 20-140 °C.

The raw material formulations were weighed into high-volume stainless steel pans, and then distilled water equivalent of twice the weight of the raw materials in the pans was added. Pans were sealed and allowed to hydrate overnight at room temperature for at least 12 hours to allow for moisture migration and a more homogenous sample. DSC was preformed according to Gibson et al. (2015) and Chanvrier et al. (2007) with a few modifications. Raw sample materials (8-10 mg) were hermetically sealed with an o-ring in the lid. The contents were heated at a rate of 10 °C/min until reaching 180 °C with an empty pan as a reference (43 Q100 V9.9 Build 303, TA Instruments, New Castle, DE) and data was analyzed with Universal Analysis Program, V4.5A (TA Instruments).

2.2.3.5 Dimensional Distortion of 3D Printed Samples

For evaluation of dimensional and shape fidelity of 3D printed objects, methods from other studies were reviewed, and ideas and methodologies from those research papers were gleaned for optimum printing accuracy methods for this study (Afarani et al., 2022). In the past, manual measurement tools such as a caliper, micrometers, and rulers have been used for evaluating the accuracy and shape analysis of 3D printed materials with simple geometries

through measurement of dimensions such as diameter, height, width, and length (Asadi-Eydivand et al., 2016; Hao et al., 2010).

For this study, three replicate prints of frozen products before drying for each experimental treatment were measured for their diameter and height as shown in Figure 3.2 using digital calipers. Measurements for each of the two dimensions were done in triplicate to capture unevenness in printing within a given samples. The measured dimensions were used to compare against the dimensions of the .stl file used for printing. The dimensional distortion ratio (DDR), as defined by Asadi-Eydivand et al. (2016), was calculated to quantify the distortion or asymmetry of printed models, as follows.

$$DDR = \frac{A_d}{A_h} - \frac{stl_d}{stl_h}$$

where A_d and A_h are respectively the averaged measured values of diameters (d) and height (h) from the 3D printed model, and stl_d and stl_h are the corresponding computer aided design model diameter and height that are used in the .stl file, When there is no deviation from the .stl file after printing, the DDR is equal to zero. Non-zero numbers mean height and/ or diameter distortion, positive if the actual height is less than designed while diameter is higher (squatter product) and negative if the actual diameter is less than designed while height is more (elongated product).

2.2.3.6 Moisture Content

Moisture content of the formulations for 3D printing was determined using the method AACC 44-19.01. Approximately $2 \text{ g} \pm 0.1$ of each sample of the dry ingredients for the food ink formulations for food 3D printing were evaporated at 135°C for 2 hrs. Analysis of samples was completed in triplicate.

2.2.3.7 Statistical Analysis:

Statistical analysis was performed using a two-way ANOVA test conducted using SAS software (SAS, Cary, NC, USA) and an ANOVA model with Tukey's test with a significance level of 5% to determine the means and significant differences between experimental treatments. Pair-wise differences were analyzed with a general linear model (GLM) and least square mean comparison. Significantly different values were denoted with different lower-cased letters.

2.3 Results and Discussion

2.3.1 Raw Material Characteristics

2.3.1.1 Water Absorption Index:

Water Absorption Index (WAI) is a measured quantity indicating the activity that occurs in raw formulations at which certain concentrations of starch materials (2.5 g in 30 ml distilled water) would react in certain ways with distilled water. The WAI is an indicator of the ability of flour to absorb water and swell for desirable consistency in food systems, which improves yield and consistency, and gives volume to the food (Choi et al., 2012). This same principle can be applied to how water gives volume to structures for food 3D printing

The treatments for both wheat and corn showed a general trend that as sucrose was included in the formulation, a clear decreasing trend in WAI with significant differences ($p < 0.0001$) occurred. This is shown in Figure 2.3. This coincides with other studies that indicate an increase of sucrose levels in starch mixtures (for applications in baking, extrusion or just as raw flour combined with sucrose) decreases water absorption due to the available hydroxyl groups of sugars to interact with unbound water and formation of intermolecular hydrogen bonds, limiting the availability of water for binding with starch (Pourmohammadi et. Al, 2018; Hedayati et al.,

2016; Spies et al, 1982). Therefore, chemical and physical reactions of water with other components are decreased with the addition of sucrose, along with the reduction of water reactivity when higher levels of sucrose are in starch mixtures. (Li et. al, 2015). Those same authors also discovered that all of the saccharides (ribose, fructose, glucose, sucrose, lactose, trehalose, maltose, raffinose and stachyose) have a protection effect on starch crystalline structure, and that the protection effect increased with the increase of sucrose concentration.

The wheat base formulation had the highest WAI value for the wheat flour treatments, with decreasing values for higher sucrose inclusion levels that were all significant from one another ($p = < 0.0001$). For the corn treatments, the 4% sucrose corn treatment was the highest WAI value, and within the corn treatments, as higher levels of sucrose were included in the solution, this also led to a decrease in WAI. It was observed that two competing effects of sugar occurred within the treatments; where it was able to absorb moisture, but then negatively impact overall absorption for the formulation as it was included in higher amounts. This could explain the WAI values in the corn base and 4% sucrose corn treatment. It is also important to note that this method shows the amount of water absorbed by insoluble material, not the sugar that was solubilized into the distilled water, as per the procedure used in the study, the soluble material was discarded in the supernatant, which lowers the WAI measurement since some of the original solids would no longer be present in the sediment (Osen et al. 2014). A corrected WAI can be interpreted as follows:

$$WAI = W_{gel} / W_{sample} - W_{soulution}$$

Where W_{gel} is the weight of the gel and W_{sample} is the weight of the dry sample and $W_{soulution}$ is the weight of the supernatant.

The importance of WAI when it comes to 3D food printing performance is it is used as an indication for the interaction of raw formulations for starch-based food ink mixes. Starch-based food ink formulations with a 0.7-0.8 WAI exhibited the best printing performance when compared to other treatments, both the 8% sucrose inclusion level and wheat base formulation fell into this range, which could indicate that a WAI value within this range would be optimal for starch-based food ink mixes.

2.3.1.2 Rapid-Visco Analysis:

Rapid Visco Analyzer (RVA) was used to study the pasting properties of starch in the food 3D printing ink formulation. In general, RVA can be used to determine the physiochemical and, in particular, the pasting properties of cereal starches (Ravi et. al, 1999). The results of RVA include peak viscosity (cP) and pasting temperature (°C) and were measured and recorded. The results showed sugar level had a significant effect ($p = <.0001$ for both wheat and corn) on both peak viscosity and pasting temperature with a clear decreasing trend in the former, and increasing trend in the latter as sugar increased from 0 to 12% in the case of corn and wheat based formulations, which are reported in Figure 2.4 and 2.5 respectively. Many studies investigating the inclusion of sugar in corn-starch systems found similar results of a decreasing trend of peak viscosity values with inclusion of sucrose (Kose, 2020)

In general when it comes to flours for various starch sources, there's a difference between how different types of flours from different grains develop viscosity, both in terms of peak viscosity and temperature needed for pasting. Normal cereal starches (such as wheat and corn) need >2 minutes to have initial viscosity development during RVA pasting (Lawton et al., 1999). In terms of peak viscosity differences for corn vs. wheat treatments, the corn displayed higher peak viscosity values when compared to wheat treatments. This corresponds with

previous literature as the thickening power of wheat starch is less than that of corn starch, with its higher relative starch content in flour compared to wheat, and its ability to swell and act like a sponge, absorbing liquid and expanding; however, paste texture, clarity, and strength are about the same (Knight et al., 1984).

Additionally it should be noted that WAI data relates to RVA data as the WAI is an indication of what interactions occur between starch-flour systems with water at room temperature. At least for the low temperatures in RVA, both tests use an abundance of water with a dry flour sample to determine values during their respective tests.. This is what occurred in the RVA tests that were conducted on starch-based food ink formulations.

Pasting temperature is the temperature at which the viscosity begins to rapidly increase during the heating process (Kumar et al., 2017). For both corn and wheat, the pasting temperature increased with sugar inclusion which lines up with other studies investigating the effect of sugar on starch systems (Woodbury et. al, 2023; Pourmohammadi et. Al, 2018). The reasoning behind this is to do with how sucrose has a plasticizing effect in starch-systems when subjected to heat and shear (Ployetchara et al., 2018 ; Yoo et al., 2005).. The higher the sucrose inclusion in food-inks for food 3D printing, the more the viscosity build-up will be delayed

The significance of this for food 3D printing with starch-based formulations correlates with how viscosity is built-up during the water bath ‘cooking’ step for the food inks and the physical state and transformation potential of starch granules during various applications and stages including 3D printing and setting. During the water bath ‘cooking’ step for the 3D printing process, food inks began to thicken and become gel-like around their respective pasting temperatures that were recorded in the RVA tests. This information could be used to understand

what ideal temperatures would be for food 3D printing inks based on their pasting temperatures from RVA tests

2.3.1.3 Phase Transition Analysis:

Phase transition analysis was used to predict and assess thermodynamic flow behaviors of the starch-based formulations for 3D Printing. PTA was conducted on raw materials for the formulations, and the data of corn and wheat food ink formulations for 3D printing softening and flow temperatures are shown in Figure 2.5 and 2.6 respectively showing a clear decreasing trend for the corn values and increasing trend for wheat values in both figures. T_s of the formulations varied from 40 to 55 °C, while T_f of the formulations varied from 91 to 147 °C. The wheat and corn treatments were statistically significant from one another ($p = <0.0001$), and a positive interaction effect occurred between different starch types and sucrose inclusion level treatments. However, it should be noted, that while a consistent trend occurred for both the wheat and corn treatments, there was no significance related to the effect of sucrose inclusion on PTA flow point for both wheat and corn treatments.

PTA is often used in application for extrusion related research as the softening point and flow point temperatures (°C) are a predictive indication of the behavior of how certain raw materials will process under extrusion because they are related to biopolymer interactions and rheological properties of the material (Xie, 2012). The data collected from these tests can reasonably be connected this principle and be valid based on the raw material being tested, as it compares to similar PTA tests from another study involving degermed corn base as well (Karkle et al., 2012). Within that study, blends of corn flour and apple pomace were combined to obtain ratios of 100:0, 83:17, 78:22 and 72:28 (corn flour: apple pomace), and the blends were analyzed on a Phase Transition Analyzer to determine the softening and flow temperatures.

The softening temperature (T_s) represents the temperature obtained from the mid-point between onset and end of softening (identified by chamber displacement over the threshold of 0.0106 mm/°C). Before this part of the PTA, the starch granule has a highly ordered structure, with crystalline and amorphous regions that require moisture and heat to be plasticized (Perry et al., 2000). The low moisture content needed for the phase transition experiments (~18%) is needed to allow for granule hydration without causing flow errors within the PTA testing, and allow therefore interactions between starch molecules to remain intact. When starch is replaced with sucrose, these interactions are reduced and the material is more easily compacted during the PTA test.

The flow temperature (T_f) represents the temperature at which the blend is plasticized enough that it flows through a capillary orifice. The (T_f) is related to shear viscosity and resistance to flow (Liu et al., 2011). In addition to lower starch content, the plasticizing effect sucrose has on starch systems may have also played a role in the values from the PTA. As stated previously when tested with RVA, for both the corn and wheat treatments, the pasting temperature increased with sugar inclusion which lines up with other studies investigating the effect of sugar on starch systems (Woodbury et. al, 2023; Pourmohammadi et. Al, 2018). Sucrose has a plasticizing effect in starch-systems when subjected to heat and shear (Ploypetchara et al., 2018). Sugars such as sucrose are known to affect on rheological properties and recrystallization, and when tested on PTA, the samples undergo massive heat treatment in order to obtain values from the test (Smits et al., 2003; Yoo et al., 2005).

Reduction in starch content with the addition of sucrose in the formulations limited viscosity development for the corn treatments, but seemed to have an opposite effect on the wheat treatments. Other studies have suggested that sugar molecules interact with the amorphous

regions of the starch granules, and require more energy (higher temperature) to melt the starch granules, this could have effected the wheat treatments and caused their trend (Evans et al., 1996). But it should be stated that there are two competing phenomenon occurring within the heat exchange of the PTA when looking at the starch-based formulations for food ink 3D printing, one being the plasticizing effect of sucrose and the fact that there is less water available for the plasticizing for starch due the hydrophobic effect of sugar (Zhang et al., 2008). Additionally, the difference in composition and physicochemical properties of corn and wheat starch and flour have been investigated in other studies and shown to have major differences in water absorption and pasting characteristics of due to measurable differences in molecular and granular level starch structures (Li et al., 2020). These differences led a strong interaction response, when the effect of starch depended on the level of sucrose present in the treatments (p-value = < 0.001).

In terms of relevance of this for food 3D printing with starch-based formulations is that these results show how the reduction of starch content with the addition of sucrose in the formulations limited viscosity development in the melt for the corn treatments, causing a serious decline in viscosity available for the food ink to hold structure after being printed. This also relates to WAI and RVA data as well, confirming similar trends with viscosity build-up and how molecules interact with the amorphous regions of the starch granules, and require more energy (higher temperature) to melt the starch granules (Evans et al., 1996).

For the corn treatments, when comparing the ratios of base mix to sucrose (100:0, 96:4, 92:8 and 88:12), the higher base mix formulations that had lower sucrose inclusion could have led to more starch degradation, which has an opposite effect on the PTA flow and softening temperatures. As starch is degraded in the PTA test, the molecular size is broken down into

smaller pieces that allow it to flow more easily through the orifice and require less thermal energy to break down (Webb et al., 2021).

2.3.1.4 Differential Scanning Calorimetry:

Differential Scanning Calorimetry (DSC) was used to determine the properties of the starch-based 3D printing food ink formulations, including starch gelatinization through heat flow changes. There was a significant difference found in the peak gelatinization temperatures of the corn and wheat treatments ($p = <0.0001$) and among sucrose inclusion levels ($p = 0.0180$). Peak gelatinization values ranged from 73.55 to 77.17 °C for corn treatments from the base formulation to the 12% sucrose inclusion level respectively, while for wheat treatments the values ranged from 64.54 to 68.99 °C from the base formulation to the 12% sucrose inclusion level respectively. It has been previously been reported that wheat starch has a lower gelatinization temperature than corn starch, and this phenomenon occurred in this study as well, and will be expanded upon later in this section (Liu et al., 2006; Knight et al., 1984).

There was also a significant difference found in the enthalpy values and onset temperatures of the corn and wheat treatments ($p = 0.0319$ and $p = < 0.0001$), but no statistically significance in the enthalpy values for the sucrose inclusion level treatments ($p = 0.5564$). Enthalpy values ranged from 11.15 to 8.95 J/g for corn treatments from the base formulation to the 12% sucrose inclusion level respectively, while for wheat treatments the values ranged from 7.85 to 9.295 J/g from the base formulation to the 12% sucrose inclusion level respectively. Onset temperature values ranged from 66.58 to 70.48 °C for corn treatments from the base formulation to the 12% sucrose inclusion level respectively, while for wheat treatments the values ranged from 57.49 to 60.92 °C from the base formulation to the 12% sucrose inclusion level respectively. Onset temperatures for gelatinization or peak gelatinization temperatures in

the DSC tests for the starch-based 3D printing food ink formulations that are reported in Figure 2.9. The main takeaway from the results presented in the figures was the noticeable trend for the corn and wheat starch-based 3D printing food ink formulation for onset temperatures for gelatinization and peak gelatinization temperatures; as sucrose inclusion levels increased, onset temperatures for gelatinization and peak gelatinization temperatures increased as well.

DSC enthalpy represents a net sum of all endothermic processes that take place during heating. Salt (sodium chloride) and sugar (sucrose) are two commonly used food ingredients, and it is generally known that both sodium chloride and sucrose increase the starch gelatinization temperature and decrease the degree of gelatinization, especially at low ($<50 \text{ g kg}^{-1}$) concentrations (Ratnayake, 2012). Sugar alters starch gelatinization temperatures and also affects the pasting behavior and granule swelling of cereal starch (Woodbury et. al, 2023; Pourmohammadi et. Al, 2018). This coincides with other studies that indicate an increase of sucrose levels in starch mixtures (for applications in baking, extrusion or just as raw flour combined with sucrose) decreases water absorption due to the available hydroxyl groups of sugars to interact with unbound water and formation of intermolecular hydrogen bonds, limiting the availability of water for binding with starch with effect starch gelatinization (Pourmohammadi et. Al, 2018; Hedayati et al., 2016; Spies et al, 1982). Therefore, chemical and physical reactions of water with other components are decreased with the addition of sucrose, along with the reduction of water reactivity when higher levels of sucrose are in starch mixtures. (Li et. al, 2015). Those same authors also discovered that all of the saccharides (ribose, fructose, glucose, sucrose, lactose, trehalose, maltose, raffinose and stachyose) have a protection effect on starch crystalline structure during gelatinization, and that the protection effect increased with the increase of sucrose concentration.

Furthermore, it has been reported in previous literature that wheat starch has a lower gelatinization temperature than corn starch, and this phenomenon occurred here as it did in previous literature (Liu et al., 2006; Knight et al., 1984). Water also plays an important role in the process of thermal starch gelatinization as the gelatinization temperature decreases with increasing water content of starch suspensions (Donmez et al., 2021). Additionally the presence of salts, sugars, lipids, alcohol, organic acids and their salts etc. also influence starch gelatinization by rupturing hydrogen bonds within the starch granule, or by forming soluble complexes with starch (Eliasson, 2017). Due to their ability to bond with starch chains, sucrose can penetrate the amorphous regions of starch granules, thereby stabilizing these areas and reducing the water absorption and, consequently, the water activity of starch granules. This suppressed interaction between starch chains and water molecules can influence the starch gelatinization (Mohamed, 2021). They have an impact on the gelatinization temperature and therefore affect the extent of gelatinization. It is also important to consider, the flour from the dry ingredients were not just starch, but also contained other components such as protein from the corn and wheat as well. It has been found in previous studies that zein and the gluten protein matrix also effect DSC results for corn and wheat, respectively (Chanvrier et al., 2005). However, no discernable peak was found in the tests where wheat or corn protein has been reported to exhibit a peak in DSC tests during the experimentation.

It is important to note that the gelatinization temperature of corn vs. wheat starch has been evaluated in previous literature, where typically onset and peak temperatures for corn are higher than wheat (Roos et al, 2015; King et al., 2009). In addition, corn starch requires a larger thermal energy for gelatinization per gram of dry starch. Additionally, thermally induced gelatinization required higher temperatures and exhibit larger enthalpy change for corn flour than

wheat flour suggesting a more stable crystalline structure for corn starch (Roos et al, 2015; King et al., 2009).

2.3.2 Post-Printing Analysis

2.3.2.1 Dimensional Distortion Ratio:

With increase in sucrose level from 0 to 12%, 3D printed products using corn-based inks maintained dimension fidelity better (DDR absolute values of 0.002 to 0.056) as compared to those based on wheat-based inks (DDR absolute values of 0.092 to 0.156) (Figures 2.11 and 2.12). The wheat-based products also showed a trend of greater shape and dimensional distortion (increasing DDR) as sugar level increased. No such trend was observed for corn-based samples. It was clear that products using corn-based inks were more resilient to distortion effects due to sucrose at the ink moisture level of 50% wet basis, at which the 3D printing experiment was conducted.

On the other hand, with increase in moisture level from 50 to 62% wet basis, 3D printed products using corn-based inks had much poorer dimensional fidelity (DDR absolute values of 0.032 to 1.110) as compared to those based on wheat-based inks (DDR absolute values of 0.040 to 0.092). The corn-based products clearly showed a trend of high shape and dimensional distortion (increasing DDR) as moisture content increased. Surprisingly, a reverse trend was observed for wheat-based samples, although the differences in DDR were not substantial. Hence, products using wheat-based inks were more resilient to distortion effects due to increase in moisture content. Higher moisture levels increased flow of materials out of the nozzle head of the 3D printer, but worsened printing accuracy in the case of corn-based inks. Higher moisture levels especially impacted the ability for the model to accurately print along the z-axis most (A_h). Printed models of starch-based food ink formulations with higher moisture levels were less

stable, and prone to collapsing or not printing to their full height in many cases, as can be seen from the picture of 58% and 62% moisture corn-ink based products (Figure 2.19). Higher moisture levels negatively affected the food ink formulations during printing because the ink was less viscous and easier to extrude out of the nozzle head but its potential for setting on the print deck was worsened.

DDR data indicate that sucrose level would play less of a meaningful role in structure forming of starch-based food inks for 3D printing when compared to altering moisture level by the same percentage value, although the former did have a significant effect on printing performance. Additionally, sucrose was observed to lower water absorption for the samples due to sucrose effecting the water activity of the food ink material, similar results of sucrose affecting the gluten network cross-linking by competing with gluten or corn starch molecules for available moisture has been observed in other studies (Dongdong et al., 2023; Trinah et al., 2015; Mezreb, K., et al., 2006). Sucrose inclusion demonstrated a consistent effect on lowering water absorption, altering pasting properties and influencing phase transition temperatures. The observed trends later confirmed in DSC results for corn and wheat formulations align with existing literature, emphasizing the importance of considering both starch source and sucrose levels in formulation optimization for 3D printing

2.3.2.2 Printing Ink Post-Printing Rapid-Visco Analysis:

Rapid Visco Analyzer (RVA) was again used to study the pasting properties of starch in the food 3D printing ink formulation after printing; samples of the models were collected and tested. The results of RVA include peak viscosity (cP), time and temperature (°C) of peak viscosity, setback, breakdown, pasting temperature (°C) and end viscosity (cP) were measured and recorded. Testing was done in triplicate. The results showed a very clear difference between

raw samples and post printed samples of starch-based food ink formulations. One trend that was observed that carried over from the raw material analysis was that there was a significant difference between treatments as sucrose was included in the formulations with a distinct decreasing trend in peak viscosity and increasing trend in pasting temperature, which are reported in Figure 2.12 and 2.13 respectively.

It is important to note, that tests were conducted for both wheat and corn treatments, but not discernable peak viscosity, setback, breakdown, pasting temperature (°C) or end viscosity (cP) was observed in the wheat treatments. All of the wheat treatments had a slight viscosity build-up during the initial part of the test (30 sec – 1 min), but then leveled out around a range of 0-15 cp for the remainder of the test, with no slope or gradual viscosity development. Further tests were conducted on the wheat treatments with DSC to inform these results. However, the corn treatments did have a discernable peak viscosity, setback, breakdown, pasting temperature (°C) or end viscosity (cP) and were recorded. Again, like with the raw material analysis, the pasting temperature increased with sugar inclusion which lines up with other studies investigating the effect of sugar on starch systems (Woodbury et. al, 2023; Pourmohammadi et. Al, 2018).

Peak Viscosity for the corn treatments ranged from 770 to 218 cp from 0% to 12% sucrose inclusion level, and were significantly different ($p < 0.0001$) from treatment to treatment. Pasting temperature for the corn treatments ranged from 79.6 to 82.1 from 0% to 12% sucrose inclusion level, and were not significantly different ($p = 0.3849$) from treatment to treatment. These data are shown in Figures 2.12 and 2.13. The effect of sucrose on the starch system has been shown in previous studies. Sucrose inhibits retrogradation, increases breaking strain and has a higher plasticizing effect (Souza et al. 2012; Yoo et al., 2005). The significance of this for food

3D printing with starch-based formulations correlates with how viscosity is built-up during the water bath ‘cooking’ step for the food inks. During the water bath ‘cooking’ step for the 3D printing process, food inks began to thicken and become gel-like around their respective pasting temperatures that were recorded in the RVA tests.

2.3.2.3 Printing Ink Post-Printing Differential Scanning Calorimetry:

DSC was used as a thermal analysis test to determine the properties of biopolymers such as starch gelatinization and protein denaturation temperatures through heat flow changes in the starch-based food inks after the 3D printing process. Within this study, it was used to determine the properties of food ink formulations both before and after printing, and see how starch gelatinization was impacted during the printing process. DSC was conducted on the post-printed samples to further explore and corroborate the RVA results from those same samples for the wheat and corn treatments.

For starch-based 3D printing, the starch gelatinization characteristics of different flours can help determine the amount of energy required to completely gelatinize starch granules. A higher starch gelatinization temperature shows a greater thermal stability, and can also help determine the extent of starch gelatinization of the different food ink formulations for 3D printing. The corn treatments with lower sucrose inclusion levels in the formulation were able to reach peak gelatinization temperature more rapidly due to less interference of water activity from sucrose and more efficient heat transfer. The presence of solutes, such as sugars, are known to decrease the water activity (a_w) of starch solutions and increase the glass transition temperature (T_g) of the amorphous fraction of starch (Beleia et al., 1996). In addition to the water binding properties of sugars for retardation of starch gelatinization, sugars and other small solutes can affect the thermodynamic properties of water in solution and limit the water available to interact

with other components existing in the solution (Pourmohammadi et al., 2018). This could explain the trend that was observed with the post-printed food ink samples for the corn treatments, where a general increasing trend was evident as sucrose was included in higher percentages in the formulation.

It should be noted that for the post-printed wheat treatments, no discernible peak was observed for indicating gelatinization had occurred during the processing steps for food 3D printing. This could be explained due to the fact the gelatinization temperature of corn as opposed to wheat starch, typically have higher onset and peak temperatures (Roos et al, 2015; King et al., 2009). In addition, corn starch requires more thermal energy for gelatinization per gram of dry starch. During the processing of the food ink formulation for 3D printing, the material never exceeded temperatures of 72°C, and corn starch's peak gelatinization point has been reported to exceed that temperature in certain studies (Roos et al, 2015; King et al., 2009).

There was a significant difference found in the onset temperatures of the sucrose inclusion level treatments for corn ($p = 0.0096$) with values ranging from 66.58 to 70.81°C from the base formulation to the 12% sucrose inclusion level respectively. But, there was no significant difference found in the peak gelatinization temperatures ($p = 0.0887$) and enthalpy values ($p = 0.2082$) of the corn treatments with values ranging from 77.04 to 79.365°C from the base formulation to the 12% sucrose inclusion level for peak gelatinization temperatures, and 8.11 to 5.77J/g for the enthalpy values, respectively. This data is shown in Figures 2.14 – 2.16.

2.4 Conclusion

In conclusion, this study delved into the critical aspects of starch-based food ink formulations for 3D printing, aiming to understand their physico-chemical properties and

performance. The study utilized a 3D printer specifically for food 3D printing and explored the influence of starch source, moisture content, and sucrose as a plasticizer on the rheological behavior and printing accuracy of the materials. This approach was aided by assessing the formulations with water absorption index (WAI), rapid visco analysis (RVA), phase transition analysis (PTA) and differential scanning calorimetry (DSC), offering an overview of the starch-based formulation raw materials as well.

The results, supported by literature on starch gelatinization and how starch mixtures are effected by sucrose inclusion, show the differences between formulations. Sucrose, while initially decreasing viscosity and enhancing 3D printing performance, had a diminishing impact past an 8% inclusion level, negatively impacting printing accuracy. Moreover, higher moisture levels increased material flow but compromised printing accuracy. Sucrose inclusion demonstrated a consistent effect on lowering water absorption, altering pasting properties and influencing phase transition temperatures, which have a significant effect on the printing performance for food 3D printing.

Additionally, the difference in composition and physicochemical properties of corn and wheat starch within flour have been investigated in other studies and shown to have major differences in physico-chemical characteristics due to measurable differences in molecular and granular level starch structures (Li et al., 2020). These differences led to a different response in printing accuracy as well ($p = <0.0001$).

This research serves as a foundation toward developing high-performance food inks for 3D printing. The findings will provide essential insight for future studies aiming to refine starch-based food 3D printing, addressing challenges related to viscosity, moisture, and raw material composition of formulations. The potential applications of 3D printing in the food industry, such

as creating intricate shapes and unique textures, underscore the significance of ongoing research in this field.

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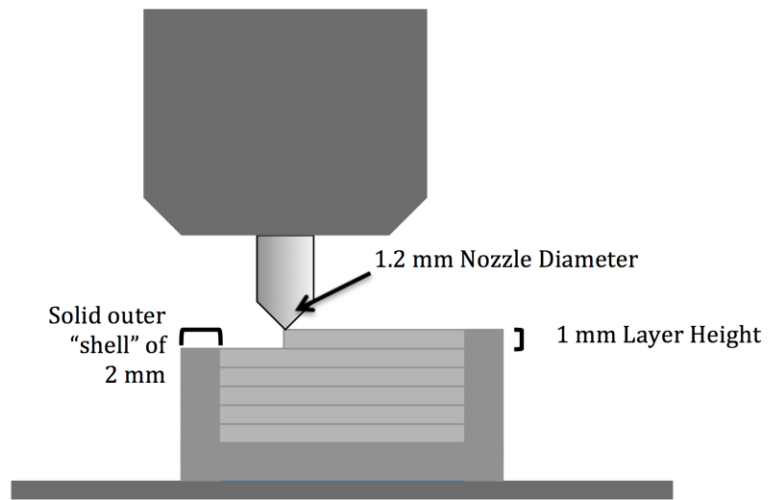


Figure 2.1. 3D Printing Starch-based materials procedure

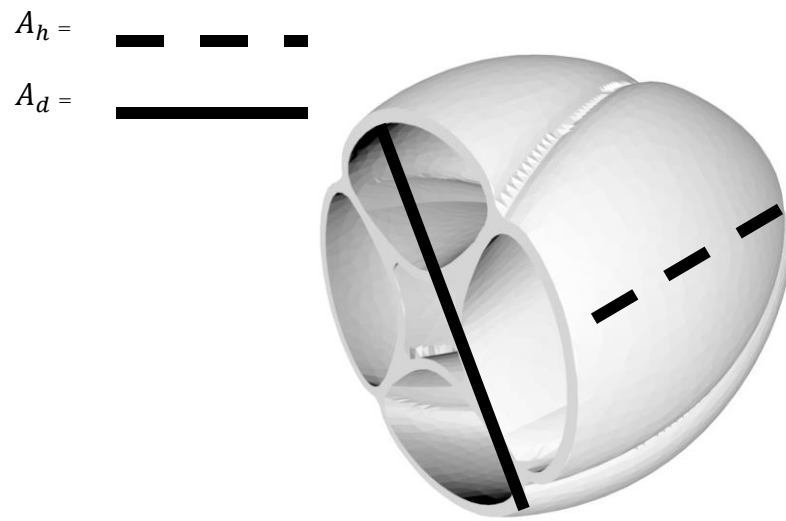


Figure 2.2. 3D printing product model. A_h = sample height (39.2 mm) and A_d = sample diameter (44.19 mm).

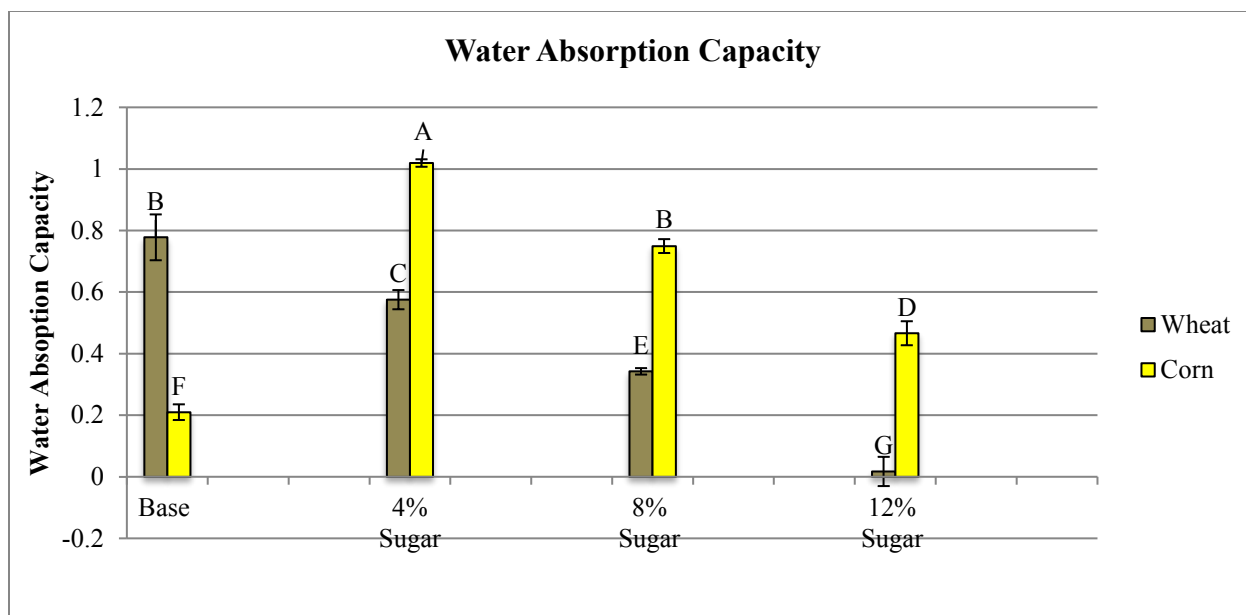


Figure 2.3. WAI Results for Sucrose Inclusion Level Treatments

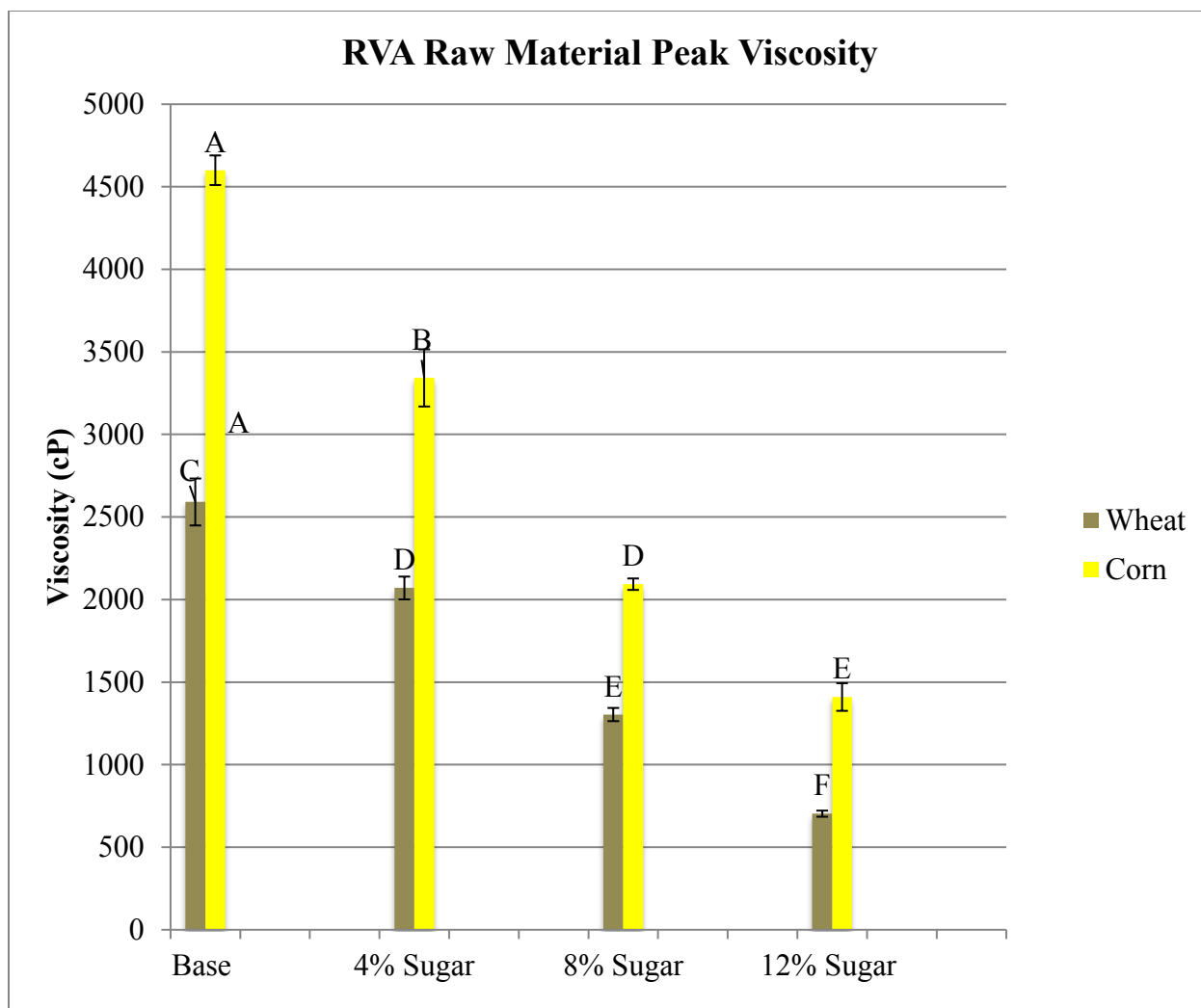


Figure 2.4. Results for Rapid Visco-Analysis for Sucrose Inclusion Level Treatments

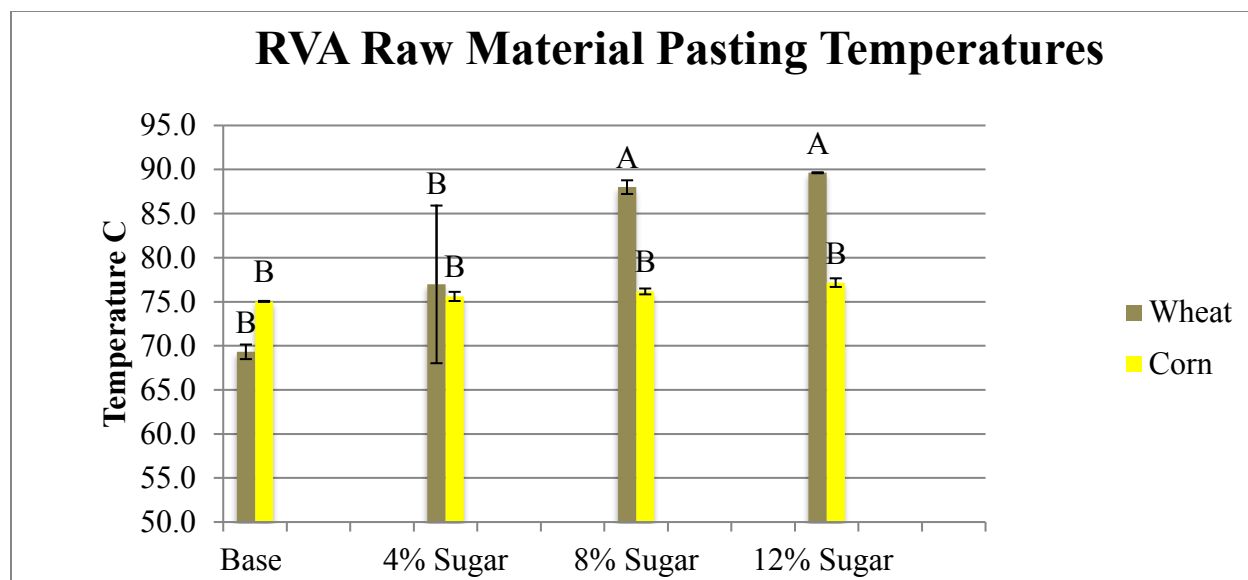


Figure 2.5. Raw Material Pasting Temperature Results for Rapid Visco-Analysis for Sucrose Inclusion Level Treatments

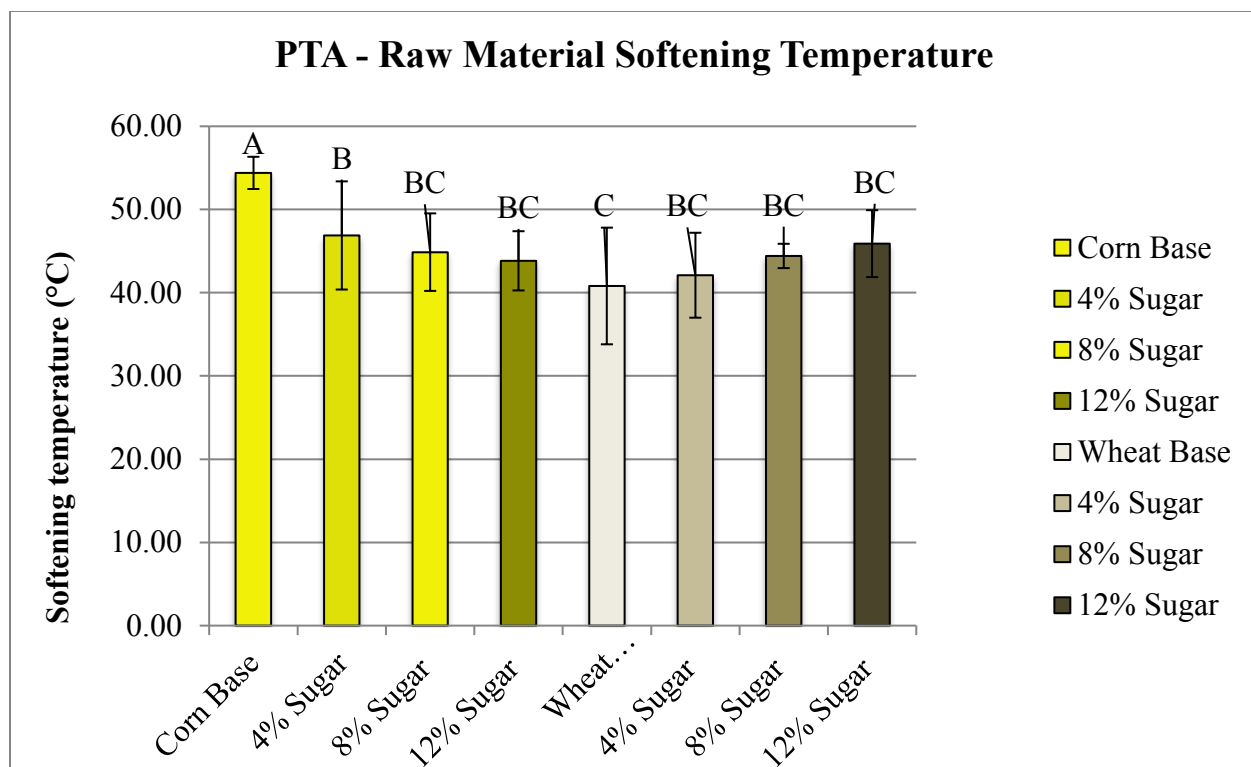


Figure 2.6. Results for Phase Transition Analysis for Sucrose Inclusion Level Treatments for Softening Temperature

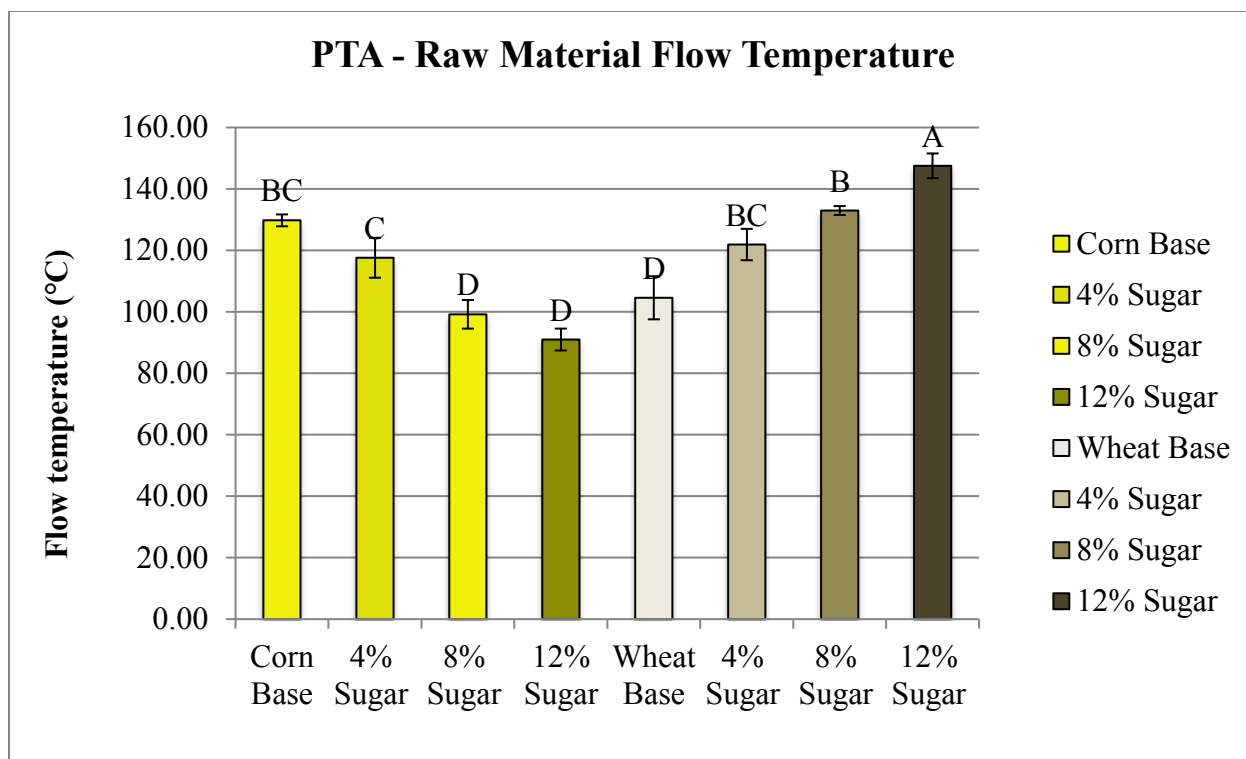


Figure 2.7. Results for Phase Transition Analysis for Sucrose Inclusion Level Treatments for Flow Temperature

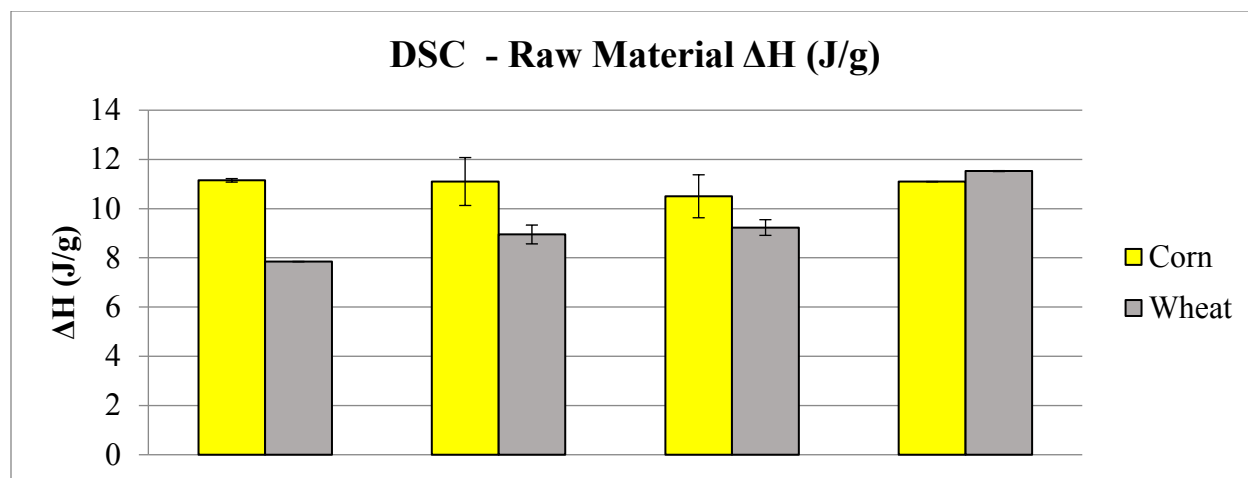


Figure 2.8. Results for Differential Scanning Calorimetry for Sucrose Inclusion Level Treatments

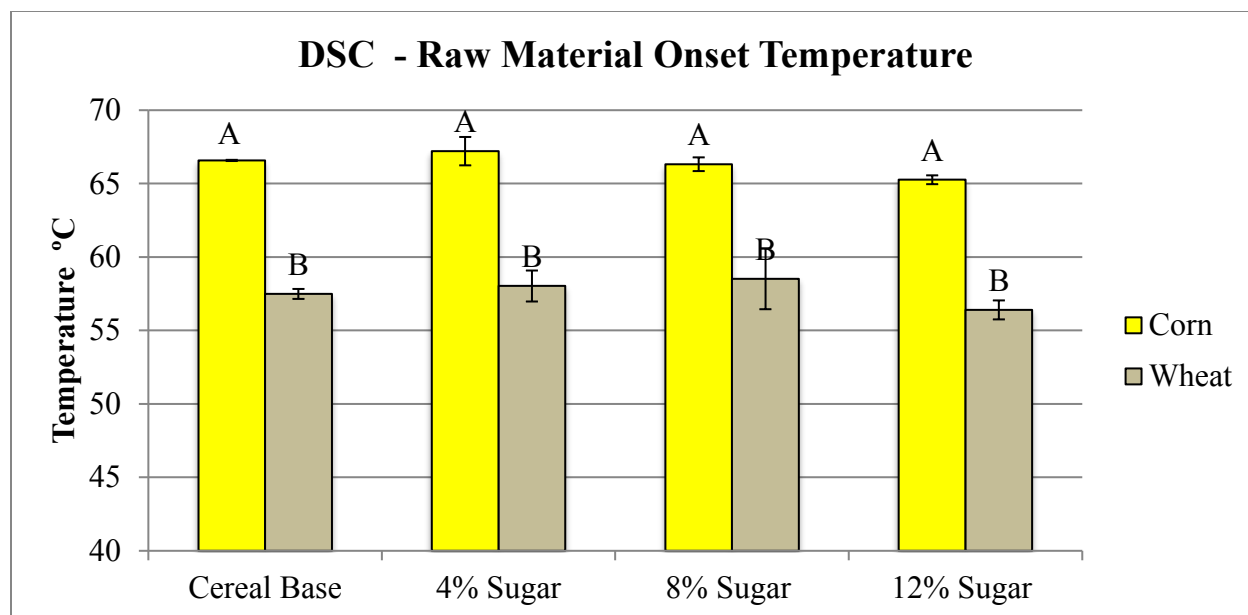


Figure 2.9. Results for Differential Scanning Calorimetry for Sucrose Inclusion Level Treatments

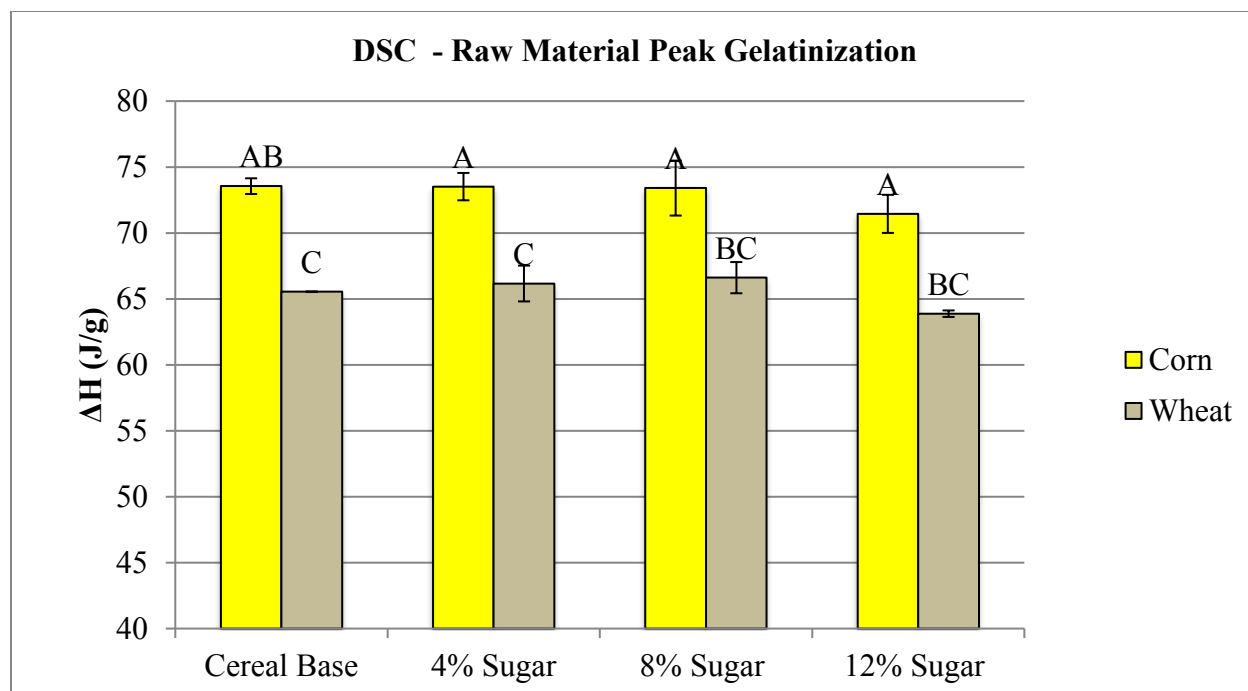


Figure 2.10. Results for Differential Scanning Calorimetry for Sucrose Inclusion Level Treatments

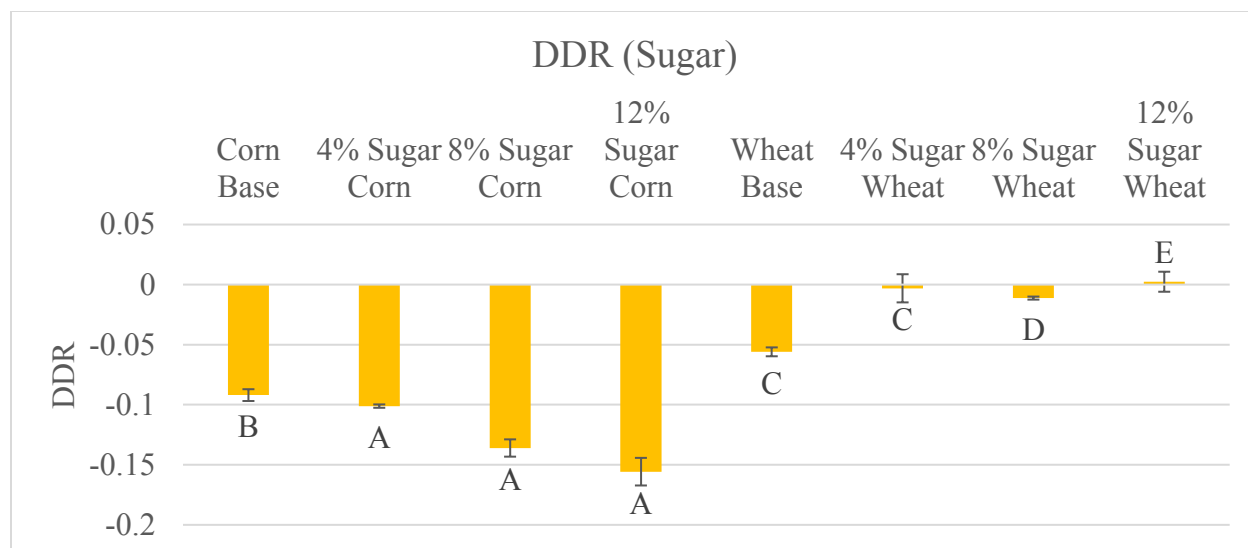


Figure 2.11. Results for DDR for Sucrose Inclusion Level Treatments

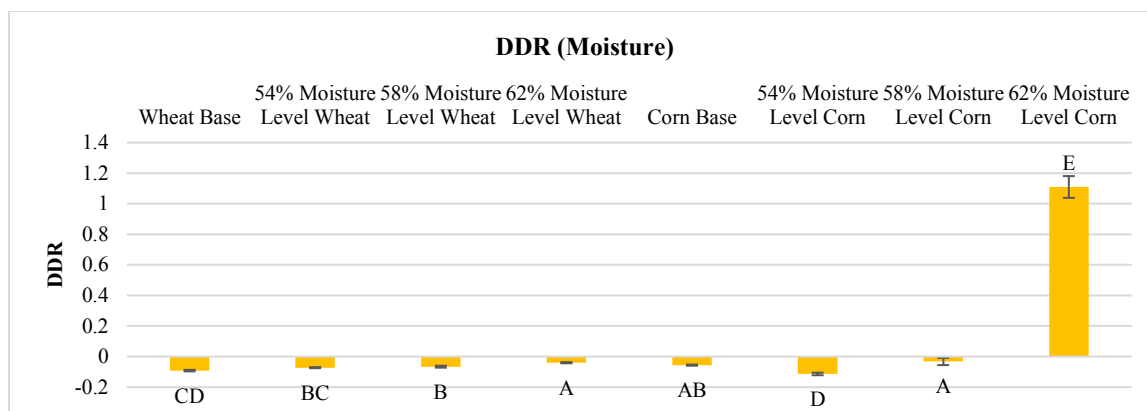


Figure 2.12. Results for DDR for Moisture Level Treatments

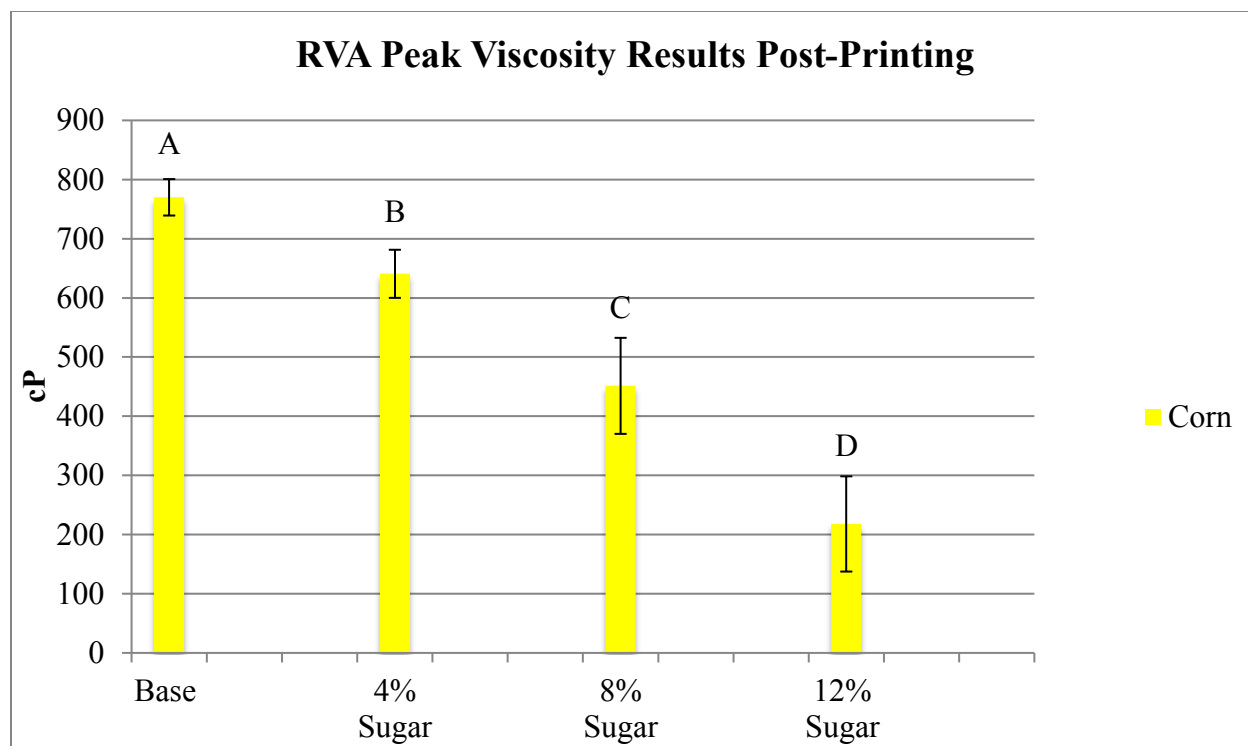


Figure 2.13. Results for Rapid Visco-Analysis for Peak Viscosity in Post-Printed Samples

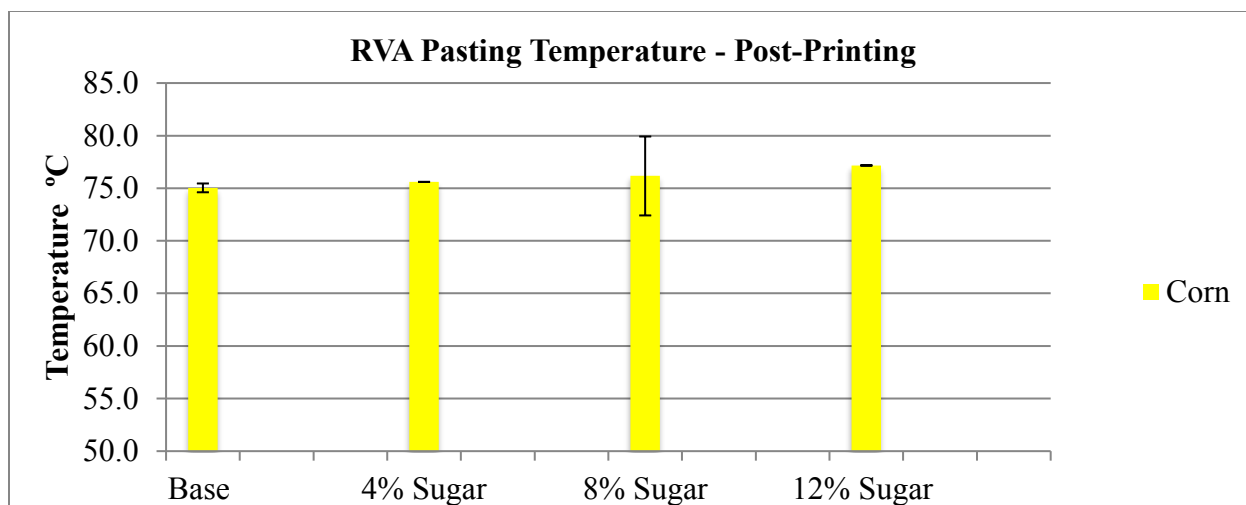


Figure 2.14. Results for Rapid Visco-Analysis for Pasting Temperature in Post-Printed Samples

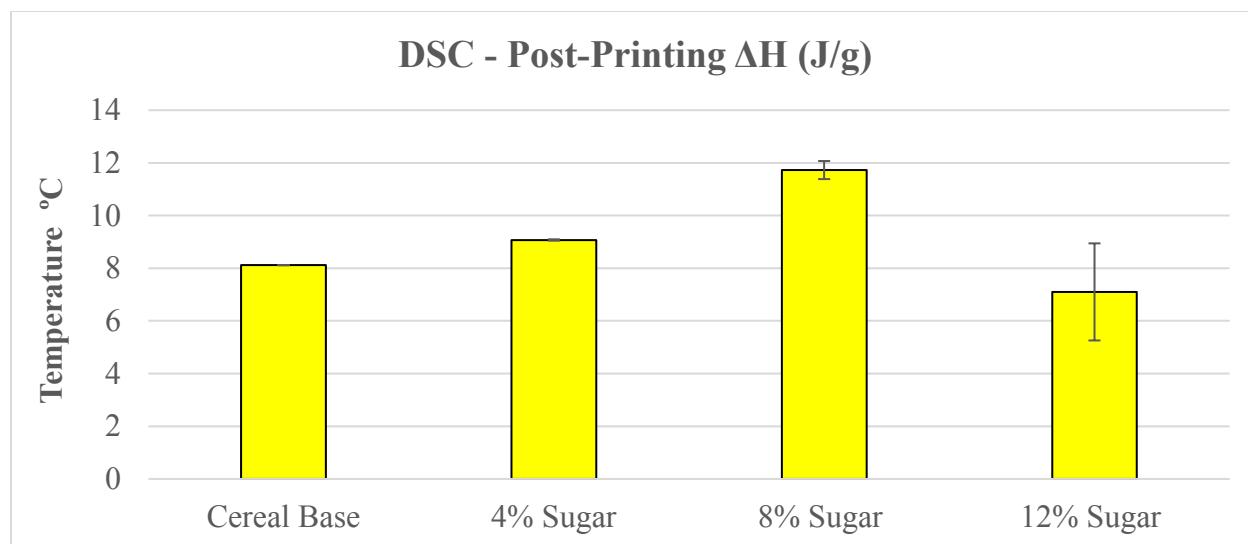


Figure 2.15. Results for Differential Scanning Calorimetry for Enthalpy Change in Post-Printed Samples

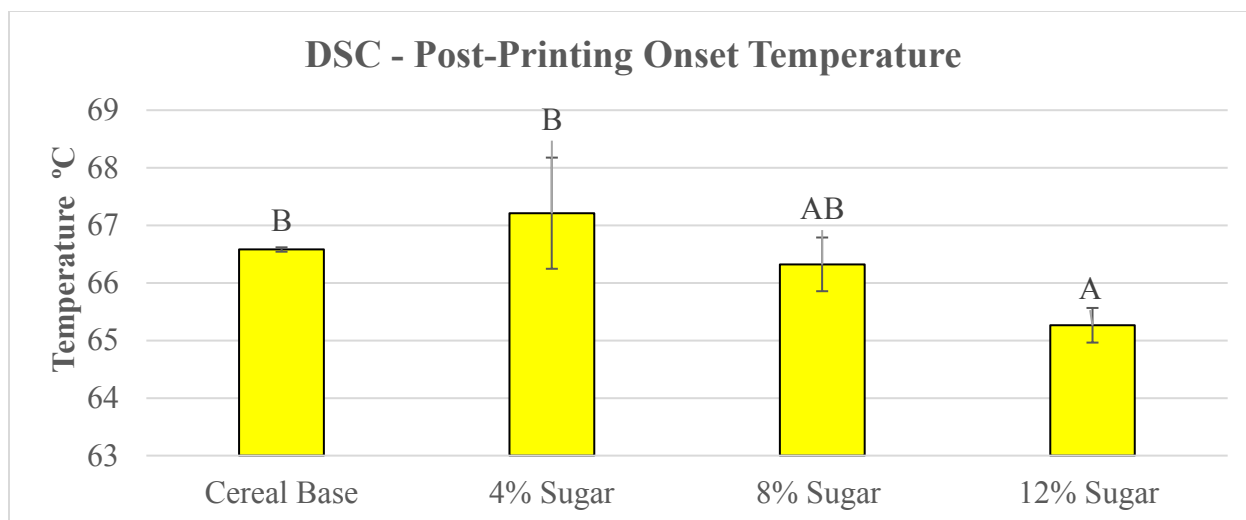


Figure 2.16. Results for Differential Scanning Calorimetry in Post-Printed Samples

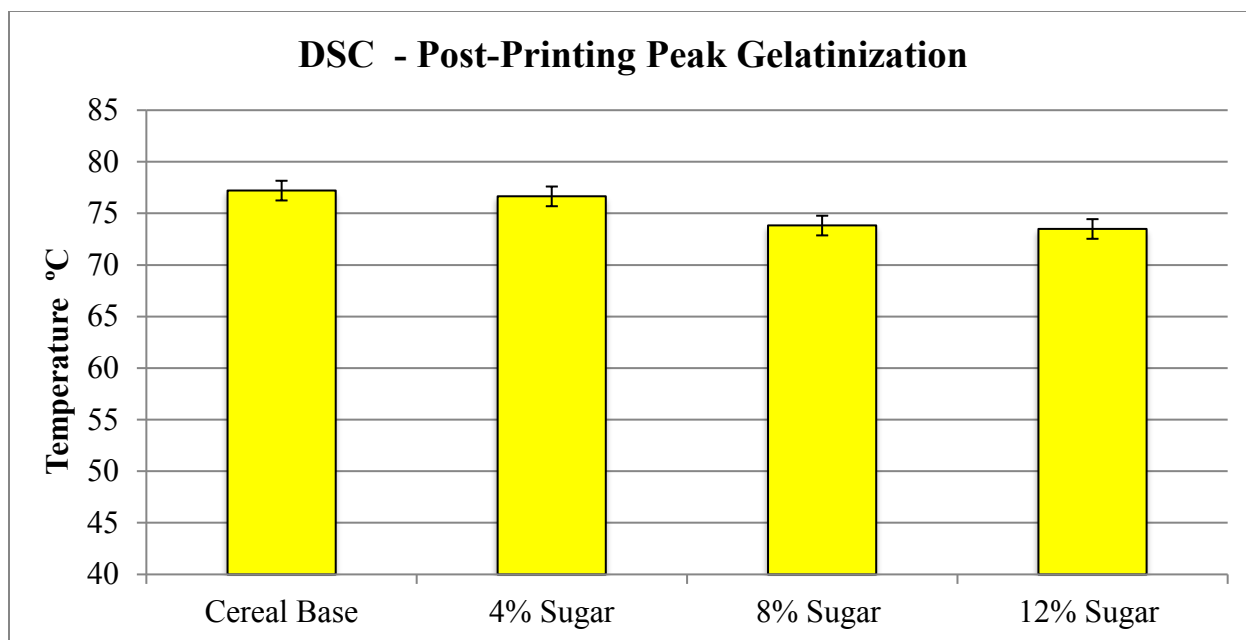


Figure 2.17. Results for Differential Scanning Calorimetry in Post-Printed Samples



Corn: **Base (0%)** **4%** **8%** **12%**



Wheat: **Base (0%)** **4%** **8%** **12%**

Figure 2.18. Examples of the Extrusion-based 3D printed samples for the Sucrose Treatments



Corn:	Base (50%)	54%	58%	62%
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Wheat:	Base (50%)	54%	58%	62%
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Figure 2.19. Examples of the Extrusion-based 3D printed samples for the Moisture Treatments

Table 2.1. Sucrose inclusion level treatment ratios

	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Base Formulation	100	96	92	88
Sucrose Inclusion Level	0	4	8	12

Table 2.2. Moisture inclusion level treatments ratios

	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Base Formulation	50	46	42	38
Distilled Water	50	54	58	62

Chapter 3 - Rheological Behavior of Starch-based Food 3D Printing

Inks and Impact of Infill Density on Printing and Product Quality

Abstract:

The performance of food inks for 3D printing and end-product quality rely on ingredient functionality and processing conditions including printing parameters. Rheological behavior of starch-based food ink formulations and infill density during printing were evaluated for their effect on dimensional fidelity and textural properties of the product under compression. Food inks were formulated with a raw degermed corn flour or wheat flour base and with 0-12% sucrose inclusion. Rheology of the inks were evaluated using a dynamic rheometer in order to understand their flow and setting behavior during 3D printing. In general, frequency sweep storage modulus (G') and loss modulus (G'') values decreased significantly for both corn and wheat-based inks with increase in sucrose, showing a plasticizing effect. The order of magnitude of G' and G'' for corn ($\sim 10^4$ - 10^5 and $\sim 10^3$ - 10^4 Pa, respectively) were higher than that for wheat-based inks ($\sim 10^3$ - 10^4 and $\sim 10^3$ Pa, respectively). Dimensional distortion ratio (DDR), which is a measure of the dimensional fidelity for corn-based ink increased significantly from 0.064 to 0.701, as 3D printing infill density was varied from 20-80%; while the DDR for wheat-based ink was more resilient with no significant differences with change in infill density (DDR ranging from -0.026 to -0.052). Increase in infill density from 20-80% significantly reinforced the structural integrity of both corn and wheat-based 3D printed products, as seen from the increase in average peak force from 4.59 and 3.75 kg, respectively, to 21.16 and 24.70 kg, respectively, determined by a texture analyzer in compression mode. In conclusion, key methodology was developed for evaluating 3D printing parameters and rheological properties and performance of

food inks. The approach outlined in this study can help to design high performance materials and fine-tuning process conditions for food 3D printing for intricate shapes and unique textures.

3.1 Introduction

Food 3D printing offers many possibilities for alternative processing and manufacturing of food products including texture modification, shape customization and nutrition personalization (Alharbi et al., 2018). Many applications exist for 3D printing including plant-based meats, meals for those who have trouble chewing and swallowing, and personalized products, but further research is needed to scale-up for industry and to improve sensory aspects of 3D printed food. It is also important to understand that for food 3D printing, rheological properties of the raw materials impact the printing performance (Jiang et al., 2019).

The rheological properties of food material ink are critical to 3D printing precision and accuracy. Generally, it is necessary that the food material inks possess low viscosity so that they can be easily ejected through the nozzle of the printhead to create accurate prints (Godoi et al., 2016). There also might be optimal rheological properties that correspond with these properties as well.

Textural properties of 3D printed foods are also critical to understand how they will impact sensory and other aspects of the end product (Fahmy et al., 2021). Foods can be textually modified for a variety of reasons, including for use as dysphagia foods which need to be nutritive, soft, safe, easy to swallow so that people with swallowing disorders enjoy the act of eating and drinking safely while their nutritional requirements are fulfilled (Bailey et al., 2022). Food textures can be modified via formulation design, food manufacturing and in post-processing (e.g., storage, cooking) (Pereira et al., 2021). As for food manufacturing methods,

new technologies such as food 3D printing are increasingly being studied in the research sector to customize nutritional aspects as well as control food texture by changing foodstuffs' structures (e.g. infill density and patterns) to meet the demands of special consumer needs (Eswaran et al., 2023).

Altering structural elements to modify the textural properties of 3D printed foods is a relatively new concept and few studies have linked the internal structures of 3D printed foodstuffs to their properties, including their texture (Zhao et al., 2021). Although the number of scientific publications covering various aspects of food 3D printing technology and its application has been on the rise in recent years, 3D printing is not popularly applied in the food industry. Therefore, the design of the 3D-printed foods and their relationship with textural properties require further research.

The printing accuracy or dimensional fidelity of 3D printed objects is an important measure of printing performance. The ideal test would utilize physical measurements of various dimensions of the printed samples and determine how well they were formed in terms of the shape and size as compared to the original .stl file used for printing. Dimensional distortion or change in the geometry of a printed model can be the consequence of various factors, including shrinkage of the material during the solidification process, uneven heat dissipation and many others. In some instances, the solidification of newer layers may cause curl or warpage in previously cured layers that lie directly underneath (Bugeda et al., 1995). Various methods for evaluation of dimensional fidelity from other studies were reviewed, and ideas and methodologies evaluated for optimum printing accuracy techniques for this study (Afarani et al., 2022). In the past, manual measurement tools such as a caliper, micrometers and rulers have been used for evaluating the accuracy and shape of 3D printed materials with simple geometries

through measurement of dimensions such as diameter, height, width, and length (Asadi-Eydivand et al., 2016; Hao et al., 2010).

Additionally, various researchers have highlighted that 3D printing technology can also construct lattice patterns internally to give structure and rigidity besides developing a three-dimensional shape, this is known as infill density (Lalegani et al., 2021). It is obvious that cost of materials will increase with higher infill density. It has also been shown that structural integrity of products improves as infill density increases, as in the case of carbon reinforced composites (Gao et al, 2023). However, research related to this aspect for 3D printed food products is scant. Although, it was hypothesized that similar to non-food materials, higher material density and closely spaced layers will increase the strength and hardness of food products, the rheology including setting properties of biopolymers such as starch might impact the dimensional fidelity and hence final texture of the product. This study was designed to This study was designed to explore the impact of rheological properties of starch-based food inks and also 3D printing infill density on product printing accuracy or distortion and textural strength.

3.2 Materials and Methods

3.2.1 Experimental Design

For dynamic rheology of food inks, a 4x2 factorial experimental design was employed with two base mixes (using degermed corn flour or refined wheat flour) and four levels of sucrose (0, 4, 8 and 12%) as shown in Table 3.1. The base mixes included 74% degermed corn flour (Lifeline Foods, St. Joseph, MO) or 74% refined wheat flour (bleached all-purpose enriched flour, Kroger, Cincinnati, OH) as the main structure forming component, 22% soybean oil (pure vegetable oil, Kroger, Cincinnati, OH), 3% tapioca starch (Cargill, Wayzata, MN) and

1% iodized salt (Kroger, Cincinnati, OH). These bases were designed based on criteria described in Chapter 2. After inclusion of sucrose at the abovementioned ratios, the final formulations were combined with distilled water in a 50:50 ratio and mixed under high shear for 30 seconds using a lab-scale food blender (Waring Laboratory Services, McConnellsburg, PA) to prepare the food inks.

For 3D printing infill density testing, a 4x2 experimental design was employed with two food inks (corn-based or wheat-based) and four levels of infill density (20, 40, 60 and 80%). The two inks were chosen from the eight described in Table 3.1, based on 3D printing performance (Chapter 2) and dynamic rheology results.

3.2.2 Dynamic Rheology of Food Inks

Dynamic rheology parameters (storage modulus G' , loss modulus G'' and loss tangent $\tan \delta$) were used to evaluate the viscoelastic properties of food inks using a StressTech Dynamic Rheometer (ATS RheoSystems, Bordentown, NJ, USA) equipped with smooth, stainless steel parallel plates of diameter 25mm and gap of 0.834 mm. Compressed air from a UN1002 tank (Matheson, Irving, TX, USA) was used to circulate air throughout the instrument system and kept constant at 0.3 MPa throughout testing (cooling and controlling the plate gap). The gap between the probe and testing plate was calibrated to 0 mm between every test. To mimic the 3D printing procedure, before rheology tests the food ink was deposited into a specialty plastic syringe along with a thermocouple temperature probe, which was then placed in a water bath at 90 °C until the geometric center of the material in the syringe reached $70^{\circ}\text{C} \pm 0.5^{\circ}$. The ink was then allowed to cool to room temperature before loading onto the rheometer. Mineral oil was applied to the sample surface to prevent samples from drying out during testing. Two metal caps were placed around the sample to further prevent air or outside forces from interfering with the

testing procedure. The testing temperature was set at 25°C and held constant throughout the process.

An oscillatory strain sweep from 0.991×10^{-2} to 0.150×10^1 was first performed on all inks at a frequency of 1 Hz to obtain the linear viscoelastic region (LVR) and the corresponding critical strain values. The critical strain was determined based on a 5% drop from the highest G' recorded. Frequency sweep tests on all food inks were then carried out from 0.1 to 100 Hz at a singular critical strain obtained after determining the LVR as described above, and G' , G'' and $\tan \delta$ recorded. RheoExplorer v5 40p9 software was used for controlling the test parameters and data output. All strain and frequency tests were done in triplicate.

3.2.3 Food 3D Printing and Infill Density

Formulations were mixed with distilled water in a 50:50 ratio using the procedure outlined in section 3.2.1 and heated to a temperature of $70^\circ\text{C} \pm 0.5^\circ\text{C}$ as described in section 3.2.2. Material was not agitated or stirred during the heating process to promote viscosity build-up. The heated formulation in the syringe was then allowed to cool at ambient temperature for 30 minutes to a temperature range of 33-38 °C before loading onto a Foodbot S2 multi-ingredient food 3D printer (Changxing Shiyin Technology Co., Ltd., Hangzhou, China). This device had a 150 x 150 x 73 mm maximum build volume and has a printing speed range 15-70 mm/s. The .stl file used for printing was programmed to have four different infill densities 20%, 40%, 60% and 80%, which varied the food ink material being printed to support the model shape from a relatively low amount to a very dense amount. The model was designed in TinkerCAD (TinkerCAD, San Francisco, CA) and exported as an .stl file to Ultimaker Cura (Zaltbommel, the Netherlands) for slicing, afterward the file was transferred to a FoodBot 3D Printer which utilizes Repetier Host 2.0.5 as its software (Repetier, Hot-World GmbH & Co. KG, Willich,

Germany). The 3D printing procedure is schematically described in Figure 3.1 and the geometrical shape of the printed object is shown in Figure 3.2. Samples were printed at a speed of 15 mm/s and on completion placed into a freezer at -9°C for 2 hours. Afterward they were carefully measured for dimensional distortion as described in the next section, and returned to the freezer for an additional 46 hours. Samples were then dried at 100°C for 3 hours and stored in a sealed container at room temperature to be later used in texture analysis.

3.2.4 Dimensional Distortion of 3D Printed Samples

Three replicate prints of frozen products before drying for each experimental treatment were measured for their diameter and height as shown in Figure 3.2 using digital calipers. Measurements for each of the two dimensions were done in triplicate to capture unevenness in printing within a given samples. The measured dimensions were used to compare against the dimensions of the .stl file used for printing. The dimensional distortion ratio (DDR), as defined by Asadi-Eydivand et al. (2016), was calculated to quantify the distortion or asymmetry of printed models, as follows.

$$DDR = \frac{A_d}{A_h} - \frac{stl_d}{stl_h}$$

where A_d and A_h are respectively the averaged measured values of diameters (d) and height (h) from the 3D printed model, and stl_d and stl_h are the corresponding computer aided design model diameter and height that are used in the .stl file, When there is no deviation from the .stl file after printing, the DDR is equal to zero. Non-zero numbers mean height and/ or diameter distortion, positive if the actual height is less than designed while diameter is higher (squatter product) and negative if the actual diameter is less than designed while height is more (elongated product).

3.2.5 Texture Analysis

Dried 3D printed samples were tested for mechanical strength using a TA-XT2 Texture Analyzer (Stable Micro Systems, Godalming, UK) operated under compression mode along the h-direction (Figure 3.2). A 38 mm cylindrical probe was used with a 1.00 mm/s pre-test speed, 1.00 mm/s test speed, and a 3.00 mm/s post-test speed. The compression was carried out for 15.00 mm past the first impact as determined by a trigger force of 5g. The force-deformation data was recorded and analyzed using the Texture Exponent 32 software (Stable Micro Systems, Godalming, UK). Three replicate tests were done for each treatment.

3.2.6 Statistical Analyses

Two-way Analysis of Variance (ANOVA) test was conducted using SAS software (SAS, Cary, NC, USA) with fixed-effects (infill density and flour type were the two factors) and the Tukey's test was used to determine significant differences between means. Significantly different means were denoted with different lower-cased letters. A significance level of 5% was used for all statistical tests ($p \leq 0.05$).

3.3 Results and Discussion

3.3.1 Dynamic Rheology

Average complex modulus (G^* , stress normalized by the strain), storage modulus (G' , corresponding to the material elastic behavior) and loss modulus (G'' , corresponding to the material viscous behavior) of the various corn and wheat flour-based inks from the strain sweep tests are shown in Figures 3.3 to 3.6. The viscoelastic parameters of the material being tested are independent of strain in the linear viscoelastic region (LVR) region. Past the critical strain or beyond the LVR, structural breakdown occurs, material deformation is no longer linear and the

moduli are a function of strain (Hajikarimi et al., 2022; Whitcomb, 2022; Melito et al., 2013; Tunick, 2011; Schramm, 1994). Based on the G' response, the LVR was determined and subsequent frequency sweep tests were conducted at a strain of 1.65×10^{-2} .

Average G^* , G' , G'' and $\tan \delta$ for the various corn and wheat flour-based inks from the frequency sweep tests are shown in Figures 3.7 to 3.12. It was clear from the trends that both G' and G'' of corn inks ($\sim 10^4$ Pa and 10^3 - 10^4 Pa, respectively) were up to an order of magnitude higher than that for wheat inks ($\sim 10^3$ - 10^4 Pa and 10^3 Pa, respectively). For both wheat and corn inks, G' and G'' had a decreasing trend with increase in sucrose from 0-12%. The $\tan \delta$ of corn inks with 0-4% sucrose was also relatively higher as compared to the corresponding wheat inks, while $\tan \delta$ for the two was similar for higher sucrose levels.

Viscoelastic data was extracted at a frequency of 0.152 Hz for comparison (Table 3.2). Both flour type and sucrose level had a significant impact on G' and G'' ($p < 0.05$). G' for corn inks (6,501 - 61,683 Pa) was 52-631% higher than that for corresponding wheat inks (4,265 - 8,441 Pa); while G'' for corn inks (1,630 - 22,610 Pa) was 67-970% higher than that for corresponding wheat inks (974 - 2,112 Pa). These differences between corn and wheat inks became less pronounced as sugar inclusion level increased. In other studies also it has been found that corn flour can exhibit greater apparent viscosity when compared to wheat flour due to various reasons including starch morphology and integrity, proteins in the matrix, and even milling characteristics (Wang et al 2020; Sun et al 2019; Guadarrama-Lezama et al., 2016; Sang et al., 2008; Martinez and El-Dahs, 1993). Previously reported results with other analyses including rapid viscoanalysis (RVA) also showed similar trends with slurry viscosity although RVA tests were done in a much higher moisture regime (Chapter 2).

Inclusion of 4-12% sucrose in corn and wheat inks, progressively decreased G' by 90 and 50%, respectively, and G'' by 93% and 54%, respectively. Similar G' and G'' value trends have been reported for wheat and corn formulation for various applications when sucrose is introduced in the formulations for food materials (Dongdong et al., 2023; Chang et al., 2004). These data were not completely consistent with other indirect measures of viscous properties of the inks reported previously (Chapter 2) using rapid viscoanalyzer (RVA) and phase transition analyzer (PTA) due to difference in moisture regimens and sample preparation before testing.

In dynamic rheology stress sweep measurements, G' and G'' values illustrate the structural strength or mechanical rigidity of the sample at rest (Rosalina et al., 2002). And within the world food 3D printing research, several studies have also related the G' and G'' values to the ability of the food ink material to support itself after printing (Maldonado-Rosas et al., 2022; Jiang et al., 2019; Zhu et al., 2019). From G^* , it appeared as though the treatment material texture was not very sensitive to frequency change, unless exposed to a higher frequency consistently. This is an ideal scenario for extrusion-based 3D printed starch materials, as one of the goals of the process is for the printed object to not collapse.

These dynamic rheology tests, combined with other physicochemical tests on wheat and corn-based inks and also food 3D printing results (Chapter 2), were used to narrow down on use of the base formulation (0% sucrose) for wheat and formulation with 4% sucrose inclusion for further 3D printing tests to study the impact of infill density as described below.

3.3.2 Dimensional Distortion Ratio of 3D Printed Samples

Example 3D printed samples are shown in Figure 3.13, and dimensional data for both corn and wheat-based samples printed at different infill densities are provided in Table 3.6. Additionally, DDR data for the 3D printed products are shown in Figure 3.14 and the

corresponding ANOVA results are given in Table 3.7. Corn ink samples had significantly higher DDR values ($p < 0.05$) compared to wheat (average absolute value of 0.401 for corn compared to that of 0.041 mm for wheat, which implied worse print quality and shape fidelity in the former. DDR for wheat-based products ranged from -0.026 to -0.053, while that for corn-based products ranged from 0.064 mm to 0.701. The large positive DDR values for the latter indicated that the samples were flatter or more squat than the CAD .stl model.

Infill density had a significant impact ($p < 0.05$) on the shape fidelity, although a significant interaction ($p < 0.05$) was observed with ink type. DDR of corn products increased significantly as infill density increased from 20 to 80%, but wheat products did not differ significantly from each other irrespective of the infill density. Thus, wheat as a starch-source in food inks had less impact on DDR of 3D printed structures when compared to corn. The phenomenon of the wheat-based products being more resilient to changes in printing parameters as compared to corn mirrored similar trends with respect to ink composition (sucrose and moisture content) that were reported previously (Chapter 2). It could be assumed with higher infill density during extrusion-based printing, more material holding the shape of the model in place would lead to more accurate prints, but for corn-based ink this was not the case. This was because the corn-based ink had a much higher viscosity compared to that of the wheat ink (as was seen from G'' data described earlier), which caused inconsistency in flow and deposition, leading to inaccuracies in the print.

3.3.3 Texture Analysis on Post-Printed Samples

Texture analysis using a simple compression test was utilized to determine and compare the textural attributes of the 3D printed infill density samples. Texture analysis results showed significant differences in peak force (kg) and work required to crush the samples between many

of the treatments. A trend occurred for both wheat and corn treatments where as the infill density increased from treatment to treatment (i.e. from 20% to 40% and 40% to 60% etc.), the average peak force also increased in value and this is outlined in Figure 3.17. For example 20% infill density in wheat and corn both exhibited an average peak force of 3.75 and 4.59 kg respectively while for 80% infill density wheat and corn both exhibited an average peak force of 24.70 and 21.16 kg respectively. Logically, it would make sense that as more material is being deposited to form a starch-based sample and support the structural integrity of the sample, the peak force required to crush it would also need to be higher as there are more beams of material that need to be compressed (Dick et al., 2020). Also, the lattice structure used to form the infill density creates smaller voids as infill density increases in percentage (Zhao et al., 2021).

Each infill density treatment (20%, 40%, 60% and 80%) was statistically significant from one another ($p < 0.05$) in terms of peak force when it came to infill density percentage, and the corn with a 60% infill density was significantly different than the wheat with that same infill density. But, in terms of starch source there was no significant difference between the 20%, 40% and 80% infill density percentages from wheat to corn.

While this trend of peak force required to crush the sample as infill density increased from treatment to treatment was not linear for either set of treatments, it did exhibit a consistent increase in peak force as infill density also increased. The increase of peak force (kg) from sample to sample for corn treatments from were also more consistent compared to wheat treatments, with a standard deviation of 1.08 kg compared to wheat's 3.91 kg respectively.

It should also be noted that the sample shape, that being three cylinders stacked on top of one another with a hollow circle in the middle as outlined in Figure 3.2, varied from treatment to treatment based on infill density % and made it difficult to test and to compress in a consistent

manner and so standard deviations were high for some treatments. And that the peak force required to crush the food samples was usually in the first peak exhibited in Figure 3.15 and 3.16 (within 9/10 replicates that was the case). When that wasn't the case it was always for the 40% and 60% infill density replicates for wheat treatments. From a subjective standpoint, while the samples were being crushed, the middle section was always the first "cylinder" to collapse, while the second "cylinder" to collapse was the bottom cylinder.

A consistent trend occurred where higher infill density samples did have a higher standard deviation in peak force when compared to those with a lower infill density (0.08 and 0.013 for Wheat and Corn 20% infill density respectively compared to 0.26 and 0.28 for Wheat and Corn 80% infill density respectively). This may have to do with the more inconsistent DDR values for the higher infill density treatments as outlined in the DDR section of the results and discussion.

To summarize the texture analyzer compression test results, increase in infill density from 20-80% significantly reinforced the structural integrity of both corn and wheat-based 3D printed products, as seen from the increase in average peak force from 4.59 and 3.75 kg, respectively, to 21.16 and 24.70 kg, respectively. In the future, texture analysis of 3D printed foods could be conducted on samples in a way that a consumer would experience the product to better characterize what optimal texture would be based on infill density. In addition to texture analysis, sensory testing could be utilized to capture aspects of texture that are difficult to capture and represent using a texture analyzer (Scheele et al., 2022).

3.4 Conclusion

In conclusion, this research study investigated the critical aspects of starch-based food ink formulations for 3D printing, aiming to understand how rheological properties and infill density impact printing performance and textural attributes. The procedures and steps used in this study were aided by the data and results from Chapter 2 and assessing formulations with dynamic rheology.

The results, supported by literature on dynamic rheology on starch-based material, texture analysis on food materials and food 3D printing, show how infill density impacts printing performance and texture. Infill density had a diminishing impact on overall quality past 20%; negatively impacting printing accuracy for both wheat and corn treatments. Moreover, it also increased the amount of peak force required to crush the samples, which could in turn negatively impact sensory attributes in future studies (Derossi et al., 2020). If sensory panelists have difficulty biting down or crushing 3D printed samples due to higher infill density values that could negatively impact the sensory experience (Shi et al., 2024).

This research serves as a foundation toward developing how 3D printing food processes may operate for high-performance food inks. The findings from this study will provide insight for future studies aiming to refine starch-based food 3D printing procedures and processes, addressing challenges related to infill density, infill patterns, texture, viscosity, elasticity, and raw material composition of formulations. The potential applications of 3D printing in the food industry, such as creating intricate shapes and unique textures, underscore the significance of ongoing research in this field.

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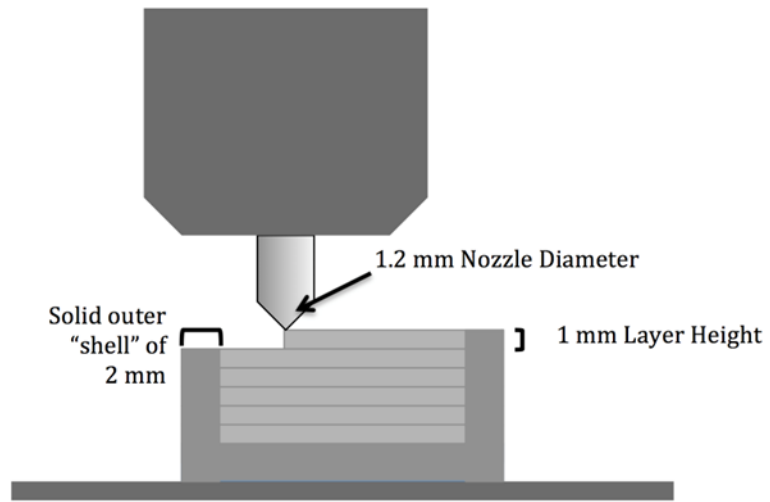


Figure 3.1. Foodbot 3D printer scheme for depositing layers

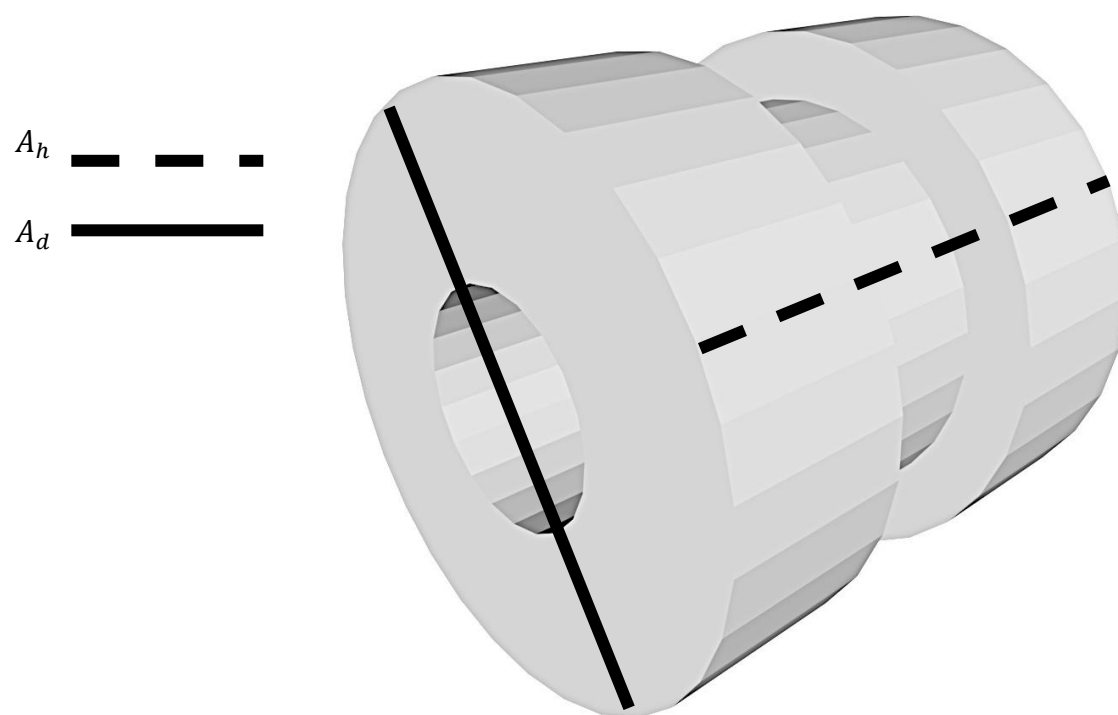


Figure 3.2. 3D printing product model. A_h = sample height (25 mm) and A_d = sample maximum diameter (30 mm).

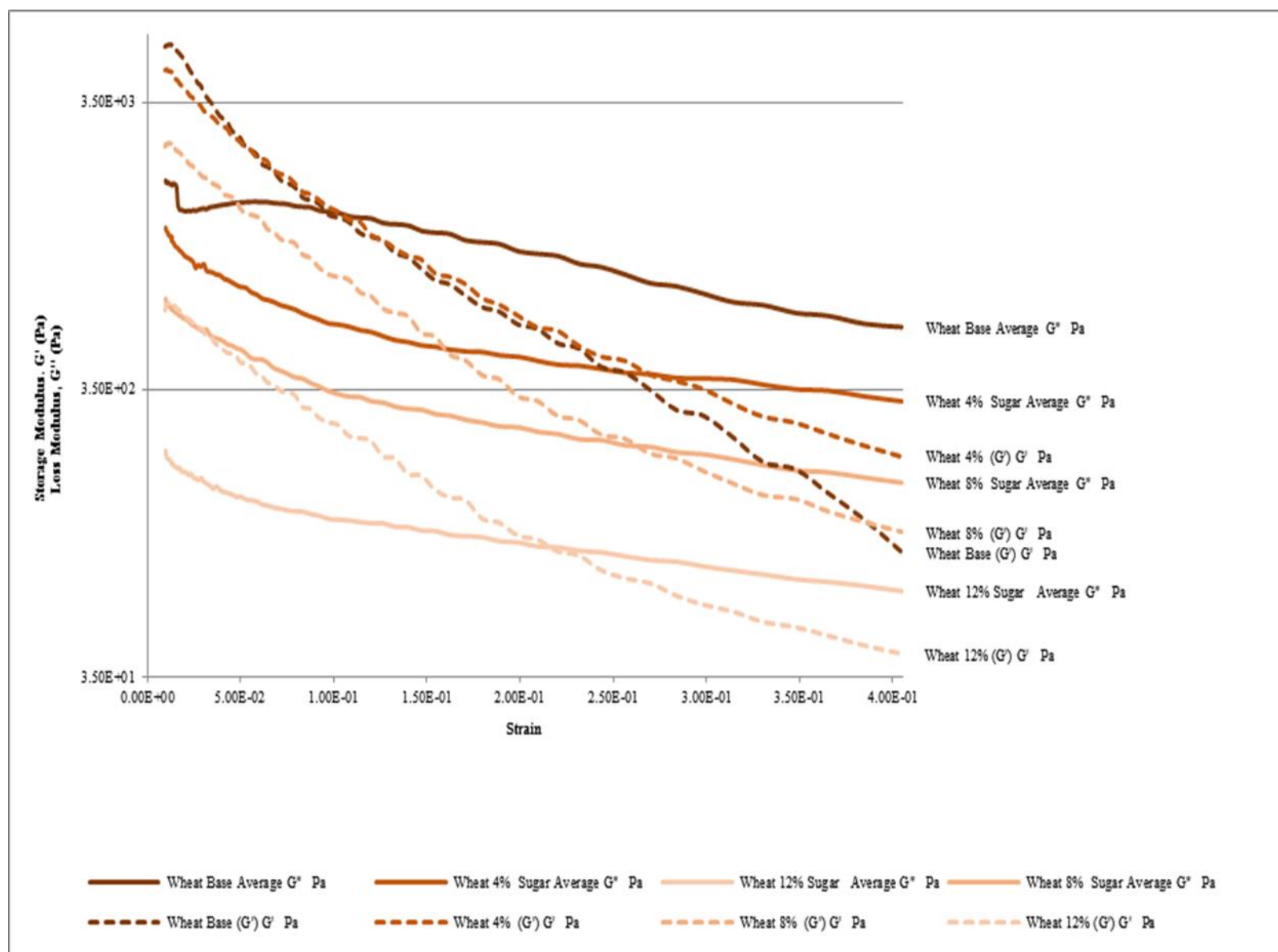


Figure 3.3. Dynamic rheology strain sweep G' and G'' for wheat-based inks.

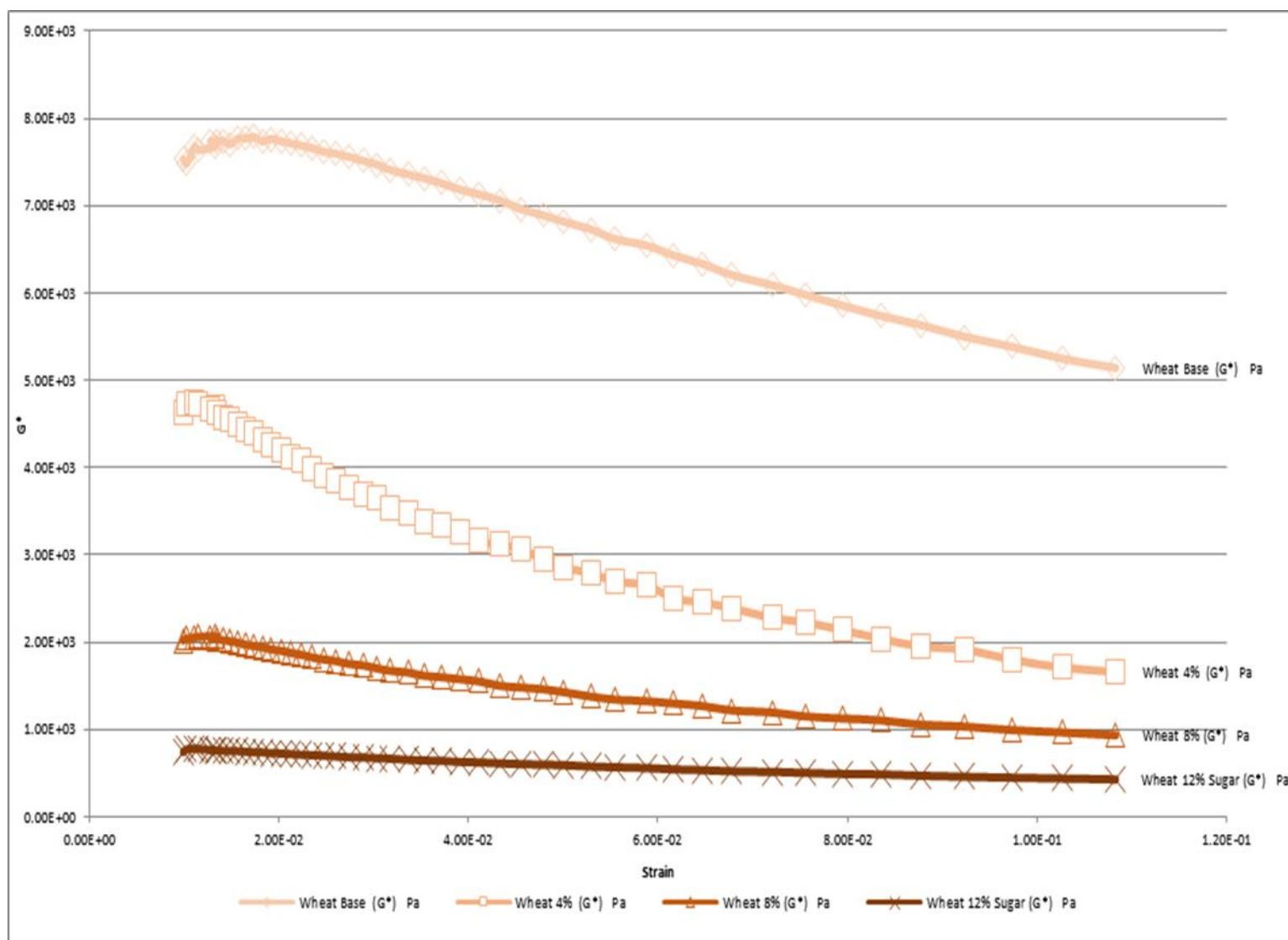


Figure 3.4. Dynamic rheology strain sweep G^* for wheat-based inks.

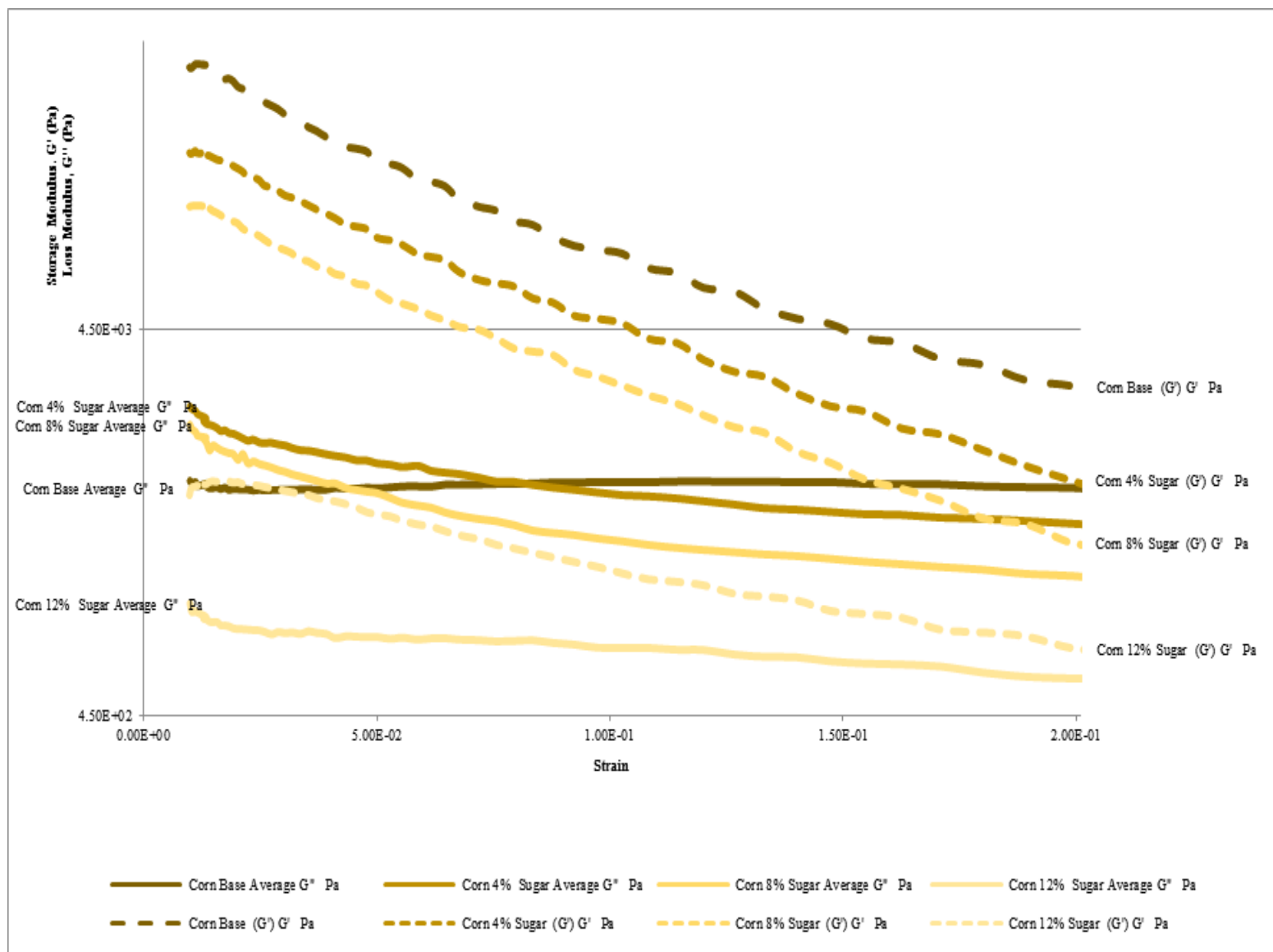


Figure 3.5. Dynamic rheology strain sweep G' and G'' for corn-based inks.

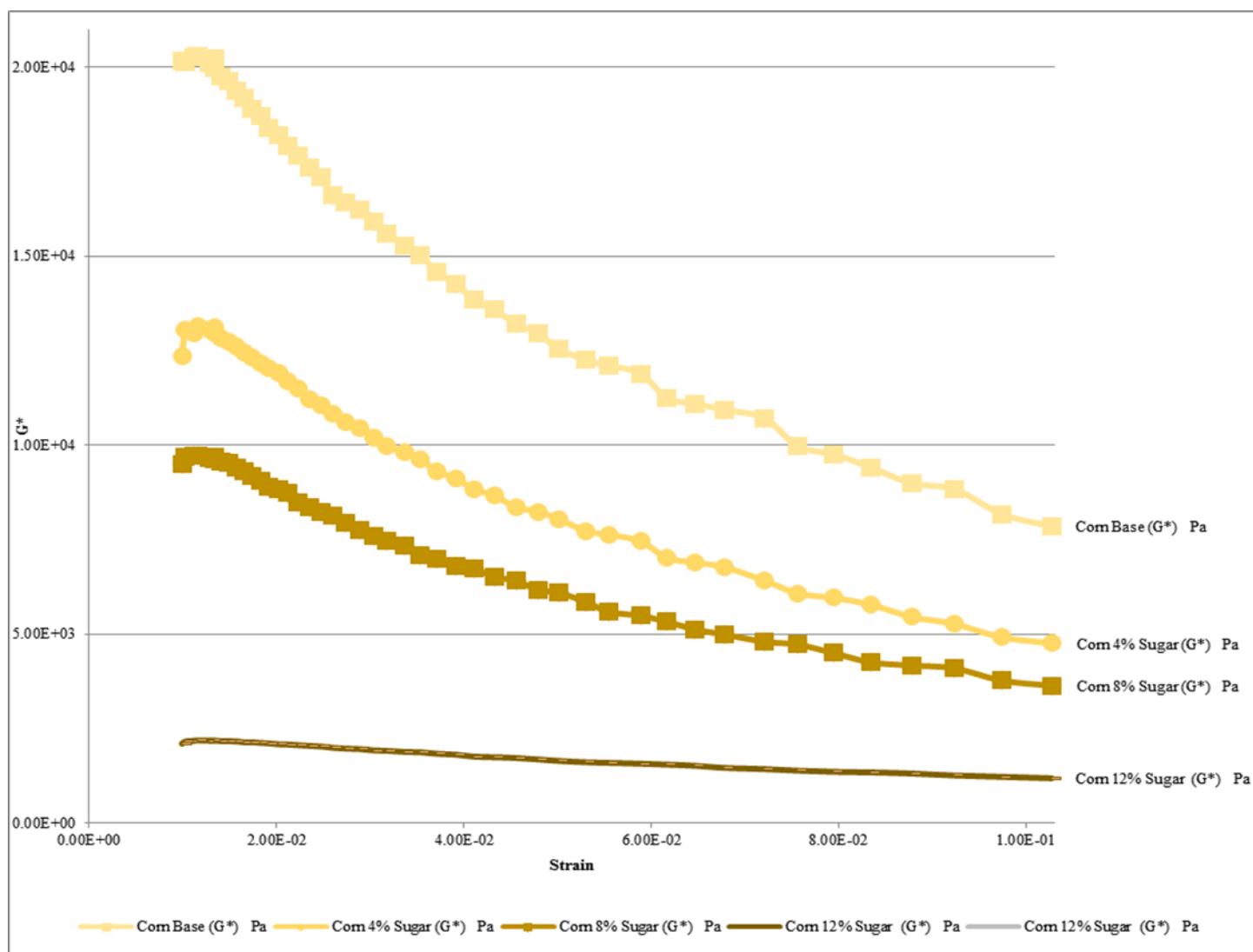


Figure 3.6. Dynamic rheology strain sweep G^* for corn-based inks.

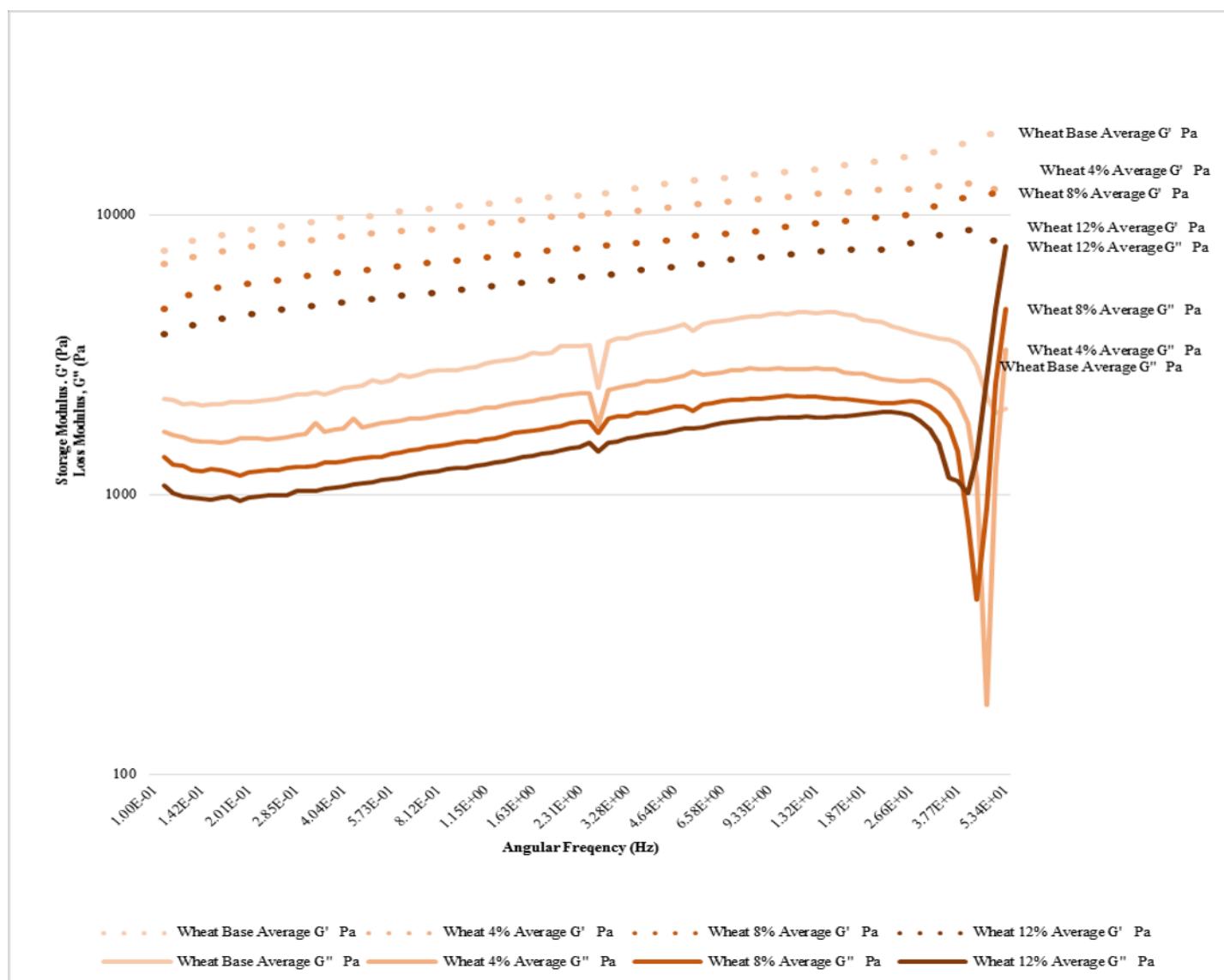


Figure 3.7. Dynamic rheology frequency sweep G' and G'' for wheat-based inks.

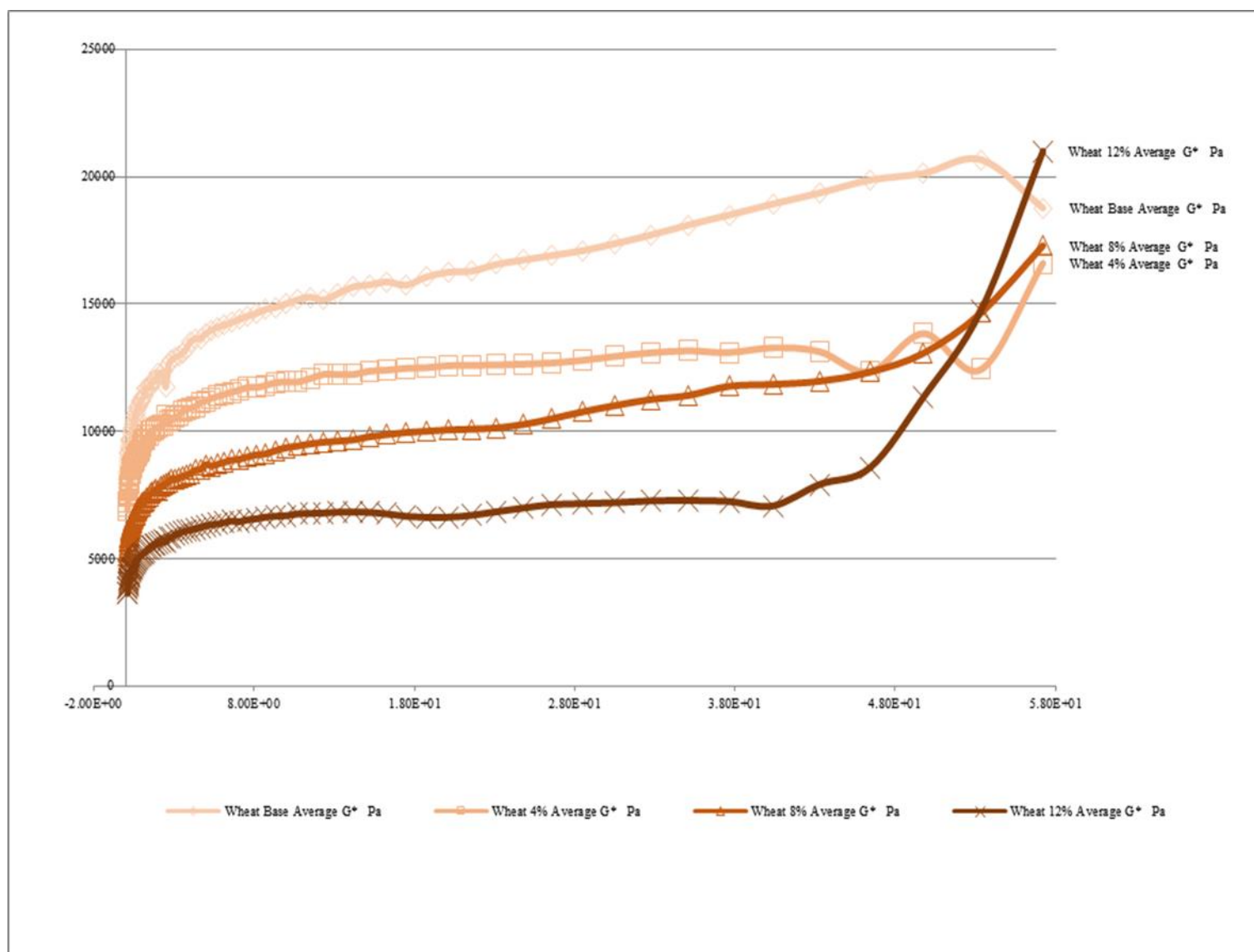


Figure 3.8. Dynamic rheology frequency sweep G^* for wheat-based inks.

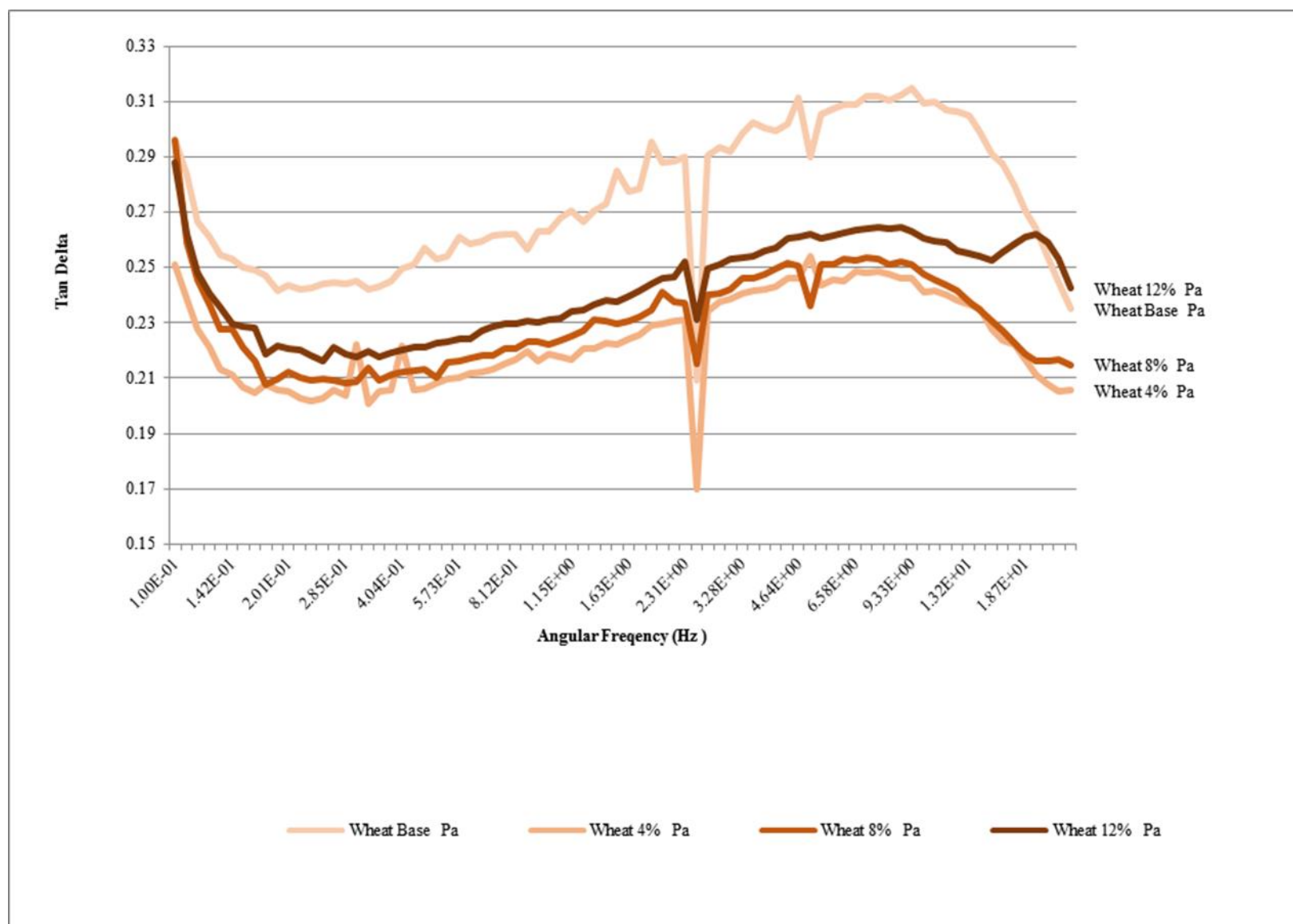


Figure 3.9. Dynamic rheology frequency sweep $\tan\delta$ for wheat-based inks.

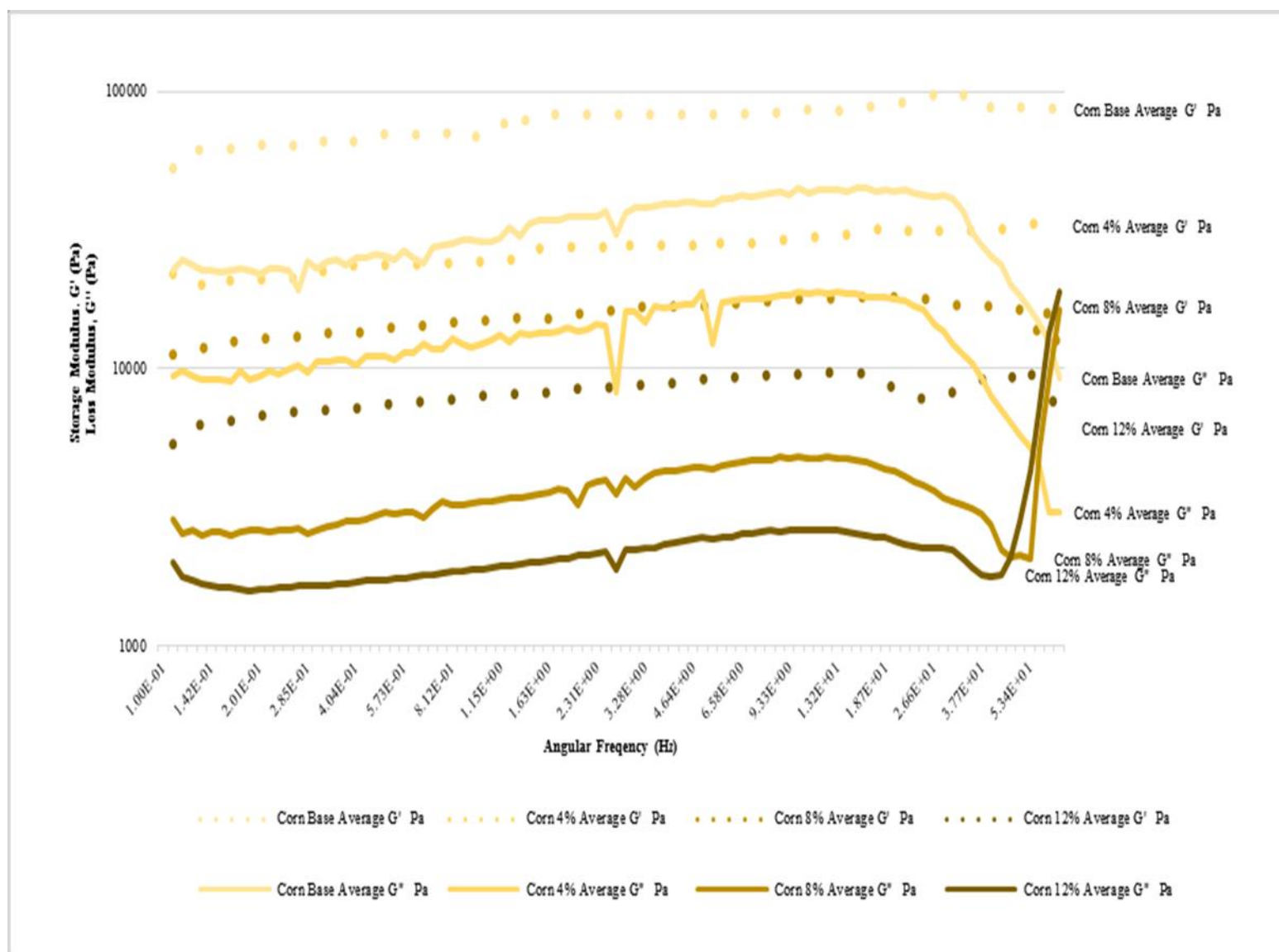


Figure 3.10. Dynamic rheology frequency sweep G' and G'' data for corn-based inks.

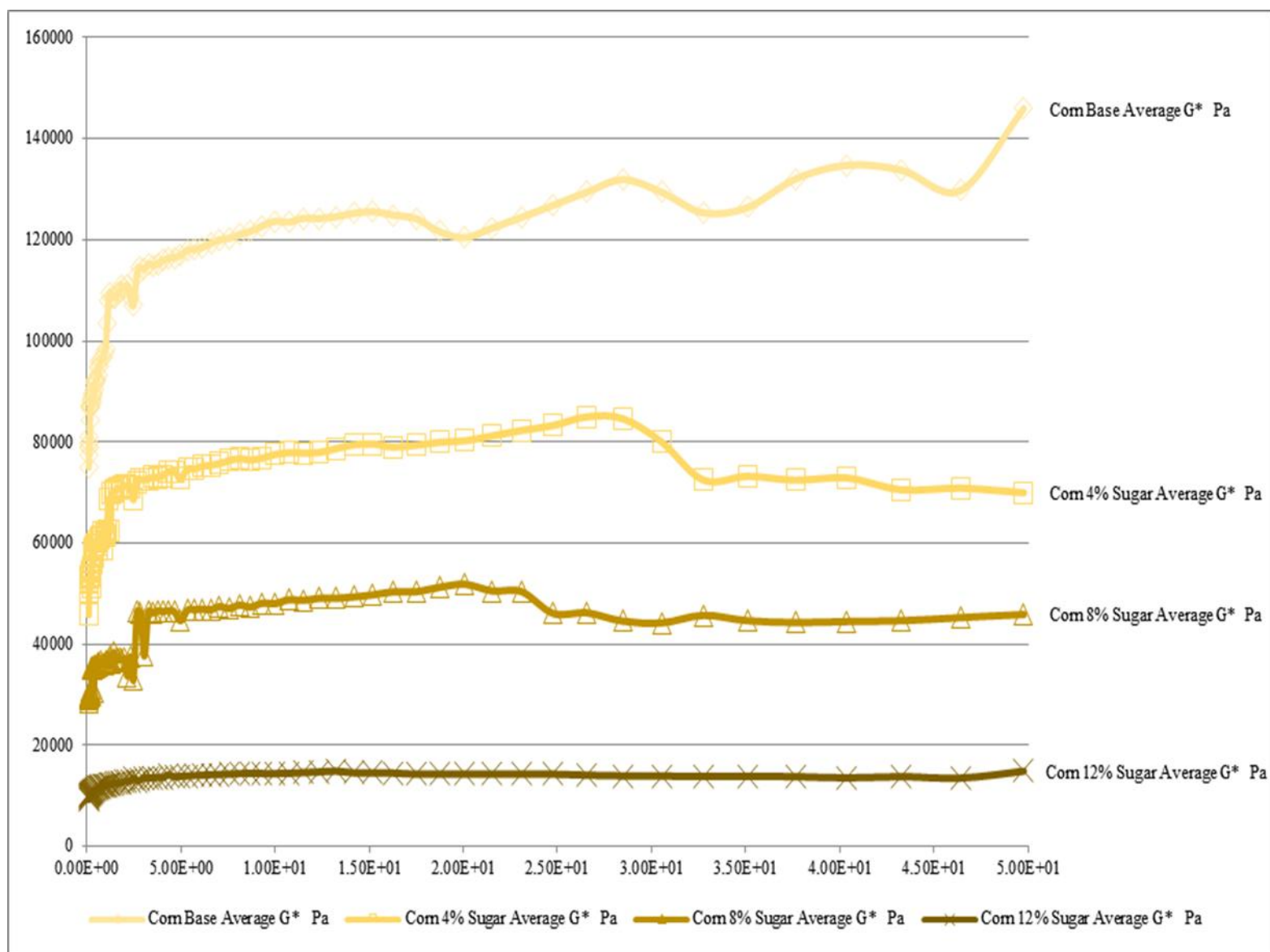


Figure 3.11. Dynamic rheology frequency sweep G^* data for corn-based inks.

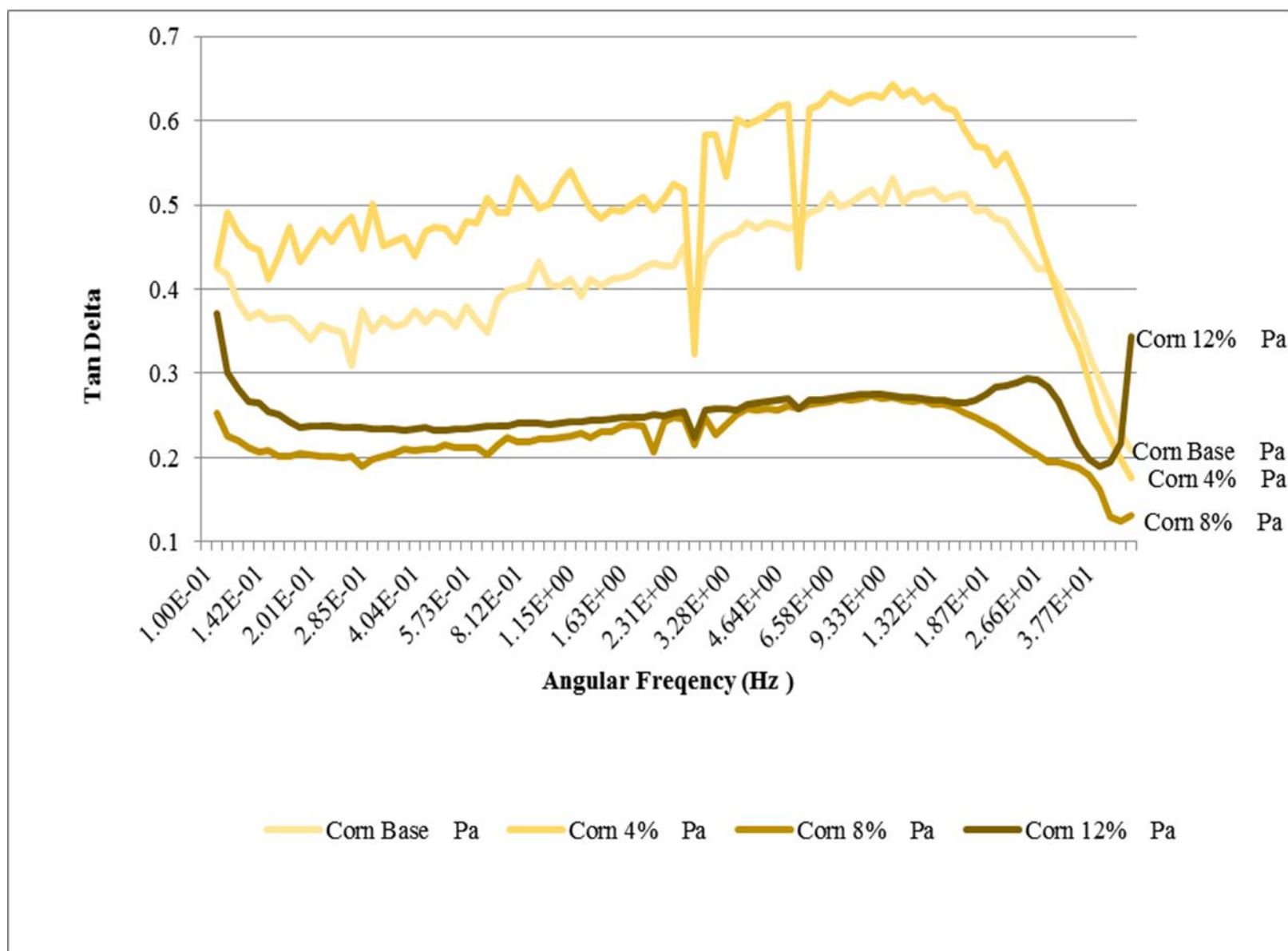


Figure 3.12. Dynamic rheology frequency sweep $\tan\delta$ data for corn-based inks.



Figure 3.13. Example 3D printed samples with different infill densities using wheat-based ink.

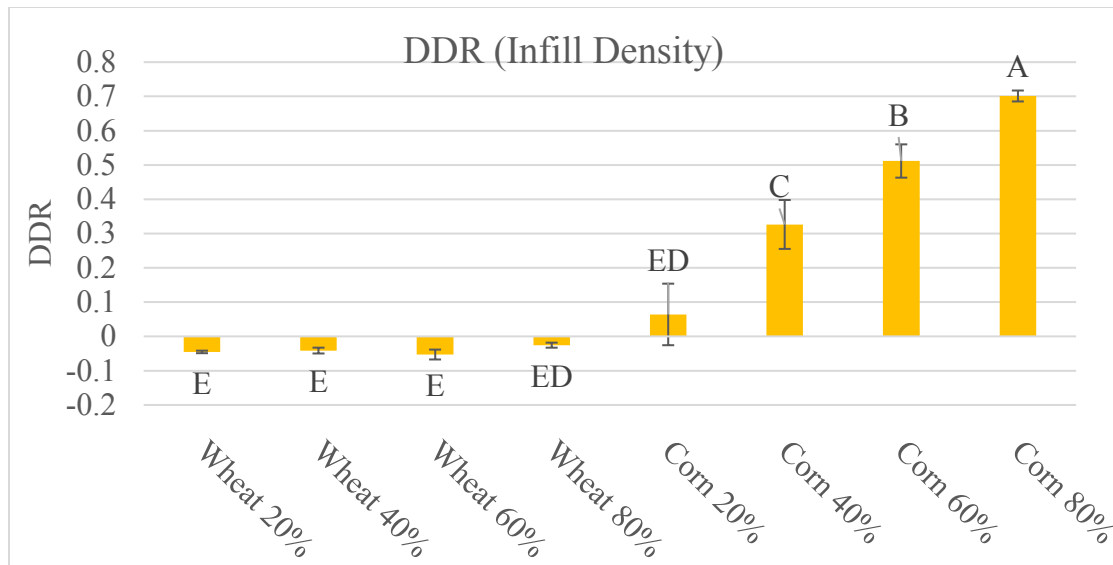


Figure 3.14. Dimensional distortion ratio (DDR) for wheat and corn-based 3D printed products as a function of infill density.

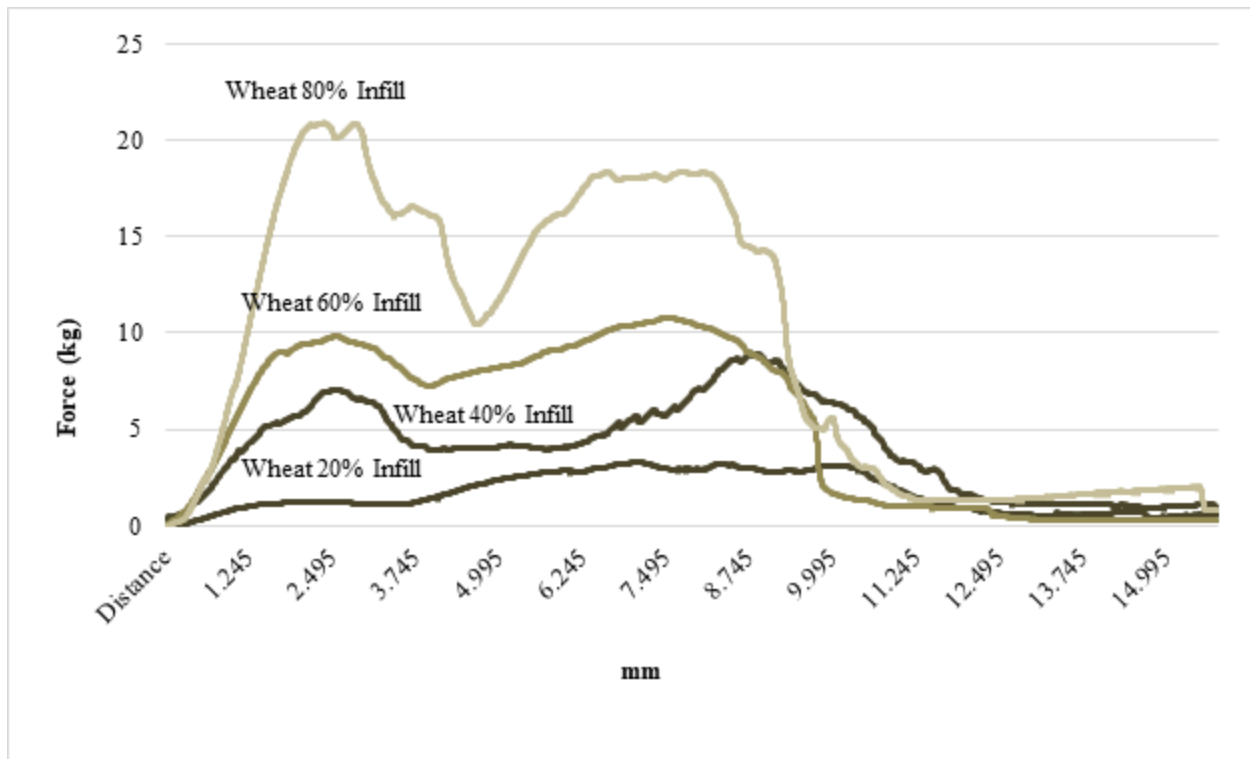


Figure 3.15. Texture analyzer average force-deformation curves for wheat-based 3D printed products with varying infill density.

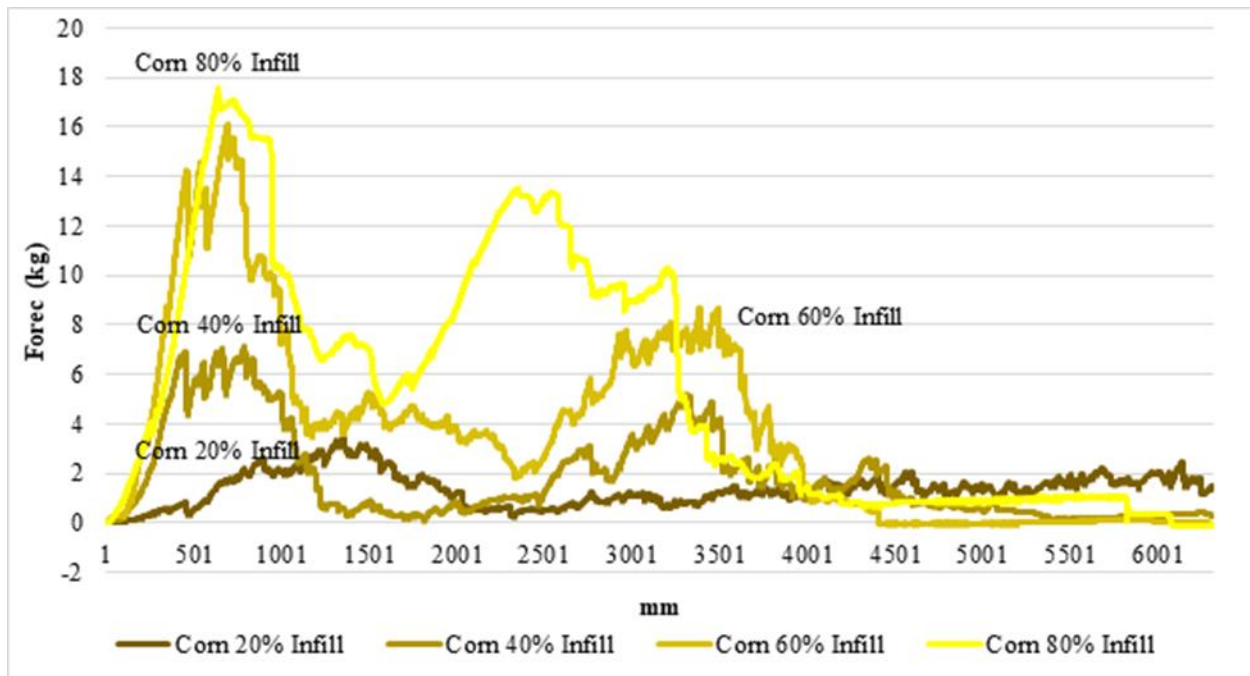


Figure 3.16. Texture analyzer average force-deformation curves for corn-based 3D printed products with varying infill density.

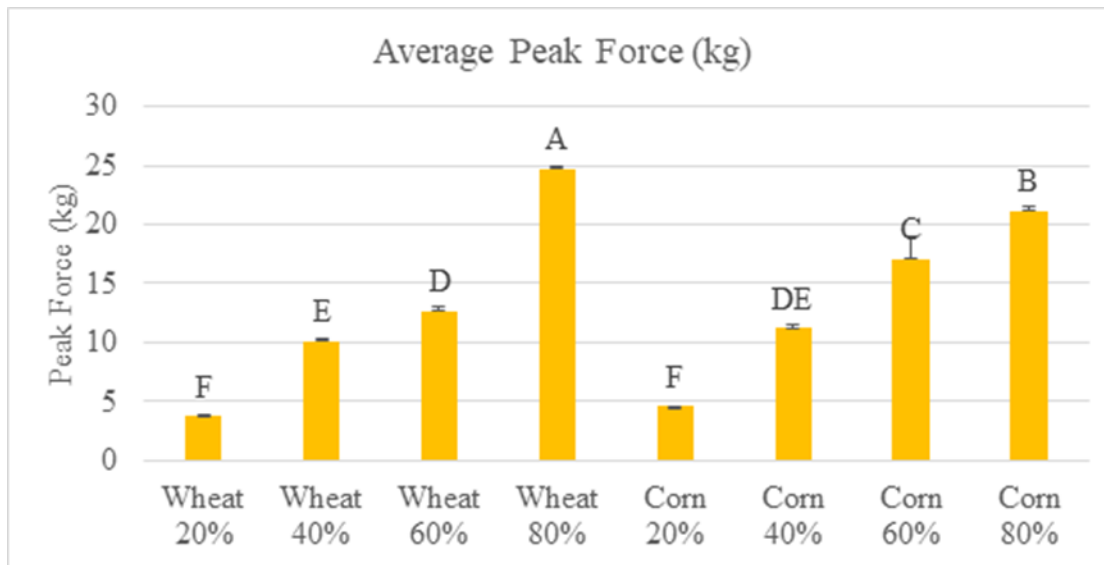


Figure 3.17. Texture analyzer peak force data for wheat and corn-based 3D printed products with varying infill density.

Table 3.1. Experimental design for dynamic rheology of food inks. The base mix contained either wheat or corn flour as the main structure forming component.

	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Base Mix	100	96	92	88
Sucrose Level	0	4	8	12

Table 3.2. Dynamic rheology frequency sweep G', G'' and G* data extracted at 0.152 Hz for wheat and corn-based inks.

		Wheat Base	Wheat 4% Sugar	Wheat 8% Sugar	Wheat 12% Sugar	Corn Base	Corn 4% Sugar	Corn 8% Sugar	Corn 12% Sugar	Frequency Used for ANOVA Results
Frequency Sweep Values	Average G* Values	9044 ± 315 B	7937 ± 112 CD	5721 ± 61 CD	5009.3 ± 174 CB	62520 ± 8226 F	21510 ± 2977 E	14310 ± 599 D	6420 ± 659 A	0.152
	Average G' Values	8441 ± 289 CD	7410 ± 87 CDE	5531 ± 54 DE	4265 ± 155 DE	61683 ± 8415 A	20560 ± 3096 B	12433 ± 613	6501 ± 694 CDE	0.152
	Average G'' Values	2112 ± 226 B	1532 ± 66 B	1223 ± 75 B	974 ± 131 B	22610 ± 6597 A	9030 ± 2700 B	2516 ± 549 B	1630 ± 648 B	0.152

Table 3.3. Two-way ANOVA results for dynamic rheology frequency sweep G' data extracted at 0.152 Hz.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Starch	2	2661684003	1330842001	472.69	< .0001
Sugar	3	3142734249	1047578083	372.08	< .0001
Starch*Sugar	3	3518469052	1172823017	416.57	< .0001

Table 3.4. Two-way ANOVA results for dynamic rheology frequency sweep G'' data extracted at 0.152 Hz.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Starch	1	322019116.4	322019116.4	21.03	0.0004
Sugar	3	446116028.5	148705342.8	9.71	0.0010
Starch*Sugar	3	373715232.1	124571744.0	8.13	0.0022

Table 3.5. Two-way ANOVA results for dynamic rheology frequency sweep G* data extracted at 0.152 Hz.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Starch	1	0.00813280	0.00813280	84.05	< .0001
Sugar	3	0.08176600	0.02725533	281.68	< .0001
Starch*Sugar	3	0.10114567	0.03371522	348.44	< .0001

Table 3.6. Dimensional measurements for wheat and corn-based 3D printed samples with varying infill density.

Flour Type	Infill Density	Average Diameter	<i>Diameter STD DEV</i>	Average Height	<i>Height STD DEV</i>
Wheat	20% Infill Density	29.77	0.12	25.78	0.05
Wheat	40% Infill Density	30.07	0.34	25.95	0.12
Wheat	60% Infill Density	29.39	0.58	25.62	0.19
Wheat	80% Infill Density	29.00	0.29	24.70	0.12
Corn	20% Infill Density	32.36	2.91	25.57	0.51
Corn	40% Infill Density	38.00	1.78	24.89	0.05
Corn	60% Infill Density	38.33	0.91	22.40	0.05
Corn	80% Infill Density	38.47	0.45	20.23	0.05

Table 3.7. Two-way ANOVA results for dimensional distortion ratio (DDR) data.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Starch	1	1.56383724	1.56383724	776.99	< .0001
InfillDensity	3	0.46284897	0.15428299	76.66	< .0001
Starch*InfillDensity	3	0.42461616	0.14153871	70.32	< .0001

Table 3.8. Two-way ANOVA results for texture analyzer peak force data.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Starch	1	3.068291	3.068291	3.57	0.0751
InfillDensity	3	1103.715403	367.905134	428.00	< .0001
Starch*InfillDensity	3	42.218711	14.072904	16.37	< .0001

Chapter 4 - Overall Conclusions

In conclusion, these studies delved into the critical aspects of starch-based food ink formulations for 3D printing, aiming to understand their physico-chemical properties and performance under various conditions. The study utilized a 3D printer and explored the influence of starch source, moisture content, sucrose, and infill density on the rheological behavior and printing accuracy of the materials. The work in this thesis can be directly used by the industry and academia. The results showed that starch source, moisture content, infill density and sucrose as a plasticizer. Have a significant impact on printing performance and rheological behavior of food inks for 3D Printing. Sucrose, while initially decreasing viscosity and enhancing 3D printing performance, had a diminishing effect past an 8% inclusion level, negatively impacting printing accuracy. Higher moisture levels were found to increase material flow but compromised printing accuracy. Additionally, higher infill levels increased peak force required in texture analysis but also negatively impacted printing accuracy past 40%.

This research serves as a foundation toward developing high-performance food inks for 3D printing. The findings will provide essential insight for future studies aiming to refine starch-based food 3D printing, addressing challenges related to viscosity, moisture, and raw material composition of formulations. Future research could be done to expand the results found in this thesis, including other starch and protein sources for ingredients in food inks for 3D Printing and more research could be done on sensory aspects of the 3D printed samples as well as how consumer acceptance for these kinds of foods.

The research conducted in this thesis focused on two primary starch sources: corn and wheat, but negated from using other starches. In future studies, rice, tapioca or potato starch could be studied using raw material analytical methods, dimensional distortion ratio tests and

texture analysis found in Chapter 2 and 3. These starches have the potential to have different results based on their differing pasting and viscosity properties. Additionally, texture profile analysis of potato starch gels has been shown to have higher hardness, cohesiveness and chewiness compared to other starches (Sandhu et al., 2010). This information could serve as the foundation for further research comparing starch bases for food inks.

In terms of texture, modifying the infill of a food design has become the common method to alter food hardness, providing opportunities for customizable food texture via 3D printing. And a focus on the feasibility and modulation of textures for starch-based inks was studied in the third chapter of the thesis, and an expansion on how these characteristics effect sensory could be carried into future research. While texture modifications of 3D printed foods are often characterized by instrumental analysis (e.g. texture analysis), data from sensory studies are still limited in current literature. Therefore, it is sometimes difficult to confirm texture modifications would result in actual changes of consumer perceptions and should be studied in future studies. The initial break of the material as observed in the texture analysis results from the third chapter may also have ramifications for consumer perceptions, and could also be studied in further research. For the next steps, combining instrumental analysis with sensory tests could provide further knowledge about how changes in food structure could impact texture.

Additionally in terms of sensory aspects of 3D printed foods, more emphasize on consumer opinions and preferences for these products could be studied in future research. Consumer acceptance is a largely underserved component to current literature for 3D printed food, yet a critically important one for further development of these products. A few studies have delved into this area of exploring how consumers view these novel products and found that food labeled as 3D printed did not change flavor, texture, or overall acceptance ratings for products

(Fang et. al, 2022). While research in this area is limited, future studies could adapt information to consumer interests and knowledge gaps that highlight relevant benefits of 3D printed food applications. The potential applications of 3D printing in the food industry, such as creating intricate shapes and unique textures, underscore the significance of ongoing research in this field.

4.1 References:

Feng, Xiaoqin, et al. "Effect of labelling and information on consumer perception of foods presented as 3D printed." *Foods* 11.6 (2022): 809.

Sandhu, Kawaljit Singh, and Maninder Kaur. "Studies on noodle quality of potato and rice starches and their blends in relation to their physicochemical, pasting and gel textural properties." *LWT-Food Science and Technology* 43.8 (2010): 1289-1293.

Appendix A - Chapter 2 and 3 Supplementary Data

Table A1 – Nutritional profile for the degermed corn flour used in the starch-based food ink formulations for extrusion-based 3D Printing

Nutrient List	Amount (per 100 grams)
	<i>ND- Not Detected</i>
Protein	5. 48
Total Carbohydrate(g)	80. 70
Total Fat (g)	1. 47
Fat, Saturated (g)	0.34
Fat, Polyunsaturated (g)	0. 80
Fat, Monounsaturated (g)	0. 33
Trans Fatty Acids (g)	ND
Moisture (g)	12. 00
Ash (g)	0. 35
Cholesterol (mg)	ND
Dietary Fiber (g)	4. 76

Fiber, Soluble (g)	ND
Fiber, Insoluble (g)	4. 76
Total Sugars (g)	1. 05
Added Sugars (g)	0. 00
Vitamin D (mcg)	0. 00
Calcium (mg)	0. 80
Potassium (mg)	120. 00
Vitamin A (mcg)	0. 00
Vitamin C (mg)	0. 00
Sodium (mg)	6. 00
Niacin (mg)	3.53 – 5.29
Iron (mg)	2.87 – 5.73
Thiamine (mg)	0.44 – 0.66
Riboflavin (mg)	0.26 – 0.40
Folic Acid (mg)	0.15 – 0.22

Table A2 - Nutritional profile for the wheat flour, white, all-purpose, enriched, bleached used in the starch-based food ink formulations for extrusion-based 3D

Printing

Nutrient List	Amount (per 100 grams) <i>ND- Not Detected</i>
Protein	10.3
Total Carbohydrate(g)	76.3
Total Fat (g)	0.98
Fat, Saturated (g)	0.155
Fat, Polyunsaturated (g)	0. 413
Fat, Monounsaturated (g)	0. 087
Trans Fatty Acids (g)	ND
Moisture (g)	11.9
Ash (g)	0. 47
Cholesterol (mg)	ND
Dietary Fiber (g)	2.7

Fiber, Soluble (g)	ND
Fiber, Insoluble (g)	2.7
Total Sugars (g)	0.27
Added Sugars (g)	0.00
Vitamin D (mcg)	0.00
Calcium (mg)	15
Potassium (mg)	107
Vitamin A (mcg)	0.00
Vitamin C (mg)	0.00
Sodium (mg)	2
Niacin (mg)	5.9
Iron (mg)	4.64
Thiamine (mg)	0.785
Riboflavin (mg)	0.494
Folic Acid (mg)	154

Table A3 – SAS output for two-way ANOVA and Tukey’s test for WAI data (see next page)

The GLM Procedure

Class Level Information		
Class	Levels	Values
Starch	2	C W
Sugar	4	0 12 4 8

Number of Observations Read	24
Number of Observations Used	24

The GLM Procedure

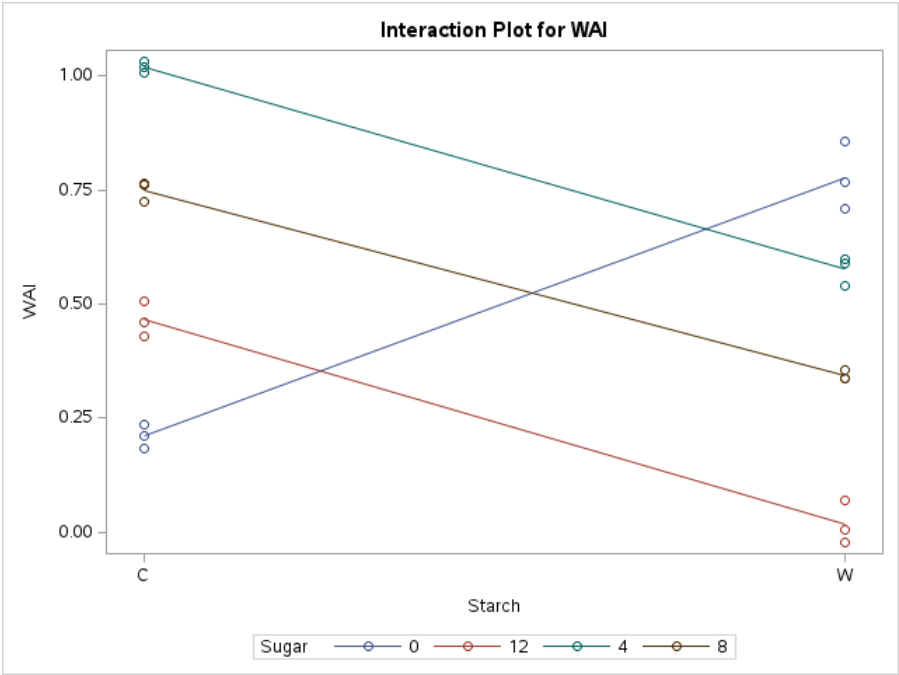
Dependent Variable: WAI

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	2.26365235	0.32337891	221.20	<.0001
Error	16	0.02339126	0.00146195		
Corrected Total	23	2.28704361			

R-Square	Coeff Var	Root MSE	WAI Mean
0.989772	7.357591	0.038236	0.519674

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Starch	1	0.20077624	0.20077624	137.33	<.0001
Sugar	3	0.93362633	0.31120878	212.87	<.0001
Starch*Sugar	3	1.12924978	0.37641659	257.48	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Starch	1	0.20077624	0.20077624	137.33	<.0001
Sugar	3	0.93362633	0.31120878	212.87	<.0001
Starch*Sugar	3	1.12924978	0.37641659	257.48	<.0001



The GLM Procedure

Tukey's Studentized Range (HSD) Test for WAI

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	16
Error Mean Square	0.001462
Critical Value of Studentized Range	2.99786
Minimum Significant Difference	0.0331

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	Starch
A	0.61114	12	C

Means with the same letter are not significantly different.			
B	Tukey Grouping	0.4629	12

The GLM Procedure

Tukey's Studentized Range (HSD) Test for WAI

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	16
Error Mean Square	0.001462
Critical Value of Studentized Range	4.04606
Minimum Significant Difference	0.0632

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	Sugar
A	0.79719	6	4
B	0.54593	6	8
B			
B	0.49381	6	0
C	0.24177	6	12

The GLM Procedure

Level of Starch	Level of Sugar	N	WAI	
			Mean	Std Dev
C	0	3	0.20982597	0.02545875
C	12	3	0.46624291	0.03898449
C	4	3	1.01907330	0.01216244
C	8	3	0.74941086	0.02249296
W	0	3	0.77778952	0.07442021
W	12	3	0.01729202	0.04745641
W	4	3	0.57530150	0.03119479
W	8	3	0.34245742	0.01049942

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

Starch	WAI LSMEAN	Standard Error	H0:LSMEAN=0	H0:LSMean1=LSMean2
			Pr > t	Pr > t
C	0.61113826	0.01103764	<.0001	<.0001
W	0.42821012	0.01103764	<.0001	

Tukey Grouping for LS-Means of Starch			
LS-means with the same letter are not significantly different.			
	WAI LSMEAN	Starch	LSMEAN Number
A	0.61113826	C	1
B	0.42821012	W	2

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

Sugar	WAI LSMEAN	Standard Error	Pr > t	LSMEAN Number
0	0.49380775	0.01560958	<.0001	1
12	0.24176747	0.01560958	<.0001	2
4	0.79718740	0.01560958	<.0001	3
8	0.54593414	0.01560958	<.0001	4

Least Squares Means for effect Sugar				
Pr > t for H0: LSMean(i)=LSMean(j)				
Dependent Variable: WAI				
i/j	1	2	3	4
1		<.0001	<.0001	0.1255
2	<.0001		<.0001	<.0001
3	<.0001	<.0001		<.0001
4	0.1255	<.0001	<.0001	

Tukey Grouping for LS-Means of Sugar			
LS-means with the same letter are not significantly different.			
	WAI LSMEAN	Sugar	LSMEAN Number
A	0.79718740	4	3
B	0.54593414	8	4
B			
B	0.49380775	0	1
C	0.24176747	12	2

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

Starch	Sugar	WAI LSMEAN	Standard Error	Pr > t	LSMEAN Number
C	0	0.20982597	0.02207528	<.0001	1
C	12	0.46624291	0.02207528	<.0001	2
C	4	1.01907330	0.02207528	<.0001	3
C	8	0.74941086	0.02207528	<.0001	4
W	0	0.77778952	0.02207528	<.0001	5
W	12	0.01729202	0.02207528	0.4449	6
W	4	0.57530150	0.02207528	<.0001	7
W	8	0.34245742	0.02207528	<.0001	8

Least Squares Means for effect Starch*Sugar								
Pr > t for H0: LSMean(i)=LSMean(j)								
Dependent Variable: WAI								
i/j	1	2	3	4	5	6	7	8
1		<.0001	<.0001	<.0001	<.0001	0.0003	<.0001	0.0110
2	<.0001		<.0001	<.0001	<.0001	<.0001	0.0472	0.0191
3	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001	<.0001
4	<.0001	<.0001	<.0001		0.9807	<.0001	0.0008	<.0001
5	<.0001	<.0001	<.0001	0.9807		<.0001	0.0002	<.0001
6	0.0003	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001
7	<.0001	0.0472	<.0001	0.0008	0.0002	<.0001		<.0001
8	0.0110	0.0191	<.0001	<.0001	<.0001	<.0001	<.0001	

Tukey Grouping for LS-Means of Starch*Sugar				
LS-means with the same letter are not significantly different.				
	WAI LSMEAN	Starch	Sugar	LSMEAN Number
A	1.01907330	C	4	3
B	0.77778952	W	0	5
B				
B	0.74941086	C	8	4
C	0.57530150	W	4	7
D	0.46624291	C	12	2
E	0.34245742	W	8	8
F	0.20982597	C	0	1
G	0.01729202	W	12	6

Table A4 – SAS output for two-way ANOVA and Tukey’s test for RVA Peak

Viscosity data (see next page)

The GLM Procedure

Class Level Information		
Class	Levels	Values
Starch	1	C
Sugar	4	0 12 4 8

Number of Observations Read	12
Number of Observations Used	12

The GLM Procedure

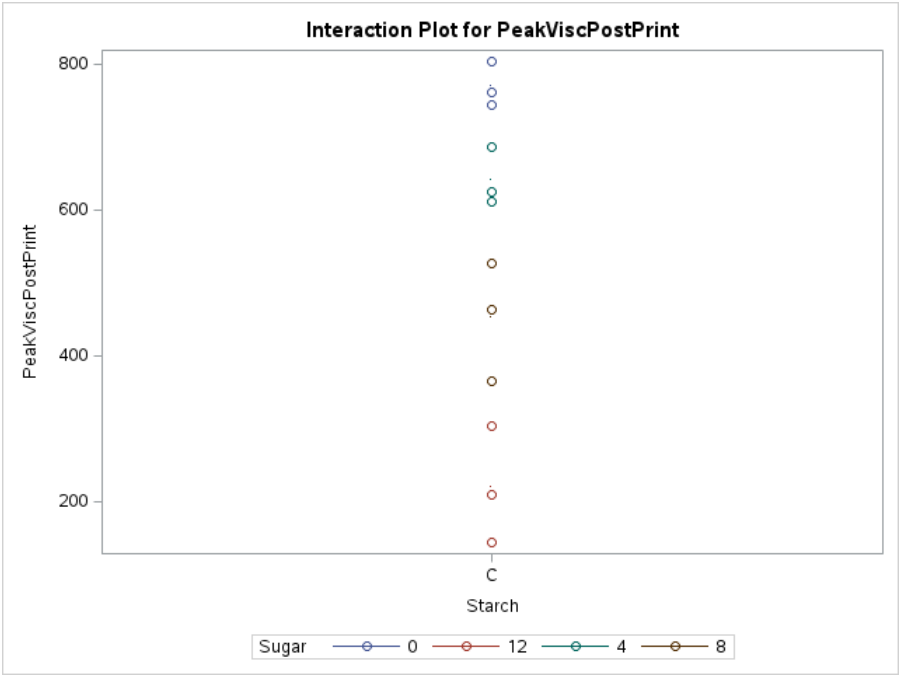
Dependent Variable: PeakViscPostPrint

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	518938.6667	172979.5556	44.19	<.0001
Error	8	31315.3333	3914.4167		
Corrected Total	11	550254.0000			

R-Square	Coeff Var	Root MSE	PeakViscPostPrint Mean
0.943089	12.03179	62.56530	520.0000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Starch	0	0.0000	.	.	.
Sugar	3	518938.6667	172979.5556	44.19	<.0001
Starch*Sugar	0	0.0000	.	.	.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Starch	0	0.0000	.	.	.
Sugar	3	518938.6667	172979.5556	44.19	<.0001
Starch*Sugar	0	0.0000	.	.	.



The GLM Procedure

Level of Starch	N	PeakViscPostPrint	
		Mean	Std Dev
C	12	520.000000	223.658425

Level of Sugar	N	PeakViscPostPrint	
		Mean	Std Dev
0	3	770.000000	30.7896086
12	3	218.000000	80.4673847
4	3	640.666667	40.6489032
8	3	451.333333	81.1315804

Level of Starch	Level of Sugar	N	PeakViscPostPrint	
			Mean	Std Dev
C	0	3	770.000000	30.7896086
C	12	3	218.000000	80.4673847
C	4	3	640.666667	40.6489032
C	8	3	451.333333	81.1315804

The GLM Procedure
Least Squares Means

Error: LINES display is not produced for effect Starch and dependent variable PeakViscPostPrint because p-values are not available for all least-squares means differences.

Starch	PeakViscPostPrint LSMEAN	Standard Error	Pr > t
C	520.000000	18.061046	<.0001

Error: LINES display is not produced for effect Starch and dependent variable PeakViscPostPrint because p-values are not available for all least-squares means differences.

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

Sugar	PeakViscPostPrint LSMEAN	Standard Error	Pr > t	LSMEAN Number
0	770.000000	36.122092	<.0001	1
12	218.000000	36.122092	0.0003	2
4	640.666667	36.122092	<.0001	3
8	451.333333	36.122092	<.0001	4

Least Squares Means for effect Sugar Pr > t for H0: LSMean(i)=LSMean(j)				
Dependent Variable: PeakViscPostPrint				
i/j	1	2	3	4
1		<.0001	0.1286	0.0011
2	<.0001		0.0002	0.0079
3	0.1286	0.0002		0.0248
4	0.0011	0.0079	0.0248	

Tukey Grouping for LS-Means of Sugar			
LS-means with the same letter are not significantly different.			
	PeakViscPostPrint LSMEAN	Sugar	LSMEAN Number
A	770.000	0	1
A			
A	640.667	4	3
B	451.333	8	4
C	218.000	12	2

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

Starch	Sugar	PeakViscPostPrint LSMEAN	Standard Error	Pr > t	LSMEAN Number
C	0	770.000000	36.122092	<.0001	1
C	12	218.000000	36.122092	0.0003	2
C	4	640.666667	36.122092	<.0001	3
C	8	451.333333	36.122092	<.0001	4

Least Squares Means for effect Starch*Sugar Pr > t for H0: LSMean(i)=LSMean(j)				
Dependent Variable: PeakViscPostPrint				
i/j	1	2	3	4
1		<.0001	0.1286	0.0011
2	<.0001		0.0002	0.0079
3	0.1286	0.0002		0.0248
4	0.0011	0.0079	0.0248	

Tukey Grouping for LS-Means of Starch*Sugar			
LS-means with the same letter are not significantly different.			
	PeakViscPostPrint LSMEAN	Starch	Sugar
			LSMEAN Number

Tukey Grouping for LS-Means of Starch*Sugar				
LS-means with the same letter are not significantly different.				
	PeakViscPostPrint LSMEAN	Starch	Sugar	LSMEAN Number
A	770.000	C	0	1
A				
A	640.667	C	4	3
B	451.333	C	8	4
C	218.000	C	12	2

Table A5 – SAS output for two-way ANOVA and Tukey’s test for RVA Pasting

Temperature data (see next page)

The GLM Procedure

Class Level Information		
Class	Levels	Values
Starch	2	C W
Sugar	4	0 12 4 8

Number of Observations Read	24
Number of Observations Used	24

The GLM Procedure

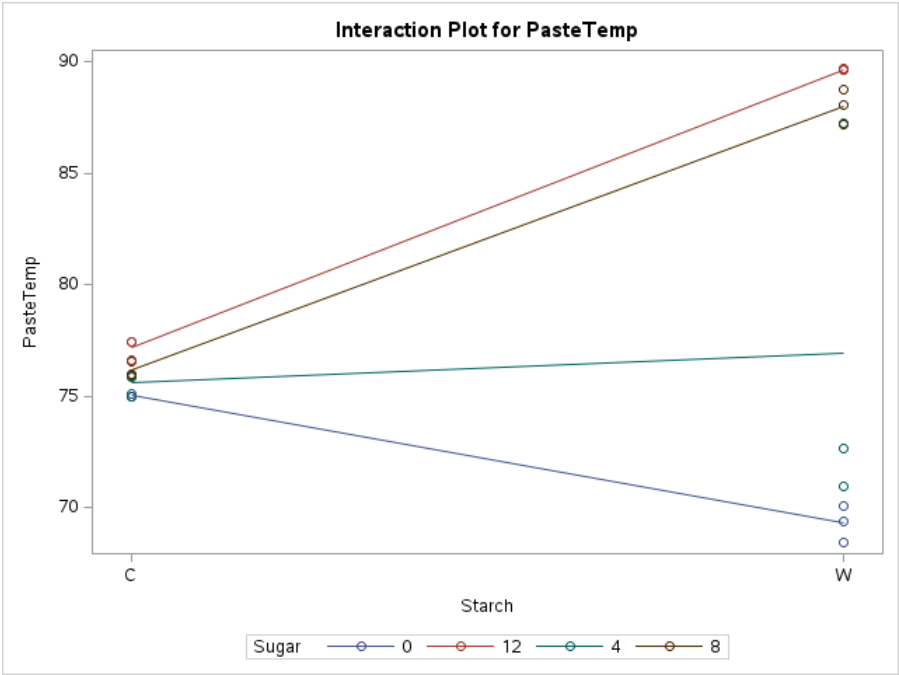
Dependent Variable: PasteTemp

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	985.602396	140.800342	13.75	<.0001
Error	16	163.815000	10.238438		
Corrected Total	23	1149.417396			

R-Square	Coeff Var	Root MSE	PasteTemp Mean
0.857480	4.076879	3.199756	78.48542

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Starch	1	149.2509375	149.2509375	14.58	0.0015
Sugar	3	490.6119792	163.5373264	15.97	<.0001
Starch*Sugar	3	345.7394792	115.2464931	11.26	0.0003

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Starch	1	149.2509375	149.2509375	14.58	0.0015
Sugar	3	490.6119792	163.5373264	15.97	<.0001
Starch*Sugar	3	345.7394792	115.2464931	11.26	0.0003



The GLM Procedure

Tukey's Studentized Range (HSD) Test for PasteTemp

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	16
Error Mean Square	10.23844
Critical Value of Studentized Range	2.99786
Minimum Significant Difference	2.7691

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	Starch
A	80.979	12	W

Means with the same letter are not significantly different.			
B	Tukey Grouping	75.992	Starch

The GLM Procedure

Tukey's Studentized Range (HSD) Test for PasteTemp

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	16
Error Mean Square	10.23844
Critical Value of Studentized Range	4.04606
Minimum Significant Difference	5.2853

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	Sugar
A	83.400	6	12
A			
A	82.083	6	8
B	76.283	6	4
B			
B	72.175	6	0

The GLM Procedure

Level of Starch	Level of Sugar	N	PasteTemp	
			Mean	Std Dev
C	0	3	75.0333333	0.05773503
C	12	3	77.1666667	0.49074773
C	4	3	75.6000000	0.51961524
C	8	3	76.1666667	0.33291641
W	0	3	69.3166667	0.82815055
W	12	3	89.6333333	0.05773503
W	4	3	76.9666667	8.94375946
W	8	3	88.0000000	0.77620873

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

Starch	PasteTemp LSMEAN	Standard Error	H0:LSMEAN=0	H0:LSMean1=LSMean2
			Pr > t	Pr > t
C	75.9916667	0.9236900	<.0001	0.0015
W	80.9791667	0.9236900	<.0001	

Tukey Grouping for LS-Means of Starch			
LS-means with the same letter are not significantly different.			
	PasteTemp LSMEAN	Starch	LSMEAN Number
A	80.97917	W	2
B	75.99167	C	1

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

Sugar	PasteTemp LSMEAN	Standard Error	Pr > t	LSMEAN Number
0	72.1750000	1.3062949	<.0001	1
12	83.4000000	1.3062949	<.0001	2
4	76.2833333	1.3062949	<.0001	3
8	82.0833333	1.3062949	<.0001	4

Least Squares Means for effect Sugar				
Pr > t for H0: LSMean(i)=LSMean(j)				
Dependent Variable: PasteTemp				
i/j	1	2	3	4
1		<.0001	0.1589	0.0003
2	<.0001		0.0069	0.8906
3	0.1589	0.0069		0.0291
4	0.0003	0.8906	0.0291	

Tukey Grouping for LS-Means of Sugar			
LS-means with the same letter are not significantly different.			
	PasteTemp LSMEAN	Sugar	LSMEAN Number
A	83.40000	12	2
A			
A	82.08333	8	4
B	76.28333	4	3
B			
B	72.17500	0	1

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

Starch	Sugar	PasteTemp LSMEAN	Standard Error	Pr > t	LSMEAN Number
C	0	75.0333333	1.8473799	<.0001	1
C	12	77.1666667	1.8473799	<.0001	2
C	4	75.6000000	1.8473799	<.0001	3
C	8	76.1666667	1.8473799	<.0001	4
W	0	69.3166667	1.8473799	<.0001	5
W	12	89.6333333	1.8473799	<.0001	6
W	4	76.9666667	1.8473799	<.0001	7
W	8	88.0000000	1.8473799	<.0001	8

Least Squares Means for effect Starch*Sugar								
Pr > t for H0: LSMean(i)=LSMean(j)								
Dependent Variable: PasteTemp								
i/j	1	2	3	4	5	6	7	8
1		0.9895	1.0000	0.9998	0.4065	0.0008	0.9941	0.0027
2	0.9895		0.9984	0.9999	0.1148	0.0040	1.0000	0.0135
3	1.0000	0.9984		1.0000	0.3019	0.0013	0.9993	0.0042
4	0.9998	0.9999	1.0000		0.2173	0.0019	1.0000	0.0064
5	0.4065	0.1148	0.3019	0.2173		<.0001	0.1311	<.0001
6	0.0008	0.0040	0.0013	0.0019	<.0001		0.0034	0.9979
7	0.9941	1.0000	0.9993	1.0000	0.1311	0.0034		0.0116
8	0.0027	0.0135	0.0042	0.0064	<.0001	0.9979	0.0116	

Tukey Grouping for LS-Means of Starch*Sugar				
LS-means with the same letter are not significantly different.				
	PasteTemp LSMEAN	Starch	Sugar	LSMEAN Number
A	89.63333	W	12	6
A				
A	88.00000	W	8	8
B	77.16667	C	12	2
B				
B	76.96667	W	4	7
B				
B	76.16667	C	8	4
B				
B	75.60000	C	4	3
B				
B	75.03333	C	0	1
B				
B	69.31667	W	0	5

**Table A6 – SAS output for two-way ANOVA and Tukey’s test for PTA Softening
Temperature data (see next page)**

The GLM Procedure

Class Level Information		
Class	Levels	Values
Starch	2	C W
Sugar	4	0 12 4 8

Number of Observations Read	24
Number of Observations Used	24

The GLM Procedure

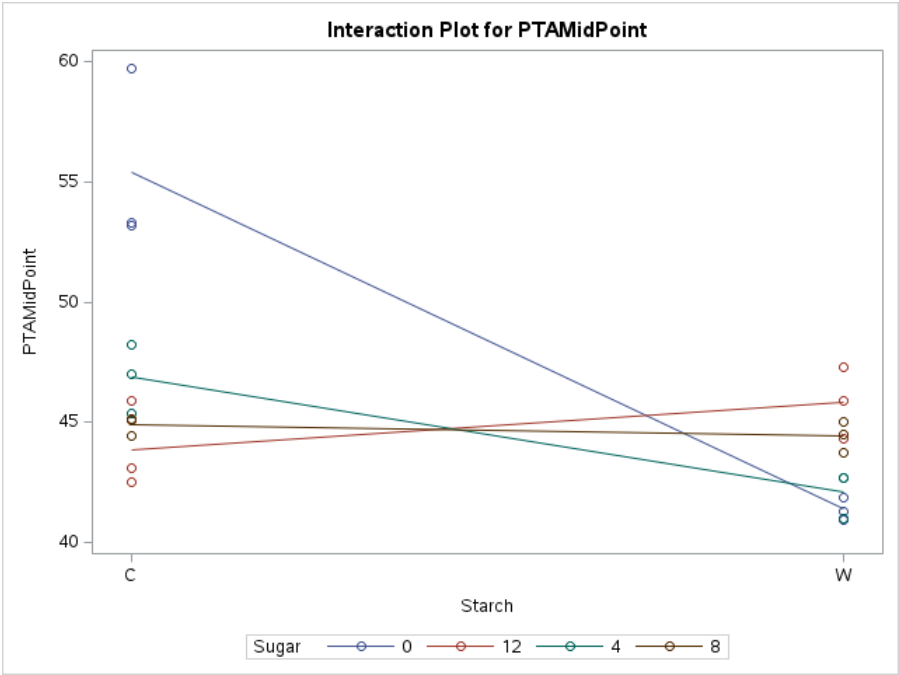
Dependent Variable: PTAMidPoint

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	397.6587833	56.8083976	19.58	<.0001
Error	16	46.4278000	2.9017375		
Corrected Total	23	444.0865833			

R-Square	Coeff Var	Root MSE	PTAMidPoint Mean
0.895453	3.735975	1.703449	45.59583

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Starch	1	111.1981500	111.1981500	38.32	<.0001
Sugar	3	62.8492833	20.9497611	7.22	0.0028
Starch*Sugar	3	223.6113500	74.5371167	25.69	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Starch	1	111.1981500	111.1981500	38.32	<.0001
Sugar	3	62.8492833	20.9497611	7.22	0.0028
Starch*Sugar	3	223.6113500	74.5371167	25.69	<.0001



The GLM Procedure

Tukey's Studentized Range (HSD) Test for PTAMidPoint

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	16
Error Mean Square	2.901738
Critical Value of Studentized Range	2.99786
Minimum Significant Difference	1.4742

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	Starch
A	47.7483	12	C

Means with the same letter are not significantly different.			
B	Tukey Grouping	43.4483	12 Starch

The GLM Procedure

Tukey's Studentized Range (HSD) Test for PTAMidPoint

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	16
Error Mean Square	2.901738
Critical Value of Studentized Range	4.04606
Minimum Significant Difference	2.8138

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	Sugar
A	48.3917	6	0
B	44.8333	6	12
B			
B	44.6483	6	8
B			
B	44.5100	6	4

The GLM Procedure

Level of Starch	Level of Sugar	N	PTAMidPoint	
			Mean	Std Dev
C	0	3	55.4000000	3.72424489
C	12	3	43.8333333	1.81475435
C	4	3	46.8833333	1.42857738
C	8	3	44.8766667	0.39551654
W	0	3	41.3833333	0.48045118
W	12	3	45.8333333	1.50111070
W	4	3	42.1366667	0.97572195
W	8	3	44.4200000	0.64583280

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

Starch	PTAMidPoint LSMEAN	Standard Error	H0:LSMEAN=0 Pr > t	H0:LSMean1=LSMean2 Pr > t
C	47.7483333	0.4917433	<.0001	<.0001
W	43.4433333	0.4917433	<.0001	

Tukey Grouping for LS-Means of Starch			
LS-means with the same letter are not significantly different.			
	PTAMidPoint LSMEAN	Starch	LSMEAN Number
A	47.74833	C	1
B	43.44333	W	2

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

Sugar	PTAMidPoint LSMEAN	Standard Error	Pr > t	LSMEAN Number
0	48.3916667	0.6954300	<.0001	1
12	44.8333333	0.6954300	<.0001	2
4	44.5100000	0.6954300	<.0001	3
8	44.6483333	0.6954300	<.0001	4

Least Squares Means for effect Sugar				
Pr > t for H0: LSMean(i)=LSMean(j)				
Dependent Variable: PTAMidPoint				
i/j	1	2	3	4
1		0.0111	0.0057	0.0076
2	0.0111		0.9873	0.9975
3	0.0057	0.9873		0.9990
4	0.0076	0.9975	0.9990	

Tukey Grouping for LS-Means of Sugar			
LS-means with the same letter are not significantly different.			
	PTAMidPoint LSMEAN	Sugar	LSMEAN Number
A	48.39167	0	1
B	44.83333	12	2
B			
B	44.64833	8	4
B			
B	44.51000	4	3

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

Starch	Sugar	PTAMidPoint LSMEAN	Standard Error	Pr > t	LSMEAN Number
C	0	55.4000000	0.9834866	<.0001	1
C	12	43.8333333	0.9834866	<.0001	2
C	4	46.8833333	0.9834866	<.0001	3
C	8	44.8766667	0.9834866	<.0001	4
W	0	41.3833333	0.9834866	<.0001	5
W	12	45.8333333	0.9834866	<.0001	6
W	4	42.1366667	0.9834866	<.0001	7
W	8	44.4200000	0.9834866	<.0001	8

Least Squares Means for effect Starch*Sugar								
Pr > t for H0: LSMean(i)=LSMean(j)								
Dependent Variable: PTAMidPoint								
i/j	1	2	3	4	5	6	7	8
1		<.0001	0.0003	<.0001	<.0001	<.0001	<.0001	<.0001
2	<.0001		0.4040	0.9936	0.6515	0.8273	0.9143	0.9998
3	0.0003	0.4040		0.8250	0.0195	0.9933	0.0548	0.6459
4	<.0001	0.9936	0.8250		0.2578	0.9962	0.5281	1.0000
5	<.0001	0.6515	0.0195	0.2578		0.0811	0.9991	0.4090
6	<.0001	0.8273	0.9933	0.9962	0.0811		0.2053	0.9650
7	<.0001	0.9143	0.0548	0.5281	0.9991	0.2053		0.7209
8	<.0001	0.9998	0.6459	1.0000	0.4090	0.9650	0.7209	

Tukey Grouping for LS-Means of Starch*Sugar					
LS-means with the same letter are not significantly different.					
	PTAMidPoint LSMEAN	Starch	Sugar	LSMEAN Number	
A	55.40000	C	0	1	
B	46.88333	C	4	3	
B					
C B	45.83333	W	12	6	
C B					
C B	44.87667	C	8	4	
C B					
C B	44.42000	W	8	8	
C B					
C B	43.83333	C	12	2	
C B					
C B	42.13667	W	4	7	
C					
C	41.38333	W	0	5	

Table A7– SAS output for two-way ANOVA and Tukey’s test for PTA Flow

Temperature data (see next page)

The GLM Procedure

Class Level Information		
Class	Levels	Values
Starch	2	C W
Sugar	4	0 12 4 8

Number of Observations Read	22
Number of Observations Used	22

The GLM Procedure

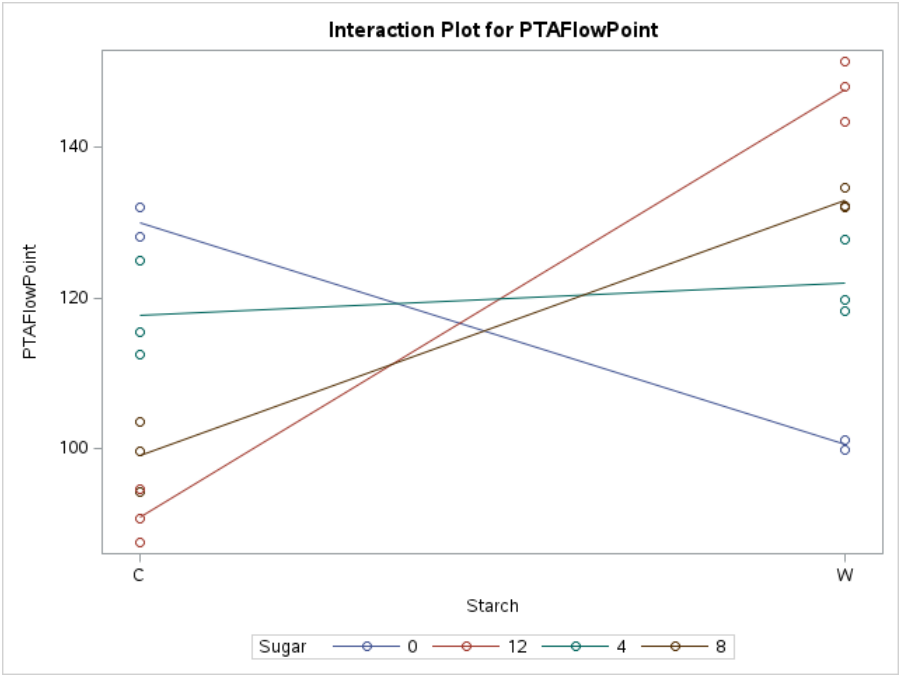
Dependent Variable: PTAFlowPoint

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	7478.274695	1068.324956	59.83	<.0001
Error	14	249.975800	17.855414		
Corrected Total	21	7728.250495			

R-Square	Coeff Var	Root MSE	PTAFlowPoint Mean
0.967654	3.586750	4.225567	117.8105

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Starch	1	2298.886914	2298.886914	128.75	<.0001
Sugar	3	78.971845	26.323948	1.47	0.2643
Starch*Sugar	3	5100.415936	1700.138645	95.22	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Starch	1	1414.696059	1414.696059	79.23	<.0001
Sugar	3	78.971845	26.323948	1.47	0.2643
Starch*Sugar	3	5100.415936	1700.138645	95.22	<.0001



The GLM Procedure

Tukey's Studentized Range (HSD) Test for PTAFlowPoint

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	14
Error Mean Square	17.85541
Critical Value of Studentized Range	3.03319
Minimum Significant Difference	3.8645

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	Starch
A	128.033	11	W

Means with the same letter are not significantly different.			
B	Tukey Grouping	109.456	Starch

The GLM Procedure

Tukey's Studentized Range (HSD) Test for PTAFlowPoint

Note: This test controls the Type I experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	14
Error Mean Square	17.85541
Critical Value of Studentized Range	4.11036

Comparisons significant at the 0.05 level are indicated by ***.			
Sugar Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
4 - 12	0.495	-6.596	7.586
4 - 8	3.668	-3.422	10.759
4 - 0	4.487	-3.441	12.414
12 - 4	-0.495	-7.586	6.596
12 - 8	3.173	-3.917	10.264
12 - 0	3.992	-3.936	11.919
8 - 4	-3.668	-10.759	3.422
8 - 12	-3.173	-10.264	3.917
8 - 0	0.818	-7.109	8.746
0 - 4	-4.487	-12.414	3.441
0 - 12	-3.992	-11.919	3.936
0 - 8	-0.818	-8.746	7.109

The GLM Procedure

Level of Starch	Level of Sugar	N	PTAFlowPoint	
			Mean	Std Dev
C	0	2	130.000000	2.68700577
C	12	3	91.000000	3.55949435
C	4	3	117.613333	6.49909481
C	8	3	99.210000	4.65940983
W	0	2	100.550000	0.91923882
W	12	3	147.533333	4.02036483
W	4	3	121.910000	5.10336164
W	8	3	132.976667	1.45925780

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

Starch	PTAFlowPoint LSMEAN	Standard Error	H0:LSMEAN=0	H0:LSMean1=LSMean2
			Pr > t	Pr > t
C	109.455833	1.293810	<.0001	<.0001
W	125.742500	1.293810	<.0001	

Tukey Grouping for LS-Means of Starch			
LS-means with the same letter are not significantly different.			
	PTAFlowPoint LSMEAN	Starch	LSMEAN Number
A	125.743	W	2
B	109.456	C	1

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey-Kramer

Sugar	PTAFlowPoint LSMEAN	Standard Error	Pr > t	LSMEAN Number
0	115.275000	2.112783	<.0001	1
12	119.266667	1.725080	<.0001	2

Sugar	PTAFlowPoint LSMEAN	Standard Error	Pr > t	LSMEAN Number
4	119.761667	1.725080	<.0001	3
8	116.093333	1.725080	<.0001	4

Least Squares Means for effect Sugar Pr > t for H0: LSMean(i)=LSMean(j)				
Dependent Variable: PTAFlowPoint				
i/j	1	2	3	4
1		0.4836	0.3869	0.9902
2	0.4836		0.9969	0.5773
3	0.3869	0.9969		0.4613
4	0.9902	0.5773	0.4613	

Tukey-Kramer Grouping for LS-Means of Sugar			
LS-means with the same letter are not significantly different.			
	PTAFlowPoint LSMEAN	Sugar	LSMEAN Number
A	119.762	4	3
A			
A	119.267	12	2
A			
A	116.093	8	4
A			
A	115.275	0	1

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey-Kramer

Starch	Sugar	PTAFlowPoint LSMEAN	Standard Error	Pr > t	LSMEAN Number
C	0	130.000000	2.987927	<.0001	1
C	12	91.000000	2.439632	<.0001	2
C	4	117.613333	2.439632	<.0001	3
C	8	99.210000	2.439632	<.0001	4
W	0	100.550000	2.987927	<.0001	5
W	12	147.533333	2.439632	<.0001	6
W	4	121.910000	2.439632	<.0001	7
W	8	132.976667	2.439632	<.0001	8

Least Squares Means for effect Starch*Sugar Pr > t for H0: LSMean(i)=LSMean(j)								
Dependent Variable: PTAFlowPoint								
i/j	1	2	3	4	5	6	7	8
1		<.0001	0.0869	<.0001	0.0001	0.0081	0.4601	0.9921
2	<.0001		<.0001	0.3203	0.2799	<.0001	<.0001	<.0001
3	0.0869	<.0001		0.0020	0.0100	<.0001	0.9043	0.0095
4	<.0001	0.3203	0.0020		1.0000	<.0001	0.0002	<.0001
5	0.0001	0.2799	0.0100	1.0000		<.0001	0.0014	<.0001
6	0.0081	<.0001	<.0001	<.0001	<.0001		<.0001	0.0145
7	0.4601	<.0001	0.9043	0.0002	0.0014	<.0001		0.0874
8	0.9921	<.0001	0.0095	<.0001	<.0001	0.0145	0.0874	

Tukey-Kramer Grouping for LS-Means of Starch*Sugar				
LS-means with the same letter are not significantly different.				
	PTAFlowPoint LSMEAN	Starch	Sugar	LSMEAN Number
A	147.533	W	12	6
B	132.977	W	8	8
B				
C B	130.000	C	0	1
C B				
C B	121.910	W	4	7
C				
C	117.613	C	4	3
D	100.550	W	0	5
D				
D	99.210	C	8	4
D				

Tukey-Kramer Grouping for LS-Means of Starch*Sugar					
LS-means with the same letter are not significantly different.					
		PTAFlowPoint LSMEAN	Starch	Sugar	LSMEAN Number
	D	91.000	C	12	2

Table A8 – SAS output for two-way ANOVA and Tukey’s test for DSC Raw

Material ΔH data (see next page)

The GLM Procedure

Class Level Information		
Class	Levels	Values
Starch	2	C W
Sugar	4	0 12 4 8

Number of Observations Read	15
Number of Observations Used	15

The GLM Procedure

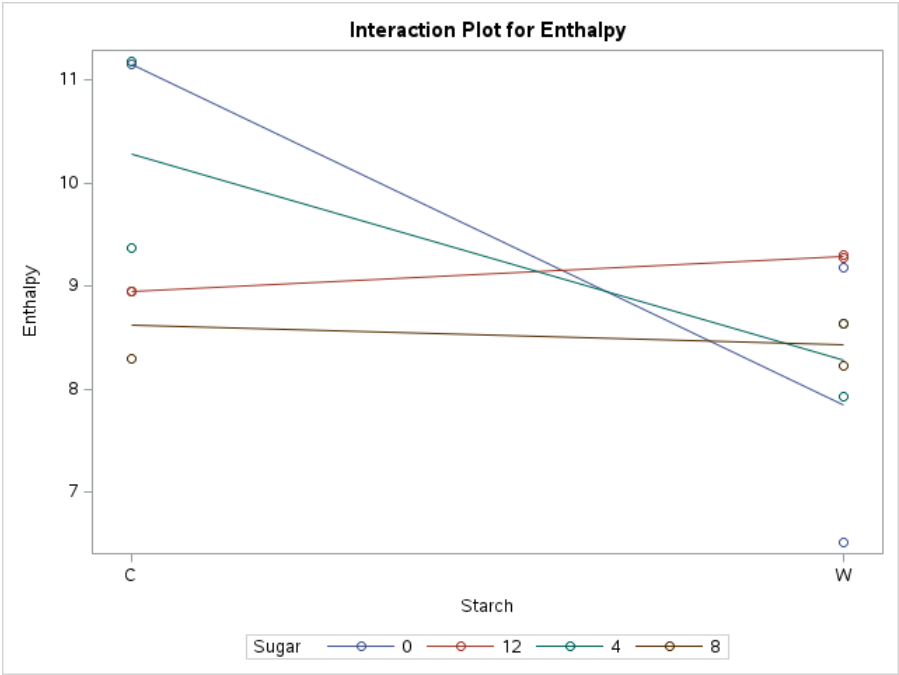
Dependent Variable: Enthalpy

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	12.63880873	1.80554410	2.20	0.1597
Error	7	5.73997700	0.81999671		
Corrected Total	14	18.37878573			

R-Square	Coeff Var	Root MSE	Enthalpy Mean
0.687685	10.09127	0.905537	8.973467

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Starch	1	4.38644214	4.38644214	5.35	0.0540
Sugar	3	1.30842045	0.43614015	0.53	0.6748
Starch*Sugar	3	6.94394614	2.31464871	2.82	0.1166

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Starch	1	5.85960556	5.85960556	7.15	0.0319
Sugar	3	1.84349141	0.61449714	0.75	0.5564
Starch*Sugar	3	6.94394614	2.31464871	2.82	0.1166



The GLM Procedure

Tukey's Studentized Range (HSD) Test for Enthalpy

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	7
Error Mean Square	0.819997
Critical Value of Studentized Range	3.34401
Minimum Significant Difference	1.1082
Harmonic Mean of Cell Sizes	7.466667

Note: Cell sizes are not equal.

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	Starch
A	9.5516	7	C
A			
A	8.4676	8	W

The GLM Procedure

Tukey's Studentized Range (HSD) Test for Enthalpy

Note: This test controls the Type I experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	7
Error Mean Square	0.819997
Critical Value of Studentized Range	4.68121

Comparisons significant at the 0.05 level are indicated by ***.			
Sugar Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
4 - 12	0.1608	-1.9588	2.2803
4 - 0	0.3338	-1.9555	2.6232
4 - 8	0.7515	-1.3680	2.8710
12 - 4	-0.1608	-2.2803	1.9588
12 - 0	0.1731	-2.1162	2.4624
12 - 8	0.5907	-1.5288	2.7103
0 - 4	-0.3338	-2.6232	1.9555
0 - 12	-0.1731	-2.4624	2.1162
0 - 8	0.4177	-1.8717	2.7070
8 - 4	-0.7515	-2.8710	1.3680
8 - 12	-0.5907	-2.7103	1.5288
8 - 0	-0.4177	-2.7070	1.8717

The GLM Procedure

Level of Starch	Level of Sugar	N	Enthalpy	
			Mean	Std Dev
C	0	1	11.1500000	.
C	12	2	8.9505000	0.00070711
C	4	2	10.2795000	1.27491353
C	8	2	8.6255000	0.45891230
W	0	2	7.8495000	1.88868221
W	12	2	9.2950000	0.02121320
W	4	2	8.2875000	0.50275292
W	8	2	8.4385000	0.28920667

The GLM Procedure

Least Squares Means

Adjustment for Multiple Comparisons: Tukey-Kramer

Starch	Enthalpy LSMEAN	Standard Error	H0:LSMEAN=0	H0:LSMean1=LSMean2
			Pr > t	Pr > t
C	9.75137500	0.35794481	<.0001	0.0319
W	8.46762500	0.32015557	<.0001	

Tukey-Kramer Grouping for LS-Means of Starch			
LS-means with the same letter are not significantly different.			
	Enthalpy LSMEAN	Starch	LSMEAN Number
A	9.751375	C	1
B	8.467625	W	2

The GLM Procedure

Least Squares Means

Adjustment for Multiple Comparisons: Tukey-Kramer

Sugar	Enthalpy LSMEAN	Standard Error	Pr > t	LSMEAN Number
0	9.49975000	0.55452571	<.0001	1
12	9.12275000	0.45276835	<.0001	2
4	9.28350000	0.45276835	<.0001	3
8	8.53200000	0.45276835	<.0001	4

Least Squares Means for effect Sugar Pr > t for H0: LSMean(i)=LSMean(j)				
Dependent Variable: Enthalpy				
i/j	1	2	3	4
1		0.9499	0.9896	0.5628
2	0.9499		0.9939	0.7943
3	0.9896	0.9939		0.6604
4	0.5628	0.7943	0.6604	

Tukey-Kramer Grouping for LS-Means of Sugar			
LS-means with the same letter are not significantly different.			
	Enthalpy LSMEAN	Sugar	LSMEAN Number
A	9.49975	0	1
A			
A	9.28350	4	3
A			
A	9.12275	12	2
A			
A	8.53200	8	4

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey-Kramer

Starch	Sugar	Enthalpy LSMEAN	Standard Error	Pr > t	LSMEAN Number
C	0	11.1500000	0.9055367	<.0001	1
C	12	8.9505000	0.6403111	<.0001	2
C	4	10.2795000	0.6403111	<.0001	3
C	8	8.6255000	0.6403111	<.0001	4
W	0	7.8495000	0.6403111	<.0001	5
W	12	9.2950000	0.6403111	<.0001	6
W	4	8.2875000	0.6403111	<.0001	7
W	8	8.4385000	0.6403111	<.0001	8

Least Squares Means for effect Starch*Sugar Pr > t for H0: LSMean(i)=LSMean(j)								
Dependent Variable: Enthalpy								
i/j	1	2	3	4	5	6	7	8
1		0.5459	0.9887	0.4105	0.1884	0.7040	0.2959	0.3435
2	0.5459		0.8036	0.9999	0.9037	0.9999	0.9923	0.9983
3	0.9887	0.8036		0.6251	0.2638	0.9409	0.4437	0.5215
4	0.4105	0.9999	0.6251		0.9818	0.9919	0.9999	1.0000
5	0.1884	0.9037	0.2638	0.9818		0.7422	0.9994	0.9961
6	0.7040	0.9999	0.9409	0.9919	0.7422		0.9344	0.9697
7	0.2959	0.9923	0.4437	0.9999	0.9994	0.9344		1.0000
8	0.3435	0.9983	0.5215	1.0000	0.9961	0.9697	1.0000	

Tukey-Kramer Grouping for LS-Means of Starch*Sugar				
LS-means with the same letter are not significantly different.				
	Enthalpy LSMEAN	Starch	Sugar	LSMEAN Number
A	11.1500	C	0	1
A				
A	10.2795	C	4	3
A				
A	9.2950	W	12	6
A				
A	8.9505	C	12	2
A				
A	8.6255	C	8	4
A				
A	8.4385	W	8	8
A				
A	8.2875	W	4	7
A				

Tukey-Kramer Grouping for LS-Means of Starch*Sugar				
LS-means with the same letter are not significantly different.				
	Enthalpy LSMEAN	Starch	Sugar	LSMEAN Number
A	7.8495	W	0	5

**Table A9 – SAS output for two-way ANOVA and Tukey’s test for DSC Raw
Material Onset Temperature data (see next page)**

The GLM Procedure

Class Level Information		
Class	Levels	Values
Starch	2	C W
Sugar	4	0 12 4 8

Number of Observations Read	22
Number of Observations Used	22

The GLM Procedure

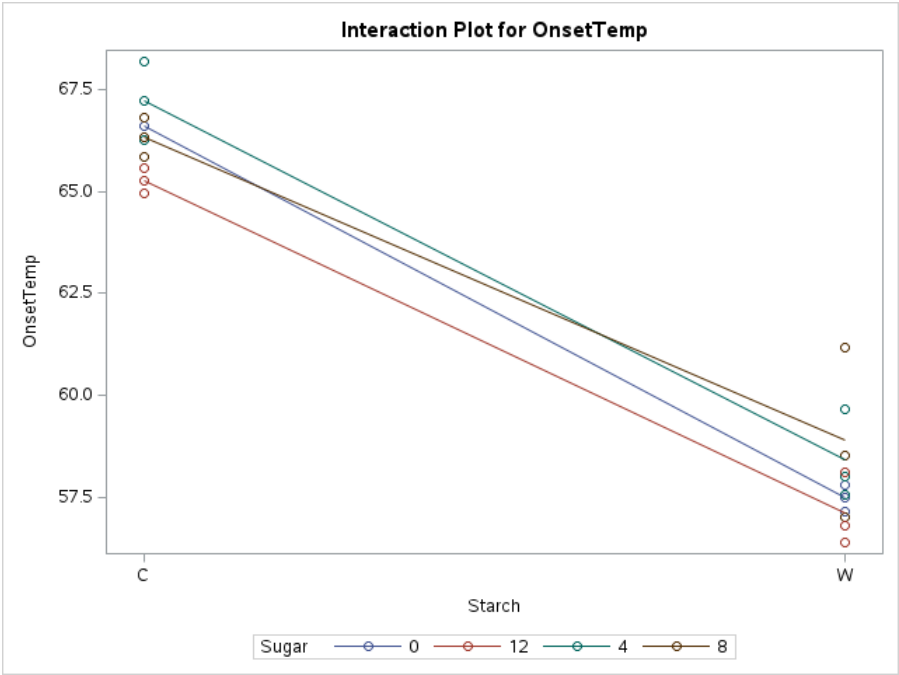
Dependent Variable: OnsetTemp

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	388.6519767	55.5217110	49.80	<.0001
Error	14	15.6085941	1.1148996		
Corrected Total	21	404.2605707			

R-Square	Coeff Var	Root MSE	OnsetTemp Mean
0.961390	1.709552	1.055888	61.76402

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Starch	1	376.7173674	376.7173674	337.89	<.0001
Sugar	3	9.8799594	3.2933198	2.95	0.0690
Starch*Sugar	3	2.0546499	0.6848833	0.61	0.6170

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Starch	1	335.2844792	335.2844792	300.73	<.0001
Sugar	3	9.4954776	3.1651592	2.84	0.0760
Starch*Sugar	3	2.0546499	0.6848833	0.61	0.6170



The GLM Procedure

Tukey's Studentized Range (HSD) Test for OnsetTemp

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	14
Error Mean Square	1.1149
Critical Value of Studentized Range	3.03319
Minimum Significant Difference	0.9697
Harmonic Mean of Cell Sizes	10.90909

Note: Cell sizes are not equal.

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	Starch
A	66.2970	10	C
B	57.9865	12	W

The GLM Procedure

Tukey's Studentized Range (HSD) Test for OnsetTemp

Note: This test controls the Type I experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	14
Error Mean Square	1.1149
Critical Value of Studentized Range	4.11036

Comparisons significant at the 0.05 level are indicated by ***.				
Sugar Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
4 - 8	0.1959	-1.5759	1.9677	
4 - 12	1.6230	-0.1489	3.3948	
4 - 0	3.0526	1.0716	5.0336	***
8 - 4	-0.1959	-1.9677	1.5759	
8 - 12	1.4271	-0.3448	3.1989	
8 - 0	2.8567	0.8757	4.8377	***
12 - 4	-1.6230	-3.3948	0.1489	
12 - 8	-1.4271	-3.1989	0.3448	
12 - 0	1.4296	-0.5513	3.4106	
0 - 4	-3.0526	-5.0336	-1.0716	***
0 - 8	-2.8567	-4.8377	-0.8757	***
0 - 12	-1.4296	-3.4106	0.5513	

The GLM Procedure

Level of Starch	Level of Sugar	N	OnsetTemp	
			Mean	Std Dev
C	0	1	66.5800000	.
C	12	3	65.2638889	0.30092593
C	4	3	67.2107843	0.96568627
C	8	3	66.3221154	0.46634615
W	0	3	57.4900000	0.34000000
W	12	3	57.1203704	0.89627286
W	4	3	58.4194118	1.10830570
W	8	3	58.9162582	2.10153880

The GLM Procedure

Least Squares Means

Adjustment for Multiple Comparisons: Tukey-Kramer

Starch	OnsetTemp LSMEAN	Standard Error	H0:LSMEAN=0	H0:LSMean1=LSMean2
			Pr > t	Pr > t
C	66.3441971	0.3733128	<.0001	<.0001
W	57.9865101	0.3048086	<.0001	

Tukey-Kramer Grouping for LS-Means of Starch			
LS-means with the same letter are not significantly different.			
	OnsetTemp LSMEAN	Starch	LSMEAN Number
A	66.34420	C	1
B	57.98651	W	2

The GLM Procedure

Least Squares Means

Adjustment for Multiple Comparisons: Tukey-Kramer

Sugar	OnsetTemp LSMEAN	Standard Error	Pr > t	LSMEAN Number
0	62.0350000	0.6096173	<.0001	1
12	61.1921296	0.4310645	<.0001	2
4	62.8150980	0.4310645	<.0001	3
8	62.6191868	0.4310645	<.0001	4

Least Squares Means for effect Sugar Pr > t for H0: LSMean(i)=LSMean(j)				
Dependent Variable: OnsetTemp				
i/j	1	2	3	4
1		0.6785	0.7269	0.8612
2	0.6785		0.0778	0.1356
3	0.7269	0.0778		0.9880
4	0.8612	0.1356	0.9880	

Tukey-Kramer Grouping for LS-Means of Sugar			
LS-means with the same letter are not significantly different.			
	OnsetTemp LSMEAN	Sugar	LSMEAN Number
A	62.81510	4	3
A			
A	62.61919	8	4
A			
A	62.03500	0	1
A			
A	61.19213	12	2

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey-Kramer

Starch	Sugar	OnsetTemp LSMEAN	Standard Error	Pr > t	LSMEAN Number
C	0	66.5800000	1.0558881	<.0001	1
C	12	65.2638889	0.6096173	<.0001	2
C	4	67.2107843	0.6096173	<.0001	3
C	8	66.3221154	0.6096173	<.0001	4
W	0	57.4900000	0.6096173	<.0001	5
W	12	57.1203704	0.6096173	<.0001	6
W	4	58.4194118	0.6096173	<.0001	7
W	8	58.9162582	0.6096173	<.0001	8

Least Squares Means for effect Starch*Sugar Pr > t for H0: LSMean(i)=LSMean(j)								
Dependent Variable: OnsetTemp								
i/j	1	2	3	4	5	6	7	8
1		0.9512	0.9993	1.0000	<.0001	<.0001	0.0002	0.0004
2	0.9512		0.3768	0.9103	<.0001	<.0001	<.0001	<.0001
3	0.9993	0.3768		0.9613	<.0001	<.0001	<.0001	<.0001
4	1.0000	0.9103	0.9613		<.0001	<.0001	<.0001	<.0001
5	<.0001	<.0001	<.0001	<.0001		0.9998	0.9515	0.7135
6	<.0001	<.0001	<.0001	<.0001	0.9998		0.7924	0.4678
7	0.0002	<.0001	<.0001	<.0001	0.9515	0.7924		0.9987
8	0.0004	<.0001	<.0001	<.0001	0.7135	0.4678	0.9987	

Tukey-Kramer Grouping for LS-Means of Starch*Sugar				
LS-means with the same letter are not significantly different.				
	OnsetTemp LSMEAN	Starch	Sugar	LSMEAN Number
A	67.21078	C	4	3
A				
A	66.58000	C	0	1
A				
A	66.32212	C	8	4
A				
A	65.26389	C	12	2
B	58.91626	W	8	8
B				
B	58.41941	W	4	7
B				
B	57.49000	W	0	5
B				

Tukey-Kramer Grouping for LS-Means of Starch*Sugar				
LS-means with the same letter are not significantly different.				
	OnsetTemp LSMEAN	Starch	Sugar	LSMEAN Number
B	57.12037	W	12	6

**Table A9 – SAS output for two-way ANOVA and Tukey’s test for DSC Raw
Material Peak Gelatinization data (see next page)**

The GLM Procedure

Class Level Information		
Class	Levels	Values
Starch	2	C W
Sugar	4	0 12 4 8

Number of Observations Read	15
Number of Observations Used	15

The GLM Procedure

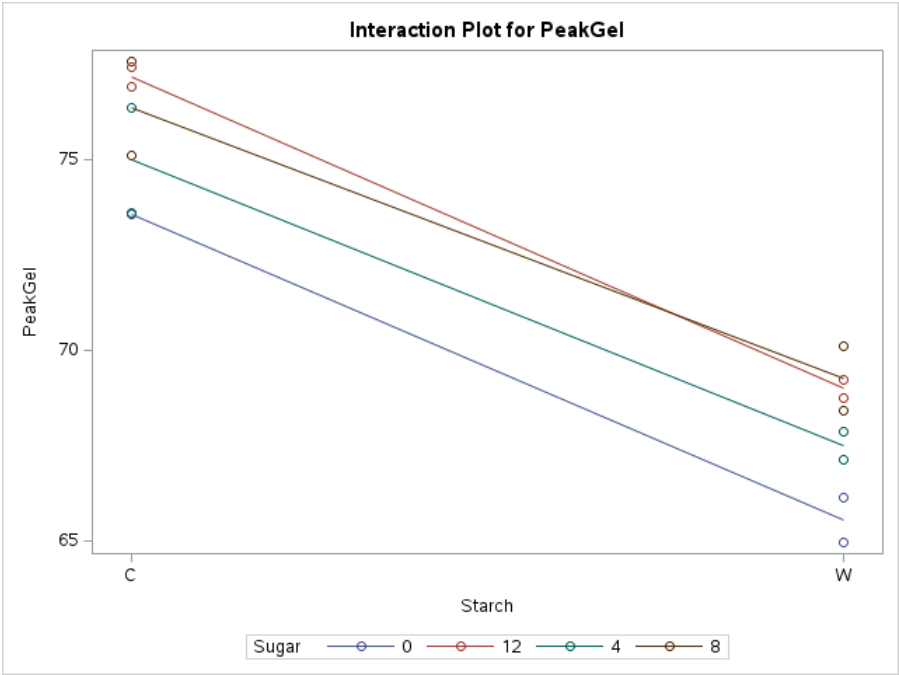
Dependent Variable: PeakGel

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	265.0878400	37.8696914	27.81	0.0001
Error	7	9.5334000	1.3619143		
Corrected Total	14	274.6212400			

R-Square	Coeff Var	Root MSE	PeakGel Mean
0.965285	1.631225	1.167011	71.54200

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Starch	1	236.8472400	236.8472400	173.91	<.0001
Sugar	3	27.5148364	9.1716121	6.73	0.0180
Starch*Sugar	3	0.7257636	0.2419212	0.18	0.9082

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Starch	1	209.9883556	209.9883556	154.19	<.0001
Sugar	3	25.3306909	8.4435636	6.20	0.0221
Starch*Sugar	3	0.7257636	0.2419212	0.18	0.9082



The GLM Procedure

Tukey's Studentized Range (HSD) Test for PeakGel

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	7
Error Mean Square	1.361914
Critical Value of Studentized Range	3.34401
Minimum Significant Difference	1.4282
Harmonic Mean of Cell Sizes	7.466667

Note: Cell sizes are not equal.

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	Starch
A	75.7900	7	C
B	67.8250	8	W

The GLM Procedure

Tukey’s Studentized Range (HSD) Test for PeakGel

Note: This test controls the Type I experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	7
Error Mean Square	1.361914
Critical Value of Studentized Range	4.68121

Comparisons significant at the 0.05 level are indicated by ***.				
Sugar Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
12 - 8	0.2700	-2.4615	3.0015	
12 - 4	1.8400	-0.8915	4.5715	
12 - 0	4.8642	1.9138	7.8145	***
8 - 12	-0.2700	-3.0015	2.4615	
8 - 4	1.5700	-1.1615	4.3015	
8 - 0	4.5942	1.6438	7.5445	***
4 - 12	-1.8400	-4.5715	0.8915	
4 - 8	-1.5700	-4.3015	1.1615	
4 - 0	3.0242	0.0738	5.9745	***
0 - 12	-4.8642	-7.8145	-1.9138	***
0 - 8	-4.5942	-7.5445	-1.6438	***
0 - 4	-3.0242	-5.9745	-0.0738	***

The GLM Procedure

Level of Starch	Level of Sugar	N	PeakGel	
			Mean	Std Dev
C	0	1	73.5500000	.
C	12	2	77.1650000	0.37476659
C	4	2	74.9850000	1.95868578
C	8	2	76.3400000	1.73948268
W	0	2	65.5450000	0.84145707
W	12	2	68.9900000	0.32526912
W	4	2	67.4900000	0.53740115
W	8	2	69.2750000	1.19501046

The GLM Procedure

Least Squares Means

Adjustment for Multiple Comparisons: Tukey-Kramer

Starch	PeakGel LSMEAN	Standard Error	H0:LSMEAN=0	H0:LSMean1=LSMean2
			Pr > t	Pr > t
C	75.5100000	0.4613015	<.0001	<.0001
W	67.8250000	0.4126006	<.0001	

Tukey-Kramer Grouping for LS-Means of Starch			
LS-means with the same letter are not significantly different.			
	PeakGel LSMEAN	Starch	LSMEAN Number
A	75.510	C	1
B	67.825	W	2

The GLM Procedure

Least Squares Means

Adjustment for Multiple Comparisons: Tukey-Kramer

Sugar	PeakGel LSMEAN	Standard Error	Pr > t	LSMEAN Number
0	69.5475000	0.7146453	<.0001	1
12	73.0775000	0.5835054	<.0001	2
4	71.2375000	0.5835054	<.0001	3
8	72.8075000	0.5835054	<.0001	4

Least Squares Means for effect Sugar Pr > t for H0: LSMean(i)=LSMean(j)				
Dependent Variable: PeakGel				
i/j	1	2	3	4
1		0.0260	0.3349	0.0376
2	0.0260		0.2047	0.9869
3	0.3349	0.2047		0.3077
4	0.0376	0.9869	0.3077	

Tukey-Kramer Grouping for LS-Means of Sugar			
LS-means with the same letter are not significantly different.			
	PeakGel LSMEAN	Sugar	LSMEAN Number
A	73.0775	12	2
A			
A	72.8075	8	4
A			
B A	71.2375	4	3
B			
B	69.5475	0	1

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey-Kramer

Starch	Sugar	PeakGel LSMEAN	Standard Error	Pr > t	LSMEAN Number
C	0	73.5500000	1.1670108	<.0001	1
C	12	77.1650000	0.8252013	<.0001	2
C	4	74.9850000	0.8252013	<.0001	3
C	8	76.3400000	0.8252013	<.0001	4
W	0	65.5450000	0.8252013	<.0001	5
W	12	68.9900000	0.8252013	<.0001	6
W	4	67.4900000	0.8252013	<.0001	7
W	8	69.2750000	0.8252013	<.0001	8

Least Squares Means for effect Starch*Sugar Pr > t for H0: LSMean(i)=LSMean(j)								
Dependent Variable: PeakGel								
i/j	1	2	3	4	5	6	7	8
1		0.3133	0.9593	0.5614	0.0101	0.1466	0.0432	0.1852
2	0.3133		0.6039	0.9937	0.0003	0.0028	0.0010	0.0034
3	0.9593	0.6039		0.9208	0.0011	0.0163	0.0046	0.0211
4	0.5614	0.9937	0.9208		0.0005	0.0052	0.0017	0.0065
5	0.0101	0.0003	0.0011	0.0005		0.1938	0.7070	0.1456
6	0.1466	0.0028	0.0163	0.0052	0.1938		0.8794	1.0000
7	0.0432	0.0010	0.0046	0.0017	0.7070	0.8794		0.7747
8	0.1852	0.0034	0.0211	0.0065	0.1456	1.0000	0.7747	

Tukey-Kramer Grouping for LS-Means of Starch*Sugar				
LS-means with the same letter are not significantly different.				
	PeakGel LSMEAN	Starch	Sugar	LSMEAN Number
A	77.165	C	12	2
A				
A	76.340	C	8	4
A				
A	74.985	C	4	3
A				
B A	73.550	C	0	1
B				
B C	69.275	W	8	8
B C				
B C	68.990	W	12	6
C				
C	67.490	W	4	7
C				

Tukey-Kramer Grouping for LS-Means of Starch*Sugar				
LS-means with the same letter are not significantly different.				
	PeakGel LSMEAN	Starch	Sugar	LSMEAN Number
C	65.545	W	0	5

Table A10 – SAS output for two-way ANOVA and Tukey’s test for DDR Sucrose data (see next page)

The GLM Procedure

Class Level Information		
Class	Levels	Values
Starch	2	Corn Wheat
SugarLevel	4	0% 12% 4% 8%

Number of Observations Read	32
Number of Observations Used	32

The GLM Procedure

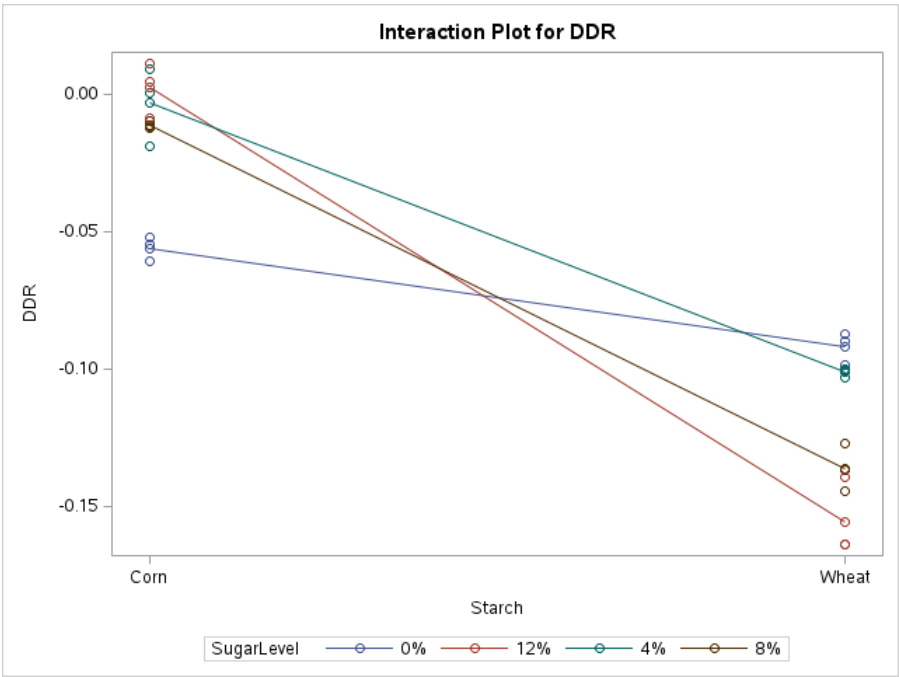
Dependent Variable: DDR

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	0.10608333	0.01515476	280.66	<.0001
Error	24	0.00129592	0.00005400		
Corrected Total	31	0.10737925			

R-Square	Coeff Var	Root MSE	DDR Mean
0.987931	-10.63559	0.007348	-0.069091

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Starch	1	0.08692927	0.08692927	1609.90	<.0001
SugarLevel	3	0.00312080	0.00104027	19.27	<.0001
Starch*SugarLevel	3	0.01603326	0.00534442	98.98	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Starch	1	0.08692927	0.08692927	1609.90	<.0001
SugarLevel	3	0.00312080	0.00104027	19.27	<.0001
Starch*SugarLevel	3	0.01603326	0.00534442	98.98	<.0001



The GLM Procedure

Tukey's Studentized Range (HSD) Test for DDR

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	24
Error Mean Square	0.000054
Critical Value of Studentized Range	2.91879
Minimum Significant Difference	0.0054

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	Starch
A	-0.016971	16	Corn

Means with the same letter are not significantly different.			
B	Tukey Grouping	-0.12121157	Wheat

The GLM Procedure

Tukey's Studentized Range (HSD) Test for DDR

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	24
Error Mean Square	0.000054
Critical Value of Studentized Range	3.90126
Minimum Significant Difference	0.0101

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	SugarLevel
A	-0.052108	8	4%
B	-0.073623	8	8%
B			
B	-0.073971	8	0%
B			
B	-0.076662	8	12%

The GLM Procedure

Level of Starch	Level of SugarLevel	N	DDR	
			Mean	Std Dev
Corn	0%	4	-0.05596489	0.00368720
Corn	12%	4	0.00239087	0.00832850
Corn	4%	4	-0.00307751	0.01172331
Corn	8%	4	-0.01123127	0.00126629
Wheat	0%	4	-0.09197803	0.00490742
Wheat	12%	4	-0.15571467	0.01149438
Wheat	4%	4	-0.10113849	0.00141030
Wheat	8%	4	-0.13601509	0.00719602

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

Starch	DDR LSMEAN	Standard Error	H0:LSMEAN=0	H0:LSMean1=LSMean2
			Pr > t	Pr > t
Corn	-0.01697070	0.00183706	<.0001	<.0001
Wheat	-0.12121157	0.00183706	<.0001	

Tukey Grouping for LS-Means of Starch			
LS-means with the same letter are not significantly different.			
	DDR LSMEAN	Starch	LSMEAN Number
A	-0.016970701	Corn	1
B	-0.121211570	Wheat	2

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

SugarLevel	DDR LSMEAN	Standard Error	Pr > t	LSMEAN Number
0%	-0.07397146	0.00259800	<.0001	1
12%	-0.07666190	0.00259800	<.0001	2
4%	-0.05210800	0.00259800	<.0001	3
8%	-0.07362318	0.00259800	<.0001	4

Least Squares Means for effect SugarLevel				
Pr > t for H0: LSMean(i)=LSMean(j)				
Dependent Variable: DDR				
ij	1	2	3	4
1		0.8832	<.0001	0.9997
2	0.8832		<.0001	0.8411
3	<.0001	<.0001		<.0001
4	0.9997	0.8411	<.0001	

Tukey Grouping for LS-Means of SugarLevel			
LS-means with the same letter are not significantly different.			
	DDR LSMEAN	SugarLevel	LSMEAN Number
A	-0.052108000	4%	3
B	-0.073623181	8%	4
B			
B	-0.073971461	0%	1
B			
B	-0.076661900	12%	2

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

Starch	SugarLevel	DDR LSMEAN	Standard Error	Pr > t	LSMEAN Number
Corn	0%	-0.05596489	0.00367412	<.0001	1
Corn	12%	0.00239087	0.00367412	0.5214	2
Corn	4%	-0.00307751	0.00367412	0.4105	3
Corn	8%	-0.01123127	0.00367412	0.0054	4
Wheat	0%	-0.09197803	0.00367412	<.0001	5
Wheat	12%	-0.15571467	0.00367412	<.0001	6
Wheat	4%	-0.10113849	0.00367412	<.0001	7
Wheat	8%	-0.13601509	0.00367412	<.0001	8

Least Squares Means for effect Starch*SugarLevel								
Pr > t for H0: LSMean(i)=LSMean(j)								
Dependent Variable: DDR								
ij	1	2	3	4	5	6	7	8
1		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
2	<.0001		0.9607	0.1965	<.0001	<.0001	<.0001	<.0001
3	<.0001	0.9607		0.7630	<.0001	<.0001	<.0001	<.0001
4	<.0001	0.1965	0.7630		<.0001	<.0001	<.0001	<.0001
5	<.0001	<.0001	<.0001	<.0001		<.0001	0.6489	<.0001
6	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001	0.0171
7	<.0001	<.0001	<.0001	<.0001	0.6489	<.0001		<.0001
8	<.0001	<.0001	<.0001	<.0001	<.0001	0.0171	<.0001	

Tukey Grouping for LS-Means of Starch*SugarLevel				
LS-means with the same letter are not significantly different.				
	DDR LSMEAN	Starch	SugarLevel	LSMEAN Number
A	0.0023908688	Corn	12%	2
A				
A	-0.0030775113	Corn	4%	3
A				
A	-0.0112312718	Corn	8%	4
B	-0.0559648890	Corn	0%	1
C	-0.0919780338	Wheat	0%	5
C				
C	-0.1011384890	Wheat	4%	7
D	-0.1360150903	Wheat	8%	8
E	-0.1557146683	Wheat	12%	6

Table A11 – SAS output for two-way ANOVA and Tukey’s test for DDR Moisture data (see next page)

The GLM Procedure

Class Level Information		
Class	Levels	Values
Starch	2	Corn Wheat
MoistureLevel	4	50% 54% 58% 62%

Number of Observations Read	32
Number of Observations Used	32

The GLM Procedure

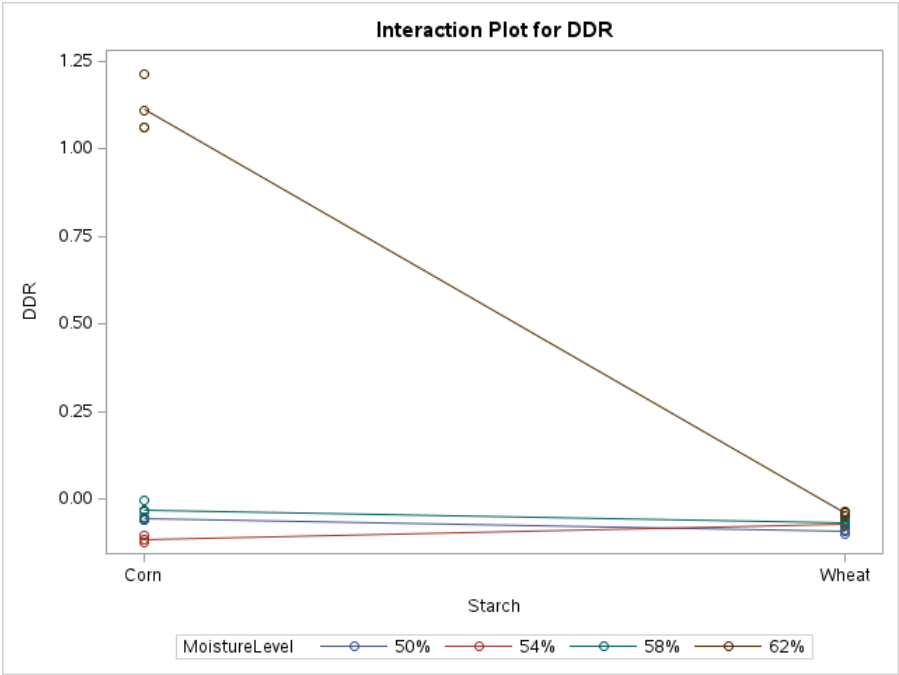
Dependent Variable: DDR

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	4.88667136	0.69809591	958.16	<.0001
Error	24	0.01748583	0.00072858		
Corrected Total	31	4.90415719			

R-Square	Coeff Var	Root MSE	DDR Mean
0.996434	33.80571	0.026992	0.079845

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Starch	1	0.69706978	0.69706978	956.76	<.0001
MoistureLevel	3	2.22404333	0.74134778	1017.53	<.0001
Starch*MoistureLevel	3	1.96555825	0.65518608	899.27	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Starch	1	0.69706978	0.69706978	956.76	<.0001
MoistureLevel	3	2.22404333	0.74134778	1017.53	<.0001
Starch*MoistureLevel	3	1.96555825	0.65518608	899.27	<.0001



The GLM Procedure

Tukey's Studentized Range (HSD) Test for DDR

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	24
Error Mean Square	0.000729
Critical Value of Studentized Range	2.91879
Minimum Significant Difference	0.0197

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	Starch
A	0.227437	16	Corn

Means with the same letter are not significantly different.			
B	Tukey Grouping	-0.06747	8

The GLM Procedure

Tukey's Studentized Range (HSD) Test for DDR

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	24
Error Mean Square	0.000729
Critical Value of Studentized Range	3.90126
Minimum Significant Difference	0.0372

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	MoistureLevel
A	0.53567	8	62%
B	-0.04913	8	58%
B			
C	-0.07397	8	50%
C			
C	-0.09319	8	54%

The GLM Procedure

Level of Starch	Level of MoistureLevel	N	DDR	
			Mean	Std Dev
Corn	50%	4	-0.05596601	0.00368720
Corn	54%	4	-0.11384889	0.00838738
Corn	58%	4	-0.03211241	0.02351738
Corn	62%	4	1.11167551	0.07127727
Wheat	50%	4	-0.09197884	0.00490742
Wheat	54%	4	-0.07252212	0.00335281
Wheat	58%	4	-0.06614277	0.00627681
Wheat	62%	4	-0.04034494	0.00603555

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

Starch	DDR LSMEAN	Standard Error	H0:LSMEAN=0	H0:LSMean1=LSMean2
			Pr > t	Pr > t
Corn	0.22743705	0.00674804	<.0001	<.0001
Wheat	-0.06774716	0.00674804	<.0001	

Tukey Grouping for LS-Means of Starch			
LS-means with the same letter are not significantly different.			
	DDR LSMEAN	Starch	LSMEAN Number
A	0.227437053	Corn	1
B	-0.067747165	Wheat	2

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

MoistureLevel	DDR LSMEAN	Standard Error	Pr > t	LSMEAN Number
50%	-0.07397242	0.00954317	<.0001	1
54%	-0.09318550	0.00954317	<.0001	2
58%	-0.04912759	0.00954317	<.0001	3
62%	0.53566529	0.00954317	<.0001	4

Least Squares Means for effect MoistureLevel				
Pr > t for H0: LSMean(i)=LSMean(j)				
Dependent Variable: DDR				
i/j	1	2	3	4
1		0.4975	0.2796	<.0001
2	0.4975		0.0162	<.0001
3	0.2796	0.0162		<.0001
4	<.0001	<.0001	<.0001	

Tukey Grouping for LS-Means of MoistureLevel			
LS-means with the same letter are not significantly different.			
	DDR LSMEAN	MoistureLevel	LSMEAN Number
A	0.535665287	62%	4
B	-0.049127589	58%	3
B			
C B	-0.073972421	50%	1
C			
C	-0.093185501	54%	2

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

Starch	MoistureLevel	DDR LSMEAN	Standard Error	Pr > t	LSMEAN Number
Corn	50%	-0.05596601	0.01349608	0.0004	1
Corn	54%	-0.11384889	0.01349608	<.0001	2
Corn	58%	-0.03211241	0.01349608	0.0256	3
Corn	62%	1.11167551	0.01349608	<.0001	4
Wheat	50%	-0.09197884	0.01349608	<.0001	5
Wheat	54%	-0.07252212	0.01349608	<.0001	6
Wheat	58%	-0.06614277	0.01349608	<.0001	7
Wheat	62%	-0.04034494	0.01349608	0.0064	8

Least Squares Means for effect Starch*MoistureLevel								
Pr > t for H0: LSMean(i)=LSMean(j)								
Dependent Variable: DDR								
i/j	1	2	3	4	5	6	7	8
1		0.0898	0.9081	<.0001	0.5719	0.9864	0.9993	0.9903
2	0.0898		0.0053	<.0001	0.9394	0.4052	0.2426	0.0149
3	0.9081	0.0053		<.0001	0.0725	0.4325	0.6365	0.9998
4	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001
5	0.5719	0.9394	0.0725	<.0001		0.9667	0.8687	0.1690
6	0.9864	0.4052	0.4325	<.0001	0.9667		1.0000	0.6957
7	0.9993	0.2426	0.6365	<.0001	0.8687	1.0000		0.8695
8	0.9903	0.0149	0.9998	<.0001	0.1690	0.6957	0.8695	

Tukey Grouping for LS-Means of Starch*MoistureLevel				
LS-means with the same letter are not significantly different.				
	DDR LSMEAN	Starch	MoistureLevel	LSMEAN Number
A	1.111675513	Corn	62%	4
B	-0.032112408	Corn	58%	3
B				
B	-0.040344939	Wheat	62%	8
B				
C B	-0.055966007	Corn	50%	1
C				
C B	-0.066142769	Wheat	58%	7
C				
C B	-0.072522116	Wheat	54%	6
C				
C B	-0.091978836	Wheat	50%	5
C				
C	-0.113848886	Corn	54%	2

Table A12 – SAS output for two-way ANOVA and Tukey’s test for RVA Peak
Viscosity Post-Printing data (see next page)

The GLM Procedure

Class Level Information		
Class	Levels	Values
Starch	1	C
Sugar	4	0 12 4 8

Number of Observations Read	12
Number of Observations Used	12

The GLM Procedure

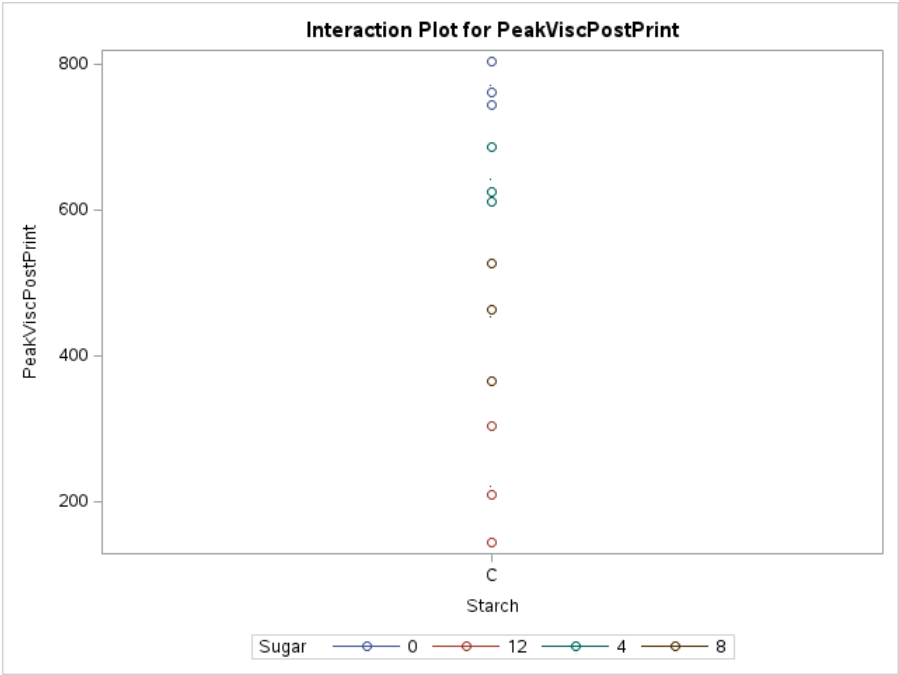
Dependent Variable: PeakViscPostPrint

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	518938.6667	172979.5556	44.19	<.0001
Error	8	31315.3333	3914.4167		
Corrected Total	11	550254.0000			

R-Square	Coeff Var	Root MSE	PeakViscPostPrint Mean
0.943089	12.03179	62.56530	520.0000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Starch	0	0.0000	.	.	.
Sugar	3	518938.6667	172979.5556	44.19	<.0001
Starch*Sugar	0	0.0000	.	.	.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Starch	0	0.0000	.	.	.
Sugar	3	518938.6667	172979.5556	44.19	<.0001
Starch*Sugar	0	0.0000	.	.	.



The GLM Procedure

Level of Starch	N	PeakViscPostPrint	
		Mean	Std Dev
C	12	520.000000	223.658425

Level of Sugar	N	PeakViscPostPrint	
		Mean	Std Dev
0	3	770.000000	30.7896086
12	3	218.000000	80.4673847
4	3	640.666667	40.6489032
8	3	451.333333	81.1315804

Level of Starch	Level of Sugar	N	PeakViscPostPrint	
			Mean	Std Dev
C	0	3	770.000000	30.7896086
C	12	3	218.000000	80.4673847
C	4	3	640.666667	40.6489032
C	8	3	451.333333	81.1315804

The GLM Procedure
Least Squares Means

Error: LINES display is not produced for effect Starch and dependent variable PeakViscPostPrint because p-values are not available for all least-squares means differences.

Starch	PeakViscPostPrint LSMEAN	Standard Error	Pr > t
C	520.000000	18.061046	<.0001

Error: LINES display is not produced for effect Starch and dependent variable PeakViscPostPrint because p-values are not available for all least-squares means differences.

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

Sugar	PeakViscPostPrint LSMEAN	Standard Error	Pr > t	LSMEAN Number
0	770.000000	36.122092	<.0001	1
12	218.000000	36.122092	0.0003	2
4	640.666667	36.122092	<.0001	3
8	451.333333	36.122092	<.0001	4

Least Squares Means for effect Sugar Pr > t for H0: LSMean(i)=LSMean(j)				
Dependent Variable: PeakViscPostPrint				
i/j	1	2	3	4
1		<.0001	0.1286	0.0011
2	<.0001		0.0002	0.0079
3	0.1286	0.0002		0.0248
4	0.0011	0.0079	0.0248	

Tukey Grouping for LS-Means of Sugar			
LS-means with the same letter are not significantly different.			
	PeakViscPostPrint LSMEAN	Sugar	LSMEAN Number
A	770.000	0	1
A			
A	640.667	4	3
B	451.333	8	4
C	218.000	12	2

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

Starch	Sugar	PeakViscPostPrint LSMEAN	Standard Error	Pr > t	LSMEAN Number
C	0	770.000000	36.122092	<.0001	1
C	12	218.000000	36.122092	0.0003	2
C	4	640.666667	36.122092	<.0001	3
C	8	451.333333	36.122092	<.0001	4

Least Squares Means for effect Starch*Sugar Pr > t for H0: LSMean(i)=LSMean(j)				
Dependent Variable: PeakViscPostPrint				
i/j	1	2	3	4
1		<.0001	0.1286	0.0011
2	<.0001		0.0002	0.0079
3	0.1286	0.0002		0.0248
4	0.0011	0.0079	0.0248	

Tukey Grouping for LS-Means of Starch*Sugar			
LS-means with the same letter are not significantly different.			
	PeakViscPostPrint LSMEAN	Starch	Sugar
			LSMEAN Number

Tukey Grouping for LS-Means of Starch*Sugar				
LS-means with the same letter are not significantly different.				
	PeakViscPostPrint LSMEAN	Starch	Sugar	LSMEAN Number
A	770.000	C	0	1
A				
A	640.667	C	4	3
B	451.333	C	8	4
C	218.000	C	12	2

Table A13 – SAS output for two-way ANOVA and Tukey’s test for RVA Pasting

Temperature Post-Printing data (see next page)

The GLM Procedure

Class Level Information		
Class	Levels	Values
Starch	1	C
Sugar	4	0 12 4 8

Number of Observations Read	12
Number of Observations Used	12

The GLM Procedure

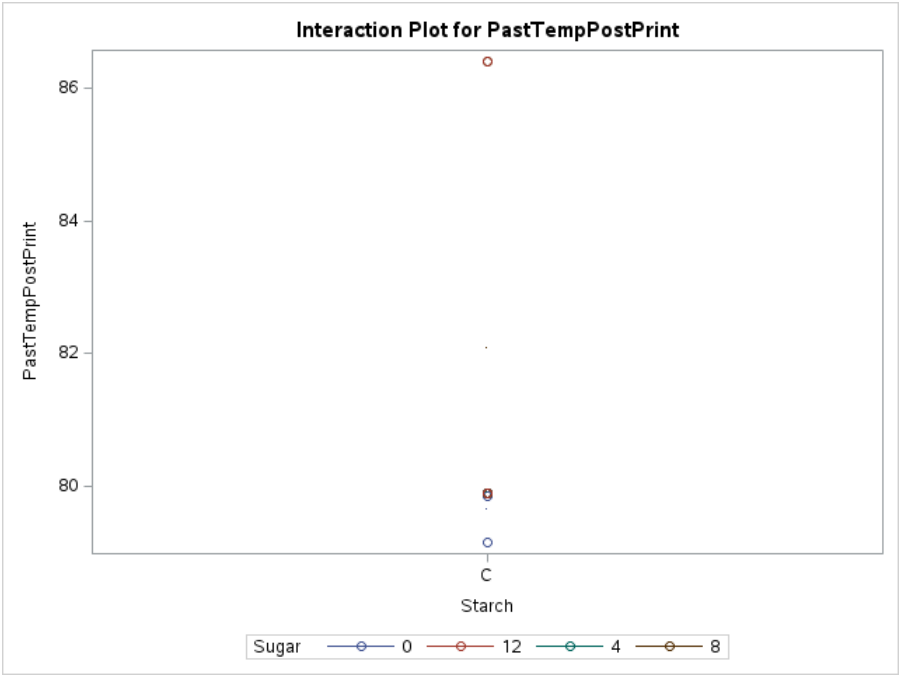
Dependent Variable: PastTempPostPrint

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	15.97666667	5.32555556	0.75	0.5515
Error	8	56.68500000	7.08562500		
Corrected Total	11	72.66166667			

R-Square	Coeff Var	Root MSE	PastTempPostPrint Mean
0.219878	3.289661	2.661884	80.91667

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Starch	0	0.00000000	.	.	.
Sugar	3	15.97666667	5.32555556	0.75	0.5515
Starch*Sugar	0	0.00000000	.	.	.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Starch	0	0.00000000	.	.	.
Sugar	3	15.97666667	5.32555556	0.75	0.5515
Starch*Sugar	0	0.00000000	.	.	.



The GLM Procedure

Level of Starch	N	PastTempPostPrint	
		Mean	Std Dev
C	12	80.9166667	2.57013736

Level of Sugar	N	PastTempPostPrint	
		Mean	Std Dev
0	3	79.6333333	0.41932485
12	3	82.0666667	3.75277675
4	3	79.9000000	0.00000000
8	3	82.0666667	3.75277675

Level of Starch	Level of Sugar	N	PastTempPostPrint	
			Mean	Std Dev
C	0	3	79.6333333	0.41932485
C	12	3	82.0666667	3.75277675
C	4	3	79.9000000	0.00000000
C	8	3	82.0666667	3.75277675

The GLM Procedure
Least Squares Means

Error: LINES display is not produced for effect Starch and dependent variable PastTempPostPrint because p-values are not available for all least-squares means differences.

Starch	PastTempPostPrint LSMEAN	Standard Error	Pr > t
C	80.9166667	0.7684196	<.0001

Error: LINES display is not produced for effect Starch and dependent variable PastTempPostPrint because p-values are not available for all least-squares means differences.

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

Sugar	PastTempPostPrint LSMEAN	Standard Error	Pr > t	LSMEAN Number
0	79.6333333	1.5368393	<.0001	1
12	82.0666667	1.5368393	<.0001	2
4	79.9000000	1.5368393	<.0001	3
8	82.0666667	1.5368393	<.0001	4

Least Squares Means for effect Sugar Pr > t for H0: LSMean(i)=LSMean(j)				
Dependent Variable: PastTempPostPrint				
i/j	1	2	3	4
1		0.6886	0.9993	0.6886
2	0.6886		0.7555	1.0000
3	0.9993	0.7555		0.7555
4	0.6886	1.0000	0.7555	

Tukey Grouping for LS-Means of Sugar			
LS-means with the same letter are not significantly different.			
	PastTempPostPrint LSMEAN	Sugar	LSMEAN Number
A	82.06667	12	2
A			
A	82.06667	8	4
A			
A	79.90000	4	3
A			
A	79.63333	0	1

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

Starch	Sugar	PastTempPostPrint LSMEAN	Standard Error	Pr > t	LSMEAN Number
C	0	79.6333333	1.5368393	<.0001	1
C	12	82.0666667	1.5368393	<.0001	2
C	4	79.9000000	1.5368393	<.0001	3
C	8	82.0666667	1.5368393	<.0001	4

Least Squares Means for effect Starch*Sugar Pr > t for H0: LSMean(i)=LSMean(j)				
Dependent Variable: PastTempPostPrint				
i/j	1	2	3	4
1		0.6886	0.9993	0.6886
2	0.6886		0.7555	1.0000
3	0.9993	0.7555		0.7555
4	0.6886	1.0000	0.7555	

Tukey Grouping for LS-Means of Starch*Sugar			
LS-means with the same letter are not significantly different.			
	PastTempPostPrint LSMEAN	Starch	Sugar
			LSMEAN Number

Tukey Grouping for LS-Means of Starch*Sugar				
LS-means with the same letter are not significantly different.				
	PastTempPostPrint LSMEAN	Starch	Sugar	LSMEAN Number
A	82.06667	C	12	2
A				
A	82.06667	C	8	4
A				
A	79.90000	C	4	3
A				
A	79.63333	C	0	1

**Table A8 – SAS output for two-way ANOVA and Tukey's test for DSC Raw
Material ΔH Post-Printing data (see next page)**

The GLM Procedure

Class Level Information		
Class	Levels	Values
Starch	1	C
Sugar	4	0 12 4 8

Number of Observations Read	8
Number of Observations Used	8

The GLM Procedure

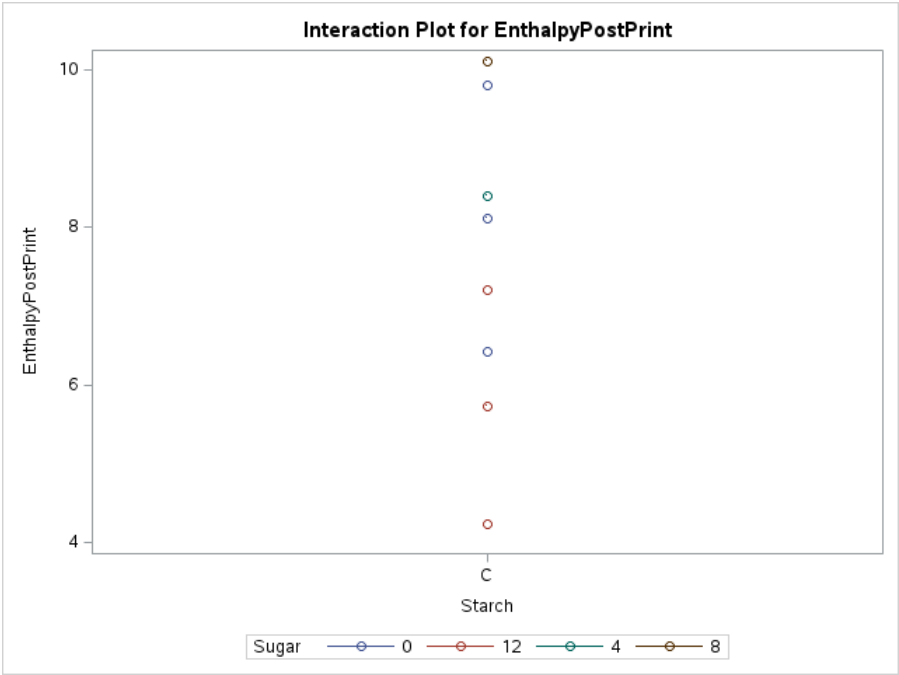
Dependent Variable: EnthalpyPostPrint

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	18.20349538	6.06783179	2.40	0.2082
Error	4	10.10118500	2.52529625		
Corrected Total	7	28.30468038			

R-Square	Coeff Var	Root MSE	EnthalpyPostPrint Mean
0.643127	21.17307	1.589118	7.505375

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Starch	0	0.00000000	.	.	.
Sugar	3	18.20349538	6.06783179	2.40	0.2082
Starch*Sugar	0	0.00000000	.	.	.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Starch	0	0.00000000	.	.	.
Sugar	3	18.20349538	6.06783179	2.40	0.2082
Starch*Sugar	0	0.00000000	.	.	.



The GLM Procedure

Level of Starch	N	EnthalpyPostPrint	
		Mean	Std Dev
C	8	7.50537500	2.01085200

Level of Sugar	N	EnthalpyPostPrint	
		Mean	Std Dev
0	3	8.1175000	1.68550000
12	3	5.7265000	1.48650000
4	1	8.4010000	.
8	1	10.1100000	.

Level of Starch	Level of Sugar	N	EnthalpyPostPrint	
			Mean	Std Dev
C	0	3	8.1175000	1.68550000
C	12	3	5.7265000	1.48650000
C	4	1	8.4010000	.
C	8	1	10.1100000	.

The GLM Procedure
Least Squares Means

Error: LINES display is not produced for effect Starch and dependent variable EnthalpyPostPrint because p-values are not available for all least-squares means differences.

Starch	EnthalpyPostPrint LSMEAN	Standard Error	Pr > t
C	8.08875000	0.64875474	0.0002

Error: LINES display is not produced for effect Starch and dependent variable EnthalpyPostPrint because p-values are not available for all least-squares means differences.

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey-Kramer

Sugar	EnthalpyPostPrint LSMEAN	Standard Error	Pr > t	LSMEAN Number
0	8.1175000	0.9174777	0.0009	1
12	5.7265000	0.9174777	0.0034	2
4	8.4010000	1.5891181	0.0061	3
8	10.1100000	1.5891181	0.0031	4

Least Squares Means for effect Sugar Pr > t for H0: LSMean(i)=LSMean(j)				
Dependent Variable: EnthalpyPostPrint				
i/j	1	2	3	4
1		0.3742	0.9985	0.7157
2	0.3742		0.5326	0.2211
3	0.9985	0.5326		0.8684
4	0.7157	0.2211	0.8684	

Tukey-Kramer Grouping for LS-Means of Sugar			
LS-means with the same letter are not significantly different.			
	EnthalpyPostPrint LSMEAN	Sugar	LSMEAN Number
A	10.1100	8	4
A			
A	8.4010	4	3
A			
A	8.1175	0	1
A			
A	5.7265	12	2

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey-Kramer

Starch	Sugar	EnthalpyPostPrint LSMEAN	Standard Error	Pr > t	LSMEAN Number
C	0	8.1175000	0.9174777	0.0009	1
C	12	5.7265000	0.9174777	0.0034	2
C	4	8.4010000	1.5891181	0.0061	3
C	8	10.1100000	1.5891181	0.0031	4

Least Squares Means for effect Starch*Sugar Pr > t for H0: LSMean(i)=LSMean(j)				
Dependent Variable: EnthalpyPostPrint				
i/j	1	2	3	4
1		0.3742	0.9985	0.7157
2	0.3742		0.5326	0.2211
3	0.9985	0.5326		0.8684
4	0.7157	0.2211	0.8684	

Tukey-Kramer Grouping for LS-Means of Starch*Sugar				
LS-means with the same letter are not significantly different.				
	EnthalpyPostPrint LSMEAN	Starch	Sugar	LSMEAN Number

Tukey-Kramer Grouping for LS-Means of Starch*Sugar				
LS-means with the same letter are not significantly different.				
	EnthalpyPostPrint LSMEAN	Starch	Sugar	LSMEAN Number
A	10.1100	C	8	4
A				
A	8.4010	C	4	3
A				
A	8.1175	C	0	1
A				
A	5.7265	C	12	2

**Table A9 – SAS output for two-way ANOVA and Tukey’s test for DSC Raw
Material Onset Temperature Post-Printing data (see next page)**

The GLM Procedure

Class Level Information		
Class	Levels	Values
Starch	1	C
Sugar	4	0 12 4 8

Number of Observations Read	10
Number of Observations Used	10

The GLM Procedure

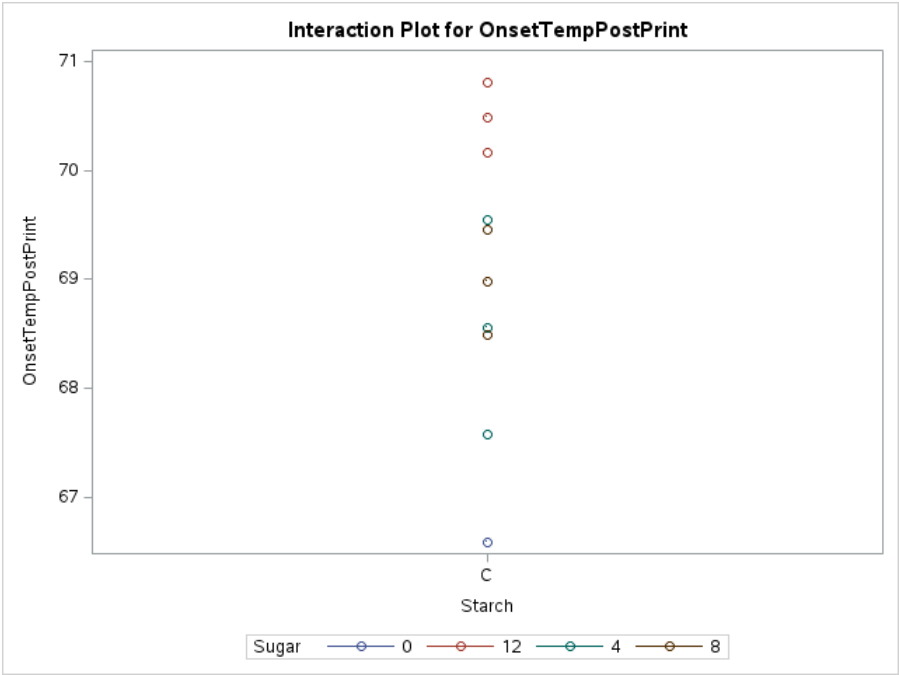
Dependent Variable: OnsetTempPostPrint

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	13.02896250	4.34298750	9.94	0.0096
Error	6	2.62215000	0.43702500		
Corrected Total	9	15.65111250			

R-Square	Coeff Var	Root MSE	OnsetTempPostPrint Mean
0.832462	0.957218	0.661079	69.06250

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Starch	0	0.00000000	.	.	.
Sugar	3	13.02896250	4.34298750	9.94	0.0096
Starch*Sugar	0	0.00000000	.	.	.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Starch	0	0.00000000	.	.	.
Sugar	3	13.02896250	4.34298750	9.94	0.0096
Starch*Sugar	0	0.00000000	.	.	.



The GLM Procedure

Level of Starch	N	OnsetTempPostPrint	
		Mean	Std Dev
C	10	69.0625000	1.31871623

Level of Sugar	N	OnsetTempPostPrint	
		Mean	Std Dev
0	1	66.5800000	.
12	3	70.4850000	0.32500000
4	3	68.5550000	0.98500000
8	3	68.9750000	0.48500000

Level of Starch	Level of Sugar	N	OnsetTempPostPrint	
			Mean	Std Dev
C	0	1	66.5800000	.
C	12	3	70.4850000	0.32500000
C	4	3	68.5550000	0.98500000
C	8	3	68.9750000	0.48500000

The GLM Procedure
Least Squares Means

Error: LINES display is not produced for effect Starch and dependent variable OnsetTempPostPrint because p-values are not available for all least-squares means differences.

Starch	OnsetTempPostPrint LSMEAN	Standard Error	Pr > t
C	68.6487500	0.2337266	<.0001

Error: LINES display is not produced for effect Starch and dependent variable OnsetTempPostPrint because p-values are not available for all least-squares means differences.

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey-Kramer

Sugar	OnsetTempPostPrint LSMEAN	Standard Error	Pr > t	LSMEAN Number
0	66.5800000	0.6610787	<.0001	1
12	70.4850000	0.3816739	<.0001	2
4	68.5550000	0.3816739	<.0001	3
8	68.9750000	0.3816739	<.0001	4

Least Squares Means for effect Sugar Pr > t for H0: LSMean(i)=LSMean(j)				
Dependent Variable: OnsetTempPostPrint				
i/j	1	2	3	4
1		0.0087	0.1414	0.0731
2	0.0087		0.0439	0.1097
3	0.1414	0.0439		0.8618
4	0.0731	0.1097	0.8618	

Tukey-Kramer Grouping for LS-Means of Sugar			
LS-means with the same letter are not significantly different.			
	OnsetTempPostPrint LSMEAN	Sugar	LSMEAN Number
A	70.485	12	2
A			
B	68.975	8	4
B			
B	68.555	4	3
B			
B	66.580	0	1

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey-Kramer

Starch	Sugar	OnsetTempPostPrint LSMEAN	Standard Error	Pr > t	LSMEAN Number
C	0	66.5800000	0.6610787	<.0001	1
C	12	70.4850000	0.3816739	<.0001	2
C	4	68.5550000	0.3816739	<.0001	3
C	8	68.9750000	0.3816739	<.0001	4

Least Squares Means for effect Starch*Sugar Pr > t for H0: LSMean(i)=LSMean(j)				
Dependent Variable: OnsetTempPostPrint				
i/j	1	2	3	4
1		0.0087	0.1414	0.0731
2	0.0087		0.0439	0.1097
3	0.1414	0.0439		0.8618
4	0.0731	0.1097	0.8618	

Tukey-Kramer Grouping for LS-Means of Starch*Sugar				
LS-means with the same letter are not significantly different.				
	OnsetTempPostPrint LSMEAN	Starch	Sugar	LSMEAN Number

Tukey-Kramer Grouping for LS-Means of Starch*Sugar					
LS-means with the same letter are not significantly different.					
		OnsetTempPostPrint LSMEAN	Starch	Sugar	LSMEAN Number
	A	70.485	C	12	2
	A				
B	A	68.975	C	8	4
B					
B		68.555	C	4	3
B					
B		66.580	C	0	1

**Table A9 – SAS output for two-way ANOVA and Tukey’s test for DSC Raw
Material Peak Gelatinization Post-Printing data (see next page)**

The GLM Procedure

Class Level Information		
Class	Levels	Values
Starch	1	C
Sugar	4	0 12 4 8

Number of Observations Read	8
Number of Observations Used	8

The GLM Procedure

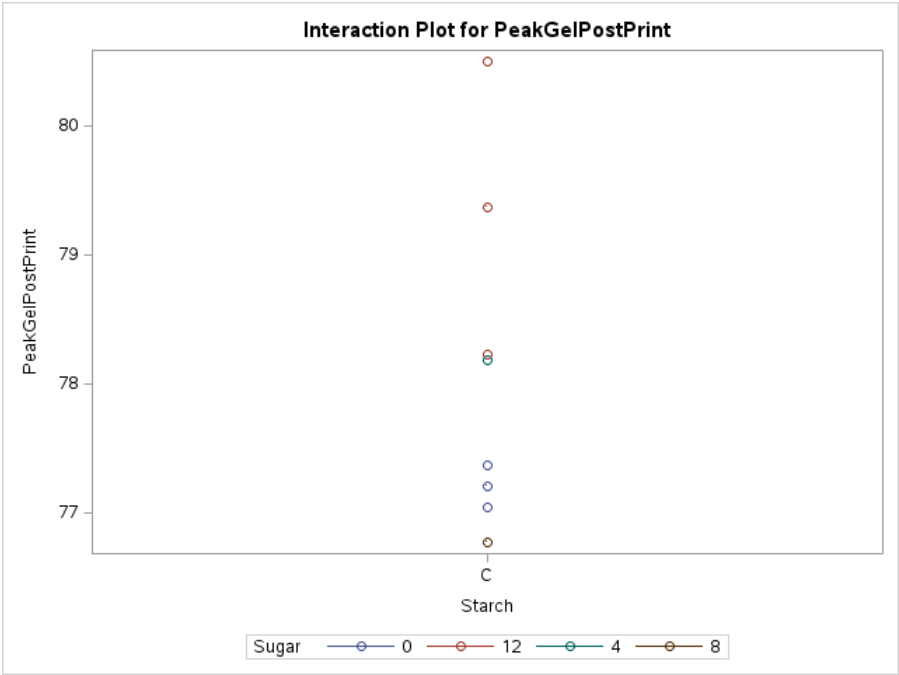
Dependent Variable: PeakGelPostPrint

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	8.97660000	2.99220000	4.55	0.0887
Error	4	2.63090000	0.65772500		
Corrected Total	7	11.60750000			

R-Square	Coeff Var	Root MSE	PeakGelPostPrint Mean
0.773345	1.038648	0.811002	78.08250

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Starch	0	0.00000000	.	.	.
Sugar	3	8.97660000	2.99220000	4.55	0.0887
Starch*Sugar	0	0.00000000	.	.	.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Starch	0	0.00000000	.	.	.
Sugar	3	8.97660000	2.99220000	4.55	0.0887
Starch*Sugar	0	0.00000000	.	.	.



The GLM Procedure

Level of Starch	N	PeakGelPostPrint	
		Mean	Std Dev
C	8	78.0825000	1.28771669

Level of Sugar	N	PeakGelPostPrint	
		Mean	Std Dev
0	3	77.2050000	0.16500000
12	3	79.3650000	1.13500000
4	1	78.1800000	.
8	1	76.7700000	.

Level of Starch	Level of Sugar	N	PeakGelPostPrint	
			Mean	Std Dev
C	0	3	77.2050000	0.1650000
C	12	3	79.3650000	1.1350000
C	4	1	78.1800000	.
C	8	1	76.7700000	.

The GLM Procedure
Least Squares Means

Error: LINES display is not produced for effect Starch and dependent variable PeakGelPostPrint because p-values are not available for all least-squares means differences.

Starch	PeakGelPostPrint LSMEAN	Standard Error	Pr > t
C	77.8800000	0.3310904	<.0001

Error: LINES display is not produced for effect Starch and dependent variable PeakGelPostPrint because p-values are not available for all least-squares means differences.

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey-Kramer

Sugar	PeakGelPostPrint LSMEAN	Standard Error	Pr > t	LSMEAN Number
0	77.2050000	0.4682325	<.0001	1
12	79.3650000	0.4682325	<.0001	2
4	78.1800000	0.8110025	<.0001	3
8	76.7700000	0.8110025	<.0001	4

Least Squares Means for effect Sugar Pr > t for H0: LSMean(i)=LSMean(j)				
Dependent Variable: PeakGelPostPrint				
i/j	1	2	3	4
1		0.0983	0.7382	0.9631
2	0.0983		0.6253	0.1538
3	0.7382	0.6253		0.6433
4	0.9631	0.1538	0.6433	

Tukey-Kramer Grouping for LS-Means of Sugar			
LS-means with the same letter are not significantly different.			
	PeakGelPostPrint LSMEAN	Sugar	LSMEAN Number
A	79.365	12	2
A			
A	78.180	4	3
A			
A	77.205	0	1
A			
A	76.770	8	4

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey-Kramer

Starch	Sugar	PeakGelPostPrint LSMEAN	Standard Error	Pr > t	LSMEAN Number
C	0	77.2050000	0.4682325	<.0001	1
C	12	79.3650000	0.4682325	<.0001	2
C	4	78.1800000	0.8110025	<.0001	3
C	8	76.7700000	0.8110025	<.0001	4

Least Squares Means for effect Starch*Sugar Pr > t for H0: LSMean(i)=LSMean(j)				
Dependent Variable: PeakGelPostPrint				
i/j	1	2	3	4
1		0.0983	0.7382	0.9631
2	0.0983		0.6253	0.1538
3	0.7382	0.6253		0.6433
4	0.9631	0.1538	0.6433	

Tukey-Kramer Grouping for LS-Means of Starch*Sugar			
LS-means with the same letter are not significantly different.			
	PeakGelPostPrint LSMEAN	Starch	Sugar
			LSMEAN Number

Tukey-Kramer Grouping for LS-Means of Starch*Sugar				
LS-means with the same letter are not significantly different.				
	PeakGelPostPrint LSMEAN	Starch	Sugar	LSMEAN Number
A	79.365	C	12	2
A				
A	78.180	C	4	3
A				
A	77.205	C	0	1
A				
A	76.770	C	8	4

Table A10 – Procedure and data for determination of LVR and critical strain for wheat- and corn-based 3D printing inks

[illegible]

Table A11 – SAS output for two-way ANOVA and Tukey’s test for dynamic rheology frequency sweep G’ data (see next page)

The GLM Procedure

Class Level Information		
Class	Levels	Values
Starch	2	C W
Sugar	4	12 4 8 B

Number of Observations Read	21
Number of Observations Used	21

The GLM Procedure

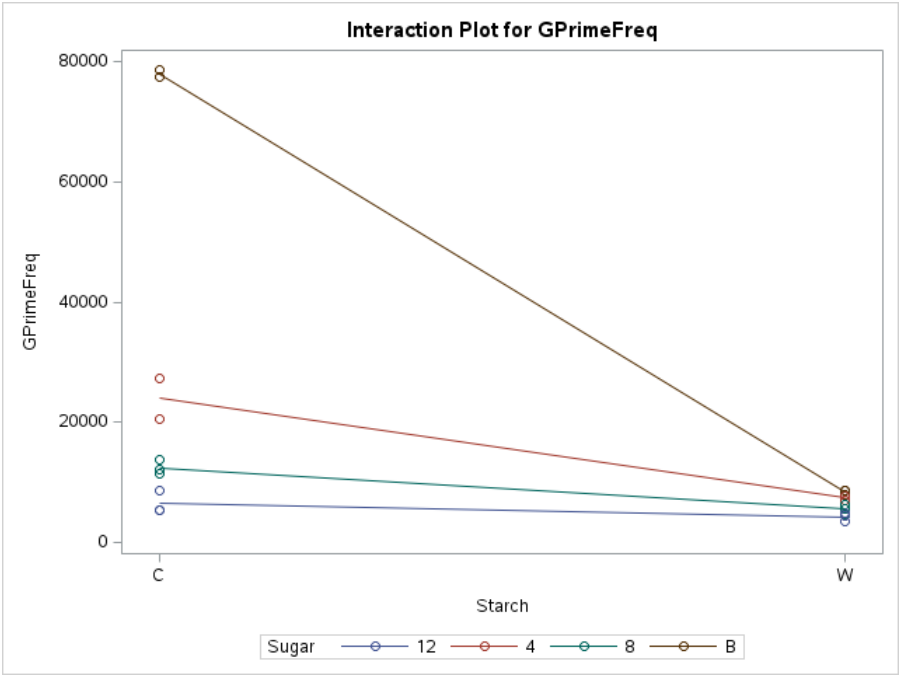
Dependent Variable: GPrimeFreq

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	9183945714	1311992245	474.02	<.0001
Error	13	35981467	2767805		
Corrected Total	20	9219927181			

R-Square	Coeff Var	Root MSE	GPrimeFreq Mean
0.996097	10.57579	1663.672	15730.95

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Starch	1	2044689563	2044689563	738.74	<.0001
Sugar	3	3468006794	1156002265	417.66	<.0001
Starch*Sugar	3	3671249358	1223749786	442.14	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Starch	1	2866824758	2866824758	1035.78	<.0001
Sugar	3	4501955409	1500651803	542.18	<.0001
Starch*Sugar	3	3671249358	1223749786	442.14	<.0001



The GLM Procedure

Tukey's Studentized Range (HSD) Test for GPrimeFreq

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	13
Error Mean Square	2767805
Critical Value of Studentized Range	3.05508
Minimum Significant Difference	1570.3
Harmonic Mean of Cell Sizes	10.47619

Note: Cell sizes are not equal.

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	Starch
A	26080.0	10	C
B	6322.7	11	W

The GLM Procedure

Tukey's Studentized Range (HSD) Test for GPrimeFreq

Note: This test controls the Type I experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	13
Error Mean Square	2767805
Critical Value of Studentized Range	4.15087

Comparisons significant at the 0.05 level are indicated by ***.				
Sugar Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
B - 4	20561.0	17285.3	23836.7	***
B - 8	27282.7	24325.8	30239.5	***
B - 12	30882.7	27925.8	33839.5	***
4 - B	-20561.0	-23836.7	-17285.3	***
4 - 8	6721.7	3569.7	9873.7	***
4 - 12	10321.7	7169.7	13473.7	***
8 - B	-27282.7	-30239.5	-24325.8	***
8 - 4	-6721.7	-9873.7	-3569.7	***
8 - 12	3600.0	780.8	6419.2	***
12 - B	-30882.7	-33839.5	-27925.8	***
12 - 4	-10321.7	-13473.7	-7169.7	***
12 - 8	-3600.0	-6419.2	-780.8	***

The GLM Procedure

Level of Starch	Level of Sugar	N	GPrimeFreq	
			Mean	Std Dev
C	12	3	6500.0000	1908.42867
C	4	2	24000.0000	4808.32611
C	8	3	12433.3333	1137.24814
C	B	2	78000.0000	707.10678
W	12	3	4266.6667	670.99429
W	4	2	7410.0000	523.25902
W	8	3	5533.3333	740.09008
W	B	3	8443.3333	332.31511

The GLM Procedure

Least Squares Means

Adjustment for Multiple Comparisons: Tukey-Kramer

Starch	GPrimeFreq LSMEAN	Standard Error	H0:LSMEAN=0	H0:LSMean1=LSMean2
			Pr > t	Pr > t
C	30233.3333	536.9479	<.0001	<.0001
W	6413.3333	509.3935	<.0001	

Tukey-Kramer Grouping for LS-Means of Starch			
LS-means with the same letter are not significantly different.			
	GPrimeFreq LSMEAN	Starch	LSMEAN Number
A	30233	C	1
B	6413	W	2

The GLM Procedure

Least Squares Means

Adjustment for Multiple Comparisons: Tukey-Kramer

Sugar	GPrimeFreq LSMEAN	Standard Error	Pr > t	LSMEAN Number
12	5383.3333	679.1913	<.0001	1
4	15705.0000	831.8361	<.0001	2
8	8983.3333	679.1913	<.0001	3
B	43221.6667	759.3590	<.0001	4

Least Squares Means for effect Sugar Pr > t for H0: LSMean(i)=LSMean(j)				
Dependent Variable: GPrimeFreq				
i/j	1	2	3	4
1		<.0001	0.0114	<.0001
2	<.0001		0.0002	<.0001
3	0.0114	0.0002		<.0001
4	<.0001	<.0001	<.0001	

Tukey-Kramer Grouping for LS-Means of Sugar			
LS-means with the same letter are not significantly different.			
	GPrimeFreq LSMEAN	Sugar	LSMEAN Number
A	43222	B	4
B	15705	4	2
C	8983	8	3
D	5383	12	1

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey-Kramer

Starch	Sugar	GPrimeFreq LSMEAN	Standard Error	Pr > t	LSMEAN Number
C	12	6500.0000	960.5216	<.0001	1
C	4	24000.0000	1176.3939	<.0001	2
C	8	12433.3333	960.5216	<.0001	3
C	B	78000.0000	1176.3939	<.0001	4
W	12	4266.6667	960.5216	0.0007	5
W	4	7410.0000	1176.3939	<.0001	6
W	8	5533.3333	960.5216	<.0001	7
W	B	8443.3333	960.5216	<.0001	8

Least Squares Means for effect Starch*Sugar Pr > t for H0: LSMean(i)=LSMean(j)								
Dependent Variable: GPrimeFreq								
i/j	1	2	3	4	5	6	7	8
1		<.0001	0.0127	<.0001	0.7191	0.9983	0.9950	0.8284
2	<.0001		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
3	0.0127	<.0001		<.0001	0.0008	0.0779	0.0038	0.1424
4	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001
5	0.7191	<.0001	0.0008	<.0001		0.4777	0.9767	0.1143
6	0.9983	<.0001	0.0779	<.0001	0.4777		0.9067	0.9962
7	0.9950	<.0001	0.0038	<.0001	0.9767	0.9067		0.4389
8	0.8284	<.0001	0.1424	<.0001	0.1143	0.9962	0.4389	

Tukey-Kramer Grouping for LS-Means of Starch*Sugar				
LS-means with the same letter are not significantly different.				
	GPrimeFreq LSMEAN	Starch	Sugar	LSMEAN Number
A	78000	C	B	4
B	24000	C	4	2
C	12433	C	8	3
C				
D C	8443	W	B	8
D C				
D C	7410	W	4	6
D				
D	6500	C	12	1
D				
D	5533	W	8	7
D				

Tukey-Kramer Grouping for LS-Means of Starch*Sugar				
LS-means with the same letter are not significantly different.				
	GPrimeFreq LSMEAN	Starch	Sugar	LSMEAN Number
D	4267	W	12	5

Table A12 – SAS output for two-way ANOVA and Tukey’s test for dynamic rheology frequency sweep G'' data (see next page)

The GLM Procedure

Class Level Information		
Class	Levels	Values
Starch	2	C W
Sugar	4	12 4 8 B

Number of Observations Read	22
Number of Observations Used	22

The GLM Procedure

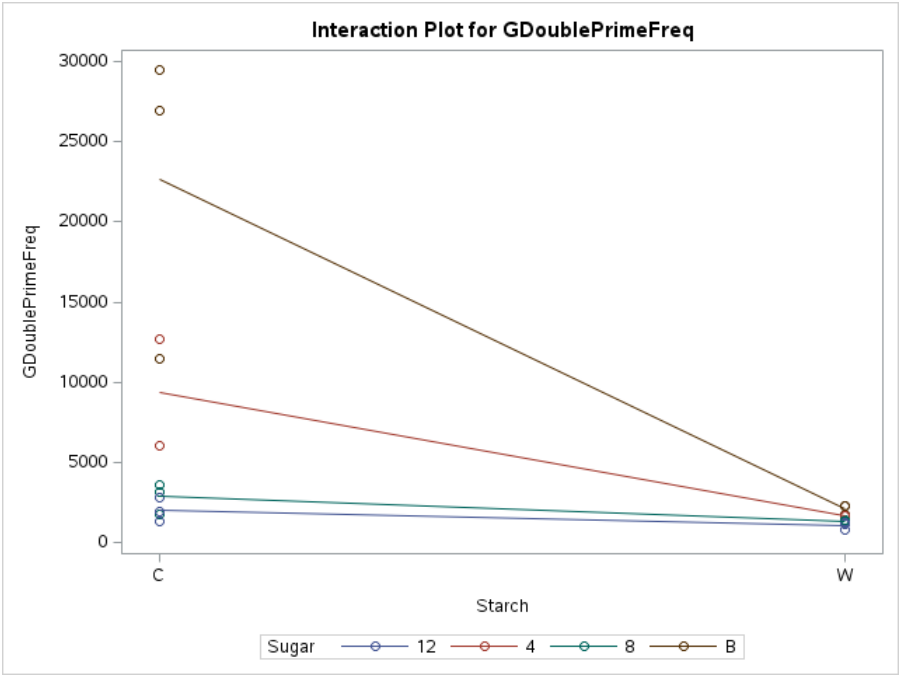
Dependent Variable: GDoublePrimeFreq

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	1141850377	163121482	10.65	0.0001
Error	14	214413304	15315236		
Corrected Total	21	1356263681			

R-Square	Coeff Var	Root MSE	GDoublePrimeFreq Mean
0.841909	72.80810	3913.469	5375.045

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Starch	1	322019116.4	322019116.4	21.03	0.0004
Sugar	3	446116028.5	148705342.8	9.71	0.0010
Starch*Sugar	3	373715232.1	124571744.0	8.13	0.0022

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Starch	1	312623402.8	312623402.8	20.41	0.0005
Sugar	3	446116028.5	148705342.8	9.71	0.0010
Starch*Sugar	3	373715232.1	124571744.0	8.13	0.0022



The GLM Procedure

Tukey's Studentized Range (HSD) Test for GDoublePrimeFreq

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	14
Error Mean Square	15315236
Critical Value of Studentized Range	3.03319
Minimum Significant Difference	3579

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	Starch
A	9201	11	C

Means with the same letter are not significantly different.			
B	Tukey Grouping	Mean	Starch

The GLM Procedure

Tukey's Studentized Range (HSD) Test for GDoublePrimeFreq

Note: This test controls the Type I experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	14
Error Mean Square	15315236
Critical Value of Studentized Range	4.11036

Comparisons significant at the 0.05 level are indicated by ***.				
Sugar Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
B - 4	6839	-503	14181	
B - 8	10258	3691	16825	***
B - 12	10837	4270	17403	***
4 - B	-6839	-14181	503	
4 - 8	3419	-3923	10761	
4 - 12	3997	-3345	11339	
8 - B	-10258	-16825	-3691	***
8 - 4	-3419	-10761	3923	
8 - 12	578	-5989	7145	
12 - B	-10837	-17403	-4270	***
12 - 4	-3997	-11339	3345	
12 - 8	-578	-7145	5989	

The GLM Procedure

Level of Starch	Level of Sugar	N	GDoublePrimeFreq	
			Mean	Std Dev
C	12	3	1990.0000	739.12110
C	4	2	9385.0000	4688.11796
C	8	3	2856.6667	944.79275
C	B	3	22633.3333	9728.99447
W	12	3	1080.3333	209.42859
W	4	2	1680.0000	42.42641
W	8	3	1370.0000	60.00000
W	B	3	2110.0000	277.12813

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

Starch	GDoublePrimeFreq LSMEAN	Standard Error	H0:LSMEAN=0	H0:LSMean1=LSMean2
			Pr > t	Pr > t
C	9216.25000	1198.25013	<.0001	0.0005
W	1560.08333	1198.25013	0.2139	

Tukey Grouping for LS-Means of Starch			
LS-means with the same letter are not significantly different.			
	GDoublePrimeFreq LSMEAN	Starch	LSMEAN Number
A	9216.3	C	1
B	1560.1	W	2

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey-Kramer

Sugar	GDoublePrimeFreq LSMEAN	Standard Error	Pr > t	LSMEAN Number
12	1535.1667	1597.6668	0.3529	1
4	5532.5000	1956.7343	0.0134	2

Sugar	GDoublePrimeFreq LSMEAN	Standard Error	Pr > t	LSMEAN Number
8	2113.3333	1597.6668	0.2071	3
B	12371.6667	1597.6668	<.0001	4

Least Squares Means for effect Sugar Pr > t for H0: LSMean(i)=LSMean(j)				
Dependent Variable: GDoublePrimeFreq				
i\j	1	2	3	4
1		0.4190	0.9939	0.0014
2	0.4190		0.5464	0.0717
3	0.9939	0.5464		0.0023
4	0.0014	0.0717	0.0023	

Tukey-Kramer Grouping for LS-Means of Sugar				
LS-means with the same letter are not significantly different.				
	GDoublePrimeFreq LSMEAN	Sugar	LSMEAN Number	
A	12372	B		4
A				
B A	5533	4		2
B				
B	2113	8		3
B				
B	1535	12		1

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey-Kramer

Starch	Sugar	GDoublePrimeFreq LSMEAN	Standard Error	Pr > t	LSMEAN Number
C	12	1990.0000	2259.4421	0.3933	1
C	4	9385.0000	2767.2401	0.0044	2
C	8	2856.6667	2259.4421	0.2268	3
C	B	22633.3333	2259.4421	<.0001	4
W	12	1080.3333	2259.4421	0.6399	5
W	4	1680.0000	2767.2401	0.5535	6
W	8	1370.0000	2259.4421	0.5540	7
W	B	2110.0000	2259.4421	0.3662	8

Least Squares Means for effect Starch*Sugar Pr > t for H0: LSMean(i)=LSMean(j)								
Dependent Variable: GDoublePrimeFreq								
i\j	1	2	3	4	5	6	7	8
1		0.4750	1.0000	0.0003	1.0000	1.0000	1.0000	1.0000
2	0.4750		0.6139	0.0363	0.3452	0.5319	0.3840	0.4936
3	1.0000	0.6139		0.0005	0.9989	1.0000	0.9997	1.0000
4	0.0003	0.0363	0.0005		0.0002	0.0008	0.0002	0.0003
5	1.0000	0.3452	0.9989	0.0002		1.0000	1.0000	1.0000
6	1.0000	0.5319	1.0000	0.0008	1.0000		1.0000	1.0000
7	1.0000	0.3840	0.9997	0.0002	1.0000	1.0000		1.0000
8	1.0000	0.4936	1.0000	0.0003	1.0000	1.0000	1.0000	

Tukey-Kramer Grouping for LS-Means of Starch*Sugar				
LS-means with the same letter are not significantly different.				
	GDoublePrimeFreq LSMEAN	Starch	Sugar	LSMEAN Number
A	22633	C	B	4
B	9385	C	4	2
B				
B	2857	C	8	3
B				
B	2110	W	B	8
B				
B	1990	C	12	1
B				
B	1680	W	4	6
B				
B	1370	W	8	7
B				

Tukey-Kramer Grouping for LS-Means of Starch*Sugar				
LS-means with the same letter are not significantly different.				
	GDoublePrimeFreq LSMEAN	Starch	Sugar	LSMEAN Number
B	1080	W	12	5

Table A13 – SAS output for two-way ANOVA and Tukey’s test for dynamic rheology frequency sweep G^* data (see next page)

The GLM Procedure

Class Level Information		
Class	Levels	Values
Starch	2	C W
Sugar	4	12 4 8 B

Number of Observations Read	24
Number of Observations Used	24

The GLM Procedure

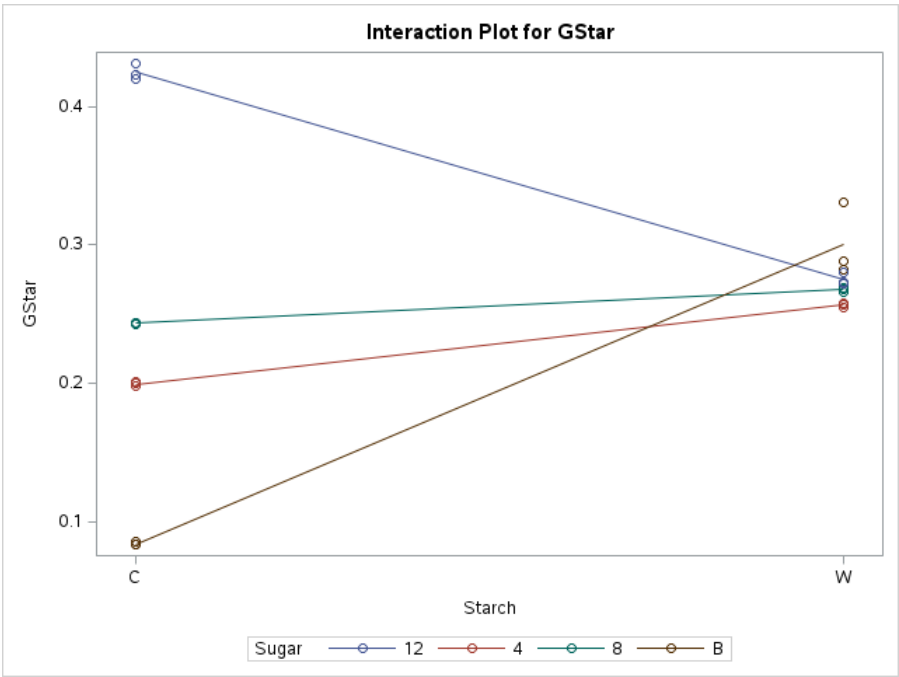
Dependent Variable: GStar

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	0.19104448	0.02729207	282.06	<.0001
Error	16	0.00154816	0.00009676		
Corrected Total	23	0.19259264			

R-Square	Coeff Var	Root MSE	GStar Mean
0.991961	3.834833	0.009837	0.256508

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Starch	1	0.00813280	0.00813280	84.05	<.0001
Sugar	3	0.08176600	0.02725533	281.68	<.0001
Starch*Sugar	3	0.10114567	0.03371522	348.44	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Starch	1	0.00813280	0.00813280	84.05	<.0001
Sugar	3	0.08176600	0.02725533	281.68	<.0001
Starch*Sugar	3	0.10114567	0.03371522	348.44	<.0001



The GLM Procedure

Tukey's Studentized Range (HSD) Test for GStar

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	16
Error Mean Square	0.000097
Critical Value of Studentized Range	2.99786
Minimum Significant Difference	0.0085

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	Starch
A	0.274917	12	W

Means with the same letter are not significantly different.			
B	Tukey Grouping	0.2381000	12 Starch

The GLM Procedure

Tukey's Studentized Range (HSD) Test for GStar

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	16
Error Mean Square	0.000097
Critical Value of Studentized Range	4.04606
Minimum Significant Difference	0.0162

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	Sugar
A	0.349833	6	12
B	0.255667	6	8
C	0.228167	6	4
D	0.192367	6	B

The GLM Procedure

Level of Starch	Level of Sugar	N	GStar	
			Mean	Std Dev
C	12	3	0.42466667	0.00568624
C	4	3	0.19966667	0.00152753
C	8	3	0.24366667	0.00057735
C	B	3	0.08440000	0.00103923
W	12	3	0.27500000	0.00435890
W	4	3	0.25666667	0.00152753
W	8	3	0.26766667	0.00152753
W	B	3	0.30033333	0.02672702

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

Starch	GStar LSMEAN	Standard Error	H0:LSMEAN=0	H0:LSMean1=LSMean2
			Pr > t	Pr > t
C	0.23810000	0.00283960	<.0001	<.0001
W	0.27491667	0.00283960	<.0001	

Tukey Grouping for LS-Means of Starch			
LS-means with the same letter are not significantly different.			
	GStar LSMEAN	Starch	LSMEAN Number
A	0.27491667	W	2
B	0.23810000	C	1

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

Sugar	GStar LSMEAN	Standard Error	Pr > t	LSMEAN Number
12	0.34983333	0.00401580	<.0001	1
4	0.22816667	0.00401580	<.0001	2
8	0.25566667	0.00401580	<.0001	3
B	0.19236667	0.00401580	<.0001	4

Least Squares Means for effect Sugar				
Pr > t for H0: LSMean(i)=LSMean(j)				
Dependent Variable: GStar				
i/j	1	2	3	4
1		<.0001	<.0001	<.0001
2	<.0001		0.0009	<.0001
3	<.0001	0.0009		<.0001
4	<.0001	<.0001	<.0001	

Tukey Grouping for LS-Means of Sugar			
LS-means with the same letter are not significantly different.			
	GStar LSMEAN	Sugar	LSMEAN Number
A	0.34983333	12	1
B	0.25566667	8	3
C	0.22816667	4	2
D	0.19236667	B	4

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

Starch	Sugar	GStar LSMEAN	Standard Error	Pr > t	LSMEAN Number
C	12	0.42466667	0.00567920	<.0001	1
C	4	0.19966667	0.00567920	<.0001	2
C	8	0.24366667	0.00567920	<.0001	3
C	B	0.08440000	0.00567920	<.0001	4
W	12	0.27500000	0.00567920	<.0001	5
W	4	0.25666667	0.00567920	<.0001	6
W	8	0.26766667	0.00567920	<.0001	7
W	B	0.30033333	0.00567920	<.0001	8

Least Squares Means for effect Starch*Sugar								
Pr > t for H0: LSMean(i)=LSMean(j)								
Dependent Variable: GStar								
i/j	1	2	3	4	5	6	7	8
1		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
2	<.0001		0.0010	<.0001	<.0001	<.0001	<.0001	<.0001
3	<.0001	0.0010		<.0001	0.0217	0.7338	0.1181	<.0001
4	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001
5	<.0001	<.0001	0.0217	<.0001		0.3585	0.9803	0.0880
6	<.0001	<.0001	0.7338	<.0001	0.3585		0.8580	0.0011
7	<.0001	<.0001	0.1181	<.0001	0.9803	0.8580		0.0157
8	<.0001	<.0001	<.0001	<.0001	0.0880	0.0011	0.0157	

Tukey Grouping for LS-Means of Starch*Sugar				
LS-means with the same letter are not significantly different.				
	GStar LSMEAN	Starch	Sugar	LSMEAN Number
A	0.42466667	C	12	1
B	0.30033333	W	B	8
C	0.27500000	W	12	5
C	0.26766667	W	8	7
C	0.25666667	W	4	6
D	0.24366667	C	8	3
E	0.19966667	C	4	2
F	0.08440000	C	B	4

Table A14 – SAS output for two-way ANOVA and Tukey’s test for DDR data (see next page)

The GLM Procedure

Class Level Information		
Class	Levels	Values
Starch	2	Corn Wheat
InfillDensity	4	20% 40% 60% 80%

Number of Observations Read	32
Number of Observations Used	32

The GLM Procedure

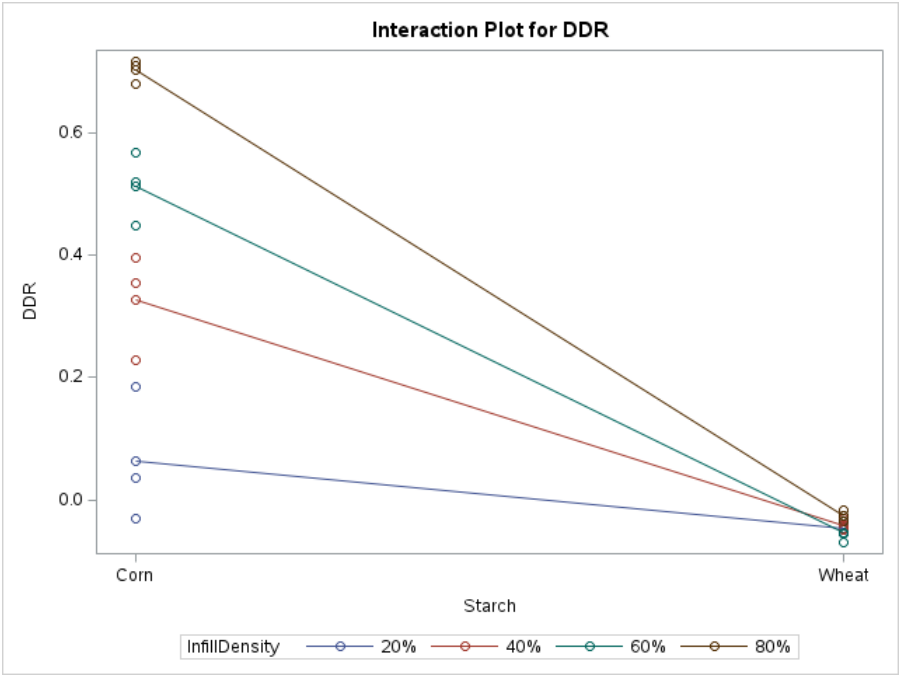
Dependent Variable: DDR

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	2.45130235	0.35018605	173.99	<.0001
Error	24	0.04830460	0.00201269		
Corrected Total	31	2.49960695			

R-Square	Coeff Var	Root MSE	DDR Mean
0.980675	24.95286	0.044863	0.179791

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Starch	1	1.56383724	1.56383724	776.99	<.0001
InfillDensity	3	0.46284897	0.15428299	76.66	<.0001
Starch*InfillDensity	3	0.42461614	0.14153871	70.32	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Starch	1	1.56383724	1.56383724	776.99	<.0001
InfillDensity	3	0.46284897	0.15428299	76.66	<.0001
Starch*InfillDensity	3	0.42461614	0.14153871	70.32	<.0001



The GLM Procedure

Tukey's Studentized Range (HSD) Test for DDR

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	24
Error Mean Square	0.002013
Critical Value of Studentized Range	2.91879
Minimum Significant Difference	0.0327

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	Starch
A	0.40086	16	Corn

Means with the same letter are not significantly different.			
B	Tukey Grouping	-0.04127	18

The GLM Procedure

Tukey's Studentized Range (HSD) Test for DDR

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	24
Error Mean Square	0.002013
Critical Value of Studentized Range	3.90126
Minimum Significant Difference	0.0619

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	InfillDensity
A	0.33784	8	80%
B	0.22936	8	60%
C	0.14257	8	40%
D	0.00939	8	20%

The GLM Procedure

Level of Starch	Level of InfillDensity	N	DDR	
			Mean	Std Dev
Corn	20%	4	0.06406958	0.08975436
Corn	40%	4	0.32648691	0.07130779
Corn	60%	4	0.51159672	0.04856914
Corn	80%	4	0.70127289	0.01594747
Wheat	20%	4	-0.04527988	0.00372613
Wheat	40%	4	-0.04134165	0.00845777
Wheat	60%	4	-0.05288080	0.01441944
Wheat	80%	4	-0.02559482	0.00736660

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

Starch	DDR LSMEAN	Standard Error	H0:LSMEAN=0	H0:LSMean1=LSMean2
			Pr > t	Pr > t
Corn	0.40085652	0.01121576	<.0001	<.0001
Wheat	-0.04127429	0.01121576	0.0012	

Tukey Grouping for LS-Means of Starch			
LS-means with the same letter are not significantly different.			
	DDR LSMEAN	Starch	LSMEAN Number
A	0.400856525	Corn	1
B	-0.041274287	Wheat	2

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

InfillDensity	DDR LSMEAN	Standard Error	Pr > t	LSMEAN Number
20%	0.00939485	0.01586148	0.5592	1
40%	0.14257263	0.01586148	<.0001	2
60%	0.22935796	0.01586148	<.0001	3
80%	0.33783904	0.01586148	<.0001	4

Least Squares Means for effect InfillDensity				
Pr > t for H0: LSMean(i)=LSMean(j)				
Dependent Variable: DDR				
i/j	1	2	3	4
1		<.0001	<.0001	<.0001
2	<.0001		0.0038	<.0001
3	<.0001	0.0038		0.0003
4	<.0001	<.0001	0.0003	

Tukey Grouping for LS-Means of InfillDensity			
LS-means with the same letter are not significantly different.			
	DDR LSMEAN	InfillDensity	LSMEAN Number
A	0.3378390351	80%	4
B	0.2293579602	60%	3
C	0.1425726336	40%	2
D	0.0093948454	20%	1

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

Starch	InfillDensity	DDR LSMEAN	Standard Error	Pr > t	LSMEAN Number
Corn	20%	0.06406958	0.02243152	0.0087	1
Corn	40%	0.32648691	0.02243152	<.0001	2
Corn	60%	0.51159672	0.02243152	<.0001	3
Corn	80%	0.70127289	0.02243152	<.0001	4
Wheat	20%	-0.04527988	0.02243152	0.0548	5
Wheat	40%	-0.04134165	0.02243152	0.0777	6
Wheat	60%	-0.05288080	0.02243152	0.0269	7
Wheat	80%	-0.02559482	0.02243152	0.2651	8

Least Squares Means for effect Starch*InfillDensity								
Pr > t for H0: LSMean(i)=LSMean(j)								
Dependent Variable: DDR								
i/j	1	2	3	4	5	6	7	8
1		<.0001	<.0001	<.0001	0.0372	0.0488	0.0217	0.1348
2	<.0001		0.0001	<.0001	<.0001	<.0001	<.0001	<.0001
3	<.0001	0.0001		<.0001	<.0001	<.0001	<.0001	<.0001
4	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001
5	0.0372	<.0001	<.0001	<.0001		1.0000	1.0000	0.9982
6	0.0488	<.0001	<.0001	<.0001	1.0000		0.9999	0.9996
7	0.0217	<.0001	<.0001	<.0001	1.0000	0.9999		0.9870
8	0.1348	<.0001	<.0001	<.0001	0.9982	0.9996	0.9870	

Tukey Grouping for LS-Means of Starch*InfillDensity				
LS-means with the same letter are not significantly different.				
	DDR LSMEAN	Starch	InfillDensity	LSMEAN Number
A	0.701272890	Corn	80%	4
B	0.511596719	Corn	60%	3
C	0.326486913	Corn	40%	2
D	0.064069575	Corn	20%	1
D				
E D	-0.025594820	Wheat	80%	8
E				
E	-0.041341646	Wheat	40%	6
E				
E	-0.045279885	Wheat	20%	5
E				
E	-0.052880799	Wheat	60%	7

Table A15 – SAS output for two-way ANOVA and Tukey’s test for peak force data
(see next page)

The GLM Procedure

Class Level Information		
Class	Levels	Values
Starch	2	C W
InfillDensity	4	20 40 60 80

Number of Observations Read	26
Number of Observations Used	26

The GLM Procedure

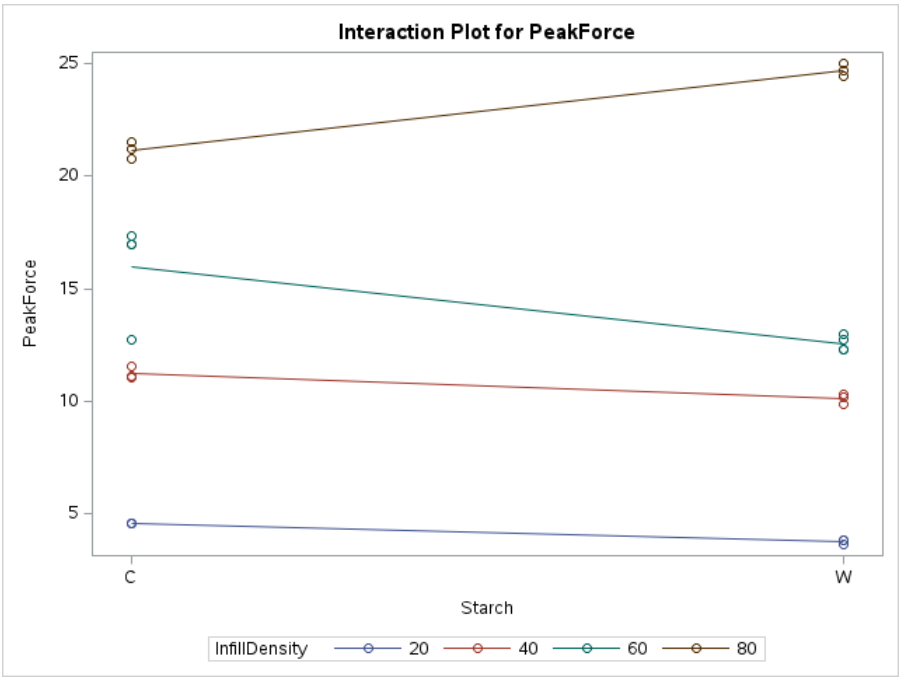
Dependent Variable: PeakForce

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	1149.002405	164.143201	190.96	<.0001
Error	18	15.472499	0.859583		
Corrected Total	25	1164.474904			

R-Square	Coeff Var	Root MSE	PeakForce Mean
0.986713	7.067263	0.927137	13.11876

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Starch	1	3.068291	3.068291	3.57	0.0751
InfillDensity	3	1103.715403	367.905134	428.00	<.0001
Starch*InfillDensity	3	42.218711	14.072904	16.37	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Starch	1	1.345119	1.345119	1.56	0.2270
InfillDensity	3	1103.715403	367.905134	428.00	<.0001
Starch*InfillDensity	3	42.218711	14.072904	16.37	<.0001



The GLM Procedure

Tukey's Studentized Range (HSD) Test for PeakForce

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	18
Error Mean Square	0.859583
Critical Value of Studentized Range	2.97105
Minimum Significant Difference	0.764

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	Starch
A	13.4623	13	C

Means with the same letter are not significantly different.			
A	Tukey Grouping	12.79240	Starch

The GLM Procedure

Tukey's Studentized Range (HSD) Test for PeakForce

Note: This test controls the Type I experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	18
Error Mean Square	0.859583
Critical Value of Studentized Range	3.99697

Comparisons significant at the 0.05 level are indicated by ***.				
InfillDensity Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
80 - 60	8.6468	7.2317	10.0620	***
80 - 40	12.2353	10.7224	13.7482	***
80 - 20	18.7560	17.2431	20.2688	***
60 - 80	-8.6468	-10.0620	-7.2317	***
60 - 40	3.5885	2.1733	5.0036	***
60 - 20	10.1092	8.6940	11.5243	***
40 - 80	-12.2353	-13.7482	-10.7224	***
40 - 60	-3.5885	-5.0036	-2.1733	***
40 - 20	6.5207	5.0078	8.0335	***
20 - 80	-18.7560	-20.2688	-17.2431	***
20 - 60	-10.1092	-11.5243	-8.6940	***
20 - 40	-6.5207	-8.0335	-5.0078	***

The GLM Procedure

Level of Starch	Level of InfillDensity	N	PeakForce	
			Mean	Std Dev
C	20	3	4.5945676	0.01684433
C	40	3	11.2506667	0.28376810
C	60	4	15.9995040	2.19356592
C	80	3	21.1586636	0.35457597
W	20	3	3.7557939	0.10279150
W	40	3	10.1410133	0.21360775
W	60	4	12.5691653	0.34414167
W	80	3	24.7036363	0.27977745

The GLM Procedure

Least Squares Means

Adjustment for Multiple Comparisons: Tukey

Starch	PeakForce LSMEAN	Standard Error	H0:LSMEAN=0	H0:LSMean1=LSMean2
			Pr > t	Pr > t
C	13.2508504	0.2591427	<.0001	0.2270
W	12.7924022	0.2591427	<.0001	

Tukey Grouping for LS-Means of Starch			
LS-means with the same letter are not significantly different.			
	PeakForce LSMEAN	Starch	LSMEAN Number
A	13.25085	C	1
A			
A	12.79240	W	2

The GLM Procedure

Least Squares Means

Adjustment for Multiple Comparisons: Tukey-Kramer

InfillDensity	PeakForce LSMEAN	Standard Error	Pr > t	LSMEAN Number
20	4.1751807	0.3785021	<.0001	1
40	10.6958400	0.3785021	<.0001	2

InfillDensity	PeakForce LSMEAN	Standard Error	Pr > t	LSMEAN Number
60	14.2843347	0.3277925	<.0001	3
80	22.9311499	0.3785021	<.0001	4

Least Squares Means for effect InfillDensity Pr > t for H0: LSMean(i)=LSMean(j)				
Dependent Variable: PeakForce				
i/j	1	2	3	4
1		<.0001	<.0001	<.0001
2	<.0001		<.0001	<.0001
3	<.0001	<.0001		<.0001
4	<.0001	<.0001	<.0001	

Tukey-Kramer Grouping for LS-Means of InfillDensity			
LS-means with the same letter are not significantly different.			
	PeakForce LSMEAN	InfillDensity	LSMEAN Number
A	22.931150	80	4
B	14.284335	60	3
C	10.695840	40	2
D	4.175181	20	1

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey-Kramer

Starch	InfillDensity	PeakForce LSMEAN	Standard Error	Pr > t	LSMEAN Number
C	20	4.5945676	0.5352829	<.0001	1
C	40	11.2506667	0.5352829	<.0001	2
C	60	15.9995040	0.4635686	<.0001	3
C	80	21.1586636	0.5352829	<.0001	4
W	20	3.7557939	0.5352829	<.0001	5
W	40	10.1410133	0.5352829	<.0001	6
W	60	12.5691653	0.4635686	<.0001	7
W	80	24.7036363	0.5352829	<.0001	8

Least Squares Means for effect Starch*InfillDensity Pr > t for H0: LSMean(i)=LSMean(j)								
Dependent Variable: PeakForce								
i/j	1	2	3	4	5	6	7	8
1		<.0001	<.0001	<.0001	0.9467	<.0001	<.0001	<.0001
2	<.0001		<.0001	<.0001	<.0001	0.8150	0.5905	<.0001
3	<.0001	<.0001		<.0001	<.0001	<.0001	0.0012	<.0001
4	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001	0.0037
5	0.9467	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001
6	<.0001	0.8150	<.0001	<.0001	<.0001		0.0483	<.0001
7	<.0001	0.5905	0.0012	<.0001	<.0001	0.0483		<.0001
8	<.0001	<.0001	<.0001	0.0037	<.0001	<.0001	<.0001	

Tukey-Kramer Grouping for LS-Means of Starch*InfillDensity				
LS-means with the same letter are not significantly different.				
	PeakForce LSMEAN	Starch	InfillDensity	LSMEAN Number
A	24.703636	W	80	8
B	21.158664	C	80	4
C	15.999504	C	60	3
D	12.569165	W	60	7
D				
E D	11.250667	C	40	2
E				
E	10.141013	W	40	6
F	4.594568	C	20	1
F				

Tukey-Kramer Grouping for LS-Means of Starch*InfillDensity				
LS-means with the same letter are not significantly different.				
	PeakForce LSMEAN	Starch	InfillDensity	LSMEAN Number
F	3.755794	W	20	5