Investigating the Attenuation of Starch Hydrolysis by Synergistic Interaction of Xanthan and Guar Gum Fortification during In Vitro Digestion

by

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ABSTRACT

INVESTIGATING THE ATTENUATION OF STARCH HYDROLYSIS BY SYNERGISTIC INTERACTION OF XANTHAN AND GUAR GUM FORTIFICATION DURING IN VITRO DIGESTION

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The effects of a simulated in vitro digestion model on the solution viscosity and ability to reduce the rate of glucose absorption and diffusivity of 0.81%, xanthan, 1.0% guar and 0.86% (80:20 ratio) guar-xanthan-fortified 4% pre-gelatinized starch solutions was examined in this study. Solutions were formulated with comparable apparent viscosities (50^1 s) after simulated digestion (without the addition of digestion enzymes) then passed throughout a 30 minute gastric and 2 hour small intestinal simulated digestion protocol. After 60, 90 and 120 min of simulated intestinal digestion, the xanthan gum-treated solution demonstrated a pronounced ability to retain its viscosity after 2 h of simulated intestinal digestion when compared to guar and guar-xanthan. Additionally, despite having dissimilar viscosities, the guar-xanthan and xanthan gum solutions both demonstrated the greatest suppressive effect on glucose release (GOPOD) and diffusion (dialysis) perhaps implying the significance of non-viscosity-related mechanisms influencing the release and diffusivity of glucose.
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List of Acronyms and Symbols

XG     Xanthan gum
GG     Guar gum
CS     Control solution
CVD    Cardiovascular disease
OG     Oat gum
T2D    Type 2 diabetes
RDS    Rapidly digestible starch
NSP    Non-starch polysaccharide
FRAP   Fluorescence recovery after photobleaching
SEM    Scanning electron microscopy
SGF    Simulated gastric fluid
SIF    Simulated intestinal fluid
G'     Storage modulus
G"     Loss modulus
tan δ   Loss tangent
γ      Shear rate
σ      Shear stress
K      Consistency index
n      Flow behaviour index
Τ      Shear stress
A      Absorbance
1.0 Introduction

Due to the increasing prevalence of cardiovascular diseases, obesity, and diabetes in modern western society, many researchers and health officials have turned to investigating ways to improve the dietary habits of humans to mediate and perhaps help prevent these chronic health conditions. The importance of maintaining healthy diet and active lifestyle has been increasingly highlighted as one of the primary preventative measures against these diseases. Benefits of following a healthy diet that is balanced, varied, and moderate can lead to improved health and wellness, not only physically, but also mentally. Implementation of various health programs promoting these healthy lifestyles by governments and public agencies across Canada and the world have continued to grow in importance as the research continues to demonstrate the invaluable benefits of maintaining a healthy diet.

A large degree of research has illustrated the health benefits of the regular consumption of healthy food components like anti-oxidants and dietary fiber. Ensuring these healthy food components are consumed regularly, research has pushed forward into improving our understanding of the complex ways that our bodies handle food components like dietary fiber as there is still much to be learned about the human digestive system. Dietary fiber, while being a critical component of a healthy diet, is still drastically under-consumed since it has been reported that most Canadians do not consume the recommended daily amount of dietary fiber (25 g and 38 g for women and men respectively) (Health Canada,
This trend is not limited to Canadians, making it a relevant issue around the globe.

Dietary fiber can come from many sources, is naturally present in many foods or it can be added to foods in the form of hydrocolloids. These polysaccharides have demonstrated great functionality in food formulations, being added to increase viscosity, alter mouth feel and texture, colloidal stability, and induce gelation among many other applications. The ability to add dietary fiber to a food system allows for food development professionals to create delicious, more satisfying foods, while being healthier at the same time. The functionality of dietary fiber to add viscosity and interact within food systems has been thought to aid in its beneficial health properties that have been witnessed in high fiber-food experiments and trials. Its intake has been associated with numerous health benefits (to be further detailed in later chapters), including the ability to attenuate postprandial glycaemia, something which is incredibly relevant for preventing the development of the aforementioned ailments like cardiovascular diseases, cancer, and type two diabetes (Health Canada, 2010; Abdullah et al., 2015; Diabetes Canada, 2017).

Overall, great achievements have been made in fiber research as the health benefits and potential impact on digestion have been well established but greater focus is still required to better understand in detail why and how these physiological responses or benefits occur. More recent studies of dietary fiber have focused on better understanding the direct mechanisms behind the attenuation of post-prandial glycaemia and starch hydrolysis.
The proposed mechanisms and ability for various sources of dietary fiber to illicit certain health benefits have been linked to numerous factors. Some of the primary factors relevant to the attenuating of postprandial glycemic responses include viscosity-related mechanisms that, for example, lower enzymatic accessibility to starch molecules effectively delaying hydrolysis, thicken the unstirred water layer at the level the mucosal barrier membrane slowing glucose diffusion and uptake, or modify gastric emptying and transit rates and more (Guerin et al., 2001; Gunness & Gidley, 2010; Wanders et al., 2011; Repin et al., 2017). Other related mechanisms include inherent physiochemical properties of dietary fiber sources that can interact with starch molecules and again affect the accessibility, digestibility and uptake of glucose-containing foods. Many of these proposed mechanisms and literature observations are referenced in the following chapters and evaluated with the findings of this study.

1.1 Research Hypothesis & Objectives

The research objective of the following work focuses on the enhancement of knowledge regarding the ability for soluble dietary fiber sources, specifically xanthan gum and guar gum, to attenuate starch hydrolysis and glucose release when mixed together in a starch solution. Based on prior research on these two notable dietary fiber sources and their significant ability to attenuate starch hydrolysis and glucose release (Fabek & Goff, 2015), along with the well-recognized synergistic relationship of the two gums, the research question of whether these synergistic properties may also be applicable under simulated
digestion conditions and contribute to further attenuate starch hydrolysis and available glucose release was investigated.

Both xanthan gum and guar gum individually, along with a combination of the two, were mixed in a 4% waxy maize starch solution at comparable apparent viscosities after a simulated digestion dilution treatment and exposed to a 2.5-hour, 2-stage simulated digestion procedure during which rheological and available glucose measurements were recorded. Additionally, a dialysis system was also utilized under similar conditions to measure the diffusivity and available glucose of the samples.

The initial working hypothesis was that the combined gums, because of their synergistic structural nature, would allow for enhanced attenuation of starch hydrolysis and available glucose release compared to each gum individually.

With the beneficial effects of the stability of xanthan gum in alkaline and acidic conditions, as witnessed by Fabek & Goff (2015), and the collective gelling strength of these two gums combined, it was suggested that these properties and synergistic effects may carry-over into the application of simulated digestion of starch. Furthermore, it was proposed that the potentially enhanced retention of viscosity throughout simulated digestion conditions may be able to attenuate the overall diffusivity and glucose release in a dialysate membrane system.

Based on these working hypotheses, the following goals of the present research are summarized below.
1) To formulate solutions of xanthan gum, guar gum, and xanthan-guar gum mixture with comparable apparent viscosities after simulated digestion (without the addition of enzymes)

2) To analyze rheological behaviour of the fiber-fortified solutions throughout \textit{in vitro} digestion

3) To analyze available glucose in solution after \textit{in vitro} digestion

4) To determine the ability for dietary fiber-fortified solutions to affect diffusivity after complete \textit{in vitro} digestion using a dialysis system

These goals were designed and executed with the intention of observing and characterizing the dynamics of xanthan and guar gum-fortified starch solutions within simulated digestion conditions, and overall, advancing the existing literature of these systems for future fiber research.

\textbf{2.0 Literature Review}

The following literature review aims to focus on further exploring the structure-function relationship relevant to dietary fiber-starch solutions and the impact of dietary fiber-fortification on carbohydrate digestion and absorption. Outlined briefly is the current state of cardiovascular health in society, followed by an overview of carbohydrate digestion. Additionally, the classification and importance of dietary fiber, with regards to health and carbohydrate/starch digestion will also be touched upon. Based on these concepts, the review concludes by briefly evaluating the current research methods and theory available in the literature featuring some of the commonly proposed mechanisms
and evaluation techniques of the impact of dietary fiber on starch hydrolysis and glucose release in *in vitro* and *in vivo* digestion models.

### 2.1 Cardiovascular Diseases and Diabetes

According to the World Health Organization, worldwide, there are approximately 400 million adults who are obese and another 1 billion who are considered “overweight” (World Health Organization [WHO], 2015). Those who are overweight are prone to develop hypertension, atherosclerosis and diabetes, while these and the numerous other cardiovascular diseases (CVD) are primarily influenced by diet, stress and physical inactivity (WHO, 2015).

In 2012, the World Cancer Research Fund International (2012) estimated that there were 14.1 million cancer cases fought by individuals around the world. In just the United States and Canada, it is estimated that in 2015 there was approximately 589,430 and 78,000 cancer cases and cancer-related deaths, respectfully (American Cancer Society, 2015; Canadian Cancer Society, 2015). Additionally, CVD and cancer make up the top two causes of death, in both these countries, accounting for 30% of all deaths in Canada (American Cancer Society, 2015; Canadian Cancer Society, 2015). Again, the rate of cancer prevalence is thought to be linked to diet, obesity, and healthy lifestyle choices (or lack thereof).

Perhaps most relevant to research on dietary fiber is diabetes mellitus, specifically type 2 diabetes (T2D). Diabetes involves the chronic abnormal situation wherein the body cannot produce sufficient insulin or properly use the
insulin it produces. Insulin is a critical hormone that regulates the uptake of sugar into the blood which is used as energy or stored as fat. With type 1 diabetes, the insulin-producing pancreatic β cells are destroyed causing instances of high blood sugar levels; this is dangerous and can lead to serious organ and nerve damage. With T2D, the body does not produce or react to insulin appropriately, again leading to abnormal blood sugar levels (Diabetes Canada, 2017). There are 11 million Canadians that have diabetes or a form of prediabetes which truly makes it a national concern when it comes to healthcare. Affecting almost 30% of the Canadian population, developing new methods to prevent, treat, and hopefully cure diabetes should be one of the nation’s major healthcare goals. Methods to help treat and prevent diabetes involve promoting a healthy lifestyle involving adequate physical activity and following healthy diet. A fiber rich diet can have significant impact on those at-risk or living with diabetes for numerous reasons that will be touched upon later in this review.

2.2 Carbohydrate Digestion and Absorption

The human diet is one of the most critical components to living a healthy lifestyle. Carbohydrate consumption plays a significant role in determining caloric intake levels due to its prevalence in societies’ daily diets and thus its high energy contribution (4 kcal/g). Carbohydrates can be categorized in many different ways, such as by degree of polymerization (i.e. mon/di-saccharides, oligosaccharaides, and polysaccharides) or by function (i.e. sugar, starch, or fiber), along with many other categorical classification constituents (Cummings & Stephen, 2007). Sugar, most simply is a mono- or disaccharide molecule of
glucose, fructose or galactose. Starch typically refers to a polymeric chain of glycosidically bonded glucose molecules, and is made up of two molecules, amylose and amylopectin. The term starch is often broadly used to describe polymeric chains of complex carbohydrates. Starch is produced by many plants and is the primary form of glucose storage. It also can be modified in its amylose and amylopectin proportions through the genetic manipulation of plants in order to alter digestibility or other processing purposes. Important to distinguish is the difference between amylose and amylopectin. Amylose is a dense, primarily linear arrangement of bonded α 1-4-linked D-glucose units with few branched chains while amylopectin is typically a larger, more branched series of α 1-4-linked D-glucose units in addition to α 1-6 linkages at the many branching sections (Hasjim et al., 2010). Starch that cannot be digested and/or absorbed in the small intestine can be considered resistant starch or dietary fiber, both of which provide many physiological benefits as mentioned previously and will be explored further in later chapters.

Figure 2.1 Chemical structure of Amylose (a) and amylopectin (b) polymers of D-glucose (Adapted from A. Vazquez et al., 2012).
Health Canada’s recommended daily energy requirement for adults ranges from 1900-3000 kcal depending on activity level, gender, and age. In a 2000-calorie reference diet, approximately 45-65% of the calories should come from carbohydrates (Health Canada, 2007). Carbohydrates are essential for short-term energy, brain and organ functioning, and thus the ability of the body to digest and absorb glucose is one of utmost importance (Galgani & Ravussin, 2008; Manninen, 2004).

Due to the incredibly complex intricacies involved in the digestion process and for the sake of brevity in this review, the basic, most relevant details of carbohydrate digestion are outlined below. Also worthy to note is the great level of variability all these digestion processes and phases have from individual to individual. Innumerable physiological, physical, and environmental factors account for the variation in these processes from person to person. For example, the length of each digestion phase, pH content of each phase, transit time and rate of secretion can depend on factors such as time of day, state of satiety, general health condition, and so on (Cheeke & Dierenfeld, 2010).

The majority of carbohydrate digestion occurs in the small intestine, but the digestion process actually begins in the mouth where salivary α-amylase coats the food bolus before progressing to the stomach, beginning the digestion of carbohydrates. Depending on the time the food initially spends in the mouth during chewing or swallowing, this oral phase can be brief and relatively insignificant in actual carbohydrate digestion (Cheeke & Dierenfeld, 2010). During the gastric phase, gastric secretions of HCl acid reduce the pH of the food
bolus as well as dilute the system drastically. According to Johnson (1991), the stomach secretes approximately 2 L of gastric juices per day. Due to the acidic nature of the stomach, any starch-digesting enzymes from the oral phase are deactivated, resulting in no further carbohydrate digestion during this phase.

After sufficient time in the stomach and the addition of gastric fluid components like sodium chloride and pepsin (Minekus et al., 2014), the carbohydrate chyme is peristaltically transferred into the small intestine duodenum (Ferrua & Singh, 2010). Once inside the duodenum, the acidic chyme of the stomach is neutralized with alkaline bicarbonate intestinal fluid secretions. Additionally, cholecystokinin from the pancreas activates the release of pancreatic enzymes and bile. In the jejunum, pancreatic amylase or amylglucosidase act to break down starch into smaller polysaccharides and di- and monosaccharides by attacking the α-1-4-bonds of the starch molecules. Products of this process are typically maltose, glucose and limit dextrins. Additional disaccharidases, like maltase or isomaltase, also aid in the digestion of 1-6 α D-glucosidic bonds when necessary, releasing free glucose (Cheeke & Dierenfeld, 2010). With the help of regular contractile segmentation and peristalsis by the smooth muscles of the small intestine, once these free glucose fragments come in contact with these existing brush border enzymes in the jejunum, they can be absorbed at active transport sites and be introduced into the blood stream.
The three main membrane carrier systems for glucose absorption involve SGLF1, GLUT5, and GLUT2. These carrier systems allow for glucose to be passively and actively transported across enterocytes and pores in the brush border membrane (Johnson, 1991). Once absorbed into the blood, glucose is transported to the liver to be regulated throughout the body. This process is shown in Figure 2.2, where sugar components like fructose and glucose are taken in by their respective transporter cells at the brush border of the lumen and transported to the blood through the basolateral membrane via GLUT2. Fructose is absorbed by facilitated diffusion via GLUT5, while glucose requires active transportation by the SGLT1 and 2 Na⁺ molecules then again exiting using GLUT2.
2.3 Blood Glucose levels

Once absorbed into the blood, glucose is transported throughout the body to be converted into energy. The transportation of glucose into target cells occurs by facilitated diffusion or cotransporter system (Cheeke & Dierenfeld 2010). GLUT4 is an example of another transporter protein which sends glucose to muscle and adipose tissue, helping in regulating the postprandial utilization of glucose in the blood stream (Mueckler & Thorens, 2013; Cheeke & Dierenfeld 2010).

Typical postprandial blood glucose patterns in humans are directly influenced by available carbohydrate content present in food consumed. After consumption of carbohydrate containing food, blood glucose levels rise as the body begins to digest and breakdown the sugar and starch components, transporting them to the blood. Due to postprandial hormonal stimulation of glucagon from pancreatic α cells, the concentration of glucose in the blood increases. Once blood glucose levels become too high for the body to handle, the corresponding pancreatic β cells trigger the release of insulin, initiating the cellular storage of glucose from the blood, into the muscles, liver and adipose tissue, effectively lowering the blood glucose levels back to normal range. A normal range while fasting for healthy individuals can be considered anywhere from 6.9 and 5.5 mmol/L (American Diabetes Association, 2013). For individuals with diabetes, as outlined previously, these regulatory hormones do not respond appropriately. Typically, those with diabetes will feature very slow blood glucose regulation ability, resulting in prolonged high (or low) blood glucose levels.
Glucose tolerance tests which help diagnose diabetes or pre-diabetes conditions through monitoring blood glucose levels after standardized consumption of glucose are commonly utilized. As seen in Figure 2.3, normal blood glucose curves feature lower peaks and faster response times when it comes to lowering blood glucose levels.

**Figure 2.3** An example of glucose tolerance test featuring blood glucose curves of normal and diabetic subject after administering 1g glucose/kg of bodyweight (Adapted from Cheeke & Dierenfeld, 2010).

Depending on the carbohydrate that is consumed, blood glucose levels can exhibit different patterns. Carbohydrates that are easily digested and absorbed lead to higher postprandial glycemic responses. The opposite response can be seen for foods that are more slowly digested. The glycemic
index was developed according to this principal wherein the glycemic impact of a food can be mathematically determined and indexed, providing a critical tool to dieticians and the food industry worldwide. Hundreds of food items have been tested, scored, and indexed. Foods that score higher on the index (≥ 70) lead to higher peaks of blood glucose levels and thus produce a greater insulin demand. Increased stress on this hormonal system can seriously affect those susceptible to developing diabetes and CVD through the development of insulin resistance and pancreatic β cell fatigue (Foster-Powell et al., 2002; American Diabetes Association, 2013). Conversely, foods that score lower on the GI with lower

**Figure 2.4** Proposed mechanism whereby high-glycemic diets result in the development of T2D (Adapted from Willett et al., 2002).
readily available starch content generally put lower stress on the hormonal system, and thus non-starch polysaccharides (NSPs) and fiber-fortified foods can be critical to minimizing postprandial blood glucose spikes (Foster-Powell et al., 2002).

2.4 Dietary Fiber Classification

According to Health Canada (2012), dietary fiber is defined as “(1) carbohydrates with a degree of polymerization of 3 or more that naturally occur in foods of plant origin and that are not digested and absorbed by the small intestine; and (2) Accepted novel fibres.” Furthermore, these accepted novel fibers must be “synthetically produced or are obtained from natural sources which have no history of safe use as dietary fiber or which have been processed so as to modify the properties of the fiber contained therein. Accepted novel fiber have at least one physiological effect demonstrated by generally accepted scientific evidence.” (Health Canada, 2012). The major physiological effects include, but are not limited to, the improvement of laxation or regularity by increasing stool bulk, reducing low density lipoprotein (LDL) cholesterol, reducing blood glucose and/or insulin levels after consumption, and providing energy-yielding metabolites through colonic fermentation (Health Canada, 2012). This definition serves as a guideline for manufacturers and is the basis for the policy on labeling and advertising of dietary fiber products in Canada.

Conventionally, dietary fiber can be classified as soluble or insoluble. Soluble fibers are typically utilized in industry to alter or control viscosity of a food
product through the formation of gels, while also having the ability to change the mouth-feel and texture of food products (Sworn, 2010; Delcour & Poutanen, 2013). Typically soluble fiber sources include pectin, carrageenan, inulin, guar gum, xanthan gum, and fructans and are thought to have the ability to slow digestion via their increased viscosity, which may explain their effects on starch hydrolysis and metabolism (Brennan, 2010; Delcour & Poutanen, 2013). Insoluble fiber sources are those which cannot be dissolved in water, including lignin, cellulose and some β-glucans, and are thought to reduce the digesta transit time in the intestinal tract and directly influence enzyme activity and nutrient absorption (Woolnough et al., 2010; Delcour & Poutanen, 2013). The viscosity-increasing effect that a source of fiber may possess within a system depends on factors such as degree of polymerisation, molecular weight, structural composition and chemical nature of the fiber source. Various dietary fiber sources can be found naturally in fruits, oats, nuts etc., and some fiber sources are commonly used as thickeners or additives in salad dressings, sauces, candies, ice creams etc., to improve mouth-feel, viscosity, or overall nutritional value (Delcour & Poutanen, 2013).

2.5 Fiber Intake Health Benefits

Since 1953, when the term was first used by Eben Hipsley, dietary fiber has been linked to numerous health benefits including those mentioned above and many more (Brownlee, 2011). According to many epidemiological studies, a high-fiber diet is associated with the reduction in the prevalence of T2D (Anderson et al., 2009) due to a hypoglycemic effect which is thought to slow the
absorption of glucose. High fiber intake has been linked to having anti-cancer as well as preventative effects on diabetes, coronary heart disease and improved weight management (Kendall et al., 2010; Lattimer & Haub, 2010; Biliaderis & Izydorczyk, 2007). Table 2.1 outlines many more of these anti-cancer benefits, as adapted from Biliaderis & Izydorczyk (2007).

**Table 2.1** Mechanisms by which Fiber Can Protect against the Development of Cancer (Biliaderis & Izydorczyk, 2007).

<table>
<thead>
<tr>
<th>Mechanism</th>
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<tbody>
<tr>
<td>- Increased stool bulk</td>
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<tr>
<td>o Decrease of transit time</td>
</tr>
<tr>
<td>- Dilution of carcinogens</td>
</tr>
<tr>
<td>- Binds with bile acids or other potential carcinogens</td>
</tr>
<tr>
<td>- Lower fecal pH</td>
</tr>
<tr>
<td>- Inhibition of bacterial degradation of normal food constituents to potential carcinogens</td>
</tr>
<tr>
<td>- Changes in microflora</td>
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<tr>
<td>- Fermentation by fecal flora to short-chain fatty acids</td>
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<tr>
<td>o Decrease in colonic pH</td>
</tr>
<tr>
<td>- Inhibition of carcinogens</td>
</tr>
<tr>
<td>- Increase in luminal antioxidants</td>
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<tr>
<td>- Peptide growth factors</td>
</tr>
<tr>
<td>- Alteration of sex hormone status</td>
</tr>
<tr>
<td>- Change in satiety resulting in lowered body weight</td>
</tr>
<tr>
<td>- Alterations in insulin sensitivity and glucose metabolism</td>
</tr>
</tbody>
</table>

Due to the previously mentioned fact that most Canadians do not consume enough dietary fiber to fully reap said attributed preventative health
benefits (Health Canada, 2012), it is a continuous challenge for governments and public health authorities to ensure that these fiber sources are regularly incorporated in diets. With the many sources of fiber available in industry today, this challenge can be met with further product development and research involving evaluating the dynamics of these fiber inclusive foods.

2.5.1 Proposed Mechanisms

To recap, viscosity of dietary fiber is believed by many to contribute to the attenuation of postprandial glycaemia primarily through mechanisms such as changes in neurohumoral mediation, modifying transit and gastric emptying rates (Butt et al., 2007; Jenkins et al., 1977), as well as a “bulking effect”, inhibiting diffusivity of enzymes, substrates and nutrients (Butt et al., 2007; El Kossori et al., 2000; Kim et al., 1996). While satiety and the reported effects of dietary fiber on digesta transit and gastric emptying rates are relevant to the end result of lowered postprandial glycaemia, particularly important to this study are the latter mechanisms involving the relationship between viscosity and glucose digestion, motility, enzymatic function, and the resulting glycemic response. Peeling back the layers of the bulking effect of dietary fiber and these structure-function relationships unveils a complex and mystifying set of possible sub-mechanisms and variables responsible for the attenuation of starch hydrolysis and glucose release.
2.5.2 The Bulking Effect

The mechanism of the apparent “bulking” effect is thought to be influenced primarily by (1) inhibiting amylase enzyme ability to interact or come in contact with the starch molecule, and/or (2) inhibiting the ability for glucose to reach the luminal mucosal membrane to be absorbed (Chawla & Patil, 2010). While exact mechanism operations are still not conclusively established, viscosity provided by fiber-fortification has been accepted to be typically associated with the lowering of postprandial glycaemia within the literature. The influence of fiber fortification on the “bulking” effect is still being continually investigated with greater focus on quantifying and narrowing down the root cause of the attenuation so often observed in experimentation.

2.5.3 The Bulking Effect and Viscosity

Decades preceding, Jenkins et al. (1978) effectively found that supplementation of dietary fiber in glucose solutions led to lower mean peak blood glucose levels in human participants, which was correlated to the viscosity provided by the fiber-fortification. In their studies, lower viscosity glucose solution with hydrolysed guar gum added led to higher mean peak blood glucose levels compared to higher viscosity guar gum solutions. Additionally, the researchers found that there was also a positive correlation between viscosity and the delay in mouth-to-caecum transit time.

Years later, Gallaher & Schaubert (1990) evaluated the effect of various dietary fiber sources on indicators (glycated hemoglobin and renal hypertrophy)
of blood glucose control in adult diabetic rats. Rats were fed a fiber-free diet or a diet containing 8% dietary fiber (of various sources) for 28 days. Their findings indicated that the guar gum-fortified diets, in particular, significantly increased the viscosity of the intestinal contents in 3/4 of the rats and that the use of highly viscous fiber sources can be considered to improve glycemic control in insulin-dependent diabetes. Furthermore, Wood et al., (2007) through oat gum (OG) fiber-fortification of 50 g glucose solutions dosed with various concentrations of OG lead to a linear relationship between viscosity of the mixtures consumed and the corresponding glucose and insulin responses. Compared to the control solutions (without OG), the increased dosage of OG showed reduced insulin and plasma glucose responses. Overall, these examples in the literature demonstrate the effects that are witnessed on the holistic scale. More recently, research has been diving deeper into the biomechanics of the aforementioned attenuated glycemic response caused by viscous dietary fiber.

2.5.4 The Bulking Effect and Diffusivity

The viscous effect led by the ability of soluble fiber to gel and generate thick, hydrated networks is believed to affect numerous factors involved with the moderation of postprandial glycaemia. Investigations into how diffusivity is affected by high-viscosity fiber-fortification such as Shelat et al., (2011) determined through fluorescence recovery after photobleaching (FRAP) measurements that diffusion coefficients for molecular probes similar in size to digestive enzymes, decreased as viscosity increased, but this was determined not to be the only factor influencing diffusion.
Furthermore, numerous *in vitro* experiments have demonstrated the ability for dietary fiber to inhibit starch and enzyme accessibility as well as glucose diffusivity. For example, Kwong et al. (2013) determined that dietary fiber has the ability to slow transport of hydrolyzed starch and glucose, inhibiting digestion and absorption at the brush border, by examining how well fiber-fortified solutions and gels retained glucose *in vitro*. Figure 2.5 shows glucose diffusion over 2 hour incubation period and the results indicated that the gels released significantly less glucose over all time points. The authors went on to suggested that the solid structure of β-glucan gels allowed glucose to be trapped within the food matrix during the *in vitro* assay lowered the ability for glucose to diffuse and reach the membrane barrier (where absorption would occur).

**Figure 2.5** *In vitro* diffusion of glucose from test foods over a 2 h incubation period (mean, *n* = 8). Samples of no β-glucan (ND); 4 g low MW β-glucan (LD), 4 g high MW β-glucan (HD), viscoelastic gels containing 4 g low MW β-glucan (4LG), 2 g low plus 2 g high MW β-glucan (2H2LG), and 3 g high plus 1 g low MW β-glucan (3H1LG) were all tested. Values of the same time point with different letters are significantly different (*P* < 0.05) (Adapted from Kwong et al., 2013).
Fabek & Goff (2015) also examined the diffusivity of starch-fortified with various sources of dietary fiber in vitro. Dialysis and rheological examinations of these solutions after simulated digestion determined that glucose release measurements, even in low viscosity systems, were able to induce a decreased intensity of starch hydrolysis, while dialysis specifically indicated that fiber-fortified treatments featuring higher viscosities led to attenuated diffusion of glucose. Morphological analysis was also performed and through scanning electron microscopy (SEM) and light microscopy, the examination of granules throughout simulated digestion revealed indications that xanthan and guar gum-fortified granules both appeared to resist starch hydrolysis as the authors witnessed aggregation effect leading to less substantial degradation.

As research on dietary fiber sources commonly utilized in industry continues to be published, it becomes increasingly clear that an increase in the viscosity of fiber-fortified solutions alone does not equate to a greater suppressive effect on starch digestibility and glucose release. Many studies have evaluated the effect of viscosity but continually come back to the conclusion that viscosity alone is not solely responsible for the suppressive responses witnessed (Sasaki & Kohyama, 2011; Sasaki & Kohyama, 2012). More physiochemical inherent factors are likely to be necessarily considered in order to effectively gauge the ability of a dietary fiber source to attenuate starch digestibility.
2.5.5 Intermolecular Interaction & Enzyme Function

Another proposed mechanism by which viscous dietary fiber can attenuate the glycemic response is through inhibiting the function of α-amylase via “non-competitive inhibition affecting the biochemical degradation of starch”. Slaughter et al. (2002) examined the effects of water-soluble non-starch polysaccharides (NSP), particularly guar galactomannan-fortification, on starch hydrolysis and proposed that regions of α-amylase possessed an affinity for guar galactomannan influencing α-amylase activity. Direct binding of the enzyme to galactomannan resulted in the complex being inactive. Additionally, the processing of starch at low water levels was found to lower the catalytic efficiency of α-amylase (Slaughter et al., 2002).

Inherent physio-chemical properties of certain starches and dietary fiber also appear to influence starch hydrolysis. Ionic interactions between starch and gums, as well as swelling properties have been proposed as other contributing mechanisms. Electrostatic interactions between cationic starch and anionic gums lead to instant aggregations where conversely, non-ionic gums form sheet structures that only loosely wrap granules (Chaisawang & Suphantharika, 2006). Swelling behaviour has previously been demonstrated to influence starch hydrolysis and thus should also be considered when evaluating the ability of a dietary fiber to attenuate starch hydrolysis and glucose release. As different dietary fiber sources absorb water and form gel structures of varying strengths upon mixing, it is also important to consider these inherent abilities when
evaluating the potential for a dietary fiber to attenuate starch hydrolysis and the release of glucose.

2.6 Study Specific Fiber Sources

2.6.1 Guar Gum

Guar gum is a water soluble non-ionic galactomannan polysaccharide found in guar beans (*Cyamopsis tetragonoloba*) with a linear 1, 4-linked β-D-mannan backbone and a 1, 6 α-linked galactose side chain on alternating mannose units (Kawamura, 2008). Molecular weights range from 500 kDa to 8000 kDa (Kawamura, 2008). The structure of guar gum enables it to be a powerful viscosifying agent, increasing the viscosity of solutions as its concentration increases, acting as a non-Newtonian, pseudoplastic fluid. Possessing a higher galactose content, guar gum has the ability to swell and disperse in both hot and cold water, unlike other galactomannan gums like locust

![Structural representation of guar gum featuring the mannose backbone and galactose side chains (Adapted from Thombare et al., 2016).](image)
bean gum, which must be heated to ensure complete solubility (Wang et al., 2000; Dunstan et al., 2001). Alternatively, its galactomannan structure also makes it vulnerable to polymer breakage in environments of extreme pH as well as strong oxidizing agents (Fabek & Goff, 2015; Wielinga, 2010). In general, guar gum is often used in the food industry at concentrations of 1%, or lower, for emulsifying, thickening and gelling properties along with its abilities to prevent syneresis and starch retrogradation (Fabek & Goff, 2015). Molecular weights and intrinsic viscosity measurements for guar have been examined by Achayuthakan & Suphantharika (2008) who determined that the more flexible polymer chain of guar gum enables it to have a smaller intrinsic viscosity, as compared to gums like xanthan gum. Due to its strong viscosifying effect in liquids, guar gum is linked to having significant physiological effects due to its structure and viscosity increasing abilities, allowing it to attenuate post-prandial glycaemia, starch hydrolysis, and reduce plasma cholesterol and glucose levels in humans (Brennan & Tudorica, 2008; Fabek & Goff, 2015).

2.6.2 Xanthan Gum

Xanthan gum is produced by the bacteria *Xanthomonas campestris* and is commercially harvested by inducing anaerobic fermentation of pure cultures of the bacteria followed by various processing steps. Xanthan gum possesses a linear 1,4-linked α-d-glucose backbone with trisaccharide chains containing D-glucuronic acid (Garcia-Ochoa et al., 2000; Cargill, 2017). Figure 2.7 illustrates the structural composition of xanthan gum. Molecular conformation may depend on dissolution temperatures, conforming in two different temperature dependant
orders helical or random coil. Xanthan gum is highly soluble in water of any temperature resulting in a pseudoplastic or shear thinning substance. When added with starch, xanthan generates a weak 3 dimensional network with this helical structure enabling it to retain much of its viscoelastic properties (Garcia-Ochoa et al., 2000).

![Structural representation of xanthan gum](image)

**Figure 2.7** Structural representation of xanthan gum (Adapted from Sworn & Monsanto, 2000).

With a large molecular weight ranging from 2,000kDa to 20,000kDa and a helical formation providing structural protection from enzymatic hydrolysis, xanthan gum also is capable of synergistic gel-forming interactions with other gums (Nussinovitch, 1997; Garcia-Ochoa et al., 2000). The stiff chain helices of xanthan gum allow it to form complexes with other polymer molecules (Garcia-Ochoa et al., 2000). Through interaction with unsubstituted galactomannans, with just a small application of xanthan gum (<1%), the synergistic effect can lead to pronounced increases in viscosity and induce gelation, lending itself to be a greatly functional food component. When combined with galactomannans,
gelation may occur, while individually they will typically not gel. Xanthan-galactomannan interactions can depend on the mixture ratio along with pH, temperature, and ionic state of the environment (Garcia-Ochoa et al., 2000). Depending on the desired application, optimum ratios of xanthan to guar gum can range from 1:4 to 1:3 range and the synergetic interaction is thought to be at its maximum under neutral pH, and may be hindered by high salt concentrations or low pH (Sworn 2010; Ferrua & Singh et al., 2010). Xanthan gum was also noted by Sworn & Monsanto (2000) to be significantly resistant to pH extremes.

Being anionically charged, xanthan can also elicit electrostatic interactions between cationic starch molecules leading to aggregation (Singh et al., 2010). Other characteristics include its ability to inhibit swelling and prevent amylose leaching (Chaisawang & Suphantharika, 2005). The many unique properties of xanthan allow it to be popularly used in the food industry, functioning as a substitute for gluten, enabling the prevention of syneresis, increasing product stability, forming strongly pseudoplastic solutions, and altering texture and thickness properties among many other notable uses (Garcia-Ochoa et al., 2000; Sworn, 2000).

Recent studies on its applicability as a non-starch polysaccharide and dietary fiber by Fabek & Goff (2015) demonstrated xanthan gum to be effective at attenuating glucose diffusion and starch hydrolysis under simulated digestion treatments. These findings are congruent with previous literature sources outlining the potential for the healthful effects of xanthan, due to inhibiting starch
digestion, slowing digestion, increases in satiety, and reductions in cholesterol
(Brennan et al., 2008; Edwards et al., 1987; Sworn 2010).

2.7 Rheological Characterization

The use of rheological theory and analysis is instrumental to the evaluation of “flow” behaviour of fiber-fortified solutions and their structural dynamics during exposure to digestive processes, in vivo and in vitro. “Rheo” being Greek for “flow”, is a branch of physics that focuses on the study of the flow behaviour of liquids and the deformation behaviour of semi-fluid solids (Bourne, 2002). Relevant to the food industry, rheological theory and evaluations can identify characteristics about shelf-life, texture, microstructure, processing parameters and inherent nature of the liquid or semi-solid food. The basic concepts of rheology involve the classification of a substance as Newtonian or non-Newtonian, wherein the fluid is characterized by comparing proportionally shear rate (velocity of moving plate / the distance between two parallel plates) against shear stress (force of friction from fluid acting on a body in the path of that fluid) within the two-plates model. (Bourne, 2002; Mitchell & Wolf, 2011).

Equation 2.1. Shear stress formula

\[ \tau = \frac{F}{A} \]

(Where \( \tau \) = shear stress, \( F \) = force applied and \( A \) = cross-sectional area of material with area perpendicular to the applied force vector)
Matter can be considered within three rheological states, 1 (ideal) viscous, like water, 2 viscoelastic, like putty, and 3 (ideal) elastic, like gelled Jell-O, where the material returns to its initial size and shape when acting forces are removed (Mitchell & Wolf, 2011). Newtonian fluids, such as water or vegetable oil, show proportionality between shear stress ($\gamma$) and shear rate ($\sigma$), while all other fluids that may elicit a disproportioned or non-linear change in viscosity with the introduction of force to the system are considered non-Newtonian fluids (Mitchell and Wolf, 2011). Non-Newtonian fluids can be most simply described with the Power law equation, as seen below.

**Equation 2.2. The Power Law model**

$$\sigma = k \times \dot{\gamma}^n$$

(Where $\sigma$ = shear stress, $k$ = consistency coefficient, $\dot{\gamma}$ = shear rate and $n$ = the flow behaviour index)

The Power law model describes the shape of an ideal viscosity curve wherein $K$ is the consistency index (Pa), and $n$ is the flow behaviour index, which describes the apparent viscosity of a fluid at a given shear rate and the deviation from the Newtonian flow behaviour, respectively. The closer the flow behaviour index is to 1, the closer the fluid is to being Newtonian. Non-Newtonian fluids can be shear thinning ($0<n<1$) or if greater than 1, dilatant ($1>n>\infty$) (Rao, 1999). These flow behaviours are represented visually in Figure 2.8
Many other determinations can be performed using the rheological principles, and the numerous other mathematical models or formulas that exist in the realm of rheology. These determinations, as mentioned previously, can be used to extract inferential data and evaluate, for example, the structural composition and conformation of a solution under certain system conditions. With the use of rheological analysis methods, it is possible to identify the degree of (dis)order present in a solution, as well as the extent of entanglement and interaction of polysaccharide chains as it is relevant to fiber-fortified systems. Determining concepts like the entanglement of a solution provide evidence for structural assumptions to be made about why a solution may retain its viscosity.

![Figure 2.8](image)

**Figure 2.8** Schematic Ideal viscosity curves for Newtonian (ideal viscous/liquid) and non-Newtonian fluids (shear thinning and shear thickening) (Adapted from Baumert & Wessling, 2016).
under certain conditions. Capabilities like this speak to the significant role that rheological analysis plays in characterizing the structure, stability, and functionality of fiber-fortified systems.

### 2.8 In vitro Digestion Models and Methods Overview

Due in large part to the cost, labour and ethical considerations that typically come with *in vivo* experiments in food science, with regards to dietary fiber and digestion models, *in vitro* methods have proved to be useful in evaluating preliminary physio-chemical dynamics, bioaccessibility, as well as hypothesis building. Because the animal digestive system is innately complex, *in vitro* models unfortunately cannot come close to replicating the complexities of the animal digestive system. *In vitro* models are traditionally limited in their transference or predictability when compared to *in vivo* systems but very much serve a purpose in empirically demonstrating physiological and chemical reactions under simulated conditions. Differences in simulated fluid composition, enzyme composition and concentrations, pH, degree of dilution, and even mechanical or physical agitation/forces/events involved are just some of the factors that can vary within the *in vitro* models present in the carbohydrate-related simulated digestion literature. An example of this variability can be seen in the hydrolysis curves of a pre-gelatinised starch incubated at pH levels ranging from 4.0-6.9, (ranges commonly utilized in digestive trials), presented in Figure 2.9, adapted from Woolnough et al., (2008).
These findings validate the variable stability of porcine pancreatin at various pH levels and the consequential effect on hydrolysis and sugar release over time. At pH 4.0, pancreatic activity was deactivated after the introduction of a second pre-gelatinised starch treatment at 120 minutes, while samples at pH 6.9 featured relatively moderate pancreatin activity; samples at pH 5.0 featured the highest activity level. The variability of factors like pH clearly has the ability to play a significant role in affecting the functionality of different enzymes and thus when comparing experiments that utilize, for example, varying intestinal pH levels, transferability of data may be questionable.

Because of this large degree of variability in in vitro methods seen today and historically, some researchers such as Woolnough (2008) and Minekus et al. (2014) have attempted to review and standardize methods. While all research questions and experiments may not be able to follow the exact steps, compositions, ratios etc., outlined by the authors, the goal of review studies such as Minekus et al. (2014) is to provide a standard template to improve the consistency and transferability within the field of simulated digestion.

Static in vitro digestion models for carbohydrate digestion have been around for decades, being established most prominently in literature by Southgate (1969), as well as Englyst, Wiggins and Cummings in 1982, the latter of which set the outline for what is now known as the "Englyst method"; the foundation for which many in vitro methods have been built upon, especially those involving carbohydrates and resistant starch.
2.8.1 Static Digestion Models for Carbohydrates

These methods typically outline a three stage model typically consisting of an oral phase, a gastric phase and an intestinal phase in order to mimic actual physiological conditions during digestion. A schematic outline of these phases and protocol with standardized time, enzyme composition and pH values can be seen in Figure 2.10 from Minekus et al. (2014). Again, many of these conditions may fluctuate according to research questions, food-specific components involved, along with numerous other factors acknowledged in the article (Minekus et al., 2014). Based on the literature compiled by these authors, it is possible to generate an appropriate study-specific in vitro digestion protocol that is still standardized, according to relevant conditions.

Current methods of research have involved the development and use of in vitro simulated digestion instruments that mimic the in vivo digestive process, which include devices like plastic biopsy pots or temperature controlled shaking water baths that utilize simulated gastric fluids and enzymes (Fabek, 2015; Foschia et al., 2014).
Figure 2.9 Available sugars measurements under varying pH conditions over time demonstrating the impact of pH on starch digestibility and pancreatin activity (Adapted from Woolnough et al., 2008).
Figure 2.10 Overview and flow diagram of a simulated *in vitro* digestion method. SSF, SGF and SIF are Simulated Salivary Fluid, Simulated Gastric Fluid and Simulated Intestinal Fluid, respectively. Enzyme activities are in units per mL of final digestion mixture at each corresponding digestion phase (Adapted from Minekus et al., 2014).
Incubation throughout all the stages is suggested at 37°C. Mechanical agitation can be facilitated with the use of stirred or shaking water baths, as well as the addition of glass balls in glass flasks containing the food component to induce better mixing (Brighenti et al., 1995; Englyst et al., 1992; Foschia et al., 2014; Woolnough et al., 2008). The oral phase involves a brief mechanical disruption of the food bolus and dilution with simulated salivary fluid containing α-amylase at a pH of 7. This oral phase can be considered optional, depending on food composition and state (liquid or solid). The following gastric phase leads to further dilution with pepsin dissolved in a simulated gastric fluid made primarily with HCl and pepsin at a pH of 2-4. This phase can last anywhere from 30 minutes to 4 hours. The final stage features further dilution with simulated intestinal fluid made up of pancreatin, amyloglucosidase and various other hydrolyases, depending on preference or applicability. These enzyme compositions are combined with simulated bile fluid and buffer solution bringing the pH back up to 6.5-7.5. This phase can last in the range of 1 to 6 h.

With regards to analysis, rheological equipment, dialysis equipment and glucose or reducing sugar colorimetric methods are also typically utilized to measure viscosity, glucose diffusion, and reducing sugars respectively (Fabek, 2015; Foschia et al., 2014; Negrulescu et al., 2012). The measurement of glucose concentrations have also been evaluated with the D-Glucose assay kit (GOPOD) method, which has been widely accepted in food analysis and chemistry literature (Izydorczyk et al., 2008; McCleary et al., 1994). Importantly, the rapidly digestible starch (RDS) component of a food is typically thought to be
hydrolysed and absorbed within 15 to 30 minutes of simulated intestinal digestion (Guyton & Hall, 2000), therefore it is important to measure the RDS within this beginning stage.

2.8.2 Dynamic Models

As in vitro digestion models continued to grow in their use and application, new dynamic methods have also been developed, such as the human gastric simulator, developed by Kong & Singh in 2010, where the focus on peristaltic agitation and gastric emptying of the stomach contents has been developed to induce more representative conditions of the gastric phase. Another dynamic system that has been developed recently is the TIM system (TNO Nutrition and Research Institute) which features a more complex system of chambers and compartments facilitated by representative peristaltic movement of digesta through the three stages of digestion. Automated sensors and programs calculate parameters such as pH, simulated fluid secretion and flow rates of the digesta. The TIM system, while expensive to utilize, may be the most complete and complex dynamic model system currently available.

The incorporation of computer technology and a better understanding of in vivo conditions allow for dynamic models to further standardize the methods of simulated digestion protocol and improve consistency amongst different experimental objectives. Correspondingly, as modelling techniques (dynamic and static) become increasingly complex and strive to become more representative of the in vivo digestion process, the body of research on carbohydrate and starch
digestion will continue to grow. Future developments of these models are enabling the faculty of food science to enhance their understanding of carbohydrate digestion and develop novel methods or theories to curb the metabolic syndrome.

3.0 Materials and Methods

3.1 Materials

The four sample treatments formulations included two fiber fortification treatments, GUMPLETE™ XANTHAN GUM 80 MESH SRg2, GUMPLETE™ GUAR GUM, and “ULTRA-SPERSE® A” as the modified starch component, all provided by Ingredion (Bridgewater NJ, USA). ULTRA-SPERSE® A is a cold water swelling modified waxy maize starch with a wide variety of applications. Simulated gastric fluid (0.2% (w/v) Sodium Chloride in 0.7% (v/v) Hydrochloric Acid) was supplied by Ricca Chemical Company (Arlington, TX, USA) while pepsin (from porcine gastric mucosa, ≥250 units/mg solid) was supplied by Sigma Aldrich (St. Louis, Missouri, USA). Intestinal phase buffer solution was created using sodium phosphate monobasic monohydrate and sodium phosphate dibasic heptahydrate, both from Sigma Aldrich (St. Louis, Missouri, USA). Enzymatic component of Amyloglucosidase (from Aspergillus niger >260U/ml) was also supplied by Sigma Aldrich (St. Louis, Missouri, USA) while bile salts were obtained from Fisher Science Education (Hanover Park, IL, USA) with pancreatin U.S.P. supplied by MP Biomedicals (Solon, OH, USA). The pH levels of samples were balanced using 2 N hydrochloric acid and sodium
hydroxide solutions which were provided, by Fisher Scientific (Fair Lawn, New Jersey, USA). Deionized water was used to prepare all solutions and ethyl alcohol (95%) was acquired from Commercial Alcohols (Brampton, ON, Canada).

3.2 Methods

3.2.1 Sample Formulation & Preparation

Samples were prepared by first dry mixing 0.86%, 1.00%, or 0.81% (w/w) concentrations of guar gum with xanthan gum at an 80:20 ratio, guar gum, and xanthan gum, respectively, with 4% (w/w) modified starch, then adding each weighed mixture to deionized water at room temperature. The concentration of 80:20 was selected based on Saha and Bhattacharya's (2010) recommended optimum guar/xanthan gum ratio used in industry for synergistic effects. The final fiber-fortification concentrations were determined during preliminary trials and chosen within the 1% range to relate to samples typical industry usage values. The control sample for analytical comparison involved creating a non-fiber-fortified 4% (w/w) starch solution. Concentrations of fiber-fortification varied with the purpose of matching apparent viscosities at a shear rate of 50 s\(^{-1}\) after preliminary two-stage simulated digestion trials (void of any enzymatic addition and replaced with identical volumes of water). This was conducted to help evaluate the effect of pH and dilution on the sample formulations. This shear rate is the suggested approximate shear rate that is typically demonstrated during digestion (Minekus et al., 2014; Steffe, 1996). The prior mixing of dry ingredients was critical to facilitate total gelation and reduce clumping or aggregates from
forming. Samples were mixed using a magnetic stir bar and stir plate until fully dissolved, then covered and stored at 4°C for 16 h prior to use. Table 3.1 displays the various final formulations utilized. The simplified sample formulations were favorable to examine the effect that each fiber-fortified treatment alone had on starch hydrolysis and consequential glucose release.

**Table 3.1** Study treatment formulation (% w/w) for guar gum (GG) + xanthan gum (XG), guar gum, xanthan gum-fortified solutions and the control solution (CS).

<table>
<thead>
<tr>
<th></th>
<th>GG + XG (80:20)</th>
<th>GG</th>
<th>XG</th>
<th>CS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>95.14</td>
<td>95</td>
<td>95.19</td>
<td>96</td>
</tr>
<tr>
<td>Fiber</td>
<td>0.86</td>
<td>1</td>
<td>0.81</td>
<td>0</td>
</tr>
<tr>
<td>Starch</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>TOTAL</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

### 3.2.2 In Vitro Simulated Digestion Methods

The *in vitro* simulated digestion protocol was adapted from various sources in the literature, primarily from Fabek (2011), Fabek & Goff (2015), and Minekus et al. (2014). The two-stage *in vitro* methods involved simulating the effects of the gastric and small intestinal phases. Salivary phase was omitted due to the literature recommending to not including this phase unless the food source was solid in nature and preliminary digestion experiments verifying the negligible effect of the oral phase on total available glucose (Minekus et al., 2014). To
begin the two-stage digestion procedure, 15 g of each fiber-fortified and control solutions were added to Erlenmeyer flasks containing 4 glass balls, 1 cm in diameter (to mimic and induce agitation), covered, and placed in a shaking water bath (Thermo Scientific Marietta, OH, USA) at 37°C for 5 min, allowing for the samples to acclimate to the temperature prior to the addition of the simulated gastric fluid (SGF). The SGF contained 3.2 mg mL\(^{-1}\) of pepsin, dissolved in 7 mL of a 0.2% NaCl (w/w) and 0.7% HCl (w/v) solution at a pH of 1.8 (±0.1).

The combined mixture was then incubated in the shaking water bath at a speed of 160 rpm for 30 min and a temperature of 37°C. The duration of gastric digestion was supported by Minekus et al. (2014), which indicates the typical gastric digestion phase can vary from 30 min to 2 h, depending on food type and other physiological factors.

Afterwards, to mimic the small intestinal phase, 4.6 mL of simulated bile fluid comprised of 3.6 mL water and 1 mL of 2N hydrochloric acid with 8 mg mL\(^{-1}\) of bile salts was added to each of the flasks. Additionally, simulated intestinal fluid (SIF), made up of 5mg mL\(^{-1}\) of pancreatin dissolved in 14 mL of 0.5M sodium phosphate buffer (pH 6.8) was added. Table 3.2 represents the composition of these simulated digestion fluids. As utilized by Fabek & Goff (2015), under the influence of Oomen et al. (2003), the ratio of SGF: SIF: SBF was 1:2:0.5 respectively (Guyton & Hall, 2010).
Finally, 0.33 mL of amyloglucosidase (260 U/mL) was then added to each solution. The pH of the final mixture was adjusted to 6.8 (±0.1) with 1 M NaOH as observed in human small intestine by the literature (Guyton & Hall, 2010; Minekus et al., 2014). The samples continued their incubation in the shaking water bath at 160 rpm at 37°C for the next 2 h, consistent with dynamics of stomach and intestinal in vivo conditions (Minekus et al., 2014).

Glucose concentration was performed using the D-Glucose Assay Kit (GOPOD) by Megazyme International Ireland Ltd., (Wicklow, Ireland). The procedure involved adding 3.0 mL of GOPOD reagent to 0.1 mL of sample and incubating for 20 min at 45°C. The reagent, inducing a colour change, allowed for the absorbance to be read at 510 nm against the reagent blank using a spectrophotometer (Beckmand DU 7400). A glucose standard solution provided within the kit was also utilized to read against these measurements in order to determine the D-glucose concentration (ug/0.1 mL)

**Table 3.2** Composition of simulated gastric, intestinal and bile fluids used during in vitro digestion.

<table>
<thead>
<tr>
<th>Simulated Gastric Fluid</th>
<th>Simulated Intestinal Fluid</th>
<th>Simulated Bile Fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2% NaCl (w/w) in 0.7% HCl (w/v)</td>
<td>0.5M NaH2PO4</td>
<td>3.6 (water): 1 (2N HCl) (v/v)</td>
</tr>
<tr>
<td>3.2 mg mL⁻¹ pepsin</td>
<td>5 mg mL⁻¹ pancreatin</td>
<td>8 mg mL⁻¹ bile salts</td>
</tr>
</tbody>
</table>
Formula 3.1 D-glucose calculation

\[
D - Glucose \left( \frac{\mu g}{0.1 \text{mL}} \right) = \frac{\Delta A_{Sample}}{\Delta A_{D-Glucose \text{ standard} \ (100 \mu g)}} \times 100
\]

Table 3.3 GOPOD assay procedure for reagent blank, glucose standard, and sample formulation.

<table>
<thead>
<tr>
<th></th>
<th>Reagent Blank</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>GOPOD reagent</td>
<td>3.0 mL</td>
<td>3.0 mL</td>
<td>3.0 mL</td>
</tr>
<tr>
<td>D-glucose standard</td>
<td>-</td>
<td>0.1 mL</td>
<td>-</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>-</td>
<td>0.1 mL</td>
</tr>
<tr>
<td>Buffer or water</td>
<td>0.1 mL</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

3.2.3 Sampling Protocol

After the completion of the simulated gastric phase, 1mL aliquots of digesta were withdrawn at 0, 30, 60, 90 and 120 min then transferred into 50 mL centrifuge tubes filled with 4mL of absolute ethanol. Tubes were briefly vortexed immediately after sampling to minimize clumping and improve precipitation in ethanol, then centrifuged for 20 min at 2500 rpm in an IEC Centra CL2 centrifuge (Sigma Aldrich, USA). The resulting supernatant was then extracted and stored at -20°C until glucose concentration measurements were conducted.

3.2.4 Glucose Diffusion within Dialysis

To measure the rate of diffusion of the sugar release in solution, typical digestion protocol (as outlined above) was followed with the exception that upon reaching the simulated intestinal digestion phase, all contents of the digesta were
transferred into a dialysis bag. This bag was sealed, and submerged into a beaker containing 450 mL of sodium phosphate buffer, covered, and placed into the 37°C shaking waterbath at 160 rpm and incubated for 2 h. Aliquots of 0.1 mL of the buffer solution were extracted at time points of 0, 30, 60, 90, and 120 min while the equivalent volume of buffer was replaced after each withdrawal. The bag was inverted using forceps every 30 min to ensure proper exposure and movement of the digesta. Aliquots were diluted as needed and added directly to the prepared GOPOD solution where they underwent the GOPOD procedure as per manufacturer instructions.

3.3 Digesta Viscosity Matching

The *in vitro* protocol outlined in sections above was followed initially without the addition of any enzymes which were replaced with the equivalent volumes of water for rheological evaluation. This step was necessary to match the apparent viscosities at 50 s⁻¹, helping to isolate for the variables of pH and dilution, and better evaluate the effect of enzyme activity on viscosity through simulated digestion conditions. Additionally, this step helped determine the appropriate sample formulation and amount of fiber-fortification necessary to match the viscosities before undergoing simulated digestion protocol with the addition of enzymes.

3.4 Rheological Measurements

Initial rheological measurements were attained using a cone and plate geometry with a cone radius of 60 mm, truncation gap of 50.8, and an angle of
2°, while post-dilution samples were measured using a double gap rotor and 
standard concentric cylinder recessed end cup style geometry. All measurements 
were gathered using the AR 2000, TA Instruments (New Castle, DE, USA). Flow 
behavior, viscosity, and dynamic viscoelasticity data were all gathered at 3 
different instances, before digestion, after dilution and digestion (without 
enzymes), and after dilution and digestion (with enzymes). All measurements 
were performed in triplicate and at 37°C.

Flow behavior of the various NSP-fortified solutions was measured by 
applying a continuous shear rate sweep from 10 to 200 s\(^{-1}\) with 30 sample points 
at 37°C and a truncation gap of 50 µm or 200 µm for each the cone and plate, 
and cup and bob geometries, respectively. Dynamic viscoelasticity (G’ and G") 
was measured in a frequency range of 0.01 – 10 Hz at 37°C and constant stress 
(0.5 Pa). At this stress level, all solutions displayed linear viscoelastic behavior in 
the preliminary stress sweep tests.

3.5 Statistical Analysis

Statistical analysis was performed using Graphpad prism 5.0 (GraphPad 
Software, Inc., La Jolla, CA, U.S.A.). Analysis of variance (ANOVA) test 
complemented by a Tukey’s Multiple Comparison test were performed to 
determine mean and standard deviation values of solution digesta viscosity and 
available glucose values. The significance level was set at \( p < 0.05 \). Nonlinear 
regression analysis was also utilized to model flow behaviour of digesta and the
Power Law model was used to analyse the flow curve and consistency index values. All experiments were performed in triplicate.

4.0 Results and Discussion

4.1 Viscosity & Flow Behaviour Characterization

Apparent viscosity at 50 s⁻¹ and concentration of the three fiber-starch formulations at the various stages of observance are listed in Table 4.1 below. The apparent viscosities of the solutions are recorded as an average and the matched apparent viscosity value of 50 s⁻¹ was based on reported typical shear rates during digestion (Borwankar, 1992; Minekus et al., 2014).

Table 4.1 Apparent viscosity (Pa.s) at 50s⁻¹ of control, guar gum, xanthan gum, guar/xanthan gum-fortified starch solutions before, and after simulated digestion treatments.

<table>
<thead>
<tr>
<th>Fiber-fortified Solution</th>
<th>Concentration of fiber fortification</th>
<th>Apparent Viscosity at 50 s⁻¹ (Pa.s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Post-digestion</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(no enzymes)</td>
</tr>
<tr>
<td>Guar Gum</td>
<td>1.0%</td>
<td>0.133ᵃ</td>
</tr>
<tr>
<td>Xanthan Gum</td>
<td>0.81%</td>
<td>0.1408ᵃ</td>
</tr>
<tr>
<td>Guar/Xanthan mixture</td>
<td>0.86%</td>
<td>0.1493ᵃ</td>
</tr>
<tr>
<td>Control</td>
<td>0%</td>
<td>0.0081ᵇ</td>
</tr>
</tbody>
</table>

*Values with different lettering indicate significant difference (p<0.05)*

The flow behaviours of these solutions were evaluated (1) after the simulated digestion protocol without the addition of enzymes, (2) before undergoing simulated digestion, and (3) after simulated digestion with the
inclusion of enzymes. Evident from Table 4.1, apparent viscosity levels were drastically reduced after undergoing simulated digestion. Xanthan gum resulted with the highest apparent viscosity, despite having the lowest initial apparent viscosity of all the three fiber-fortified solutions. Guar/xanthan and guar gum solutions experienced more significant losses in viscosity at 50 s\(^{-1}\) after simulated digestion than the control, but ultimately were not significantly different from one another. Both the guar/xanthan and guar gum-fortified solutions did not appear to be able to slow down the enzymatic degradation of starch experienced during simulated digestion; retaining approximately just 2% and 3%, respectively, of their matched initial viscosity. On the other hand, the xanthan gum-fortified solution demonstrated the highest degree of retained viscosity after simulated digestion, retaining approximately 12% of its pre-digestion viscosity.

The post-digestion apparent viscosity values, when compared to the post-digestion (without enzymes) values for all three fiber-fortified solutions, showed a similar trend as the fiber-fortified solutions retained approximately 19% (guar gum), 61% (xanthan gum), and 26% (guar/xanthan mixture). These results illustrate the superior ability of xanthan gum to retain its viscoelasticity and significantly reduce enzymatic degradation of starch, a notion that has been demonstrated previously in the literature, specifically the works of Fabek & Goff (2015).

As illustrated in Figure 4.1, which plots, prior to simulated digestion, viscosity versus shear rate, all three fiber-fortified solutions initially exhibited non-Newtonian pseudoplastic behaviour. Upon the fiber fortification of the control
starch solution, all solutions saw significant increases in initial viscosity.

Subsequently, the resulting apparent viscosities following the simulated gastric phase and 2 h of simulated intestinal phase conditions can be seen in Figure 4.2. All solutions demonstrated lower flow curve values and apparent viscosities across the tested shear rate range, indicating further, the effects of enzymatic hydrolysis on the fiber-fortified solutions under simulated intestinal phase conditions.

![Figure 4.1 Viscosity of control (CS), guar gum (GG), xanthan gum (XG), guar/xanthan gum mixture (Mix) fortified solutions as a function of shear rate (1/s) before simulated digestion.](image-url)
Most evident from this figure is the significant retention of viscosity and shear-thinning behaviour of xanthan gum solution compared to the other two fiber-fortified solutions and the control. This shear-dependant behaviour exhibited is also later quantified in Table 4.2 with the corresponding low behaviour index value of xanthan gum. The structural properties of xanthan gum, mainly its helical structure, and larger molecular weight are likely related to its ability to retain viscosity, resist depolymerisation and overall, protect the starch complex from hydrolysis during simulated digestion. Similar results regarding the structural resiliency of xanthan gum-fortified solutions have been reported under comparable digestion treatment conditions such as those by Fabek (2011) and Dikeman et al. (2006). Less rigid, more randomized-coil fiber-fortifications, like

Figure 4.2 Viscosity of control (CS), guar gum (GG), xanthan gum (XG), guar/xanthan gum mixture (Mix) fortified solutions as a function of shear rate (1/s) after simulated digestion.
guar gum, likely contribute to the greatly reduced degree of shear-dependant behaviour. Additionally, high dilution and enzymatic hydrolysis factors experienced by guar/xanthan and guar gum solutions during simulated digestion would seemingly contribute to this loss of pseudoplasticity. At 50 s\(^{-1}\), no significant difference was determined between the apparent viscosities of guar gum and the guar/xanthan gum mix solution, possibly implying that potential synergistic combination of the guar/xanthan mixture does not appear to offer any enhanced ability to retain viscosity or pseudoplasticity after simulated intestinal digestion. Compared to the control solution, all fiber-fortified solutions still demonstrated small but significant viscosity-retaining ability after undergoing simulated digestion likely due to the presence of the unhydrolyzed gums.

Consistency index (K), flow behaviour index (n) data derived from application of the Power Law equation are shown in Table 4.2 for before, and after simulated digestion treatment.

**Table 4.2** Consistency index (K) and flow behaviour index (n) from the Power Law model of the control, guar gum-, xanthan gum-, fortified solutions before and after *in vitro* digestion.

<table>
<thead>
<tr>
<th>Solution</th>
<th>Before Digestion</th>
<th>After Digestion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K (Pa.s)</td>
<td>n</td>
</tr>
<tr>
<td>Xanthan gum</td>
<td>11.86(^{a})</td>
<td>0.0586</td>
</tr>
<tr>
<td>Guar &amp; Xanthan gum</td>
<td>2.975(^{b})</td>
<td>0.07555</td>
</tr>
<tr>
<td>Guar gum</td>
<td>3.885(^{b})</td>
<td>0.2019</td>
</tr>
<tr>
<td>Control</td>
<td>0.02955(^{c})</td>
<td>0.4777</td>
</tr>
</tbody>
</table>

*Values with different lettering indicate significant difference (p<0.05)*
The flow behaviour index values from this table demonstrate that after simulated digestion only xanthan gum and, to a lesser degree, guar/xanthan-fortified solutions behaved as pseudoplastic non-Newtonian fluids; as higher values (closer to 1) indicate lesser degree of dependence on shear thinning and thus acting more Newtonian. The xanthan gum solution remained the most shear dependant of all the fiber-fortified solutions, due to its previously described inherent structural properties. Guar gum and the control solutions both behaved Newtonian-like, while the guar/xanthan mixture was slightly less Newtonian than these other two. Consistency index (K) values demonstrate a significant loss in viscosity experienced by all solutions after simulated digestion. Again, the xanthan gum solution demonstrated the greatest retention of viscosity while the guar/xanthan mixture featured lesser amount retention ability.

Considering all solutions were formulated specifically to have similar apparent viscosities after simulated digestion (without any enzymes), it would appear that guar gum solutions, including the guar/xanthan mixture, may be more susceptible to the enzymatic hydrolysis of starch, leading to lower consistency index values, compared to the xanthan gum solution. Xanthan-fortified solutions may benefit from a superior bulking effect limiting the effect of enzymatic hydrolysis of starch through possible inherent interactions with starch granule itself; details of these potential mechanisms are discussed in Chapter 4.5.
4.2 Study Treatment Rheology

The evaluation of the storage (G') and loss moduli (G'') for each of the three fiber-fortified solutions throughout the two-stage simulated digestion phases are represented below in Figures 4.3.1 to 4.3.4. These measurements help determine the viscoelastic properties of each solution via frequency sweeps, illustrating the solid- or liquid-like properties of each solution tested. Information regarding the colloidal forces and molecular interactions of the fiber-fortified solutions can also be determined from this data.
**Figure 4.3.1** Storage ($G'$) and loss moduli ($G''$) of xanthan gum-fortified solutions for before (a) and after simulated digestion (b) as a function of frequency featuring an oscillation stress value of 0.5 Pa.
Figure 4.3.2 Storage ($G'$) and loss moduli ($G''$) of guar/xanthan-fortified solutions for before (a) and after simulated digestion (b) as a function of frequency featuring an oscillation stress value of 0.5 Pa.
Figure 4.3.3 Storage ($G'$) and loss moduli ($G''$) of guar gum-fortified solutions for before (a) and after simulated digestion (b) as a function of frequency featuring an oscillation stress value of 0.5 Pa.
Figure 4.3.4 Storage ($G'$) and loss moduli ($G''$) of control starch solutions for before (a) and after simulated digestion (b) as a function of frequency featuring an oscillation stress value of 0.5 Pa.
Initially, all three fiber-fortified solutions exhibited elastic-like characteristics, having considerably higher G’ values; guar/xanthan gum mixture representing the most elastic while the xanthan gum-fortified solution having a slightly lower G’ across the frequency sweep range.

After simulated digestion conditions, as seen in Figures 4.3.1 to 4.3.4 (b), the G’ values for all solutions except for xanthan gum, decreased below G” values, indicating a loss in previous solid-like behaviour. Notably, the G’ values of the xanthan gum-fortified solution remained higher than G”. These higher G’ and G” values indicate that the xanthan gum solution would possess a higher degree of entanglement and viscosity than the other fiber-fortified solutions tested. The higher molecular weight of xanthan gum may also be responsible for this difference, as the molecular weight of guar gum is typically lower, leading to a lesser degree of entanglements (Achayuthakan & Suphantharika, 2008). The quantification of these storage and loss moduli results, at 1 Hz, following simulated digestion, are shown in Table 4.3 below.

**Table 4.3** Mean values for viscoelastic measurements of storage (G’) and loss (G”) moduli as well as tan δ at 1 Hz for control, xanthan gum, guar/xanthan gum, and guar gum-fortified starch solutions.

<table>
<thead>
<tr>
<th>Sample</th>
<th>G’ (Pa)</th>
<th>G” (Pa)</th>
<th>Tan δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xanthan gum</td>
<td>9.749</td>
<td>3.233</td>
<td>0.3316a</td>
</tr>
<tr>
<td>Guar/xanthan gum</td>
<td>0.2600</td>
<td>0.5136</td>
<td>1.9753b</td>
</tr>
<tr>
<td>Guar gum</td>
<td>0.0193</td>
<td>0.2137</td>
<td>11.049c</td>
</tr>
<tr>
<td>Control</td>
<td>0.0006252</td>
<td>0.03684</td>
<td>58.925d</td>
</tr>
</tbody>
</table>

Values with different letters in the same column differ significantly (p<0.05)
The loss tangent (tan δ) value, which quantifies, within a solution, the ratio of energy loss and storage modulus at a certain frequency, was lowest (0.3316) for the xanthan gum solution, likely indicating a weak-gel solution with a higher concentration of polymer entanglement or more solid-like structure. A tan δ value of <0.1 typically represents a true gel. Inversely, the higher the tan δ value, the less stress is required to disrupt the inter-particle bond between the gum and starch and thus, a tan δ value >1 is characteristic of a more dilute solution. Both the guar and guar/xanthan gum solutions featured higher tan δ values, 11.049 and 1.975 respectively, with the control coming in at 58.925. In addition to this, it is worthy to note that despite its high G’ value and high viscosity before simulated digestion, the guar/xanthan solution featured the second lowest tan δ value, which may indicate a less diluted composition after simulated digestion when compared to the guar gum only solution.

Overall, the superior ability for xanthan gum to retain its viscosity and pseudoplasticity throughout simulated digestion is evident, while the proposed potential synergistic combination of guar and xanthan gum withstood the simulated digestion conditions just slightly better than guar gum fortification alone. Importantly, the rheological characterization of the three fiber-fortified solutions examined in this chapter establish some of the important physiochemical changes occurring after simulated digestion. In the chapters to come, these characterizations allow for further interpretation of their ability to influence glucose release and the attenuation of hydrolytic activity during simulated digestion conditions.
4.3 Available Glucose Release

The following Figure 4.4 represents available glucose levels of digesta throughout the 2 h simulated intestinal digestion phase protocol for the fiber-fortified solutions and their control containing just a 4% starch solution.

![Graph showing glucose release over time]

**Figure 4.4** Available glucose measurements for control (CS), guar gum (GG), xanthan gum (XG), and guar/xanthan gum-fortified (Mix) starch solutions throughout the 2 h simulated small intestinal phase. Values of the same time point with different letters are significantly different ($P < 0.05$).

The data illustrates many interesting observations, first being the significant difference between the control and all three of the fiber-fortified solutions during the first 30 min of simulated intestinal digestion ($p<0.05$). This initial reduction in available rapidly digestible sugars after the addition of fiber is congruent with previous studies of Fabek et al. (2015), which compared guar and xanthan gum-fortified starch solutions over similar conditions for 300 min of
simulated intestinal digestion. The second major observation that can be made is that after 60 min, the guar gum-fortified solution showed a lower ability to attenuate glucose release under simulated intestinal conditions and resulted in the highest available glucose level of the three fiber-fortified solutions upon completion of the 2 h simulated digestion protocol.

The third, and perhaps most intriguing, observation is that after 2 h, there was no significant difference between the guar/xanthan mixture solution and the xanthan gum solution (p>0.05). This indicates that the combination of guar and xanthan gum in the starch solution may have an impact on improving the attenuation ability of guar gum-based solutions, attributed to the addition of xanthan gum. This observation is peculiar when considered in conjunction to the previous rheological analysis where it was demonstrated that the guar/xanthan gum mixture did not effectively retain its viscosity to the same degree that the xanthan gum solution did after the simulated digestion. It would appear that with the inclusion of just 20% xanthan gum to the guar gum-fortified starch solution and despite not being able to retain its viscoelastic properties to the same degree as the xanthan gum-fortified solution, the guar/xanthan mixture solution still exhibited a similar ability to attenuate glucose release. The similar attenuation levels these two fiber-fortified solutions indicate that 1) there may be an inherent property of xanthan gum or this mixture that leads to this attenuation of hydrolysis or 2) viscosity retention is not the primary mechanism driving the attenuation effect. These proposed inherent mechanisms will be explored in further detail in Chapter 4.5.
4.4 Glucose Release with Dialysis System

The available glucose measurements from the dialysis system, measuring glucose diffusion rates over the course of the 2 h simulated intestinal phase, are seen in Figure 4.5. These results provide insight into the ability of the fiber-fortified solutions to attenuate diffusion.

![Graph showing glucose release with dialysis system](image)

**Figure 4.5** Diffusion of glucose (g/L) present in the dialysate over 120 min of *in vitro* small intestinal digestion of control, guar gum, xanthan gum, and guar/xanthan gum-fortified starch solutions. Values of the same time point with different letters are significantly different (*P* < 0.05).

Evident from Figure 4.5, all three fiber-fortified solutions demonstrated a significant reduction in the amount of available glucose in the dialysate when compared to the control. Again, xanthan gum and the guar/xanthan-fortified solutions were found to be the greatest at eliciting an attenuation effect. Glucose
diffusion for both these solutions differed significantly from the control and the guar solution, but did not differ significantly from one another. Guar gum was the least effective of the three fiber-fortified samples in this respect, however it still demonstrated a significant difference from the control solution indicating its ability to attenuate glucose diffusion into the dialysate (p<0.05). Up until the first 30 min of the simulated digestion, all three fiber-fortified solutions demonstrated a similar degree of diffusion attenuation, but at 60 min, guar gum began to show a lesser effect to attenuate glucose diffusion through the dialysis membrane. A similar delayed deviation trend was also demonstrated in the available glucose measurements previously in Chapter 4.3.

In addition to this similar deviation trend, much like the available glucose levels witnessed in the previous section, the peculiar ability for the guar/xanthan-fortified solution to have similar effects on attenuating diffusion as the xanthan gum-fortified solution did also was witnessed here in Figure 4.5. Again, comparing to the previous rheological data, it appears that viscosity is not the primary driving force behind attenuating glucose diffusion rates.

If viscosity were indeed the primary driving force of controlling glucose diffusion rates, this phenomenon would not likely be observed as the final flow behaviour viscosities of both the guar/xanthan gum and xanthan gum solutions were in fact drastically different (0.8836 and 0.4086 respectively). Despite significant differences in loss tangent, behaviour, and consistency index values for both these solutions, where the xanthan gum solution acted (1) less Newtonian, and (2) retained more of its viscosity after the simulated digestion
protocol, the guar/xanthan mixture may still be effective in binding with starch molecules, conceivably lowering the diffusion ability and rate of hydrolysis of glucose molecules.

Typically, once the viscosity and structural stability of the solution components begin to lower during simulated digestion, as represented by the lower K and n values in Table 4.3, so does their ability to suppress glucose diffusion. Again, because the guar/xanthan mixture exhibits a lower viscoelastic quality and final viscosity than xanthan gum, it is intriguing that the mixture solution appears to have a similar (if not better) ability to attenuate glucose diffusion rates. The observance of this phenomenon leads back to the conclusion that it must be due to a non-viscosity related property, perhaps inherent to xanthan gum which influences the attenuation effect that the guar/xanthan mixture demonstrates.

4.5 Proposed Mechanisms and Inherent Factors

The three major observations exhibited during both simulated digestion experiments included firstly that all three fiber-fortified solutions had a significant impact on attenuating the release of glucose, as well as the diffusion of glucose through the dialysate membrane. The second major observation from both simulated digestion experiments was that the guar gum-fortified solutions appeared to decrease their ability to elicit an attenuation response after 30 or 60 min of both diffusion and available glucose experiments, respectively. Finally, the
third, and perhaps most important, observation was that the xanthan gum and the guar/xanthan gum-fortified solutions did not differ significantly in their ability to elicit an attenuation response for both available glucose and diffusion rates, despite having different final viscosities and rheological profiles. With these three major observations considered in tandem with the rheological data and existing literature involving xanthan and guar-fortified solutions, some specific inherent properties or mechanisms may play a significant role in explaining the observations in the present study.

4.5.1 Coating and Intermolecular Interaction

The inherent ability for xanthan gum to resist degradation, associate with starch granules, and have the ability to form a protective coating around the starch granule would stand to be an effective mechanism of attenuation that could explain why these three major observations were witnessed in the present study. Xanthan gum has been noted to have inherent ability to associate with the starch granule, (Chaisaawang & Suphantharika, 2005), and could contribute to this attenuation effect. Within the guar/xanthan-fortified solution specifically, if enough of the starch can be effectively coated by the xanthan gum component of the mixture, there should be a decrease in starch diffusion and hydrolysis.

As demonstrated by Fabek & Goff (2015) in their research, xanthan gum possesses a longer-lasting effect to coat and protect the granule from hydrolysis, which was indicated by the deviation of guar gum from the other two fiber-fortified samples after 60 min for available glucose (Figure 4.4), and after 30 min for
glucose diffusion (Figure 4.5). Chaisawang & Suphantharika (2005) investigated the structural and rheological properties of xanthan and guar gum with native tapioca starch and proposed the presence of a protective component in the ability for xanthan gum to completely wrap the starch molecule, while guar gum creates a more loosely folded layer, leading to less effective protection against granule deterioration during simulated digestion. This loose wrapping can be seen in Figure 4.6 (b), (c), which involves the native tapioca starch-guar gum mixture and anionic tapioca starch-guar gum mixtures respectively. Figure 4.6 visualizes the wrapping of the granule by guar gum (b) and then the higher degree of wrapping by xanthan gum, both compared to the non-fortified tapioca starch solution (a).

Chaisawang & Suphantharika (2005) also go on to explain other potential inherent gum properties, importantly citing the role that electrostatic interactions between starch and gum molecules have on influencing starch-gum matrix structure and function. Further research by Achayuthakan and Suphantharika (2008) report that guar and xanthan differ significantly in their level of intermolecular interactions with starch, wherein xanthan gum solutions behave more as weak gels, while guar gum solutions behave more like entangled solutions. These stronger interactions by xanthan gum could contribute to the superior retention of viscosity after simulated digestion (both with and without enzymes). The ability for the xanthan gum-fortified solution to resist digestion likely contributes to the superior ability of the guar/xanthan solution to retain viscoelastic properties than the guar gum solution alone. The stronger
interaction, enabling greater structural integrity and protective coating ability, may account for the improved performance of the mixture solutions attenuation and rheological profile after simulated digestion.

**Figure 4.6** Scanning electron micrograph images of cationic tapioca starch with and without gums: (a) starch alone, (b) starch-guar gum mixture, (c) starch-xanthan gum mixture (1500x) (Adapted from Chaisawang & Suphantharika, 2005).
In a study conducted by Fuwa et al. (2016), xanthan gum-fortified rice was evaluated for its ability to lower postprandial blood sugar levels of healthy female students. The authors observed the xanthan gum-fortified rice samples to be coated with a thin layer of xanthan gum as the concentration increased to 1.5% leading to difficulty separating after cooking. Furthermore, the subjects who consumed the xanthan gum-fortified rice were observed to have significantly lower postprandial blood sugar levels than the standard non-fortified rice samples, as immediate as 15 min after consumption. They attributed these observations to the ability of xanthan gum to absorb the rice into the sol structure during cooking, creating a protective barrier from digestive enzymes and processes. These results support the notion of the ability for xanthan gum to attenuate postprandial glycemic response in humans and warrant a greater consideration into the mechanisms that make xanthan gum so effective as a fiber-fortifying agent.

The intermolecular interaction between starch molecules and fiber-fortification, in this particular study, could demonstrate a potential influence on gelatinized starch molecules trapped within the gel network, slowing diffusion, and starch hydrolysis. A gel network influenced by the hydrogen-bond-forming, ionic and coating capabilities of xanthan gum could help explain the fiber-fortified starch solution in the aforementioned attenuation effects.

Unfortunately there is limited research examining patterns of interaction and coating of guar/xanthan blends with modified gelatinized starch but research from Dartois et al. (2010), investigating the influence of guar gum on in vitro
gelatinized starch digestibility, determined that the presence of other biopolymers, like guar or xanthan gum, can modify starch digestibility through this barrier-forming capability around the starch granules. Shi and BeMillier (2002) also reported similar results of altered fiber-starch solution molecular interactions, regarding changes in starch granule gelatinization and leaching from the granule due to protective effects of anionic gums like xanthan.

An additional sub-mechanism that may directly influence the ability for both the guar/xanthan and xanthan to suppress glucose diffusion and absorbance despite having differing viscoelastic profiles, could be the potential for the gums to directly interact with amylolytic enzyme function. There may be a synergistic effect brought on by the inherent ability for guar gum to inhibit enzymatic hydrolysis through a non-competitive inhibitory effect on α-amylase and the ability for xanthan gum to coat the granule and reduce granule swelling (to be discussed in the section 4.5.2).

Dating all the way back to the early 1980s with works from Isaksson et al. (1982) along with Hansen and Schulz (1982), it has been noted in the literature that fiber-influenced inhibition of enzyme functionality may occur, limiting starch digestibility via their interaction with amylolytic enzymes. Both these authors determined that dietary fiber of different kinds have the capacity to directly inhibit pancreatic enzyme activity and α-amylase, respectively. Furthermore, many different fiber sources have been found to affect enzyme function, including guar gum, cellulose, fucoidan, and C. asiatica (Dhital et al., 2015; Cho et al., 2011; Khabir et al., 2014). As touched upon earlier, Slaughter et al. (2002) established
that guar gum has an inhibitory effect on $\alpha$-amylase due to the binding of the enzyme to the galactomannan rather than due to enzyme diffusion impairment. Moreover, Dhital et al., (2015) also observed that cellulose can non-specifically bind to amylase leading to $\alpha$-amylase inhibition while Cho et al., (2011) reported an apparent non-competitive inhibition of amyloglucosidase by oversulfated fucoidan in a starch solution. Being that xanthan gum consists of a cellulose backbone, these results by Dhital et al., (2015) are curious, albeit the trisaccharide side chains on xanthan gum may alter the potential inhibition interaction.

Furthermore, what may also be relevant to the interesting observations in this experiment could be the role that an altered, or hindered amylase or amyloglucosidase activity may play. $\alpha$-Amylase functions to break down starch molecules into maltose, maltotriose, and small oligosaccharides, by attacking $1,4$-$\alpha$-D-glucosidic linkages of the polysaccharide substrates but cannot cleave glucose terminal monomers or branches. Alternatively, amyloglucosidase functions to break down starch completely to glucose by hydrolyzing $\alpha$-1,4 and branching $\alpha$-1,6 linkages, starting from the non-reducing end of the starch substrate eventually converting starch to dextrins and glucose. Both enzymes work together to break down starch but if one were not to function fully, complete starch digestion may not occur. In the event that amyloglucosidase, but not amylase, was inhibited significantly by the dietary fiber source, i.e. xanthan gum or guar gum, it would be likely that the system would show low viscosity, in addition to low amounts of free glucose. This potential mechanism or occurrence
could explain the observed suppressive effect of the guar/xanthan-fortified solution despite having lower retained viscosity than the xanthan-fortified solution.

*Unfortunately* the literature examining the ability for xanthan gum to hold a direct inhibitory effect on α-amylase or amyloglucosidase function has seemingly yet to be performed and thus the possibility of a direct xanthan gum enzyme inhibitory effect can only be speculated. Further analysis of these enzyme kinetics are clearly still required to better understand the extent at which xanthan, guar and the combination of the two can act as inhibitors of *α-amylase* or *amyloglucosidase*.

**4.5.2 Swelling & Hydration**

Another major mechanism could be that the degree of swelling occurred by all fiber-fortified solutions may be pivotal in the inherent ability to attenuate the release of glucose during simulated digestion, a factor also noted by authors Chaisawang & Suphantharika (2006). As mentioned previously in the literature review chapter, guar gum and xanthan gum vary in swelling power (SP), which can have an effect on viscosity as well as susceptibility to hydrolysis. While a higher SP, like that typically exhibited by guar gum, may result in higher initial viscosity, it can also result in greater loss in structural integrity of the swelled granule once shear is applied to the system (Funami et al., 2005). This, combined with the susceptibility of guar gum to extreme pH conditions exhibited during digestion phases, fit the narrative demonstrated by the data in the present
study. With regards to xanthan gum-fortified solution, a lesser degree of swelling, and thus lesser susceptibility to granule deterioration, combined with inherent properties previously mentioned, could allow for the greater retained viscosity and attenuation levels witnessed.

The interesting ability of the guar/xanthan solution to elicit an attenuation of glucose release may be due to the incorporated ability of xanthan gum to limit starch granule swelling, create an initial rigid gel-like structure with guar gum, and interact with water and starch polymer molecules that may be leached, improving structural stability (Shi & BeMiller, 2002). The ability of xanthan gum to compete for water molecules in the guar/xanthan mixture solution and effectively reduce hydration in the amorphous regions of waxy starch may also help reduce the swelling that may occur with guar gum alone (Achayuthakan & Suphantharika, 2008; Weber et al., 2009).

Limiting starch granule disintegration upon gelatinization can result in a mixture wherein some granules remain within a continuous phase formed by xanthan gum, as determined by Weber et al. (2009). Ramirez et al. (2015) also studied the effect of anionic sodium alginate had on gelatinized starch during a similar two-step *in vitro* digestion model. Similar to the present study results, the authors witnessed reductions in starch hydrolysis by the alginate-fortified starch solutions, which were attributed to the starch-protecting role as well as ability to limit granule swelling during gelatinization.
In effect, the influence on swelling and hydration by xanthan gum may be a favourable compliment to guar gum-fortified starch solutions where the pairing of these two hydrocolloids create a strong initial gel network making them a greater source of dietary fiber with the potential to attenuate the release and uptake of glucose within the intestinal phase of digestion.

5.0 Conclusion & Future Directions

Within this study, guar, xanthan and guar/xanthan-fortified modified starch solutions were evaluated for their viscoelastic behaviour and ability to attenuate an in vitro glycemic response. The potential synergy for the xanthan and guar mixture to influence the attenuation of glucose hydrolysis and diffusion rates in starch solutions was postulated based on their well-recognized synergistic structural behaviour and demonstrated individual ability to be effective fiber sources.

Formulations of three fiber-fortified starch solutions were matched for viscosity at 50 s\(^{-1}\) after simulated digestion devoid of enzymes, in order to isolate the effects of the addition of enzymes to each-fortified starch solution. When compared to the control, all fiber-fortified solutions of guar gum, xanthan gum, and the mixture of gaur and xanthan gum (80:20), saw considerable reductions in viscosity upon completion of the 2-phase simulated digestion protocol, although the xanthan gum-fortified solution retained the largest degree of its viscosity, with both other fortified solutions appearing to result in significantly greater losses in
structure and viscoelastic properties. All three fiber-fortified starch solutions also demonstrated a significant ability to attenuate glucose diffusion and the release of glucose after analyzing the dialysis and available glucose data.

While the guar gum-fortified starch solution did not exhibit as effective of an attenuation response as the xanthan and guar/xanthan-fortified solution, the guar solution did appear to produce a comparable attenuation response to these two fortified solutions until 30 and 60 min of the respective glucose diffusion and available glucose simulated digestion protocols. After these times, it was speculated that significant structural degradations occur for the guar-fortified solution, resulting in more drastic losses in the ability to attenuate glucose diffusion and hydrolytic release of glucose.

Although xanthan gum demonstrated a superior ability to retain its structural integrity throughout the simulated digestion protocol, when compared to all the guar/xanthan-fortified solution, it was found to not offer superior ability to attenuate glucose diffusion and release (starch hydrolysis). This phenomena may be explained by inherent properties of the xanthan gum component, such as its specific ability to retain a portion of viscoelasticity after simulated digestion, protect the starch granules from amylolysis by forming a protective coating around starch granules, interacting with starch components, and reducing initial granule swelling. These mechanisms and properties are thought to enable the starch components of the solution to be less accessible and less mobile during degradation and hydrolysis.
Overall, the results from this study suggest that incorporating just 20% xanthan gum to a guar gum-starch solution can improve the attenuation of glucose release and starch hydrolysis thus possess a potential impact on improving postprandial glycemic responses. Further research into the structure-function relationship will continue to aid in determining the degree of influence that the various mechanisms or inherent properties may play in eliciting an attenuation response.

As novel techniques and technology continue to be developed, so will the accuracy and knowledge to which researchers can answer the important questions surrounding the impact of dietary fiber on the glycemic response. The results from this study would benefit from further investigations into the various morphological changes occurring to guar/xanthan gum-fortified mixtures during simulated digestion. Scanning electron microscopy (SEM) can help determine changes to morphological structure, intermolecular interactions, and changes within the granule and fiber-fortified matrix that may have occurred for guar/xanthan starch solution. Techniques such as fluorescence recovery after photobleaching could also be useful in evaluating mobility and diffusion rates of enzymes or glucose within the various fiber-fortified solutions. Additionally, these results would also benefit from complimentary *in vivo* data, solidifying the classification or application of guar/xanthan-fortified solutions as effective sources of dietary fiber with the ability to attenuate the postprandial glycemic response in humans.
Being that there is currently a limited amount of research that has investigated the combination of guar and xanthan gum as dietary fiber sources under simulated digestion conditions, there is a great number of unanswered questions to be explored when evaluating the potential for guar/xanthan gum-fortified starch solutions to attenuate glucose hydrolysis and diffusion. Future studies may look to investigate the optimum ratio of guar and xanthan gums in order to elicit the greatest attenuation response. Furthermore, this study hopefully sets the stage for further investigations into verifying the mechanisms and specific inherent qualities this gum mixture possess that allow for the significant attenuation of glucose diffusion and hydrolysis. Once these mechanisms or inherent properties can be better identified and associated with the glycemic response, the larger question of “which fiber sources are most superior health-wise and why” can be satisfied leading to better dietary guidance, healthier foods and reductions in major diet-influenced disease like diabetes.
6.0 References


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