Effect of *In vitro* Human Digestion on the Viscosity of Hydrocolloids in Solution: A Dietary Fibre Study

by

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A Thesis
presented to
The University of Guelph

In partial fulfilment of requirements
for the degree of
Master of Science
in
Food Science

Guelph, Ontario, Canada

ABSTRACT

EFFECT OF *IN VITRO* HUMAN DIGESTION ON THE VISCOSITY OF HYDROCOLLOIDS IN SOLUTION: A DIETARY FIBRE STUDY

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The effects of a simulated *in vitro* digestion model on the viscosity of solutions of locust bean gum, guar gum, fenugreek gum, xanthan gum, gum Arabic, psyllium, flaxseed gum and soy soluble polysaccharides (SSPS) were examined in this study. All hydrocolloid solutions were formulated for low viscosity (LV), medium viscosity (MV) and high viscosity (HV), which were subsequently subjected to 3 treatments of equal volumes each. The treatments consisted of 1) H_2O-dilutions, 2) acid and alkali in the absence of enzymes/bile and 3) an *in vitro* digestion model simulating the gastric and duodenal phases with pH changes in the presence of hydrolytic enzymes and bile salts. All hydrocolloids showed substantial reductions in viscosity, with dilutions exerting the greatest effect. Depending on the concentration, xanthan gum retained 20-50% of its initial viscosity while the other solutions were in a lower range of 1-16%, thereby showing considerable resilience to the 3 simulated conditions.

Acknowledgements

I would like to express my sincere gratitude to my advisor Dr. Douglas Goff, without whom this project would not have been possible. The experience, knowledge, and skills that I have gained through the completion of this project are invaluable and have led me to pursue further research in this field. Thank you for the guidance and never-ending support you have offered throughout my graduate degree.

Special thanks to my two advisory committee members, Dr. Alison Duncan and Dr. Milena Corredig. Your knowledge and contribution to this project is greatly appreciated. Thank you for offering me your time throughout the past two years.

I would also like to thank Keying Qian for her assistance in extracting and isolating the soluble gum fraction of flaxseed hulls.

Thanks to all my labmates and friends who have helped make the time spent on this project enjoyable. Also, thank you to Ms. Heidi Anderson, Ms. Leona Varga-Lowes and Ms. Lisa Hall for their help on the administrative side of the project. Also, special thanks to Fernanda Peyronel for her efforts in maintaining the laboratory where I conducted most of my research.

Lastly, I would like to thank my family (Martin, Marina, Martina, Baja and John) and my loving fiancée Danielle who have been nothing but supportive of me in my every endeavour.

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1. Introduction

The functions of food and everyday nutritional habits are becoming increasingly significant in maintaining and promoting health and well-being for North American consumers. Diseases and associated risk factors such as cardiovascular disease (CVD), type 2 diabetes (T2D), elevated cholesterol and blood sugar levels, obesity and cancer are mere examples of the perpetual growth of chronic ailments that afflict millions of North Americans, annually. According to a report issued in 2004 by Health Canada, T2D is considered to be one of the fastest growing diseases in this country (Health Canada, 2004). Statistics Canada reported that the number of Canadians diagnosed with T2D was approximately 1.3 million in 2004, and this number increased to over 1.6 million by 2008 (Statistics Canada, 2010) and is expected to reach over 3 million by 2020 (CDA, 2009). Concurrently, this places a heavy economic burden on Canadians. In 2000, it was estimated that the costs related to T2D were close to $6.3 billion and this value is expected to reach $16.9 billion by 2020 (CDA, 2009). Fortunately, research shows that the incidence of this ailment can be delayed or prevented by making healthy lifestyle choices, which include daily exercise and a proper diet.

The benefits of incorporating exercise and a healthy balanced diet extend far beyond physical appearance and reach the most important of human goals, those being health and longevity. A recommendation was put forth by the Public Health Agency of Canada to help prevent T2D by incorporating 30 minutes of physical activity daily, minimal alcohol consumption as well as consuming a well balanced diet that should include fruits, vegetables, minimal fat and salt intake and sufficient consumption of

dietary fibre (Public Health Agency of Canada, 2008). It has been shown that incorporation of dietary fibre in whole wheat pasta is linked to retardation of starch digestion and subsequent reduction in glycemic response (Brennan, 2008). An adequate daily intake for total fibre was put forth by the U.S. Food and Nutrition Board of the Institute of Medicine (IOM), which indicates that 25 g and 38 g for women and men, respectively, should be consumed (IOM, 2005). This recommendation is based on the intake level observed to protect against CVD. In Canada, 2002 surveys indicated that mean dietary fibre intakes generally ranged between 14.3 to 16.6 g/d for women and 16.5 to 19.4 g/d for men (Health Canada and Statistics Canada, 2004). A similar trend is seen in the US where the consumption level is found to be close to 14 g/d (ADA, 2008). These low numbers lead to continual need for fibre fortification of foods.

The deficiency of North American dietary fibre consumption may be related to the texture and palatable quality that foods rich in fibre deliver, or more importantly do not deliver. Although healthy eating warrants great interest it nevertheless has to be matched with desirable product quality. The two must be optimized wherein the fibre concentration must not compromise consumer acceptance. Viscosity is a technological feature of many hydrocolloids which, when acting as dietary fibre, have shown to promote many health benefits including reduction of post-prandial glycemia, which will be shown in Chapter 2. Unfortunately, high viscosity can lead to over-texturization of foods. Moreover, the viscous nature of any food product inadvertently changes once consumed. The viscosity of dietary fibre is believed to be an essential tool in lowering the risk factors for T2D. Nevertheless, the impact it has on glucose and insulin metabolism is yet to be ascertained. In this study various hydrocolloids such as guar gum, locust bean

gum, fenugreek gum, xanthan gum, flaxseed gum, soy-soluble polysaccharides and gum Arabic, which are novel fibre sources, were evaluated for their contribution to viscosity in various concentrations both in solution and after *in vitro* digestion, mimicking the upper gastrointestinal tract to ascertain how well the viscosity is retained through the digestion process.

1.1. Research objectives and hypotheses

The overall objective of this study was to examine the relationship of eight structurally-different hydrocolloids in solution to viscosity, specifically to observe the changes that take place when they are exposed to dilutions, pH extremes, and hydrolytic enzymes that are all present in the upper gastrointestinal tract, including the stomach and small intestine. This allowed the determination of the main biological effect that is responsible for structure breakdown and viscosity reduction of soluble dietary fibres. Guar gum, locust bean gum, fenugreek gum, xanthan gum, flaxseed gum, soy soluble polysaccharide, psyllium and gum Arabic were each dissolved in water at three concentrations. Because of structural heterogeneity and differences in apparent viscosity, their viscosity profiles were kept comparable at all three concentrations before the *in vitro* digestion. Each hydrocolloid was exposed to 3 separate treatments with equal volumes of added fluid: dilution, acidification and neutralization, and digestion in the presence of pH, hydrolytic enzymes and bile salt. Following the 4-hour long simulation, rheological analyses were conducted at shear rates from 30 to 200 s^{-1}, which have been shown to be relevant during digestion.

It was hypothesized that the gums whose core structure shows a more protective nature will resist the reduction in viscosity more so than others. Moreover, due to the

nature of dietary fibres, the presence of digestive enzymes would have less of an effect on viscosity reduction in comparison to dilutions and the influence of acid and alkali environments.

2. Literature Review

This review mainly focuses on the relationship between T2D and soluble dietary fibre. An overview of T2D including its diagnosis and prevention are initially presented, followed by current evidence on the preventative effects of soluble dietary fibre on T2D, in relation to viscosity and the ability to modulate postprandial metabolism and gut motility. Furthermore, carbohydrate digestion and metabolism are explained in detail and the impact they have on T2D is also analyzed, followed by a review of current *in vitro* digestion methods. Also, basic concepts of rheology are outlined. Lastly, the fibres utilized in the present study, namely guar gum, locust bean gum, fenugreek gum, xanthan gum, flaxseed gum, psyllium, soy-soluble polysaccharide and gum Arabic are introduced.

2.1 Type 2 diabetes

2.1.1. Diagnosis

According to the Canadian Diabetes Association, two types of tests are available and widely used for patients to be screened for pre-diabetes, where blood glucose levels are above normal signifying an increased risk of disease development, but below the level designating T2D and/or diabetes (CDA, 2009). The first procedure is known as an Oral Glucose Tolerance Test (OGTT). Here, the patient fasts for a minimum of 8 hours, during

which time there is no caloric intake. This is followed by measuring the plasma glucose levels before and 2 hours after consumption of a 75 g glucose drink. The fasting and 2-hour-post plasma glucose levels are measured and utilized for the identification of normal glucose metabolism, pre-diabetes or impaired fasting glucose, diabetes or impaired glucose tolerance, as seen in Table 2-1.

Table 2-1. Classification and diagnosis of pre-diabetes and/or diabetes based on the Oral Glucose Tolerance Test (CDA, 2009).

2-hour Plasma glucose reading (mmol/L)	Diagnosis
<7.8	Normal
7.8-10	Pre-diabetes
≥11	Diabetes

The second test, which is more convenient and less costly albeit less accurate than the OGTT, is called the Fasting Plasma Glucose (FPG) test. As the name suggests, the test is administered at least 8 hours after the patients' last consumed meal and the plasma glucose result is used for the same diagnostic purpose as the OGTT, as summarized in Table 2-2.

Table 2-2. Classification and diagnosis of pre-diabetes and/or diabetes based on Fasting Plasma Glucose test (CDA, 2009).

Plasma glucose reading (mmol/L)	Diagnosis
<6.1	Normal
6.1-6.9	Pre-diabetes
≥ 7	Diabetes

2.1.2. Prevention of diabetes

The incessant growth in the prevalence of diabetes is cause for great concern and thus has led to considerable research on the potential preventative strategies to combat or delay this ailment. Healthy lifestyle changes, such as increased physical activity, are a clear step in the direction of diabetes avoidance (Pan et al., 1997, Knower et al., 2002). Moreover, a study found that increased social support accentuates the importance of physical activity and dietary self-management (Weijman et al., 2005).

Sedentary lifestyle changes, which include consuming less fat and more dietary fibre as well as increased physical activity, are often prescribed treatment regimens for T2D. A Diabetes Prevention Program was established that sought to examine the effects of an orally-administered anti-diabetic drug known as metformin and lifestyle changes on diabetes prevention. The results of the study show that after 2.8 years the incidence of diabetes was decreased by 58 percent and 31 percent for the lifestyle changes group and metformin group, respectively (Knowler et al., 2002). The Zuni Diabetes project found that community-based involvement through exercise and weight control successfully facilitated weight loss in North American natives. After 10 weeks, the participants lost up

to 14 kg. Moreover, the researchers found that the participants of the 10 week study decreased the usage and/or dose of hypoglycaemic medication and displayed a reduction in mean fasting glucose, from 13.2 to 10.8 mmol/L, when compared to the insignificant drop from 12.6 to 12.4 mmol/L for the non-participants (Heath et al., 1991). The Malmo Feasibility Study demonstrated a similar trend which, after 5 years, resulted in a significant reduction in the male participants' mean body weight, lipids, glucose intolerance, blood pressure and overall incidence of diabetes (Eriksson & Lindgarde, 1991). In Asia, a 6-year study demonstrated that dietary intervention and exercise in tandem led to a greater than 20 percent reduction in the incidence of diabetes (Pan et al., 1997).

2.2 Carbohydrate metabolism and type 2 diabetes

2.2.1. Carbohydrate digestion

Digestion of any nutrient involves the incorporation of enzymes, specific pH environments, muscular movements and bacteria, which all aid in the digestive process and allow nutrient uptake by body cells. Digestion of glycemic carbohydrates, those containing alpha bonds and providing glucose (Cummings & Stephen, 2007), begins with chewing. This salivary phase mixes the food mainly with water but also with the starch-digesting enzyme, amylase, which begins to break down the carbohydrates into shorter chain polysaccharides and maltose. The swallowed bolus travels down to the stomach where the acidic environment produced by gastrin of the gastric parietal cells causes

amylase to lose its shape and consequently deactivating it entirely, which temporarily ceases carbohydrate digestion.

The stomach also acts as a temporary storage compartment to control the release of gastric contents into the duodenum (Fengua & Singh, 2010). The stomach can be separated into three anatomical subdivisions, the fundus, the body or corpus, and the antrum. The fundus is the reservoir for the ingested food. The body is responsible for initiating peristalsis, which will be defined later in the chapter. Finally, the antropyloric region is considered to be the site of the greatest mechanical agitation causing mixing of food with gastric secretions (Chang et al., 1996). In humans, the stomach secretes up to 2 L of gastric juices per day (Johnson, 1991). The stomach capacity ranges between 0.25 and 1.7 L and after consumption of a typical meal the average size is found to be 10 cm wide, 30 cm long and with an average capacity of approximately 0.94 L (Ferrua & Singh, 2010). Meal-stimulated acid secretion is separated into 3 phases: the cephalic phase, the gastric phase and the intestinal phase. The cephalic phase originates by the stimulus from the central nervous system, which is initiated by sight, smell or taste of food. The gastric phase, which accounts for 50% of total acid secretions, begins when food or fluid enters the gastric lumen. Distension is caused by this introduction and essentially, the greater the distension the greater the acid output (Chang et al., 1996). The intestinal phase is stimulated by food being brought to the small intestine. The gastric mucosa, comprised of epithelial cells at the luminal surface, is responsible for protecting the stomach from acidic juices. The secretion of acid is regulated by nerves and gastrointestinal hormones, gastrin, histamine and acetylcholine (Chang et al., 1996). Stomach emptying then releases the chyme towards the antrum and pyloric region of the stomach. Peristaltic

contractions, which in humans last between 2 and 20 sec with a frequency of 3 to 5 contractions per min, propel the chyme through the pylorus into the duodenum. However, depending on the ingested material and subsequent particle size, some of the bolus is pushed back into the stomach. This occurs when the induced peristaltic wave overtakes the contents being pushed down. This process, known as retropulsion, is designed to effectively mix the gastric secretions with the food before allowing smaller particles to enter the small intestine for nutrient absorption (Johnson, 1991).

In the small intestine the majority of carbohydrate digestion and subsequent absorption take place. However, absorption is a regulated mechanism. Chemoreceptors and osmoreceptors located in the proximal small intestine are in charge of controlling the motility and subsequent emptying of the stomach using a negative feedback system (Johnson, 1991). For example, a 750 mL isotonic citrate solution placed in the stomach passes into the small intestine in 20 min. However, upon introducing glucose to the solution it took much longer for the entire 750 mL to pass through (Johnson, 1991). For nutrient absorption to occur, the pancreas with the help of cholecystokinin (CCK) stimulates the release of pancreatic amylase through the pancreatic duct, which continues the digestive process by producing smaller oligo- and disaccharides. In humans, pancreatic secretions are estimated to be around 0.2-0.3 mL/min and when stimulated these values can reach 4.0-4.5 mL/min. Overall, the daily output of pancreatic juices is shown to be 2.5 L/day (Chang et al., 1996). Digestive enzymes such as maltase, sucrase, glucoamylase, dextrinase and lactase are found attached to the microvilli or the brush border of the mucosal cells lining the intestinal tract, which further break down the oligo- and disaccharides into their smallest monosaccharide components. There are two types of

movements responsible for mixing the chyme with the enzymes and pushing that mixture down throughout the small intestine; those are segmentation and peristalsis, respectively. Segmentation contractions occur when the small intestine is filled with digesta. The subsequent stretching of the muscle causes localized 1 cm long contractions that mix the chyme with the enzymes (Guyton & Hall, 2005; Tharakan et al., 2010) and brings the mixture into contact with the muscosal surface for subsequent absorption to take place. From there, peristalsis pushes the mixture in an analward direction at a rate of approximately 2 cm/s (Guyton & Hall, 2005).

Different glycemic carbohydrates are digested variably, depending on their individual monomeric units. Maltase hydrolyzes maltose into glucose, sucrase hydrolyzes sucrose into glucose and fructose, glucoamylase hydrolyzes amylopectin into glucose, dextrinase hydrolyzes dextrin into glucose and lactase hydrolyzes lactose into glucose and galactose (Whitney & Rolfes, 2005). Excluding lactose, the remaining sugars are joined via alpha glycosidic bonds that are hydrolyzable by alpha amylases. Lactose, on the other hand, is a molecule that joins glucose and galactose through a beta bond which, similar to certain indigestible dietary fibres such as cellulose, are not hydrolyzable by alpha enzymes. Unlike cellulose, lactose is hydroylyzable by an enzyme that is transcribed in a way that fits the bond and allows for complete digestion. Those with sufficient amounts of lactase avoid lactose intolerance. The final goal of digestion is absorption, which allow for the monosaccharide products to pass into the bloodstream and circulate throughout the body, where needed.

Non-glycemic carbohydrates, such as dietary fibre or resistant starch, contain beta glycosidic linkages and are thus unaffected by the enzymatic activity of the previously

mentioned human digestive enzymes. This causes them to pass through the gastrointestinal tract intact, reaching the large intestine. Here, soluble fibres undergo partial fermentation producing short-chain fatty acids such as acetate, butyrate and propionate, gases such as methane, and water (Whitney & Rolfes, 2005). The fatty acids are used as energy by the colon cells and have also been linked with the decreased risk of developing gastrointestinal disease(s), certain types of cancer and cardiovascular disease, which will be discussed in Section 2.3.

In general, both digestion and transit time are dependent upon a variety of different conditions, such as individual characteristics incorporating age, sex and overall health status, as well as the food properties such as particle size and viscosity. Dietary fibre has been the subject of considerable research for its ability to alter digestion and nutrient release in a physiologically favourable manner, as will be discussed in Section 2.2.3.

2.2.2. Glucose absorption and insulin release

Glucose is considered to be the single major source of metabolic fuel for the body and provides almost all the energy that vital organs, such as the brain, require for proper functioning (Horton et al., 2006). After consumption of a meal, blood glucose levels normally rise as the carbohydrate component of the food is broken down into its monosaccharide constituents and this absorption may potentially start in the mouth. As mentioned, the main site for glucose absorption is along the brush border of the small intestine. Glucose is transported down its concentration gradient through the basolateral membrane into the blood via an active glucose transporter, called glucose transporter 5 (GLUT5). Glucose, galactose and fructose travel to the liver via the portal vein; the latter

two are converted to glucose once they are taken up. The liver is responsible for regulating the distribution of glucose throughout the body. Glucose homeostasis, which is characterized by plasma glucose levels of approximately 9.8 mmol/L (Damodaran et al., 2008), is achieved through the action of insulin, a hormone produced by the beta cells of the pancreas that stimulates blood glucose uptake. Insulin is also responsible for stimulating the liver and muscle cells to convert excess glucose into glycogen. With the help of glucose transporter 4 (GLUT4), glucose is taken up by muscle and adipose tissue where it is phosphorylated via glucose kinase to glucose-6-phosphate, preventing it from diffusing out of the cells. Glucose-6-phosphate then goes through the entire glycolysis cycle whereby 2 molecules of adenosine triphosphate (ATP) are produced. As part of a feedback system, the production of ATP causes the ATP-controlled potassium channels to close causing depolarization. Consequently, voltage controlled calcium channels open, which creates an influx of calcium into the cells, subsequently activating phospholipase C. This activation leads to the production of inositol 1,4,5-triphosphate (IP3) and diacylglycerol. IP3 has a specific protein along the membrane of the endoplasmic reticulum to which it binds and initiates the release of more calcium ions, raising the overall calcium concentration in the cell (Berridge, 2009). In the end, this all triggers further release of the previously synthesized insulin into the blood. Here, it continues to promote euglycemia, lipogenesis, DNA replication, inhibition of lipolysis, proteolysis and gluconeogenesis (Ludwig, 2002).

2.2.3. Glycemic and insulinemic responses to meals

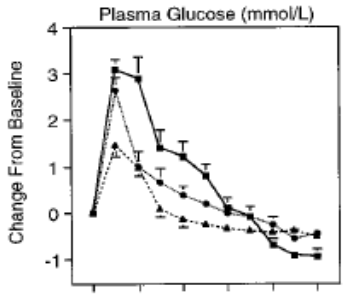
Depending on the type of food that is ingested, a different glycemic response is attained. Fast carbohydrate digestion and absorption leads to a high glycemic response and vice versa. An example can be seen in a study that examined the effect of low-, medium- , and high-GI diets on both plasma glucose and serum insulin in 12 obese teenage boys (Ludwig et al., 1999). The meal composition can be seen in Table 2-3.

Table 2-3. Meal composition of test meals in a study done to examine metabolic effect of low-, medium-, and high-GI meals on plasma glucose and serum insulin (Ludwig et al., 1999).

Low GI	Medium GI	High GI
55 g whole egg	63.9 g steel-cut oats	60.9 g instant oatmeal
45 g egg white	160 g 2% milk	160 g 2% milk (treated with lactase to increase GI of milk)
40 g low-fat cheese	15 g Half & Half cream	15 g Half & Half cream
200 g spinach	16 g fructose	19 g dextrose
30 g tomato	397 g water	0.2 g saccharine
185 g grapefruit		397 g water
115 g apple slices		

GI=Glycemic Index

Figures 2-1 and 2-2 highlight the differences seen when the participants of the study consumed a low-GI, medium-GI, and high-GI meal.



Plot symbols: square, high-GI meal; circle, medium-GI meal; triangle, low-GI meal.

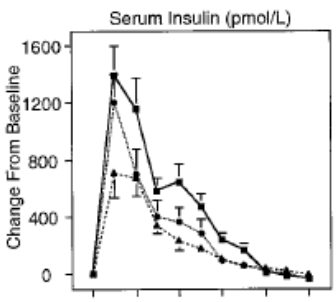


Figure 2-1. Changes in plasma glucose after test meals (Adapted from Ludwig et al., 1999).

Figure 2-2. Changes in serum insulin after test meals (Adapted from Ludwig et al., 1999).

The spike in the high-GI and medium-GI postprandial blood glucose curves signifies rapid carbohydrate digestion and subsequent absorption. On the other hand, the low-GI meal induced a much blunter curve indicating a slower and more gradual release of glucose to the blood, translating into a lower glycemic response.

The curve in Figure 2-2 shows the effect that the test meals and subsequent variation in glucose release have on serum insulin levels. The spike in plasma glucose for high-GI and medium-GI increases the release of insulin. A similar trend is seen in a second study that examined the metabolic effect of white bread and spaghetti on the participants (Granfeldt et al., 1991). What the researchers found was that the consumption of 50 g of white bread induced similar plasma glucose and serum insulin responses as did the high-GI meal in the previous study, demonstrating the white bread to be rapidly digestible allowing quicker absorption of glucose and subsequent release of insulin. On the contrary, the spaghetti was between the low- and medium-GI meals whereby the meal resulted in a slower and more gradual release of glucose into the blood (Granfeldt et al., 1991).

2.2.4. The Glycemic Index

Since different foods create varying metabolic responses, it was important to develop a method that can calculate the glycemic effect that foods have. Dr. David Jenkins of the University of Toronto developed such a strategy in 1981 whereby a simple test and equation were developed to define the glycemic index (GI) of a particular food substance (Jenkins et al., 1981). This test is based on data collected from 10 human subjects (Wolever & Jenkins, 1986). In general, it is defined as the area under the glucose response curve after consumption of a certain portion of a test food, usually 50 g, compared to that of a reference food; the latter more commonly than not is white bread or a glucose solution, with the same amount of available carbohydrates (Jenkins et al., 1986).

Equation 1-1. Equation used to calculate the GI of any food.

$$GI = \frac{Area\ under\ the\ glucose\ response\ curve\ of\ test\ food}{Area\ under\ the\ glucose\ response\ curve\ of\ reference\ food} x\ 100\%$$

Low GI foods, such as fruits, vegetables and whole grains yield values that are ≤ 55; medium-GI foods, such as most whole wheat products, show values in the range of 56-69 and the high-GI foods, such as white breads, white pastas and processed breakfast cereals have values ≥ 70 (Foster-Powell et al., 2002). A study done by a group of researchers in Australia explored the GI values of a variety of foods and food ingredients and produced tables that display numerical glycemic indices for hundreds of food items (Foster-Powell et al., 2002).

2.2.5. Influence of high-GI meals on the development of T2D

Foods that have a high GI increase the risk for developing T2D (Willett et al., 2002). Similarly, glycemic load (GL), which is essentially the GI multiplied by the amount of carbohydrate (Willett et al., 2002), can be an indicator of such a food. As summarized in Figure 2-3, a high GL, or high GI, can lead to a greater demand for insulin and an increasing concentration of counterregulatory hormones, such as glucagon, cortisol and growth hormone, which in turn results in a greater circulation of free fatty acids leading to insulin and glucose resistance. More importantly, alongside obesity, poor genetics and a lack of physical activity, prolonged consumption of high-GI/GL foods leads to a constant increase in the demand for insulin, which eventually causes beta cell failure and T2D.

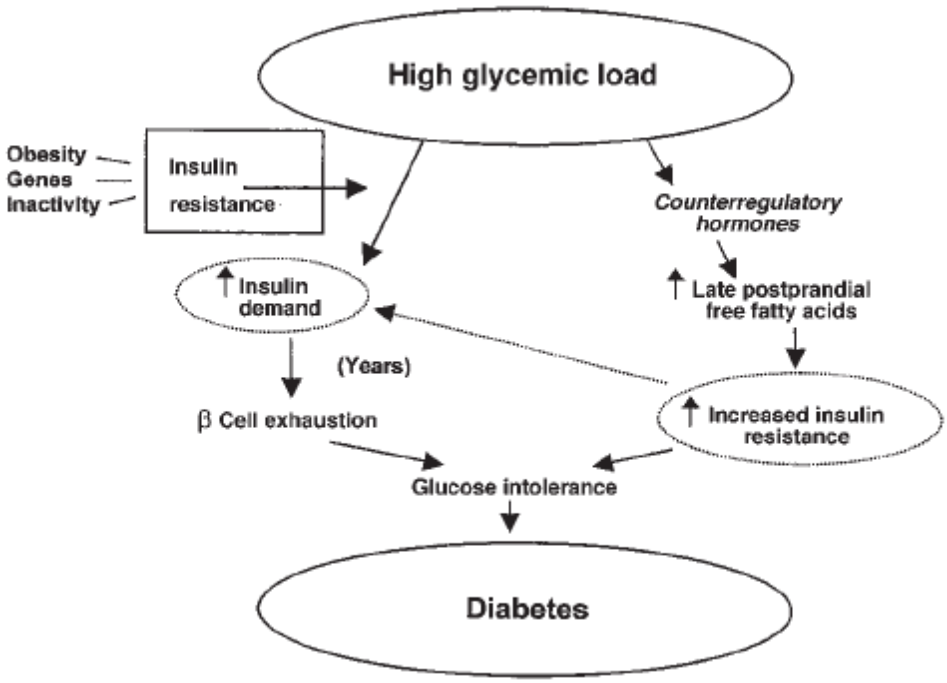


Figure 2-3. Hypothetical mechanism by which high-GL diets result in the development of T2D. (Adapted from Willett et al., 2002).

As mentioned previously in this chapter, once glucose molecules are mobilized into the pancreatic beta cells, they produce ATP via glycolysis. Unfortunately, when the concentration of these glucose molecules exceeds baseline, the glycolytic enzymes become overwhelmed pushing the glucose molecules to the autoxidation pathways, leading to the production of reactive oxygen species, such as superoxide and C-reactive protein. To beta cells, oxidation is a much more serious concern, as they have a relatively low expression of antioxidant enzymes such as catalase and glutathione peroxidase. An example of the problems associated with oxidative stress induced on beta cells can be seen with pancreatic and duodenal homeobox factor-1 (PDX-1), which is a transcription factor responsible for pancreatic development, beta cell differentiation and maintenance of normal pancreatic functioning through beta cell genes (Kajimoto & Kaneto, 2004). Upon oxidation, there is a marked decrease in the binding capability of PDX-1 to the insulin gene, resulting in impaired insulin production and secretion (Kajimoto & Kaneto, 2004).

Paradoxically, elevated blood glucose levels lead to hypoglyemia, through the over-production and stimulation of insulin secretion (Willett et al., 2002). Subsequently, this triggers the release of the previously mentioned counter-regulatory hormones, which are responsible for maintaining glucose homeostasis. Moreover, as mentioned previously, Figure 2-3 shows how hormone production increases the level of circulating fatty acids, in comparison to what is seen after ingesting a low-GI meal, causing hyperlipidemia. Due to the increased absorption of glucose, fatty acid oxidation is inhibited, causing an increase in the accumulation of the intermediate long chain fatty acyl-CoA, inadvertently causing lipotoxicity (Ludwig, 2002). Furthermore, this causes the over-production of

nitric oxide, which can induce apoptosis leading to a decrease in the number of pancreatic beta cells (Ceriello, 2005).

2.3 Dietary Fibre

2.3.1. Dietary fibre definition and classifications

All dietary carbohydrates are classified based on molecular size, which is based on the degree of polymerization (DP), the type of linkage, α or β, and the constituent monomers (Cummings & Stephen, 2007). Unfortunately, a universal definition for dietary fibre is yet to be generated. The term 'dietary fibre' was first coined by Eben Hipsley in 1953 during which time he noticed that fibre-rich foods had an inverse relationship with pregnancy toxaemia (Brownlee, 2011; Hispley, 1953). This notion of linking dietary fibre with physiological benefit(s) still applies today, despite ongoing struggles to attain a globally-accepted definition. It is widely accepted that dietary fibre is of plant origin, is resistant to human digestive enzymes and thus remains indigestible by humans in the upper GI tract (Brownlee, 2011). Health Canada has put forth a proposal to change its current definition of dietary fibre to include a wider range of carbohydrates, noting that resistant oligosaccharides and resistant starches also demonstrate the same physiological benefits, as highlighted through other definitions (Codex, 2009; Health Canada, 2010). The proposed definition reads, "Dietary fibre consists of naturally edible carbohydrates (DP>2) of plant origin that are not digested and absorbed by the small intestine and include accepted novel dietary fibres" (Health Canada, 2010). This proposal is put forth in hopes of having a positive influence on the food industry, by permitting a greater variety of dietary fibre ingredients for food enrichment, and for the consumer,

through expanding their dietary fibre intake to include a wider range of foods; without having to change or manipulate the current Nutrition Facts table in Canada (Health Canada, 2010). The importance of incorporating a DP>2 arises from observing that certain mono- and disaccharides such as fructose and lactose, as well as some sugar alcohols, such as polyols, may be slowly or incompletely absorbed leading to fermentation in the large intestine. Yet, the effect these materials have is solely an osmotic laxative one, which is not an attribute that applies to dietary fibre (Health Canada, 2010).

Dietary fibre can be further separated into water soluble and insoluble fibres, the former being fermentable and the latter not (Health Canada, 2010). Each has the ability to promote different physiological benefit(s) throughout the body. For instance, soluble fibres will display a viscous effect, which lowers blood glucose, serum insulin and cholesterol levels in the small intestine, whereas insoluble fibres will pass through unchanged allowing for subsequent action in the colon (Brownlee, 2011).

2.3.2. General health benefits of dietary fibre consumption

Regular consumption of dietary fibre is associated with a plethora of physiological benefits, including increased satiety, lowered cholesterol levels linked to improving cardiovascular health, augmentation of gastrointestinal immunity and enhanced colonic health (Brownlee, 2011).

The effects of dietary fibre on satiety, or the feeling of fullness after a meal, have been studied extensively. A study done on breakfast meals showed that 8 g supplementation of fenugreek gum led to increased reports of satiety and fullness (Slavin

et al., 2009). A second study examined the effect of low-fibre (1.6 g) and high-fibre muffins (9.0 g); concluding that the latter had a more profound influence on the sensation of fullness. Moreover, what the researchers found was that four different high-fibre muffins demonstrated varying effects on the study participants. Polydextrose had a significantly lesser effect on satiety then did the muffins supplemented with resistant starch and oat fibre; thus indicating that different fibres act differently and are not responsible for influencing all parts of digestion and absorption (Slavin & Jacobs, 2010).

Increased cholesterol levels, specifically low-density lipoprotein (LDL)-cholesterol, are associated with a greater risk for developing cardiovascular disease (CVD). Studies demonstrate that ingestion of dietary fibre reduces cholesterol levels, indirectly lowering the risk of cardiovascular disease (Brownlee, 2011). Meta-analyses of numerous observational studies including various dietary fibres, such as psyllium, demonstrated that a mean dose of 9 g/day accounted for a 7% reduction in LDL-cholesterol levels. Moreover, the effects of four sources of novel fibres, namely konjacmannan, ispaghula husk (psyllium), yeast beta-glucan and flaxseed gum, were studied for their cholesterol lowering effects. What the researchers concluded was that LDL-cholesterol was lowered by 19, 13.2, 8 and 7.6%, respectively (Jenkins et al., 2000). Furthermore, research shows that supplementing soluble fibre with soy protein leads to an increase in high-density lipoprotein (HDL-cholesterol), decrease in low-density lipoprotein (LDL-cholesterol) levels and reduced apolipoprotein B concentrations (Wilson et al., 1998). Soluble fibre has also been linked to lowering blood pressure, which is another risk factor for CVD development (Streppel et al., 2005).

The gastrointestinal tract has a range of immune functions. These include mucosal protection via the action of M cells from external antigenic compounds, luminal shear forces and the stress of other damaging agents (Allen & Flemstrom, 2005; Nicoletti, 2000). The ability of some dietary fibre sources such as alginates, wheat bran and carrageenan, to enhance the protective effects of the colonic mucus barrier when compared to cellulose, pectin and gum Arabic have been shown (Brownlee, 2011), again signifying the variation in the physiological effectiveness of different dietary fibres. Moreover, animal studies, upon analysis of gut-associated lymphoid tissue, show that after supplementing diets containing fermentable fibre, there is an increased T-cell response, which is believed to be affected by the changes in the ever-important gut microflora (Brownlee, 2011).

Fermentable dietary fibres lead to an increase in bifidobacteria, lactobacilli and other bacteria (Brownlee, 2011). Subsequent fermentation produces short-chain fatty acids such as propionate and butyrate, which are shown to be of importance in the gut epithelium (Topping & Clifton, 2001). Other studies show how high-fibre diets can lead to preventing development of diverticulosis and even reducing the risk of colon cancer (ADA, 2008; Cummings et al., 1992).

2.4. Viscous effects of soluble fibre

Many of the proposed health benefits of dietary fibre consumption, including the ones previously mentioned, are believed to be related to the effect that soluble fibres have in the gastrointestinal tract. Specifically, the belief is that it is the ability of soluble fibres to enhance the viscosity of the intestinal digesta that leads to a physiological outcome

upon consumption. The exact mechanism is yet to be ascertained. However, the postulated influence of viscosity may be due to a bulking effect, whereby viscous polysaccharides increase the luminal bulk leading to a protective barrier to pH extremes and hydrolytic enzymes. Another plausible mechanism may be due to the effect that viscosity has at the mucosa, minimizing the diffusion of nutrients across the brush border membrane. The effect that dietary fibre has on delayed gastric emptying is another observation that potentially links viscosity with physiological functionality . Lastly, as was highlighted earlier many hormones regulate gastric and intestinal secretions to stimulate carbohydrate digestion and to allow for nutrient absorption. A higher viscosity induced by the presence of viscous gums may lead to a modified hormonal feedback mechanism, triggering a depression in secretions. Nevertheless, the measurable health benefits that are believed to be linked to enhanced viscosity of the digesta include reduction in postprandial glycemia, changes in gut motility and alterations in carbohydrate digestion and subsequent absorption. It is known that viscous fibres thicken when they come in contact with fluids in the gastrointestinal tract (Dikeman et al., 2006). As a result, this leads to an increase in the viscosity of the unstirred boundary layer and a reduction in convection currents (Damodaran et al., 2008). This in turn minimizes the mixing of digestive enzymes with substrates, which all act towards lowering the rate of diffusion of carbohydrates into the epithelial cells (Jenkins et al., 1995). Brownlee (2011) noted that soluble dietary fibres are viscous and gel-forming under gastric and intestinal conditions and are therefore more capable of lowering absorption rates when compared to other less viscous dietary fibres (Brownlee, 2011).

2.4.1. Dietary fibre and lowering postprandial glycemia

A study was done that compared the effects of viscous and non-viscous dietary fibres on postprandial glucose absorption. The results of this clinical trial indicate that upon consumption of 50 g glucose tolerance tests, with and without added fibre, the most viscous fibre, guar gum, elicited the greatest reduction in both glucose and secreted insulin levels when compared to the other substances or the control (Jenkins et al., 1978). A second study was conducted which examined the glucose tolerance of healthy subjects following consumption of evening meals with differing GI values and contents of dietary fibre (Nilsson et al., 2008). The meals consisted of white wheat reference bread (WWB), WWB enriched with barley dietary fibre, spaghetti enriched with barley dietary fibre, spaghetti enriched with oat dietary fibre, barley porridge and spaghetti enriched with double amount of barley dietary fibre. The latter meal had the highest amount of dietary fibre per serving and all meals contained the same amount of starch, in g/serving. The results indicate that in the morning after consumption of a standardized breakfast meal, the evening meal with the highest amount of fibre elicited the greatest depression on the glucose response, as can be seen in Figure-2-4.

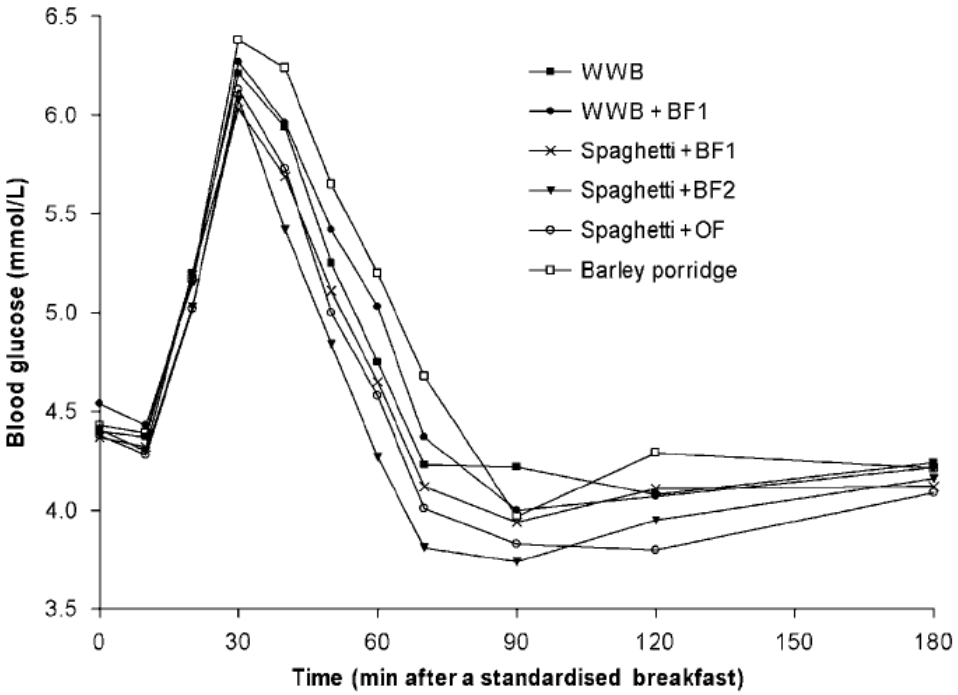


Figure 2-4. Postprandial blood glucose response after consumption of a standardized breakfast administered after consuming different evening meals with varying dietary fibre concentrations (Adapted from Nilsson et al., 2008).
WWB=White Wheat reference Bread, BF=Barley Fibre, OF=Oat Fibre

Another study showed that participants who ingested a mixed meal with 15 g fenugreek seeds had a profound reduction in postprandial glycemia (Madar et al., 1988). This trend was repeated in a 1990 study which found that supplementation of 5 g of fenugreek gum with a 100 g dose of glucose led to a significant decrease in blood glucose levels in normal subjects (Sharma & Raghuram, 1990). A third study was conducted which added guar flour and pectin to diets, which were then fed to 4 subjects. The results indicate that after an interval of 30 min, blood glucose levels fell from 6.3 to 4.7 mmol/L (Chawla & Patil, 2010). Analogous findings were made in an earlier study that supplemented bread with guar gum and marmalade with pectin. Both diets resulted in significant blood glucose reductions (Jenkins et al., 1977).

2.4.2. Dietary fibre and gut motility

As mentioned previously in section 2.2.1, the movements responsible for pushing chyme through the small intestine are segmentation and peristalsis. In a study that simulated the small intestine (Tharakan et al., 2010), researchers investigated the effects of guar gum, particularly its contribution to lumen viscosity, on the mass transfer coefficient, as well as glucose mobility. The results show that increasing concentrations of guar led to a reduction in the mass transfer coefficient; signifying a decrease in the convective mass transfer (Tharakan et al., 2010). Moreover, addition of 0.5% guar gum to starch solutions led to a 30% reduction in the glucose concentration on the recipient side of the simulated intestinal model. A second study showed that inclusion of viscous fibres in test meals resulted in reduced gastric and small intestinal transit time (Brownlee, 2011). A crossover feeding trial done on 8 healthy subjects that compared the rate of gastric emptying in the presence and absence of fruits, vegetables and whole grains, all of which are known to be rich in dietary fibre content, found that removal of the natural fibres reduced overall transit time from 232 min to 186 min (Benini et al., 1995).

Although gastric and small intestinal transit is generally measured in hours, whole gut transit encompassing the large intestine is measured in days (Brownlee, 2011). Accordingly, a study was done that examined the large intestinal transit upon intake of dietary fibre-rich foods. The results show that the meals supplemented with dietary fibre led to a reduction in overall gut transit time. This is believed to be tied to the increase in luminal bulk that arises from fibre consumption. Consequently, this leads to a reduction in toxicity of the large intestine that could arise from bacteria or some damaging factors arising from the diet and an increase in defecation frequency (Blackwood et al., 2000).

Monitoring the direct effects that viscosity of dietary fibre exerts within the GI tract may provide a clearer description as to how it is believed to be linked to any sort of physiological outcome. Marciani et al. (2000) conducted such a study whereby the researchers used echo-planar magnetic resonance imaging (EPI) to observe changes in meal viscosity with respect to gastric emptying, specifically looking at how the stomach responds to increased luminal viscosity. In short, 8 healthy volunteers consumed 50 mL of water using nasogastric tubes, after which time they were administered 1 of 4 500 mL LBG-water solutions, 0.25, 0.5, 1.0 or 1.5g/100g water. These solutions ranged from low- to high-viscosity, respectively. Their results indicate that although the viscosities of the non-nutrient meals varied 1000-fold the observed emptying rates fluctuated by a mere factor of 1.3. Despite the fact that the more viscous meal offered a more pronounced delay in gastric emptying, the researchers proposed that with the introduction of a high-viscosity meal the stomach works harder to provide intragastric dilutions to reduce the viscosity, leading to the observed minimal variation in gastric emptying rates (Marciani et al., 2000). As a follow-up to the 2000 study, the researchers combined meal viscosity with nutrient content in order to observe intragastric dilutions and emptying rates of meals. 12 healthy subjects ingested 500 mL of a low- or high-viscosity LBG test meal that contained nutrients. Those without were considered the non-nutrient control group. Essentially, the study was a four-way crossover design consisting of a low-viscosity nutrient control (LVC), high-viscosity nutrient control (HVC), low-viscosity nutrient (LVN) or high-viscosity nutrient (HVN) meal. Using single-shot EPI the results of the study indicate that gastric emptying had an inverse relationship with meal viscosity. Moreover, the presence of nutrients seemed to have a more profound influence on

delaying gastric emptying as opposed to increasing the viscosity of the nutrient meals, as seen in Figure 2-5. The total emptying rates for the HVN, LVN and HVC meals were 3.3, 4.1 and 4.7 mL/min, respectively.

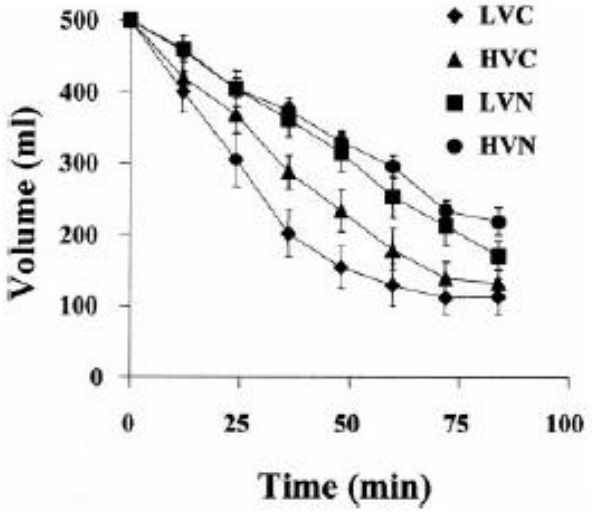


Figure 2-5. Gastric emptying curves for the 4 locust bean gum meals, with and without nutrients (Adapted from Marciani et al., 2001).

In addition to gastric emptying, the intragastric secretions were higher for the more viscous meals and highest for the HVN meal (Marciani et al., 2001). The potential mechanism for increasing intragastric secretions with increasing meal viscosity remains unclear. However, the researchers believe it could be due to enhanced salivary flow with the presence of food. Furthermore, distension of the stomach induced by an increase in viscosity could lead to greater intragastric secretions (Marciani et al., 2001).

A separate study was conducted which examined the effect that calorie load and meal volume have on gastric emptying. In particular, it was focused around the pressure-pump hypothesis, which briefly states that increasing gastric filling leads to increased

intragastric pressure and a more rapid rate of gastric emptying (Kwiatek et al., 2009). The single-blind, randomized study administered liquid nutrient test meals to 12 healthy subjects, the variation of meal compositions can be seen in Table 2-4.

Table 2-4. Combinations of 12 volume, calorie load and nutrient drinks utilized in the study (Adapted from Kwiatek et al., 2009).

Volume (mL)	Calorie load (kcal)	Nutrient content
200, 300, 400, 600, 800	200, 300, 400	41.5% carbohydrate, 41.2% fat, 17.3% protein

It was observed that in the early postprandial period, the higher the meal volume, the greater the proportion of liquid nutrient was seen passing into the duodenum thus signifying that modulation during this phase is an attribute of meal volume and not nutrient composition. Moreover, the MRI results from the study indicate that meals with a higher calorie load led to the greatest intragastric pressure, in mm Hg. This implies that nutrient feedback is responsible for changing gastrointestinal function. In response, gastric emptying was halved when the calorie load of the low volume meals was doubled, leading to a steady rate of calorie delivery. However, when the calorie load for the high volume meal was doubled, gastric emptying was only reduced by a quarter, signifying a greater nutrient delivery. Therefore, gastric emptying is a function of caloric intake, showing that the higher the caloric load the slower the rate of emptying (Kwiatek et al., 2009). Moreover, this can help explain why nutritious foods containing dietary fibre can help deliver physiological benefits such as satiety.

2.4.3. Effect of soluble fibre on carbohydrate digestion

Jenkins et al. (1987) conducted a study that sought to examine the rate of *in vitro* carbohydrate digestion of different starchy foods. They attempted to generate a

relationship between soluble fibre concentration, rate of carbohydrate digestion and the subsequent glycemic response. Different foods such as wholemeal rice, white rice, sweet potato, kidney beans, peas and lentils were used in the study, the latter 3 having the highest fibre concentration. Subsequently, 2 g carbohydrate portions were digested using pooled human saliva and pancreatic juice. The mixtures were placed in dialysis bags and the carbohydrate concentration in mmol/L was analyzed at hourly intervals over a 5-hr period (Jenkins et al., 1987). The results indicate an inverse relationship between fibre concentration and carbohydrate digestion/glucose release (Jenkins et al., 1987). In a more recent study, researchers examined the activity of α-amylase upon consumption of various sources of dietary fibre, which included guar gum and xanthan gum. The results showed that in the presence of these viscous fibres, enzyme activity was greatly reduced leading to a subsequent reduction in carbohydrate digestion (Ou et al., 2001).

2.4.4. Effect of soluble fibre on carbohydrate absorption

The assumption that lies behind the hypoglyemic effects of dietary fibres is strictly related to the belief that enhanced viscosity of the digesta is responsible for the hindrance of glucose diffusion. In a 1987 study researchers compared the effects of 3 viscous fibres on the movement of glucose across a three-compartment cell (Edwards et al., 1987). The results showed an inverse relationship with viscosity and glucose mobility whereby the most viscous polysaccharide, guar gum, elicited the most substantial decrease in the glucose concentration. A similar study was done by Wood et al. (1994) where the researchers examined the effects of a low molecular weight (MW) and a high MW oat beta-glucan solution on the postprandial glucose response after consumption of a

drink containing 10g glucose/100mL water. The findings show that with increasing MW there is an increase in viscosity and subsequently this leads to a greater reduction in postprandial glycemia (Wood et al., 1994).

A third *in vivo* study done on miniature pigs measured the effects of soluble dietary fibre on glucose absorption. Diets supplemented with 0%, 1.1%, 2.2%, 3.3% and 4.4% guar gum were perfused through the jejunum of the test pigs. Afterwards, the researchers measured the carbohydrate concentration by comparing the concentrations between the infused and recovered intestinal contents. Similar to the previous studies, there was an inverse relationship between guar gum concentration, and thus viscosity, and carbohydrate absorption. The researchers deduced that the decrease in diffusion rate was due to an increase in the luminal viscosity of the chyme, which was achieved by guar gum supplementation (Ehrlein & Stockmann, 1998).

2.5. Review of *in vitro* digestion methods

It is both costly and at times unethical to conduct *in vivo* experiments in order to reveal any effects that foods may have on the human body. More commonly, researchers design *in vitro* digestion models for foods (Hur et al., 2011). In a survey done to determine the most predominantly studied foods it was shown that out of more than 80 published journal articles, plant-based foods such as starch, rice or tea capture most of the attention (45%). This was followed by meats (18%), dairy foods (9%), marine foods (9%) and emulsions (9%). Variability is also created based on the number of steps included in the protocol, whether it includes all digestive steps from the salivary phase to the large intestine. Moreover, the composition of digestive fluids and the mechanical stresses also

vary amongst different studies. Finally, the experimental parameters that are being researched fluctuate; they can be structural changes such as those related to droplet coalescence or aggregation, chemical changes such as hydrolysis of polysaccharides and other macronutrients, or location changes as seen in studies examining release of microencapsulated components (Hur et al., 2011). Regardless of the digestion models surveyed, all showed the same temperature of 37^{o}C. However, digestion times as well as use of enzymes varied, depending on which major food component was being explored.

2.5.1. *In vitro* models applied to carbohydrate digestion

Current *in vitro* carbohydrate digestion models have been inspired by earlier work where researchers developed techniques for analysing total carbohydrate, dietary fibre and resistant starch (Woolnough et al., 2008). Carbohydrate digestion dates back to as far as 1969 where Southgate used amyloglucosidase and pullulanase to hydrolyze starch (Southgate, 1969). Since then researchers have developed, used and modified each others methods to study various applications of carbohydrate digestibility, trying to match as closely as possible to the insight that *in vivo* research would provide (Woolnough et al., 2008).

The oral phase is important as it initiates bolus formation and introduces enzyme hydrolysis via α-amylase and mechanical breakdown that normally occurs during chewing. Consequently, many researchers have incorporated this step into their *in vitro* protocol. The method by which the oral phase is administered ranges from incubation with human salivary amylase, to using sieves, food processors or choppers to mimic

chewing, and finally, some studies used volunteers to chew sample foods therefore initiating amylolysis prior to the gastric phase (Woolnough et al., 2008).

The stomach is a major site of proteolysis whereby the acidic medium and the enzyme pepsin continue food breakdown. Consequently, research with a focus on starchy foods has incorporated pepsin proteolysis in order to disrupt any starch-protein interactions that may be present. Studies using pastas such as spaghetti and wheat have included a gastric phase with pepsin incubation ranging between 30 to 60 min, a pH range of 1.5 to 2.0 and a constant temperature of 37^{o}C (Woolnough et al., 2008). The gastric phase has become an integral component of *in vitro* carbohydrate digestion as it continues the digestive process before the chyme reaches the small intestine.

As discussed in Section 2.2.1, the majority of carbohydrate digestion and absorption occur in the small intestine. Once the chyme travels through the pyloric antrum, it is propelled through the small bowel via peristaltic contractions. Enzymes in the brush-border are responsible for inducing complete starch hydrolysis. α-amylosis of carbohydrate-containing samples is a fundamental step of any *in vitro* digestion setup. Following a gastric phase an increase in pH is achieved by adding a buffer to the system. Following neutralisation with the desired buffer, enzyme mixtures are added. Englyst et al. (1992) used screw-top tubes containing the enzymes pancreatin, amyloglucosidase and invertase to digest food samples and determine the rate of starch digestibility (Englyst et al., 1992). To mimic churning, many researchers such as Jenkins et al. (1984) used dialysis tubing suspended in a stirred water bath. Other studies such as those put forth by Englyst et al. (1992) and Brighenti et al. (1995) demonstrated the efficacy of using glass balls in tubes to enhance enzyme hydrolysis of their food samples by enhancing the

mixing process (Woolnough et al., 2008). Although all of these methods provided results that give insight on starch digestibility, they nevertheless differ with respect to the manor in which their samples are being digested.

Woolnough et al. (2008) discussed the need for standardisation of *in vitro* digestion methods. Here, the researchers compared some commonly used methods for simulating digestion and compared the results of sugar release during the three digestive steps. For the oral phase, chewing was induced through the use of mincing, sieving or chopping and the 3 were compared to the rate and extent of sugar release of foods that were physically chewed by volunteers. Subsequently, the pH of all the samples was lowered to 2.5 with added pepsin to simulate gastric conditions for 30 min. Finally, the duodenal secretions incorporated pancreatin at a pH of 6 and hydrolysis continued for an additional 2 h. The foods of interest were wheat, pasta, bread and chick peas. The results indicate that when wheat was subjected to the 4 treatments in the oral phase, the mincing/sieving/chopping underestimated the actual release of available carbohydrate in comparison to the chewed samples. Bread and chick peas, whose structures are not as full-bodied, did not demonstrate any sensitivity to the method of chewing. Pasta digestion between 0 and 60 min varied considerably in terms of the amount of sugar released, noting that chewing by volunteers liberated noticeably more sugar in comparison to the mechanical treatments. However, after 1 h the differences were eliminated due to the ongoing hydrolysis induced by pancreatin (Woolnough et al., 2008). These results led the researchers to investigate which effects, if any, the addition of enzymes and pH extremes have on glycemic estimates of foods. Correspondingly, the same foods as before were chewed using a 4 mm sieve. The gastric phase was then incorporated by dividing the food

samples into 3 test categories, no pepsin, pepsin for 30 min, or pepsin for 60 min. The pH for all three was 2.5 and following proteolysis digestion continued for an additional 2 h by adding the same amount of pancreatin solution to all 3 treatments. Upon analyzing the amount of reducing sugars there was little difference in the rate of sugar release over time. These results may indicate that pepsin inclusion is not a crucial component to carbohydrate digestion. However, the results from the pasta group demonstrate variation in sugar release. At 20 min, pepsin incubation led to a greater amount of sugar being liberated, as can be seen in Figure 2-6. Pasta is known to contain a dense protein matrix and therefore these results may demonstrate the necessity of pepsin inclusion when the sample being studied contains a protein component.

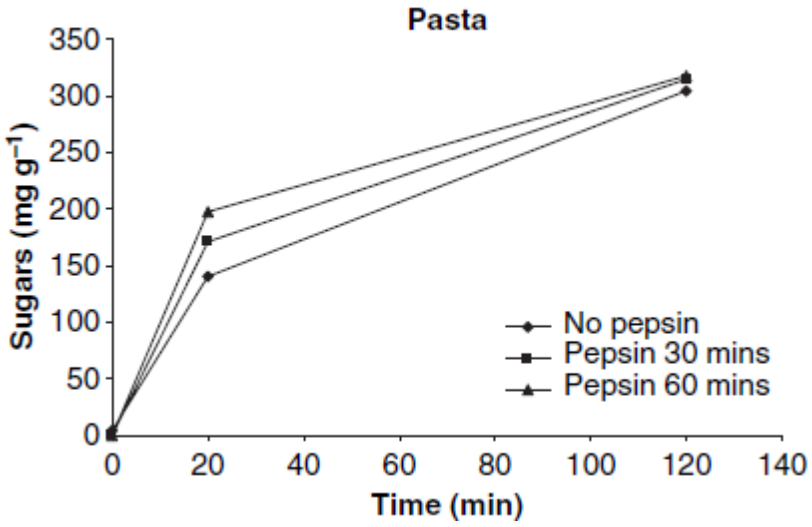


Figure 2-6. The importance of omission and inclusion of pepsin of varying duration with respect to sugar release after simulated human digestion (Adapted from Woolnough et al., 2008).

A similar approach was taken to test the variation that may arise during intestinal digestion. Here, the researchers analyzed 2.5 g of pre-gelatinised starch with pancreatin

incubation being initiated for 2 h at 5 pH values: pH 6.9, 6.0, 5.2, 5.0 and 4.0. The amount of sugar being released was subsequently measured. After the first 2 h, an additional 2.5 g of starch was added to each pH treatment and digestion proceeded for 1 h. Sugar release was again measured. The results of the study indicate that during the first 2 h of digestion, pH variation led no statistical differences in sugar release. However, once an additional 2.5 g of starch were added, respective differences arose. In particular, at pH 4.0 there seemed to be no measurable pancreatin activity, indicating deactivation of the enzyme complex. At pH 5.0 or higher starch digestion was at its peak as indicated through the sugar release curve depicted in Figure 2-7.

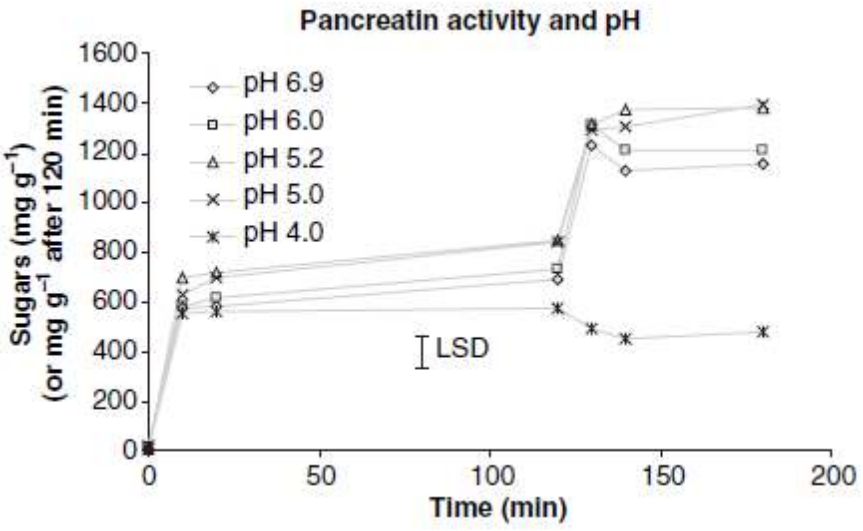


Figure 2-7. Effect of varying pH on pancreatin activity and starch digestibility/sugar release (Adapted from Woolnough et al., 2008).

The researchers of this 2008 study bring up vital questions regarding current *in vitro* digestion protocols. Differences in temperature, time, enzyme concentration, pH, and

stirring and agitation techniques all lead to considerable variations in the observed glycemic response. Furthermore, although starch digestion should incorporate α-amylase activity, the interaction of starch with proteins and lipids may require a more balanced approach to studying starch digestibility. Pepsin, bile or lipase inclusion may yield results more closely related to those elicited during *in vivo* conditions. Regardless of the foods being studied, standardisation of *in vitro* digestion methods may bring researchers closer to discovering relationships between food structure and physiological benefit by minimizing variation in the proposed techniques.

2.5.2. *In vitro* models applied to studies related to dietary fibre

Saura-Calixto et al. (2000) examined the indigestible fraction of foods such as legumes, cereals, vegetables and fruits. After preparing their samples a protocol was devised that simulated both gastric and intestinal environments. The former exposed 300 mg samples to a 0.2 mL pepsin solution containing 300 mg pepsin/1mL of 0.05 M HCl and 0.03M KCl, with a resultant pH of 1.5 for 1 h. Following gastric digestion, to starchy foods a 9-mL 0.1M Tris—maleate buffer with 1mL amylase was added and incubated for 3 h at a pH of 6.9. For nonstarchy foods amylase treatment was neglected. Following the 2-step digestion protocol the researchers proceeded to their dietary fibre determination (Saura-Calixto et al., 2000).

A second study done on beta-glucan examined the effect that *in vitro* fermentation has on the depletion of available carbohydrates from oat lines with high beta-glucan levels (Sayar et al., 2006). After sample preparation of 2 experimental oat lines, *in vitro* digestion commenced with a salivary phase, whereby 0.8 g samples were exposed to 250

mL of human salivary amylase, containing 5 mg/mL of alpha-amylase in 3.6 mM $CaCl_2$. This was stirred for 15 min. Afterwards, the pH was adjusted to 2.0 by using 6 M and 1 M HCl with 625 mL pepsin. The solution was stirred for 30 min. The small intestinal step adjusted the pH to 6.9 with 3 M and 1 M NaOH with 1.25 mL of pancreatin dissolved in a 50 mM sodium phosphate buffer. The mixture was stirred for 90 min. Following the 2-step digestion, 24 h anaerobic fermentation was induced using a batch fermentation system. Finally, the researchers were able to conduct chemical analyses for total dietary fibre composition of their oat flour digestion residues (Sayar et al., 2006).

In a 2009 study Agriculture and Agri-food Canada researchers assessed the effect of food processing on both physicochemical and physiological properties of beta-glucan, relating viscosity and molecular weight to glucose attenuation (Regand et al., 2009). At the beginning, an *in vitro* digestion step was required in order to extract beta-glucan from several batches of raw sources of oat beta-glucan. The digestion method was adopted from a previous 1997 study (Beer et al., 1997). After preparing the samples, 5 g portions were used to which solution containing 250 μL of human salivary alpha-amylase were added and stirred for 15 min. The pH was then adjusted to 2.0 using 6M and 1M HCl with added 625 μL porcine pepsin solution and incubated for 30 min. The final digestion step increased the pH to 6.9 with 3M NaOH and incorporated 0.5 mg/mL pancreatin in 20 mM sodium phosphate buffer. Subsequently, the researchers proceeded to the physicochemical analysis of beta-glucan, viscosity measurements and clinical trials (Regand et al., 2009).

2.6. Basic concepts in rheology

The term 'rheology' was first coined by Eugene Bingham in 1928 and he described the subject as "pantha rhei" meaning "everything flows" (Steffe, 1996). This early concept has developed into what we know rheology to be today, namely the science of the deformation and flow of matter. Essentially, it is the study of the manor in which materials respond to an applied stress or strain (Steffe, 1996). Stress, which can be defined as a force per unit area, can be parallel or perpendicular to the surface on which it is acting. Consequently, the resultant strain is the deformation of the material due to the applied stress. In food science, this concept can be linked to understanding and determining ingredient functionality, product quality, shelf-life, food texture and analysis of commonly used rheological equations. Many researchers studying the physicochemical and structural properties of dietary fibres apply their knowledge through the use of viscometers or rheometers whereby viscosity values and flow behaviour of the desired food material can be measured.

2.6.1. Viscosity

Fluids can either be Newtonian or non-Newtonian in nature. The former is a description used for samples that elicit a constant slope when plotting shear stress versus shear rate. Water, some fruit juices, milk, honey and vegetable oil fall under this category (Steffe, 1996). Those fluids that do not show proportionality between the two are considered to be non-Newtonian. Here, a mathematical approach can be applied to understanding how certain materials respond to increasing shear rate. An example is the power law model whereby one can attain numerical values that describe the manner in

which the material is responding. Simply put, if it is non-Newtonian then the viscosity is either increasing with increased shear eliciting a shear-thickening response, or it is decreasing, showing shear-thinning behaviour.

Equation 2-1. The Power Law model.

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

(where σ= shear stress, K= consistency coefficient, γ= shear rate and n= the flow behaviour index).

The flow behaviour index indicates the degree of dependence of shear stress or shear rate; if the 'n' value is equal to 1 the material is Newtonian. However, fluids that are non-Newtonian can either be pseudoplastic ($0<n<1$) or dilatant ($1>n>\infty$). The structure of dietary fibres and their overall state in solution dictates their apparent viscosity, which is a numerical representation of non-Newtonian viscosity at a given shear rate. Structural differences of various sources of dietary fibre lead to different apparent viscosities. Linear and branched polysaccharides and those acting as random coils or compact aggregates act differently when dispersed in water.

The physical properties of polysaccharides arise from their conformation of individual chains. In dilute solutions, disordered polysaccharides act as random coils and are concentration dependent. As the concentration in solution rises, so does the interaction of these individual coils leading to a network of overlapping chains and enhanced viscosity. Because many of the proposed health benefits of hydrocolloids arise from their ability to enhance viscosity it is crucial to examine their rheological behaviour when exposed to increasing both shear stress and shear rate. At low rates of deformation,

even concentrated/viscous polysaccharides may show Newtonian-like behaviour, allowing for the stress-induced displacements to be replaced with new entanglements and subsequently showing no change in viscosity. However, as shear rates increase there is less time for these new entanglements to form, leading to a reduction in viscosity and non-Newtonian flow. Plotting viscosity versus shear rate for solutions containing different polysaccharides or different concentrations of the same polysaccharide will elicit different maximum viscosity (η_o) values (McCleary & Prosky, 2001). Moreover, their dependence on shear rate and extent of viscosity reduction varies according to their structure. Those that consist of greater entanglements and more junction zones will allow for greater viscosity values and more importantly, a higher capability of retaining viscosity. For that reason, the ability of certain hydrocolloids to promote technological functionality, such as emulsion stability and promoting texture, as well as physiological functionality is affected. This gives rheology a great importance in exploring the physiological effects of different sources of dietary fibres that exhibit structural heterogeneity.

2.7. Study-specific soluble fibres: guar gum, locust bean gum, fenugreek gum, xanthan gum, flaxseed gum, soy-soluble polysaccharide, psyllium and gum Arabic.

2.7.1. Guar gum

The drought-resistant guar plant *Cyamopsis tetragonoloba* is a commonly cultivated legume in India. This country is responsible for approximately 80% of the total

amount of guar that is produced worldwide. However, due to the growing demand of its production, agronomy programmes have been put in place in various countries, including Brazil, Argentina, Australia and the U.S (Wielinga & Maehall, 2000). The seeds are comprised of germ, which constitutes the majority of the weight, followed by the hull constituting the least. The seeds consist of an outer aleurone layer, which contains 25% of the protein. Inside this is the galactomannan-rich endosperm, comprising more than 83% of the gum (Wielinga & Maehall, 2000). The food functional, soluble fraction is extracted from the endosperm of guar seeds. During the germination process, the endosperm absorbs close to 75% of water causing a massive depletion in the amount of galactomannans remaining. Therefore, it is crucial to store the seeds in order to avoid germination and subsequently provide a maximum yield of guar gum (Wielinga, 2010).

The functional properties of guar gum arise from its core galactomannan structure. It consists of a linear 1, 4-linked β-D-mannan backbone with attached galactose side chains that are held together via 1, 6 α-glycosidic bonds at every second mannose, as seen in Figure 2-8. This ratio of mannose to galactose is what gives guar gum its useful properties in the food industry and as will be seen later in the chapter it is what characterizes guar gum from the remainder of the galactomannans. The length of guar gum depends on the mannose backbone and ranges between 95 and 15,656 nm (Wielinga, 2010).

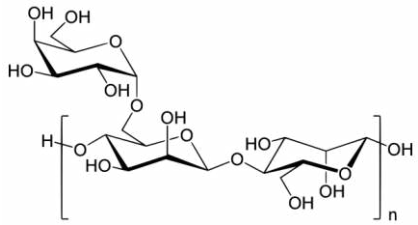


Figure 2-8. Chemical structure of guar gum (Adapted from Wielinga & Maehall, 2000)

The variability in chain length as well as the production technique creates a range of molecular weight (MW) and degree of polymerisation (DP) for guar gum. Theoretically, the MW ranges from 50-8000 kDa and the DP is 300-49,400 hexoses. In turn, this creates a range of viscosities from 5 to 100,000 mPa.s for 2% aqueous solutions. Nonetheless, it shows an increase in viscosity with increasing concentration and is considered a non-Newtonian, pseudoplastic fluid (Wielinga, 2010). Because of the high viscosity, the concentration of guar gum that is typically found in food products does not exceed 1% (Roberts, 2011). Guar gum, like other galactomannans, is susceptible to depolymerisation in the presence of strong acids, alkali and strong oxidizing agents (Wielinga & Maehall, 2000). This can potentially affect its functional and physiological properties and overall usage in food systems.

It is the ability of guar gum to change the rheology of aqueous systems that gives rise to its highly useful properties in foods. Some of these include: texture control, prevention of creaming, syneresis and retrogradation of starch products, influencing crystallisation and improving overall shelf-life. This is why it is commonly used in foods such as dairy products, ice creams, soft drinks, puddings and baby foods (Wielinga, 2010). Because of the notable variety of uses of guar gum, it is considered to be the chief galactomannan employed in the food industry, in comparison to locust bean gum or fenugreek (Roberts, 2011). Moreover, guar gum can act as a dietary fibre thus being a dual purpose ingredient, providing both technological and physiological function, much like locust bean gum. In a study comparing the effect of various viscous polysaccharides on the movement of radiolabeled glucose researchers discovered that as the

polysaccharide concentration of both guar gum and locust bean gum increased, so did the viscosity and ultimately the glucose movement across the three-compartment cell (Edwards et al., 1987). A volunteer study that incorporated 5 g of guar gum to a diet showed that the meal reduced serum cholesterol, free fatty acids and glucose concentrations (Yamatoya et al., 1997). Results of a comparative study of low-fibre versus high-fibre test meals show how supplementation of guar gum reduced postprandial glycemia, lipemia and lipoprotein composition (Redard et al., 1990). Moreover, by examining the extent of starch hydrolysis through supplementation of guar gum in wheat flour and wheat starch, researchers found that addition of guar gum led to enhanced viscosity of the starch and flour pastes and a subsequent reduction in starch hydrolysis (Brennan et al., 2008). Similar results were seen in a study whereby guar gum addition in wheat and normal maize starch led to a decrease in the amount of amylose being leached out (Tester & Sommerville, 2003). In animal studies, researchers have shown that guar gum-fed rats had improved insulin sensitivity and glucose clearance in comparison to rats fed with wheat bran (Roberts, 2011). In pigs to which either a control, guar gum, or cellulose diet was administered, blood results indicated that guar gum addition had the most substantial effect on postprandial glucose reduction. Moreover, insulin production and levels of insulin-like growth factor-1 were depressed after the pigs ingested the guar gum meal. These results are similar to those found in human studies whereby guar gum addition to breads enhanced glycemic control (Ellis et al., 1981). Additionally, meal flow rates were investigated after ingestion of low- and high-viscosity meals in the presence of glucose and starch, separately. Results indicate that the inclusion of glucose in the low- and high-viscosity test meals led to reported flow rates of 15.7 mm/s and 3.7 mm/s,

respectively; and in the presence of starch the values showed a similar trend whereby the effect of the high-viscosity meal was more profound in lowering the overall meal flow rate (Roberts, 2011). Numerous other studies have been conducted which suggest that inclusion of hydrocolloids such as guar gum into meals can lead to a blunting of postprandial glycemic and insulinemic levels (Brownlee, 2011).

2.7.2. Locust bean gum (LBG)

Evergreen carob trees *Ceratonia siliqua* found primarily in the Mediterranean produce carob seeds. The hulls of the seeds are carbonised by hot sulphuric acid, washed with water and are roasted at temperatures greater than 500^{o}C where the hulls are removed, isolating the endosperm halves (Wielinga, 2010). Similar to guar gum, it is the endosperm that contains the functional gum component.

As mentioned earlier, the difference between galactomannans is the ratio of substitution along the mannose backbone. LBG consists of a less substituted molecule, with a mannose to galactose ratio of approximately 4 to 1 (Wielinga & Maehall, 2000). Therefore, it consists of fewer galactose groups in comparison to guar gum, which is shown in Figure 2-8. Consequently, this has shown to create a stiffer galactomannan chain (Wu et al., 2011). Furthermore, unlike guar gum, the substitution is not evenly distributed in LBG, giving rise to what are known as ‘smooth’ and ‘hairy’ regions, as can be seen in the block diagram in Figure 2-9.

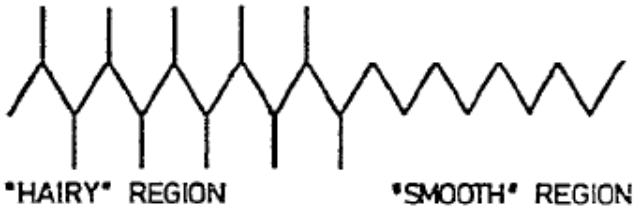


Figure 2-9. LBG block structure (Adapted from Dea et al., 1977).

The hairy regions are created due to a cluster of 4 to 1 substitutions thereby giving rise to a length of unsubstituted mannose, or the smooth portion of the molecule. Moreover, this unique feature of LBG allows for its use as a thickening and gelling agent when put in solution in the presence of cellulosic materials such as xanthan gum and carageenan (Wielinga, 2010).

Again, much like guar gum, the MW and DP depends highly on the chain length, which varies between 120 and 7410 nm, production technique and source of carob seed. Studies show that protein content, gum content, insoluble matter and intrinsic viscosity all vary depending on the origin of the carob seed. In general, the MW distribution is 50 to 3000 kDa and the DP ranges between 300 and 18 500 hexoses (Wielinga, 2010). This diversity allows for a range of viscosity values, showing an increase in viscosity with increasing DP and MW. Nonetheless, the viscosity of a 1% aqueous solution at a shear rate range of 3 -to 700 s^{-1} ranges between 20 and 3000 mPa.s, respectively. Moreover, it is pseudoplastic at all concentrations when placed in solution. LBG, much like guar gum, is said to degrade at pH extremes (Wielinga, 2010).

The structural properties of LBG make it a widely used food ingredient for much of the same reasons as guar gum. On top of those listed previously, it is also used in meat products, especially in the presence of carrageenan as studies show it is able to improve

brine retention (Tarte, 2009). Acting as a dietary fibre LBG is being placed on the forefront of human health studies. An example of this is seen in a study done to analyze the effect of adding dietary fibres to pasta products (Brennan et al., 2008). The results indicate that addition of locust bean gum led to a lower % of digested starch and ultimately a lower GI value. Moreover, as the concentration of LBG increased from 2.5 to 10%, so did the respective effect on the amount of starch being digested leading to a much slower glucose release (Brennan et al., 2008). A satiety study showed that 0.25 g-1.5 g/100 g solution significantly increased satiety levels. Moreover, as the concentration and subsequent viscosity increased so did the report on fullness (Wielinga, 2010).

2.7.3. Fenugreek gum

Because of the link between galactomannan structure and physiological wellbeing, as discussed with guar and LBG, a fairly new hydrocolloid is being investigated, namely fenugreek gum. Fenugreek (*Trigonella foenum-graecum*) is a leguminous plant native to the Eastern Mediterranean and Central Asia. It can be commonly found in Iran, China, Pakistan and India (Hannan et al., 2007). In these countries, it is widely used, in powder form, for its proposed anti-diabetic, hypocholesterolemic, anti-microbial and anti-parasitic properties (Acharya et al., 2006). Similar to guar gum and LBG, fenugreek is a galactomannan. It consists of an attached galactose side group at every mannose unit, unlike guar gum that shows a ratio of 2:1, as mentioned and illustrated in Section 2.7.1, rendering it the most hydrophilic of the galactomannans and very difficult to gel (Karsa, 1999). A study on the relationship of galactomannans with different substitution ratios showed that fenugreek, due to its 1 to 1

M/G ratio, showed a more compact conformation in comparison to guar gum and LBG allowing the formation of so-called hyperentanglements, as can be seen in Figure 2-10 (Wu et al., 2011).

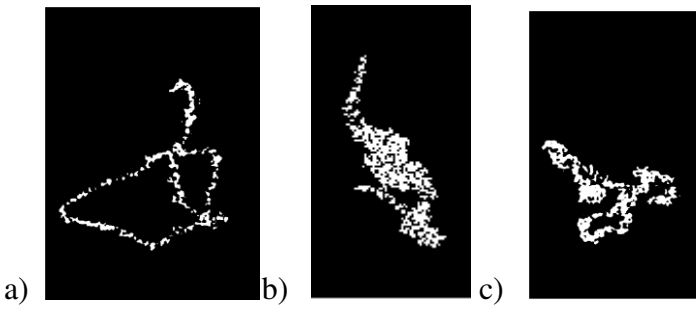


Figure 2-10. Conformation of simulated LBG (a), guar gum (b) and fenugreek gum (c) (Adapted from Wu et al., 2011)

Although more work is being done on the physicochemical properties of fenugreek, a study conducted in Ireland shows that an aqueous solution of 1.5% has a zero-shear viscosity of approximately 40 Pa.s and this value decreases as the shear rate increases, indicating its pseudoplastic nature (Doyle et al., 2009). The subsequent pH effect on this highly substituted galactomannan seems to be the same as seen in LBG and guar gum, a decrease in viscosity, as pH minimizes intermolecular association and increases electrostatic repulsion between neighbouring galactose side chains (Doyle et al., 2009). Nevertheless, fenugreek gum is becoming a much more researched polysaccharide, particularly due to its ability to act as a dietary fibre for human consumption. A 2006 study showed an inverse relationship between fenugreek consumption and postprandial blood glucose concentrations and cholesterol levels (Srinivasan, 2006). Another study done on diabetic patients showed that in both humans and animals with T2D fenugreek gum led to a pronounced hypoglyemic effect (Hannan et al., 2007). In a third study,

researchers found that supplementing 15 g/d of fenugreek in water led to lower postprandial glucose levels in T2D patients (Madar et al., 1988).

2.7.4. Xanthan gum

The production of xanthan gum is initiated by the bacteria *Xanthomonas campestris*. These bacteria can be found in various vegetables, including cabbage. However, commercial xanthan gum is produced through an aerobic fermentation process in the presence of glucose and nitrogen using a pure culture of the bacterium. Following fermentation, pasteurisation is performed to kill the bacteria and the final product is recovered by an alcohol precipitation step. Subsequently, it undergoes drying, milling and packaging for commercial use (Sworn & Monsanto, 2000).

Xanthan gum consists of a linear 1, 4 –linked β-D-glucose backbone with a trisaccharide side chain at every other glucose as well as a 1, 4-linked glucuronic acid at the terminal mannose, as seen in Figure 2-11.

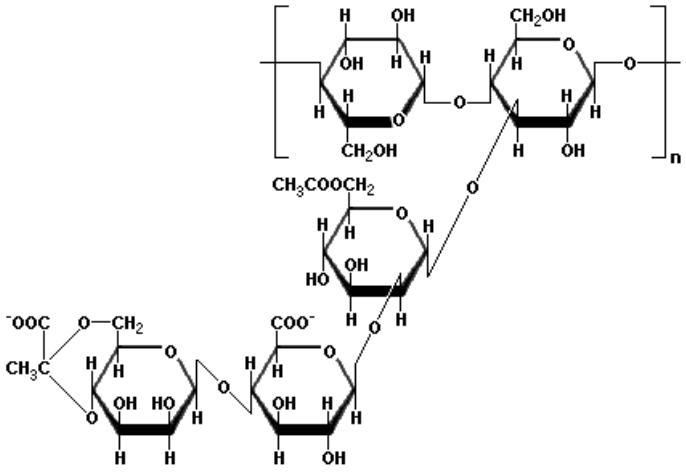


Figure 2-11. Structure of xanthan gum (Adapted from Sworn & Monsanto, 2000).

The primary structure of xanthan gum is what is believed to play a protective role against pH extremes and enzyme hydrolysis. The side chains wrap around the cellulose-like backbone inducing a helical formation thus minimizing depolymerisation (Sworn & Monsanto, 2000).

The rheological behaviour of xanthan gum solutions is multifaceted. At low shear rates the molecules are entangled resulting in what is known as lower Newtonian viscosity. However, as shear rate increases these aggregates are disrupted resulting in pseudoplastic flow. At a shear rate range found in the process of digestion, the viscosity of xanthan gum is closely related to the values found in the previously mentioned galactomannans. Finally, at extremely high shear rates, those not found during digestion, the viscosity becomes constant, reaching an upper Newtonian viscosity (Sworn, 2010). Enhanced viscosity and even gelation can be achieved through the synergism between xanthan gum and highly unsubstituted galactomannans, such as LBG. The smooth regions of LBG interact with xanthan gum and elevated viscosity can be achieved with as little as a 0.03% LBG concentration. At higher concentrations, thermally reversible elastic gels can be formed (Sworn & Monsanto, 2000). The rheological properties of xanthan gum are the reason why it is a widely used food ingredient.

The food industry employs xanthan gum for various functional attributes. Applications include baked goods, dairy products, dressings, mayonnaise, frozen foods, sauces and syrups. Prevention of syneresis, enhanced product stability, improvement of texture, and flavour release are some of the reasons why xanthan gum is found in such an array of food products (Sworn, 2010). Physiologically, xanthan gum can act as a dietary fibre making this polysaccharide a multi-functional ingredient. A study on satiety showed

that both diabetic and normal subjects who were fed 12 g/d of xanthan gum-containing muffins reported an increase in fullness (Sworn, 2010). In a study done where researchers prepared cakes using xanthan gum, up to 60% less fat was reported in the product, while maintaining other internal characteristics similar to the non-supplemented recipe (Zambrano et al., 2005). A third study investigating the effect of increasing xanthan gum concentrations on glucose mobility showed that all solutions containing xanthan gum were able to lower glucose concentrations. The more viscous/concentrated ones had the most substantial effect (Edwards et al., 1987). In a study done on dietary fibre supplementation in pasta, results were similar to that seen with LBG inclusion. Namely, addition of xanthan gum led to a blunting effect on starch digestion. As the concentration of xanthan increased so did the influence it had on starch hydrolysis and subsequent glucose release (Brennan et al., 2008).

2.7.5. Flaxseed gum

Flax (*Linum usitatissimum*) produces flaxseeds, the hulls of which have been shown to have considerable potential as food gums (Cui et al., 1994). The structure of flax consists of a hull, an embryo, an endosperm and two cotyledons (Morris, 2001). Flaxseeds contain 45% oil in the endosperm but it is the hull portion that consists of close to 37% soluble fibre, from which the gum can be extracted (Cui et al., 1996). Depending on the extraction method and source of flaxseed, one can obtain a varying composition. In a 2001 study, researchers show that the isolated mucilage consists of 50-80% carbohydrates, 4-20% protein and 3-9% ash (Cui, 2001). Flaxseed gum is composed of two types of polysaccharides (image not shown), an acidic low-molecular weight pectin-

like rhamnogalacturonan and a neutral high-molecular weight arabinoxylan (Cui et al., 1994). In the same study, the researchers showed that the neutral fraction exhibited higher apparent viscosity, with a shear-thinning behaviour elicited by the high molecular size of the arabinoxylan constituent. In contrast, the acidic fraction was less viscous and seemingly Newtonian. This flow behaviour is said to be explained by the considerably smaller molecular size of the rhamnogalacturonan (Cui et al., 1994). Similarly, a 1996 study measured the intrinsic viscosity of neutral and acidic fractions of flaxseed gum and compared them to gum Arabic, guar gum and xanthan gum. The results indicated that the viscosity of the neutral fraction was higher than the acidic one. Both were higher than that of gum Arabic but considerably lower than guar gum and xanthan gum. They explain this finding by looking at the molecular size of the polymers based on the Mark-Houwink equation, which briefly states that intrinsic viscosity is proportional to molecular size multiplied by a constant. Therefore, a lower molecular size leads to lower intrinsic viscosity due to a decrease in the hydrodynamic volume that is being occupied, as was shown through their results of gum Arabic and the acidic fraction of flaxseed mucilage (Cui et al., 1996). Although this gum is gaining popularity in food science and human health research, few studies have been published relating structure to physiological function.

In a 3-month intervention study, researchers incorporated 5 g of flaxseed gum in diets fed to sixty T2D patients. The results indicated that the fasting blood glucose levels fell from 8.6 mmol/L to 7.6 mmol/L. Moreover, cholesterol was reduced from 10.1 to 9.1 mmol/L after the 3-month study (Thakur et al., 2009). Similar studies are being

conducted focusing on nutritional functionality of flaxseed gum without impairing product quality.

2.7.6. Soy soluble polysaccharides (SSPS)

Soy-soluble polysaccharides are extracted from the internal cell wall of soybeans. Commonly associated products have been put on the market by Fuji Oil, where the name 'SOYAFIBE-S' has prevailed since 1993 (Maeda, 2000). Production begins with the manufacture of soy protein isolate. During this step, an insoluble residue known as okara is produced. Following this, okara is autoclaved in weak acidic conditions at 120^{o}C for 1.5 h. Subsequently, the autoclaved material is allowed to cool and then is centrifuged to isolate the functional mucilage. Spray-drying then produces a white-powder for food usage (Maeda, 2000). The structure of SSPS has been shown to be comprised of rhamnogalacturonans with galactan and arabinogalactan side chains, as can be seen in Figure 2-12 (European Commission Health & Consumer Protection Directorate, 2003).

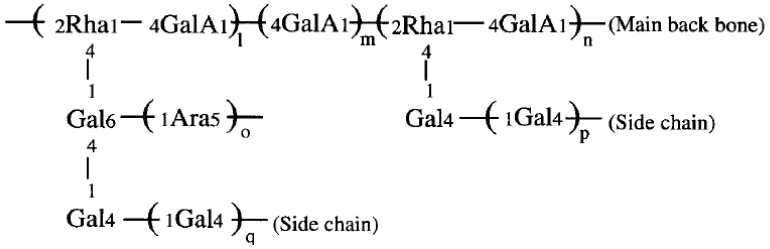


Figure 2-12. Primary structure of SSPS (Adapted from Maeda, 2000).

Furthermore, monosaccharide constituents such as fructose, xylose and glucose have been shown to be incorporated in the overall structure of SSPS (Maeda).

The dietary fibre content of SSPS, as measured by the AOAC method, is approximately 66%, while 9.2% is protein and 8.6% is ash (Maeda, 2000). The MW of the polysaccharide ranges between 5000 and 550,000 Da. The viscosity of SSPS is seemingly low in comparison to the previously mentioned gums, ranging between 20 and 10, 000 mPa.s at solution concentrations of 5% to 30%, respectively. Thus more concentrated solutions (>30%) can be prepared. However, researchers have demonstrated that by lowering the pH below 3, a drop in apparent viscosity follows.

A study was done to examine the effect that SSPS inclusion in meals has on plasma glucose and insulin levels (Tsai et al., 1987). The control diet consisted of 245 g milk, 200 g apple juice, 25 g white wheat flour and 10 g margarine; while the SSPS-fortified meal contained the same but with added 10 g SSPS. After monitoring blood levels, the results indicated that after 120 min plasma glucose levels were lower in comparison to the control. However, no effect was seen between 0 to 120 min (Tsai et al., 1987). An earlier cross-over study showed that a serving size of 25 g/d of SSPS for 17 days led to reductions in post-prandial glycemia, however no effects were shown in other parameters that were measured such as blood cholesterol levels (Tsai et al., 1983). Currently in the food industry, SSPS is being utilized for its ability to stabilize emulsions for applications such as dressings and creams, enhance foam stability, create a softening effect in products such as breads, cakes and hams, and to act as a dietary fibre (Maeda, 2000). More research is being conducted on the topic of SSPS-enriched diets and subsequent physiological effects.

2.7.7. Psyllium

Psyllium, also known as isphagula, is produced from the seed husk of plants of the *Plantago* genus mainly in the southern part of Asia (Saghir et al., 2008). The mucilaginous material consists of both soluble and insoluble fibres (Farahnaky et al., 2010). The functional polysaccharide is isolated using an alkali extraction method and it consists mainly of a highly branched, acidic arabinoxylan with β-1,4 linkages with rhamnopyranose side chains linked to galacturononic acid residues, as shown in Figure 2-13 (Fischer et al., 2004).

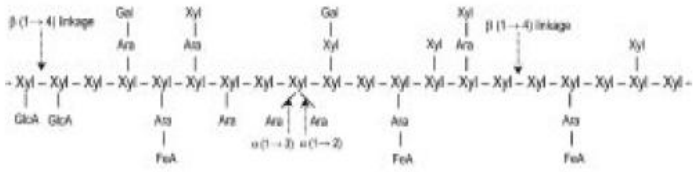


Figure 2-13. Basic structure of psyllium (Adapted from Phillips & Williams, 2000).

It is the alkali extractable polysaccharides that are responsible for the gel-forming ability of psyllium. Moreover, studies have shown that the "weak gel" property could be due to the association of rigid, ordered polysaccharide chains (Farahnaky et al., 2010; Morris, 1990). Upon acidification and neutralization at pH extremes, experimental research has shown considerable depolymerization and fewer junction zones and intermolecular cross-linking, resulting in decreased elasticity of psyllium (Farahnaky et al., 2010). This can potentially affect the functionality of psyllium in food systems and human health.

Several studies have been conducted on the efficacy of psyllium fibre as a health promoting ingredient. Metamucil® is an approved source of dietary fibre sold in Canada for its ability to gel and help with constipation, laxation and cholesterol levels. This

Health Canada approval arose from studies that were previously conducted on various biomarkers including blood lipid and glucose levels. For example, a 12-week double-blind placebo-controlled study using Plantago psyllium versus a placebo examined the effect that the treatments had on serum lipid and glucose levels (Rodriguez-Moran et al., 1998). The participants of the study were instructed to consume 5 g doses 3 times daily before a meal. The dose consisted of 79% psyllium hydrophilic mucilloid, maltodextrin, citric acid, artificial flavour, aspartame, and coloring agents. The placebo test replaced the psyllium fibre with an inert bulk fibre. The results indicated that the subjects consuming the psyllium drink showed a blood glucose reduction from 190 mg/dL to 140 mg/dL, whereas the placebo showed no significant drop. Moreover, after the 12 weeks the psyllium group had lower total cholesterol, lower LDL cholesterol and higher HDL cholesterol in comparison to the placebo and to their own values at week 0 (Rodriguez-Moran et al., 1998). Other studies have shown the use of psyllium fibre in the treatment of constipation, inflamed membranes of the intestinal canal and in the regulation of large bowel function (Saghir et al., 2008).

2.7.8. Gum Arabic

Gum Arabic, or acacia gum, is a dried, sticky exudate that is derived from the stems and branches of *Acacia* trees. The majority of these trees can be found in Africa, especially across the Sahelian belt, from Senegal to Somalia (Li et al., 2009). The isolation and purification of acacia gum includes crushing, dissolution in water, filtration, heat treatment, followed by spray-drying or roller drying (Phillips & Williams, 2000). Gum Arabic is a branched-chain heteropolysaccharide that contains a very small of

amount of protein, approximately 2%. The primary structure consists of a 1, 3-linked β-D-galactose backbone. The highly branched nature of this polysaccharide comes from the extensive sidechains of 3- and 6-linked galactose and 3-linked arabinose. The extent of branching is shown in Figure 2-13.

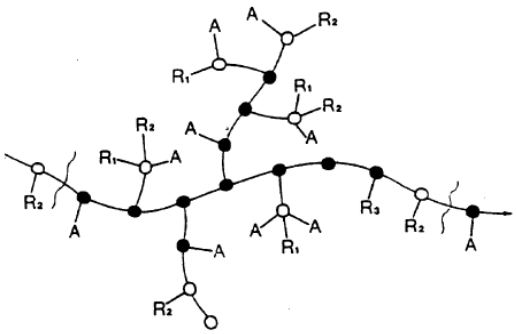


Figure 2-14. Structure of the carbohydrate component of gum Arabic (Adapted from Phillips & Williams, 2000).

The components present in the overall structure include 39-42% galactose, 24-27% arabinose, 12-16% rhamnose, 15-16% glucuronic acid and 1.5-2.6% protein (Ali et al., 2009). A common analogy used to describe the structure of gum Arabic is the 'wattle blossom model'. Essentially, it arises from having an arabinogalactan-protein (AGP) complex to which the polysaccharides are covalently linked, giving it the elongated wattle blossom-like configuration (Ali et al., 2009), as shown in Figure 2-15.

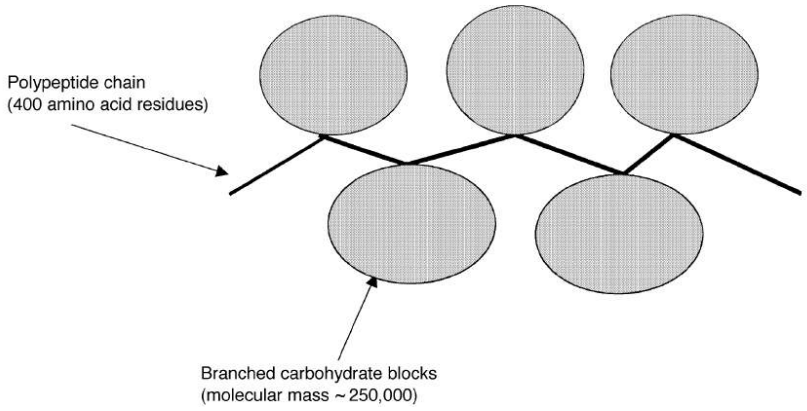


Figure 2-15. The AGP complex of gum Arabic (Adapted from Phillips & Williams, 2000).

Gum Arabic has a high MW, approximately 3.8 x 10^5 Da. Nevertheless, in comparison to other gums, its viscosity in solution is considered to be very low. When comparing the viscosity of 1% guar gum, LBG and xanthan gum in solution, gum Arabic requires a 40% concentration in water in order for it to develop a similar viscosity profile (Phillips & Williams, 2000). This viscous nature arises from its highly branched, globular structure that inhibits any cross-linking or hydrogen bonding with water. Moreover, gum Arabic solutions less than 30% in concentration appear to be Newtonian.

Despite its low viscosity, gum Arabic is a commonly used hydrocolloid in the food industry. It is very popular in confectionery, where it is said to provide texture to sugar-free systems and other products such as moulded candies, jujubes, pastilles and polyol-coated chewing gum (Phillips & Williams, 2000). It is also used as an emulsifier and stabiliser, especially for beverage emulsions, due to its AGP fraction, which allows for formation of colloidal film around oil droplets (Randall et al., 1988). Other industry usage is highlighted through its prevalence in bakery products, encapsulation technologies and wine stabilisation for both red and white wines (Phillips & Williams,

2000). Much like the previously mentioned gums, it also acts as a dietary fibre warranting a physiological response.

Gum Arabic is postulated to have an effect on various biomarkers, including blood glucose levels, effects on intestinal absorption, lipid metabolism and is said to contain anti-oxidant properties (Ali et al., 2009). In a study done on normal rabbits, researchers dispensed powdered gum Arabic at doses of 2, 3 and 4 mg/kg. They found that the blood glucose concentration levels were significantly reduced at the end of the study (Wadood et al., 1989). Other experimental designs have shown that gum Arabic improves intestinal absorption of sodium and water. In animal studies, experimental diarrhea was induced in rats and the addition of 5 and 10 g/L of the gum led to an increased rate of sodium removal from the intestinal lumen as well as a two-fold increase in water absorption (Wapnir et al., 1997). A 1983 male study showed how addition of 25 g/d of gum Arabic for 21 days led to a 6% serum cholesterol reduction, with no significant effect reported on glucose tolerance (Ross et al., 1983). Despite the fact that studies have been done to discover the health benefits of gum Arabic, little is known about the potential effects on humans.

3. Materials and Methods

3.1 The sample treatments

Twenty four sample treatments were designed and tested in the present study. These consisted of 8 hydrocolloids acting as dietary fibre. Each sample type was studied at three concentrations. The viscosities of structurally similar galactomannans, LBG, guar gum, and fenugreek gum were used as standards to which the apparent viscosities, at a shear rate range of 50 to 100 s^{-1}, of xanthan gum, gum Arabic, psyllium, flaxseed gum and SSPS were matched.

3.1.1. Materials

Simulated gastric fluid (Ricca Chemical Company, Arlington, Texas), purified pepsin (Fisher Scientific, Fair Lawn, New Jersey, USA), pancreatin U.S.P. (MP Biomedicals, Solon, Ohio, USA), bile salts (Fisher Science Education, Hanover Park, Illinois, USA), hydrochloric acid 2N solution (Fisher Scientific, Nepean, Ontario, Canada), sodium phosphate monobasic monohydrate (Fisher Scientific, Fair Lawn, New Jersey, USA), sodium phosphate dibasic anhydrous (Fisher Scientific, Fair Lawn, New Jersey, USA), guar gum (Danisco Canada Inc., Toronto, Ontario, Canada), locust bean gum (Danisco Canada Inc., Toronto, Ontario, Canada), Canafen gum® (generously donated by Dr. Cui from Agriculture and Agri-food Canada, Guelph, Ontario, Canada), xanthan gum (Sigma Chemical CO., St. Louis, Missouri, USA), Metamucil® (Procter & Gamble Inc., Toronto, Ontario, Canada), flaxseed gum (refer to Section 3.1.2.), DA-100 variety soy soluble polysaccharides (Fuji Oil Co. Ltd., Osaka, Japan), gum Arabic (Sigma-Aldrich CO., St. Louis, Missouri, USA), 1 reciprocal shaking water bath (Thermo Scientific, Marietta, Ohio, USA) and milli-Q water were purchased.

3.1.2. Flaxseed gum extraction

Omega-3 flaxseed hulls (Natunola Health Biosciences Inc., Winchester, Ontario, Canada), and deionized water were used for flaxseed gum extraction.

Three kg of flaxseed hulls were allowed to soak in 39 L of deionized water. The mixture was stirred for 18 h at 300 rpm and 20^{o}C, using an electrical stirrer (Model 4100, Burlington Pump Inc., Burlington, Ontario, Canada) to remove the soluble fibre. The extract was filtered using 4 layers of cheese cloth to separate the desired mucilage from the hulls. The mucilage was transferred into 500 mL centrifuge tubes whereby the flaxseed gum was separated from any remaining insoluble fractions. This was done by using a Beckman JA-21 centrifuge in a JA-10 Rotor (Beckman Coulter Inc., CA, USA) at 9000 rpm, 20^{o}C for 20 min. The centrifuge was then poured onto trays which were subsequently placed in a VirTis Freeze Dryer (Model FFD-42, VirTis Inc., New York, USA) and allowed to freeze dry for 72 hours. The gum was then blended into powder form using an Oster® 10 speed blender (Model 6640-33, Jarden Corporation, New York, USA). The flaxseed gum extraction procedure is listed in Figure 3-1.

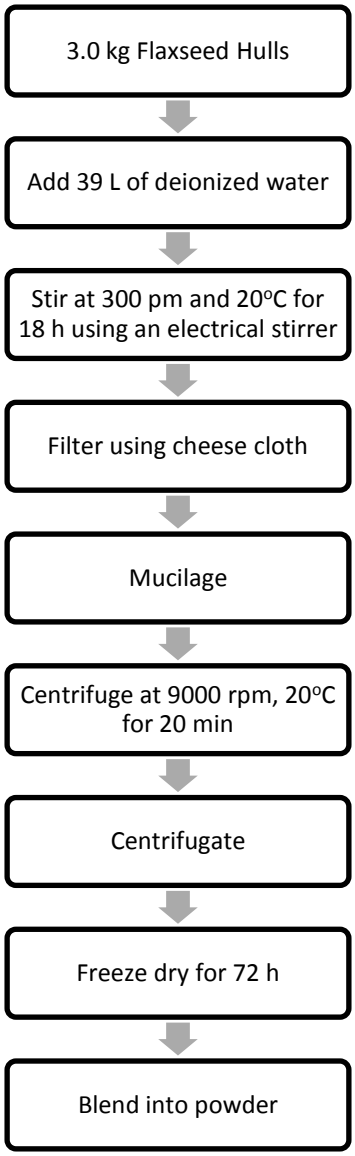


Figure 3-1. Vertical flowchart illustrating flaxseed gum extraction procedure.

3.1.3. Preparation of soluble fibre solutions

Structurally similar galactomannans (guar gum, locust bean gum and fenugreek gum) were dissolved to yield 1%, 2% and 3% concentrations of gum in water, creating low-viscosity (LV), medium-viscosity (MV), and high-viscosity (HV) solutions.

Although previous research has been done to investigate physiological effects of ≤ 0.5% solutions, this study focused on enhancing and retaining measurable viscosity after simulated digestion. Therefore the lowest concentration used was 1%, which yielded a viscosity of approximately 0.5 Pa.s at a shear rate of 50 s^{-1}. The concentrations of the remaining fibres were manipulated in a way to match their apparent viscosities to the 1%, 2% and 3% galactomannans at a shear rate range found during digestion, that being 50-100 s^{-1} (Borwankar, 1992). With the exception of psyllim which was dissolved in water at room temperature, all other hydrocolloids were completely dissolved in hot water. To begin, the desired fibre was slowly added to room temperature water until the desired concentration was reached, depending on the hydrocolloid being studied. Simultaneously, stirring was induced inside the beaker using a magnetic stirrer and a stir plate. After 10 min, the temperature of the mixture was brought to 80^{o}C and was maintained for an additional 10 min. Following solubilization the solutions were left to cool before rheological analyses.

3.2 Rheology of the sample treatments

All study treatments were made fresh on the day of experimentation. Measurements were conducted in triplicate. Rheological measurements, in particular viscosity readings in Pa.s, were obtained using a cone and plate geometry on a controlled-stress rheometer (AR 2000, TA Instruments, Delaware, USA). A 6 cm wide cone with a truncation gap of 50 μm and an angle of 2^{o} was used, as seen in Figure 3-2.

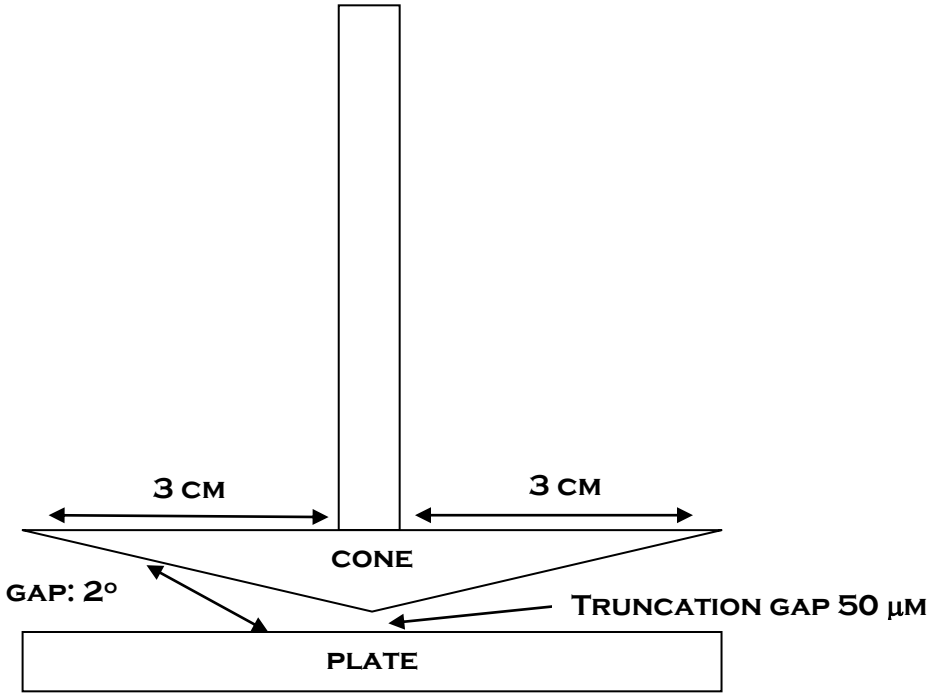


Figure 3-2. Diagram of cone and plate contrivance used to analyze flow behaviour.

3.2.1. Flow behaviour

Flow behaviour of the solutions was examined using the following procedure. Freshly made samples were allowed to cool before being analysed. At the beginning of each measurement, the Peltier plate was adjusted to 37^{o}C and this temperature was maintained throughout experimentation. The desired sample was placed on the Peltier plate and the cone was lowered to a final truncation gap of 50 μm. Subsequently, changes in viscosity were measured using shear rate sweep tests, whereby the shear rate increased from 30 to 200 s^{-1}. 22 sample points were recorded.

3.3. *In vitro* simulated human digestion protocol

The protocol was developed as a modification of 2 previously designed studies. The first sought to examine the bioaccessibility of soil contaminants by *in vitro* digestion

(Oomen et al., 2003). The second study examined changes in emulsion interfacial properties after simulated gastric-duodenal digestion (Malaki Nik et al., 2011). The current study introduced 15 g soluble fibre solutions to the following treatments. 4 glass beads with a 1 cm diameter were added to each fibre-containing flask to induce churning. Similar to both methods, the current method included a 2-step digestion procedure incorporating both a gastric and an intestinal phase while omitting salivary and large intestinal contributions as starch was not included and fermentation was not an object of study. The composition of simulated gastric fluid (SGF), simulated intestinal fluid (SIF) and simulated bile fluid (SBF) can be seen in Table 3-1.

3.3.1. Simulated gastric digestion

The effects of the stomach on digestion commence with introducing foods to an acidic environment. Correspondingly, digestion was initiated by adding 3.2 mg mL^{-1} purified pepsin to 7.0 mL of SGF. The mechanical mixing and temperature regulation were induced and maintained via a shaking water bath (Thermo Scientific, Marietta, Ohio, USA) with values of 175 rpm and 37^{o}C, respectively. Samples were mixed for 1 h at a pH of 1.8.

3.3.2. Simulated duodenal digestion

Following the gastric phase, 4.6 mL of SBF and 14 mL of SIF were concurrently added to mimic the conditions of the small intestine at a pH of 7.6. SBF was prepared by dissolving 8 mg mL^{-1} bile salts. SIF was obtained by dissolving 5 mg mL^{-1} pancreatin into the buffer solution (Table 3-1). The mixture was placed back into the 175 rpm, 37^{o}C shaking water bath for 3 h. The overall ratio of SGF: SIF: SBF was 1: 2: 1.5, as seen in

previous studies (Oomen et al., 2003). To evaluate the role of pH and dilutions, all samples were incubated with the same volume of digestive fluids in the absence of hydrolytic enzymes and bile and with the same volume of water, respectively.

Table 3-1. Composition of simulated gastric, intestinal and bile fluids used during *in vitro* human digestion

Simulated Gastric Fluid (SGF)	Simulated Intestinal Fluid (SIF)	Simulated Bile Fluid (SBF)
0.2% NaCl (w/w) in 0.7 % HCl (w/v)	0.5M NaH_2PO_4	3.6 (water): 1 (2N HCl) (v/v)
3.2 mg mL^{-1} pepsin	5 mg mL^{-1} pancreatin	8 mg mL^{-1} bile salts

3.4. Data and statistical analysis

Linear and non-linear regression analyses before and after 4 h simulated digestion were conducted using GraphPad Prism 5.0™ (GraphPad Prism Software Inc., La Jolla, CA, USA). The non-linear regression analysis was used to evaluate the Power Law model and to determine the values for the constant (K) and exponent (n) for each treatment at all concentrations. The lowest R^2 value considered was 0.8. None of the data was manipulated or transformed in any way before statistical analyses. One-way analysis of variance (ANOVA) and Tukey's Multiple Comparison testing were performed using the same software to compare the study treatments and to determine viscosity differences between all treatments groups at the specified concentrations. A significance level of $p<0.05$ was used and considered for statistical significance.

4. Results and Discussion

4.1. Study treatment rheology

To test the hypothesis that GI-induced changes in solution viscosity are dependent on the physicochemical structure of the individual hydrocolloids, the flow behaviour of each study treatment was determined at concentrations required to produce LV, MV and HV solutions.

4.1.1. Flow behaviour of low-viscosity (LV) hydrocolloid solutions

The concentrations of the 8 hydrocolloids used to represent LV dietary fibres are shown in Table 4-1 along with their reported apparent viscosities at the typical shear rate of swallowing, 50 s^{-1} (Borwankar, 1992).

Table 4-1. Concentrations of LV hydrocolloids in solution and their apparent viscosities at 50s^{-1}.

Hydrocolloid	Concentration in water (w/w)	[1]Apparent viscosity (Pa.s) at 50 s^{-1}
Locust bean gum	1%	0.67[a]
Guar gum	1%	0.54[a,b]
Fenugreek gum	1%	0.36[b,c]
Xanthan gum	1%	0.32[b]
Gum Arabic	40%	0.71[a]
Psyllium	3.5%	0.44[b]
Flaxseed gum	3.6%	0.36[b,c]
SSPS	13%	0.17[c]

[1]Data reported as mean measurements
Different superscript letters (a,b,c) within a column indicate significant difference between means ($p<0.05$)

Viscosity (Pa.s) as a function of shear rate from 30 to 200 s^{-1} for the locust bean gum, guar gum, fenugreek gum, xanthan gum, gum Arabic, psyllium, flaxseed gum and SSPS solutions are shown in Figure 4-1.

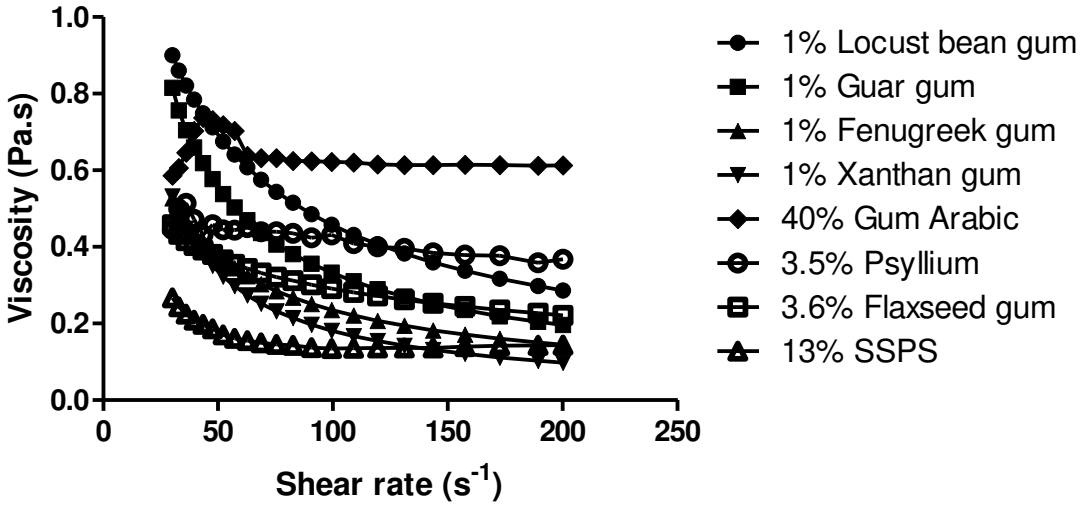


Figure 4-1. Flow curves showing viscosity (Pa.s) of the 1% locust bean gum, 1% guar gum, 1% fenugreek gum, 1% xanthan gum, 40% gum Arabic, 3.5% psyllium, 3.6% flaxseed gum, and 13% SSPS solutions as a function of shear rate (s^{-1}).

The use of 40% gum Arabic has been shown in previous studies whereby the concentration was required to reach a similar viscosity profile of 1% guar gum and 1% xanthan gum solutions (Thevenet, 2010). With the exception of gum Arabic, the remaining 7 hydrocolloids behaved as pseudoplastic non-Newtonian fluids. The Newtonian behaviour of gum Arabic is consistent with other studies where researchers observed the steady shear flow curves at concentrations ranging from 1% to 30%. They reported that at higher concentrations shear thinning was strongly reduced (Li et al., 2009). Due to a higher degree of branching, fewer entanglements are formed with minimal coil overlap therefore eliciting the observed Newtonian behaviour in Figure 4-1.

The apparent viscosities of xanthan gum, gum Arabic, psyllium, flaxseed gum and SSPS did not significantly differ from all of the galactomannans ($p>0.05$), as seen in Table 4-1. LBG and gum Arabic elicited the highest viscosity values, followed by guar gum, xanthan gum and psyllium and finally fenugreek gum, flaxseed gum and SSPS were on the lower end of promoting viscosity in solution ($p<0.05$).

4.1.2. Flow behaviour of medium-viscosity (MV) hydrocolloid solutions

The concentrations used to represent MV solutions are reported in Table 4-2, along with their apparent viscosities at 50 s^{-1}.

Table 4-2. Concentrations of MV hydrocolloid solutions and their apparent viscosities at 50s^{-1}.

Hydrocolloid	Concentration in water (w/w)	[1]Apparent viscosity (Pa.s) at 50 s^{-1}
Locust bean gum	2%	4.1[a]
Guar gum	2%	3.0[b]
Fenugreek gum	2%	1.9[c]
Xanthan gum	4%	1.8[c]
Gum Arabic	50%	2.9[b,c]
Psyllium	7%	1.9[c]
Flaxseed gum	6.5%	1.9[c]
SSPS	25%	2.4[b,c]

[1]Data reported as mean measurements
Different superscript letters (a,b,c) within a column indicate significant difference between means ($p<0.05$)

Flow curves for the MV hydrocolloid solutions are shown in Figure 4-2.

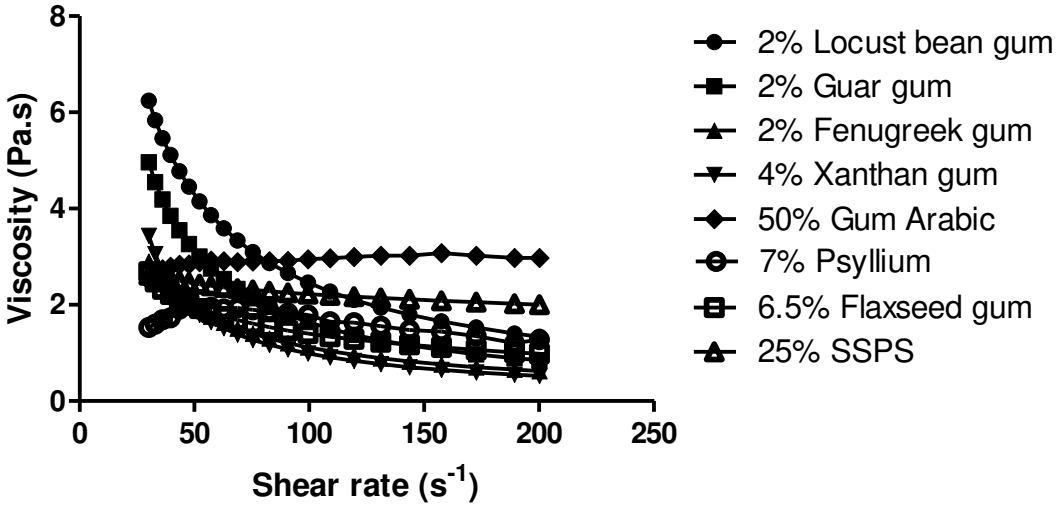


Figure 4-2. Flow curves showing viscosity (Pa.s) of the 2% locust bean gum, 2% guar gum, 2% fenugreek gum, 4% xanthan gum, 50% gum Arabic, 7% psyllium, 6.5% flaxseed gum, and 25% SSPS solutions as a function of shear rate (s^{-1}).

Again, the gum Arabic solution was the only hydrocolloid that behaved as a Newtonian fluid. The rest displayed pseudoplasticity, acting as non-Newtonian fluids. Among the 8 hydrocolloids, LBG was the only one whose apparent viscosity at 50 s^{-1} differed from all the rest ($p<0.05$), as shown in Table 4-2. However, there was no significant difference at a shear rate range $\geq 75 s^{-1}$ ($p>0.05$).

4.1.3. Flow behaviour of high-viscosity (HV) hydrocolloid solutions

The hydrocolloid concentrations required to attain viscosity profiles comparable to that seen in the HV 3% galactomannan solutions are shown in Table 4-3.

Table 4-3. Concentrations of HV hydrocolloid solutions and their apparent viscosities at 50 s^{-1}.

Hydrocolloid	Concentration in water (w/w)	[1]Apparent viscosity (Pa.s) at 50 s^{-1}
Locust bean gum	3%	10.0[a]
Guar gum	3%	5.6[b]
Fenugreek gum	3%	4.4[b]
Xanthan gum	7.%	4.3[b]
Gum Arabic	60%	3.3[b]
Psyllium	10.5%	4.9[b]
Flaxseed gum	9%	5.7[b]
SSPS	28%	4.9[b]

[1]Data reported as mean measurements
Different superscript letters (a,b,c) within a column indicate significant difference between means ($p<0.05$)

The subsequent flow curves for the HV hydrocolloid solutions are shown in Figure 4-3. LBG was again the only substrate showing a higher apparent viscosity at 50 s^{-1} in comparison to the rest ($p<0.05$). As the shear rate increased to 100 s^{-1} this difference was negated ($p>0.05$)

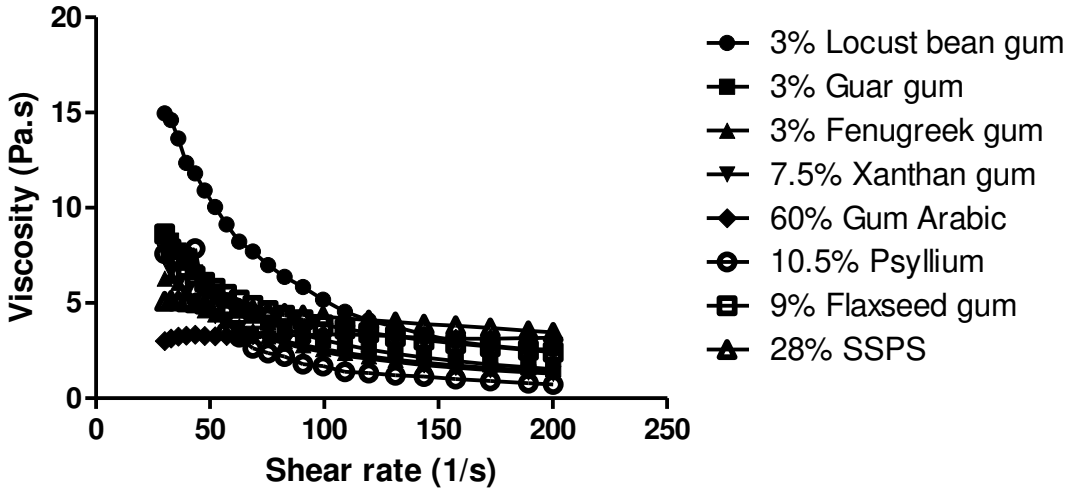


Figure 4-3. Flow curves showing viscosity (Pa.s) of the 3% locust bean gum, 3% guar gum, 3% fenugreek gum, 7.5% xanthan gum, 60% gum Arabic, 10.5% psyllium, 9% flaxseed gum, and 28% SSPS solutions as a function of shear rate (s^{-1}).

The 60% gum Arabic solution was the only one exhibiting Newtonian-like behaviour. This observation is consistent with another study, which showed that at concentrations ≥30% gum Arabic solutions are less likely to be shear-thinning. An increase in the degree of branching, as seen in gum Arabic, leads to a small degree of coil overlap and entanglement resulting in a wider range of shear rate of Newtonian behaviour (Li et al., 2009). Nonetheless, other studies have shown that concentrations ≥40% should produce a pseudoplastic gum Arabic solution with non-Newtonian behaviour (Charalambous & Doxastakis, 1989). The differences can be attributed to the source of the gum, tree age, storage conditions and processing treatments. The remaining 7 HV solutions showed pseudoplastic behaviour across the shear rate range shown in Figure 4-3.

4.2. Effect of *in vitro* digestion on the viscosity of hydrocolloids in solution

4.2.1. Effect of *in vitro* digestion on LV solutions

Apparent viscosities at 50 and 100 s^{-1} of the LV substrates after simulated human digestion are plotted in Figure 4-4. Although flow behaviours from 30 to 200 s^{-1} were used for all samples, reporting these 2 shear rates yields a better viscosity comparison at shear rates that are reported during digestion (Borwankar et al. 1992, Steffe, 1996)

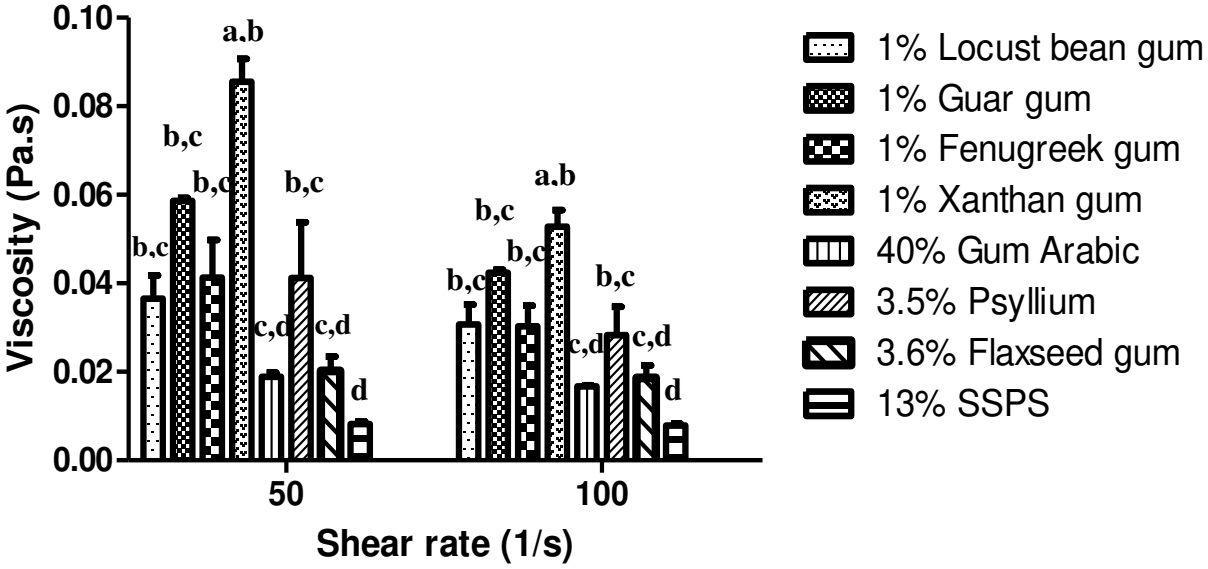


Figure 4-4. Apparent viscosities at 50 and 100 s^{-1} of the LV solutions following 4-hr simulated gastric and duodenal digestion. Error bars indicate standard error of mean. Different superscript letters (a,b,c,d) above each bar indicate significant difference between means ($p<0.05$).

The conditions in the gastric and duodenal phase of the digestion model led to a considerable viscosity reduction in all hydrocolloids studied. The extent of reduction seems to be dependent on the unique physicochemical properties possessed by each gum. With the exception of guar gum, the viscosity of xanthan gum is higher than the rest at both shear rates, as shown in Figure 4-4 ($p<0.05$). Although postulated effects of *in vitro* digestion on

xanthan gum rheology are scarcely studied, researchers have hypothesized the resilience of xanthan gum and believe it to be linked not only to its relatively high molecular weight but more so to its conformation. As mentioned in Section 2.7.4, xanthan gum possesses the ability to protect its cellulose-like backbone by having the branching units wrap around, creating a helical formation when placed in solution. This has led researchers to believe that the conformation provides greater resistance to structural degradation caused by pH extremes and enzymatic cleavage, in comparison to other gums that possess higher molecular weights, such as guar gum and locust bean gum (Valentine et al., 1995). In addition, the apparent viscosity of guar gum, at both shear rates illustrated in Figure 4-4, was maintained to a greater degree than some other hydrocolloids, namely gum Arabic, flaxseed gum, and SSPS ($P<0.05$). This finding is consistent with another study that examined the effects of acidic conditions and temperature on the viscosity and stability of high molecular weight, 1% guar gum solutions (Wang et al., 2000). The researchers of this study found that although xanthan gum has been shown to be more stable in acidic conditions, guar gum managed to promote stability at a pH value as low as 2.0. Moreover, degradation was higher for samples exposed to elevated temperatures (50^{o}C) where viscosity reduction was 23%, in comparison to those exposed to low pH levels (2.0 or 3.0) signified by a 4.9% viscosity reduction of the 1% guar gum solutions. Nonetheless, viscosity was lowered and this is believed to be attributed by the effect that the acids have on intra- and inter-molecular hydrogen bonding between guar gum and water as well as galactomannan-galactomannan linkages (Wang et al., 2000).

4.2.2. Effect of *in vitro* digestion on MV solutions

The apparent viscosities at 50 and 100 s^{-1} of the MV solutions are shown in Figure 4-5.

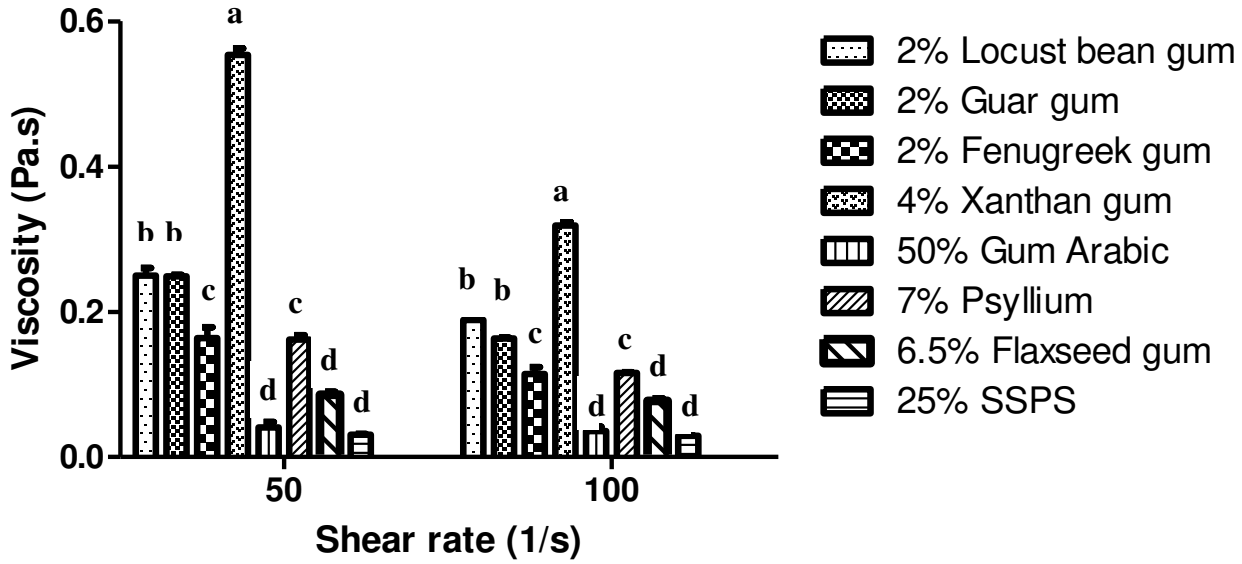


Figure 4-5. Apparent viscosities at 50 and 100 s^{-1} of the MV solutions following 4-hr simulated gastric and duodenal digestion. Error bars indicate standard error of mean. Different superscript letters (a,b,c,d) above the bars indicate a significant difference between means ($p<0.05$).

After *in vitro* digestion, xanthan gum again showed the greatest ability to retain viscosity in comparison to the other hydrocolloids, as shown in Figure 4-5. This observation is again explained by the resilient and protective nature of xanthan gum. In addition, locust bean gum and guar gum elicited higher apparent viscosities than the others. Their ability to retain an appreciable difference in viscosity in comparison to the rest is believed to be attributed to their non-ionic and neutral character. Some studies have even shown that when exposed to a pH range from 3.5 to 9.0 both gum solutions remain unchanged (Kennedy et al., 1986). However, this was not seen here as the simulated model led to a substantial decrease in overall viscosity, which may or may not be due to the pH extremes incorporated within, as will be discussed in Section 4.3. Despite the fact that fenugreek gum is a galactomannan, the reduction in apparent viscosity was more profound in comparison to locust bean or guar gum. This may be due to the fact that fenugreek gum consists of a higher substitution of

galactose along the mannose backbone. As discussed in Section 2.7 fenugreek gum is completely substituted, which renders it to be more hydrophilic and due to its branched and more compact structure there could be a greater extent of electrostatic repulsion between the neighbouring galactose side chains. Moreover, researchers have demonstrated that polysaccharides containing higher degrees of branching exert less coil overlap when placed in solution, subsequently depressing the viscosity profile (Li et al., 2009). The viscosity of psyllium was comparable to that of fenugreek gum, as shown in Figure 4-5. Previous studies have demonstrated that altering pH levels can lead to a decrease in molecular interactions due to a lowered net electrostatic repulsion, as discussed in Section 2.7. At higher pH values alkaline depolymerization results in fewer junction zones, altering the rheological behaviour of psyllium (Farahnaky et al., 2010). Furthermore, Dikeman et al. (2006) demonstrated that the viscosity of psyllium after simulated digestion was lower when compared to that of guar gum and xanthan gum, and this is consistent with the results shown here. As the hydrocolloids become more branched, as is the case with gum Arabic, flaxseed gum, and SSPS, viscosity losses are substantial, as shown in Figure 4-5 and as will be discussed in Section 4.3. Therefore, it is demonstrated that molecular weight and chain length are not the sole factors involved in determining viscosity, as some strains of gum Arabic are known to contain high molecular weights (Thevenet, 2010), and that the degree of branching and polymerization play an important role in promoting and/or retaining overall solution viscosity.

4.2.3. Effect of *in vitro* digestion on HV solutions

The apparent viscosities at 50 and 100 s^{-1} of the most concentrated HV solutions are shown in Figure 4-6.

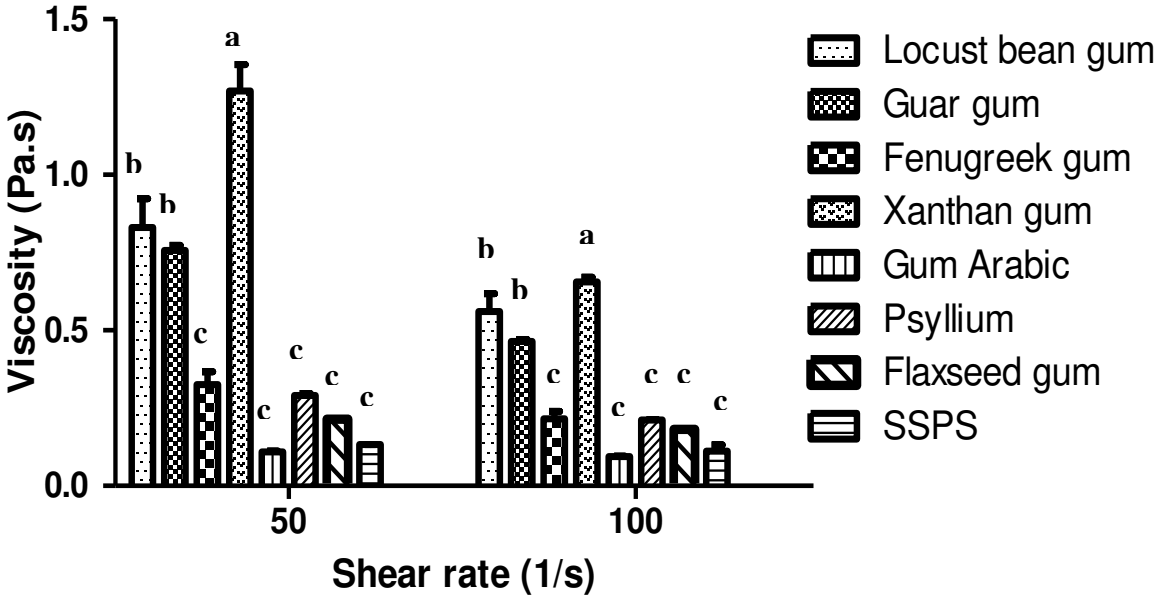


Figure 4-6. Apparent viscosities at 50 and 100 s^{-1} of the HV solutions following 4-hr simulated gastric and duodenal digestion. Error bars indicate standard error of mean. Different superscript letters (a,b,c) above the bars indicate a significant difference between means ($p<0.05$).

The findings here are very similar to those shown previously by the MV solutions in that the apparent viscosity at 50 and 100 s^{-1} of xanthan gum is higher than the rest, as shown in Figure 4-6. Guar gum and locust bean gum again demonstrated to be the second most resilient gums of the study ($p<0.05$) with the remaining 5 hydrocolloids displaying the weakest capacity for maintaining viscosity, based on their individual conformation. Following the experimental results, viscosity data were grouped and analyzed based on the power law equation (see Equation 2-1). Model development allowed for the

estimation of the consistency index, which gives a numerical value for viscosity based on shear stress and shear rate for each sample treatment. The data are shown in Table 4-4.

Table 4-4. Determination of the consistency index (K, Pa.s) of the samples after *in vitro* digestion. Different superscript letters (a,b,c) within a column indicate significant difference between means ($p<0.05$) *NS=values did not converge. Data reported as mean measurements.

Substrate	LV	MV	HV
Locust bean gum	0.09[a]	1.56[b,c]	9.78[b,c]
Guar gum	0.40[a]	3.28[b]	13.8[b]
Fenugreek gum	0.23[a]	1.51[b,c]	4.06[b,c]
Xanthan gum	1.59[a]	14.7[a]	28.4[a]
Gum Arabic	*NS	0.07[c]	0.25[c]
Psyllium	*NS	0.94[c]	1.89[c]
Flaxseed gum	*NS	0.17[c]	0.60[c]
SSPS	*NS	0.03[c]	0.42[c]

Table 4-4 demonstrates compatibility between the experimental data and the power law model that was applied. Nonlinear regression analysis shows that for all 3 concentration levels after 4 hr *in vitro* digestion, the viscosity of xanthan gum was higher than all other fibre solutions ($p<0.05$). Similar to this, Dikeman et al. (2006) showed that after simulated digestion xanthan gum elicited significantly higher non-linear regression parameters than other dietary fibres, such as psyllium and guar gum. Physicochemical properties such as molecular weight, chain length, the presence or absence of charged groups, degree of branching and degree of polymerization all dictate the overall conformation of a gum when dispersed in water. Figures 4-4 to 4-6 demonstrate that the properties discussed in Section 2.7 are responsible for not only the viscous character but

also the ability of each gum to withstand the conditions in the GI tract, where the fibres are exposed to dilutions, pH extremes and hydrolytic enzymes.

4.3. Effect of *in vitro* digestion, dilution, and acid and alkali environments in the absence of hydrolytic enzymes, on the viscosity of hydrocolloids

As foods pass through the GI tract, mechanical breakdown is responsible for allowing nutrient absorption to take place. Consequently, this causes a physical disruption on the food matrix creating the loss in viscosity presented in Section 4.2. However, this reduction can be attributed by a variety of different factors, including the effect of water that is produced in the stomach and small intestine, acid and alkali environments, or the latter in the presence of hydrolytic enzymes and bile salts, as all 3 are essential for nutrient assimilation (Johnson, 1991). Subsequently, three conditions were simulated in order to deduce which, if any, is the most significant gastrointestinal element involved in lowering the viscosity of dietary fibres.

4.3.1. Effect of dilution, pH, and *in vitro* digestion on the viscosity of locust bean gum

Viscosity reductions after simulated digestion, dilution, and acid and alkali environments of LV locust bean gum solutions are plotted in Figure 4-7.

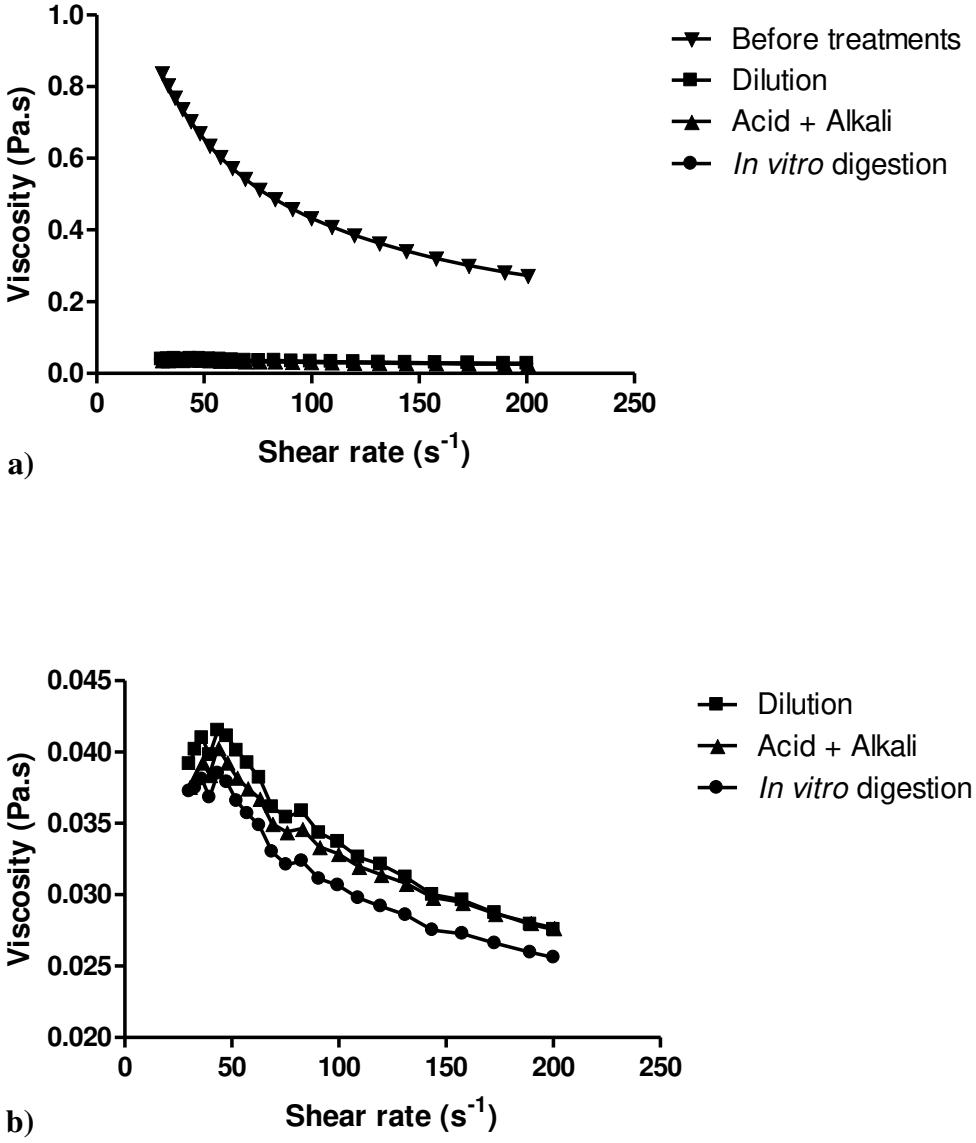


Figure 4-7. Comparison of the viscosity profiles of LV locust bean gum solutions after exposure to simulated digestion, dilution and pH a) with pre-treatment rheology and b) amongst the treatments. Data reported as mean measurements.

Figure 4-7 a illustrates that there is a substantial drop in viscosity that arises after the locust bean gum solution is exposed to the 3 treatments. Nevertheless, there seems to be no difference shown on the effect that *in vitro* digestion or acid and alkali treatments have on viscosity, as illustrated in Figure 4-7 b. This suggests that 1% locust bean gum is

resistant to acidification and neutralization in the presence or absence of digestive enzymes and that a dilution effect is the cause of the loss in structural integrity.

The viscosity profiles of the MV locust bean gum solutions following the 3 treatments are plotted in Figure 4-8.

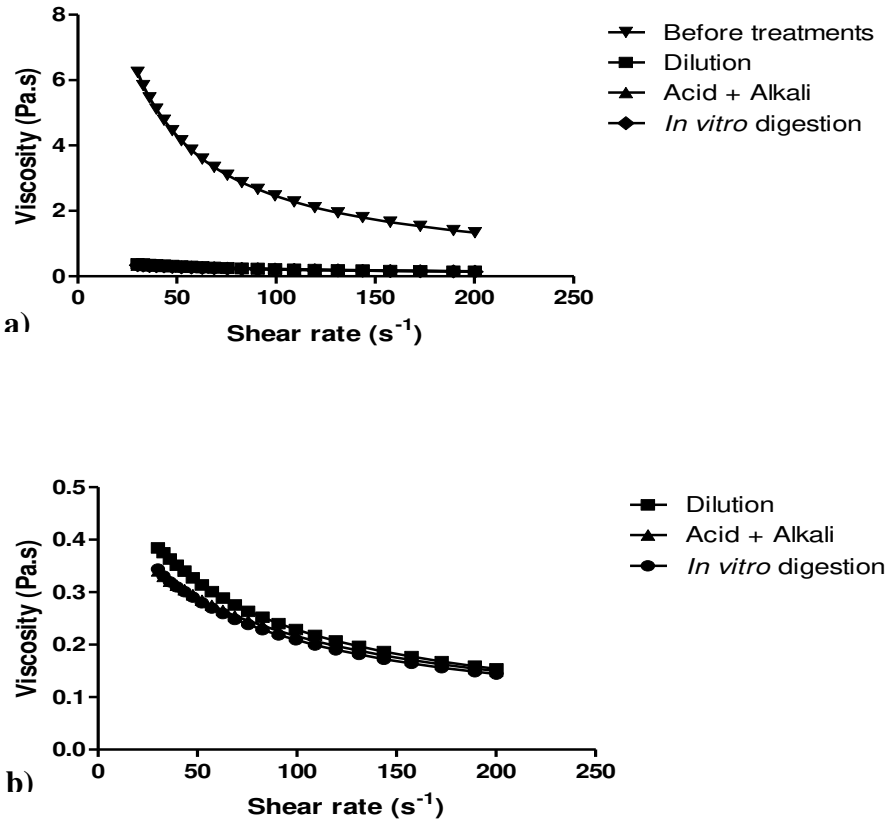


Figure 4-8. Comparison of the viscosity profiles of MV locust bean gum solutions after exposure to simulated digestion, dilution and pH a) with pre-treatment rheology and b) amongst treatments. Data reported as mean measurements.

Again, the extent of viscosity reduction when the gum is exposed to the treatments is apparent in Figure 4-8 a, continually demonstrating no change compared to simple

dilution when the solution is exposed to extreme pH in the presence or absence of enzymes.

The viscosity following the 3 treatments of the most concentrated HV gums are shown in Figure 4-9.

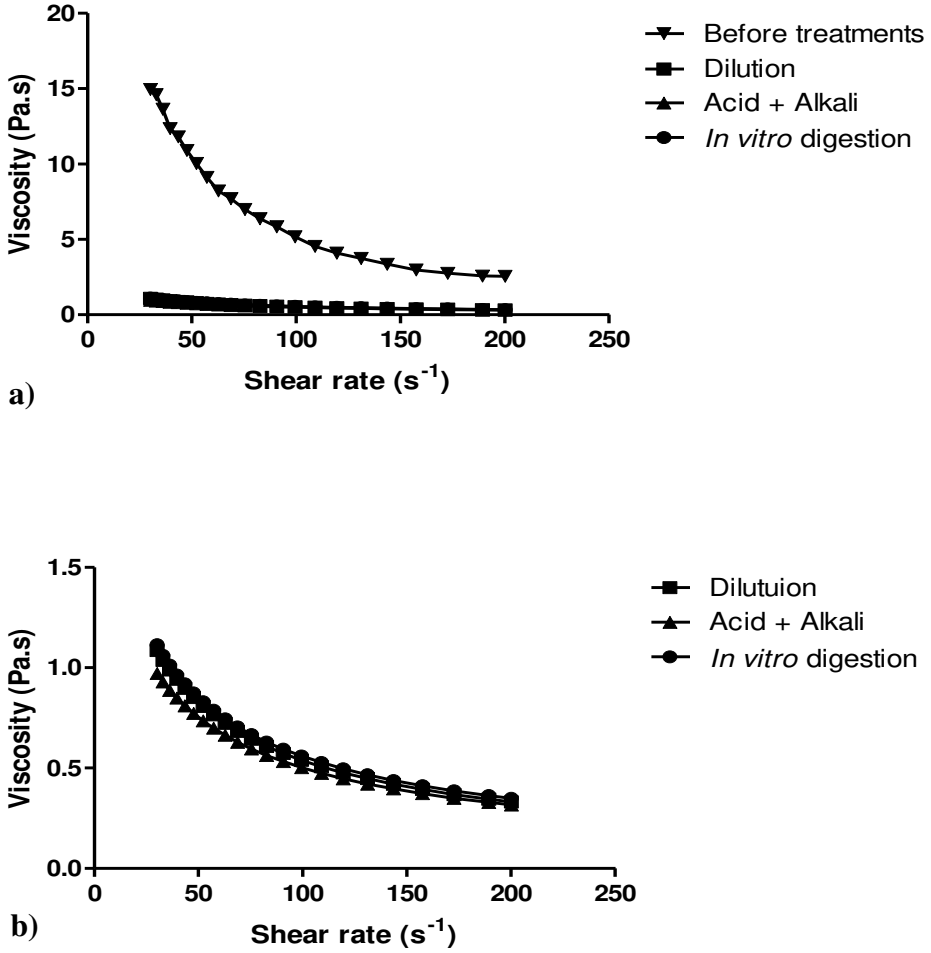


Figure 4-9. Comparison of the viscosity profiles of HV locust bean gum solutions after exposure to simulated digestion, dilution and pH a) with pre-treatment rheology and b) amongst treatments. Data reported as mean measurements.

The data of the HV solutions are consistent with the previous sets, as post-treatment rheology is showing a substantial drop in viscosity with no difference between the 3 treatments, again highlighting the resistance of locust bean gum to enzyme hydrolysis

and pH changes that occur throughout digestion. Viscosities after each simulation were compared to the pre-treatment values, as shown in Table 4-5.

Table 4-5. Viscosity values calculated as a % of the initial viscosity after LV, MV, and HV locust bean gum solutions were subjected to simulated digestion, dilution and pH extremes (acid and alkali). Values were calculated at 100 s^{-1}.

Treatment	[1]Remaining viscosity (%)		
	LV	MV	HV
Dilution	7.8[a]	9.3[a]	10.4[a]
Acid + Alkali	7.6[a]	8.8[a]	9.7[a]
In vitro digestion	7.1[a]	8.5[a]	10.8[a]

[1]Data reported as mean values.
Different superscript letters within a column indicate significant difference between means ($p<0.05$)

Dilutions in this model represent the watery secretions that are produced during the process of digestion, which was mentioned and discussed in Section 2.1. Consequently, they appear to be the most important factor in reducing gum viscosity, as there is no significant difference between the effects of dilution, and *in vitro* digestion and acid and alkali, which were all added with the same volume, as shown in Table 4-5 ($p>0.05$). This suggests that the chemical structure of locust bean gum is inherently protected against enzyme hydrolysis and pH changes. This of course is in conjunction with the current definition of dietary fibre, which briefly states that it cannot be digested or absorbed by the human small intestine, as part of the definition that was given in Section 2.3.1. Moreover, previous studies have reported on the stability of locust bean gum to varying pH, allowing it to be a popular food ingredient in ice cream, cheese and sauces, where it acts as a stabiliser and thickener (Dea & Morrison, 1975; Garcia-Ochoa & Casas, 1992).

However, as water is added to the system, mimicking the secretions of the stomach and small intestine, the number of junction zones and extent of entanglements that were present in the 3 locust bean gum solutions are minimized. Consequently, this change in physical structure may be what is affecting solution viscosity and, as an extension, may influence both technological and physiological functionality.

4.3.2. Effect of dilution, pH, and *in vitro* digestion on the viscosity of guar gum

Reductions in viscosity of the LV guar gum solutions are plotted in Figure 4-10.

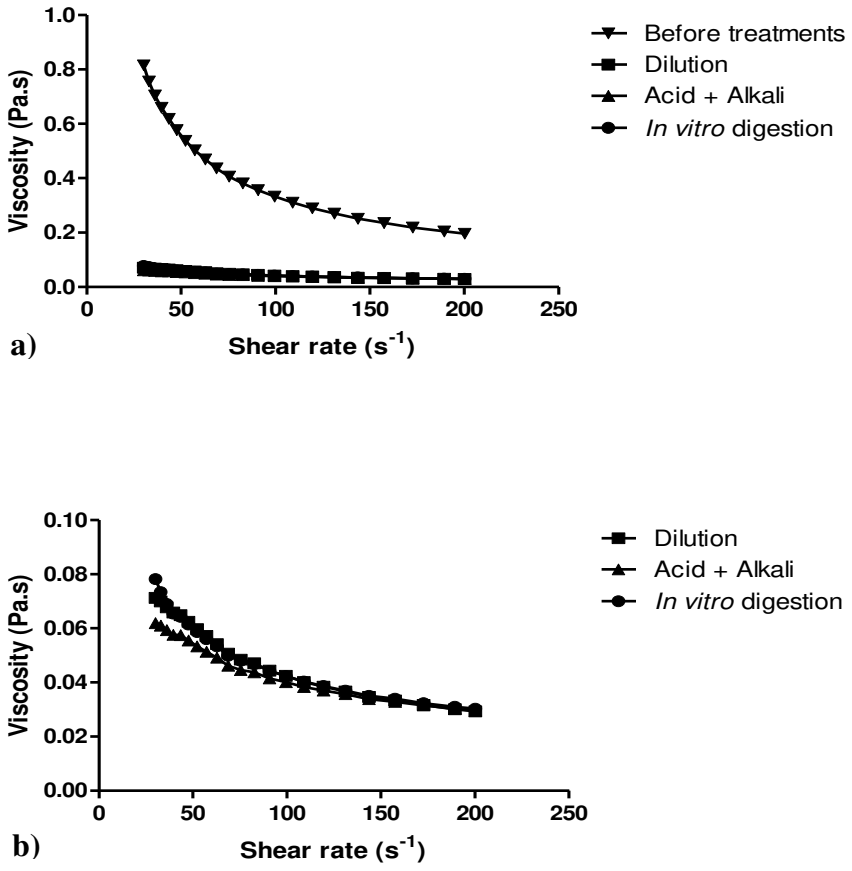


Figure 4-10. Comparison of the viscosity profiles of LV guar gum solutions after exposure to simulated digestion, dilution and pH a) with pre-treatment rheology and b) amongst treatments. Data reported as mean measurements.

Figure 4-10 a illustrates the profound reduction in the viscosity of guar gum after the solution was exposed to the 3 simulations. Figure 4-10 b demonstrates that the loss in viscosity is attributed by the effect of dilution of the fibre solution and not the presence of pH or hydrolytic enzymes.

The relationship between the rheology of MV solutions and treatment type is plotted in Figure 4-11.

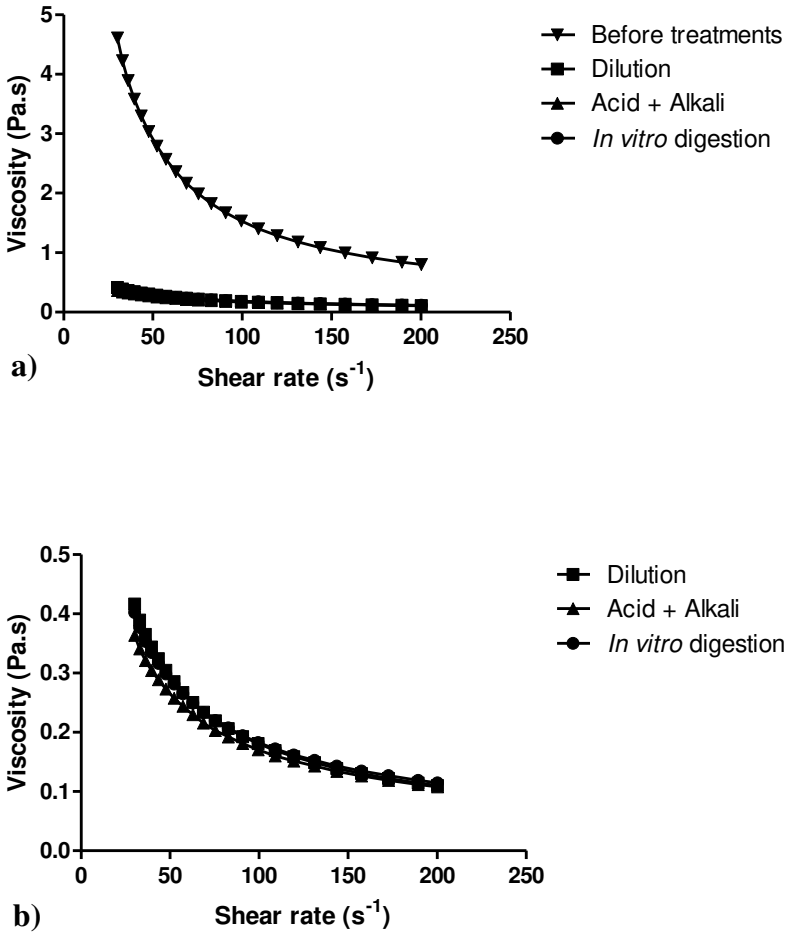


Figure 4-11. Comparison of the viscosity profiles of MV guar gum solutions after exposure to simulated digestion, dilution and pH a) with pre-treatment rheology and b) amongst treatments. Data reported as mean measurements.

Figure 4-11 shows the consistency of galactomannan rheology, whereby a significant drop in viscosity is seen. This effect is attributed by dilutions alone, as there is no effect on viscosity in the presence of acid and alkali or digestive enzymes, as shown in the previous locust bean gum and the LV guar gum solutions.

The post-treatment viscosity profile of the HV guar gum is plotted in Figure 4-12.

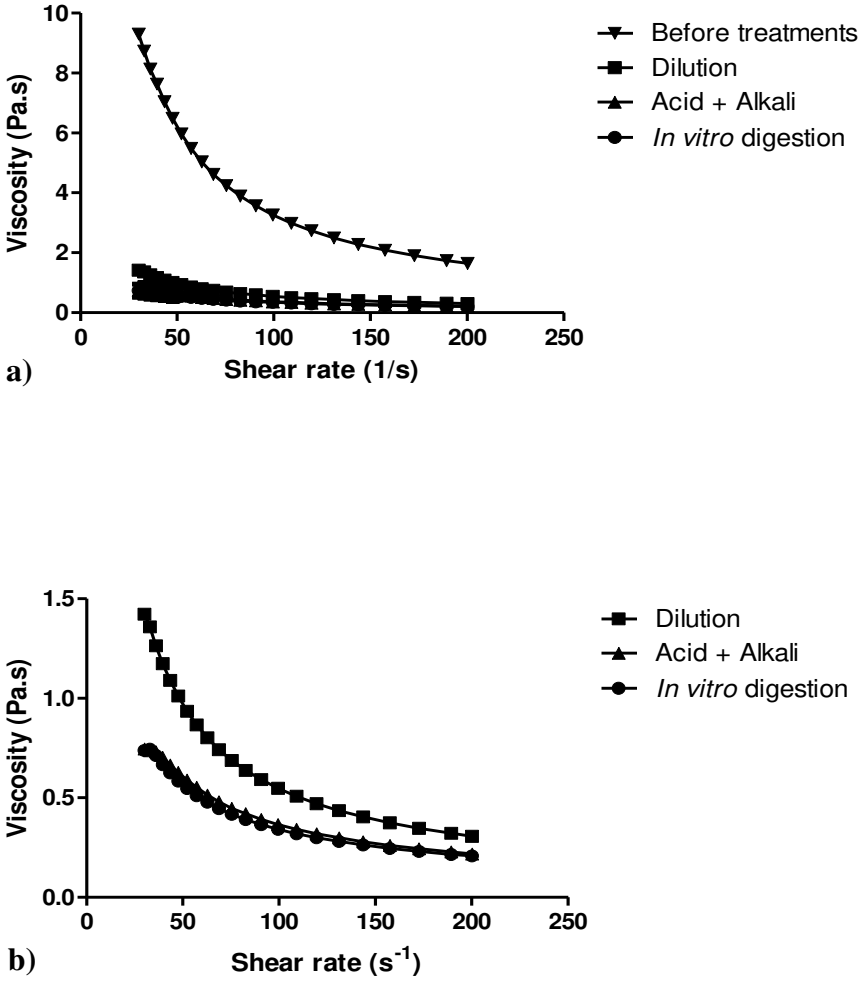


Figure 4-12. Comparison of the viscosity profiles of HV guar gum solutions after exposure to simulated digestion, dilution and pH a) with pre-treatment rheology and b) amongst treatments. Data reported as mean measurements.

Figure 4-12 a highlights the consistent drop in viscosity that arises after guar gum is subjected to the simulations. Interestingly, there seems to be a difference in the effect that the treatments have on the extent of viscosity reduction. Acidifying and then neutralizing the sample seems to reduce viscosity of the 3% guar gum solution more than dilution alone. This is made apparent in Table 4-6, which shows the drop in viscosity after exposure of guar gum to each treatment.

Table 4-6. Viscosity values calculated as a % of the initial viscosity after LV, MV, and HV guar gum solutions were subjected to simulated digestion, dilution and pH extremes (acid and alkali). Values were calculated at 100 s^{-1}.

Treatment	[1]Remaining viscosity (%)		
	LV	MV	HV
Dilution	12.7[a]	11.8[a]	16.8[b]
Acid + Alkali	12.1[a]	11.1[a]	11.2[a]
In vitro digestion	12.7[a]	11.9[a]	10.5[a]

[1]Data reported as mean values.
Different superscript letters (a,b) within a column indicate significant difference between means ($p<0.05$)

At all concentration levels, both locust bean gum and guar gum are consistently susceptible to structural changes upon dilution. These results indicate that water, which is a major component of salivary (>99%), gastric and intestinal secretions (Johnson, 1991), substantially lowers the viscous nature of galactomannans such as guar gum. However, as the concentration of guar gum in solution increases, so does its dependence on pH. Table 4-6 shows that there is a perceptible drop in viscosity when HV guar gum is exposed to pH extremes in comparison to the effect exerted by dilutions ($p<0.05$). No statistical

difference is seen between the effects of the *in vitro* digestion model, which incorporated an acidification and neutralization step in the presence of hydrolytic enzymes and bile, and the pH treatment in the absence of enzymes ($p>0.05$). Unlike starch, which is hydrolyzed from the mouth downwards, guar gum lacks the α bond that is the target of amylase enzymes. Specific galactosidase enzymes, such as α- and β-galactosidase, are required to cleave the galactose-mannose bond. They are found in many intestinal bacteria, such as *Bifidobacterium*, and can also be isolated from *Escherichia coli* (Paeschke & Aimutis, 2011). Moreover, with the absence of fat and protein in these guar gum solutions, minimal to no effect should be attributed by the action of pepsin in the stomach or pancreatin in the small intestine. Correspondingly, the effect that the present *in vitro* model had on the structural integrity of guar gum is insignificant, as shown in the viscosity curves across the 3 concentrations. Despite the fact that dilutions exerted the greatest effect on the flow behaviour of guar gum, by exposing the solutions to acid and alkali the entanglements that promoted guar gum viscosity were degraded. This is consistent with another study, which showed that the presence of strong acids and alkali lead to the depolymerisation of guar gum (Goswami, 1999; Swartz et al, 2000), ultimately affecting its functionality as a food ingredient. For example, many companies depend on such treatments in order to properly extract the gum from the seed and to isolate the functional ingredient for human consumption. Yet some of these depolymerised guar products are not given an E-number, which is required in order for any fibre product, such as Benefiber® and Sunfiber®, to be sold in the EU market, as the molecular weight and viscosity were shown to be significantly reduced (Wielinga, 2010).

As an extension, it may be hypothesized that physiological functionality may also be affected by such changes, as will be proposed in Chapter 5.

4.3.3. Effect of dilution, pH, and *in vitro* digestion on the viscosity of fenugreek gum

The changes in viscosity after subjecting LV fenugreek solutions to the prescribed treatments are shown in Figure 4-13.

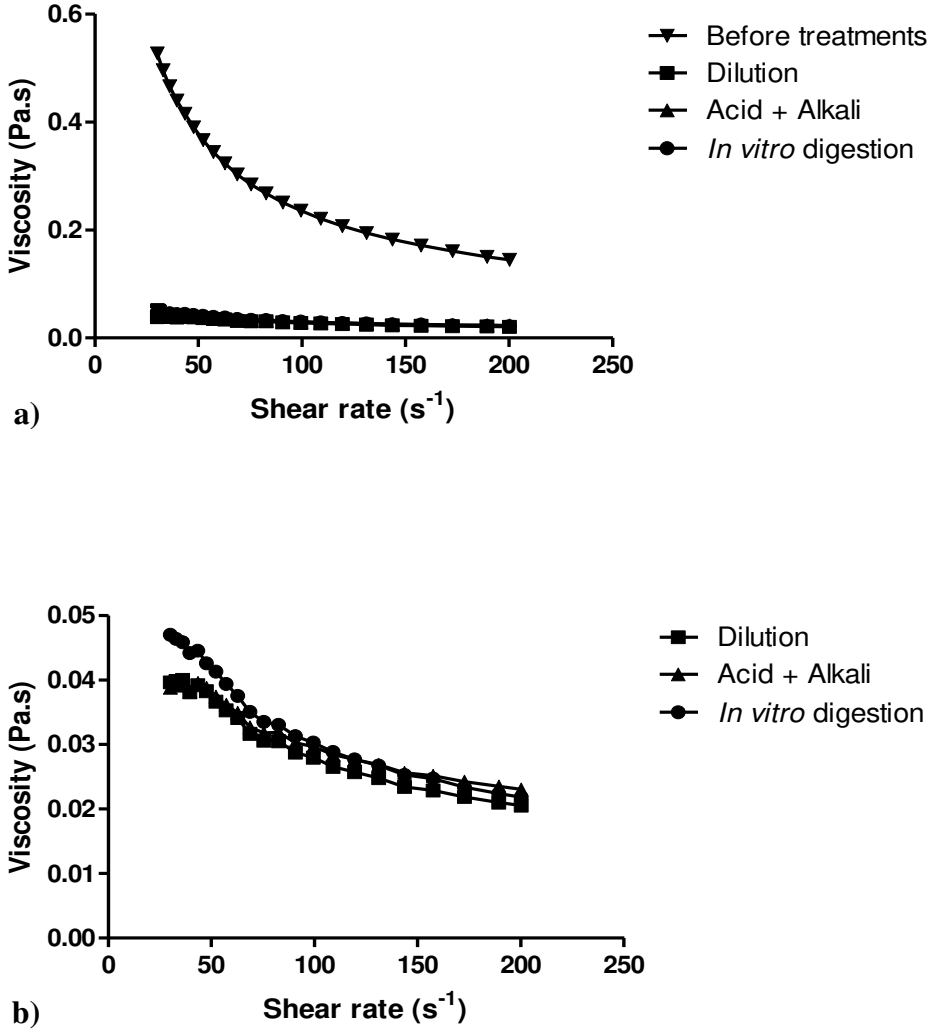


Figure 4-13. Comparison of the viscosity profiles of LV fenugreek gum solutions after exposure to simulated digestion, dilution and pH a) with pre-treatment rheology and b) amongst treatments. Data reported as mean measurements.

In vitro digestion, dilution, and the induction of acid and alkali into the system all led to a substantial decrease in the flow behaviour of fenugreek. There is no difference between the effects of each treatment ($p>0.05$), as shown in Figure 4-13. The susceptibility of fenugreek to incur physical changes with the addition of water is again shown through the substantial reduction in the viscosity of MV solutions, illustrated in Figure 4-14 a.

Figure 4-14 shows the flow behaviour of treated MV fenugreek gum.

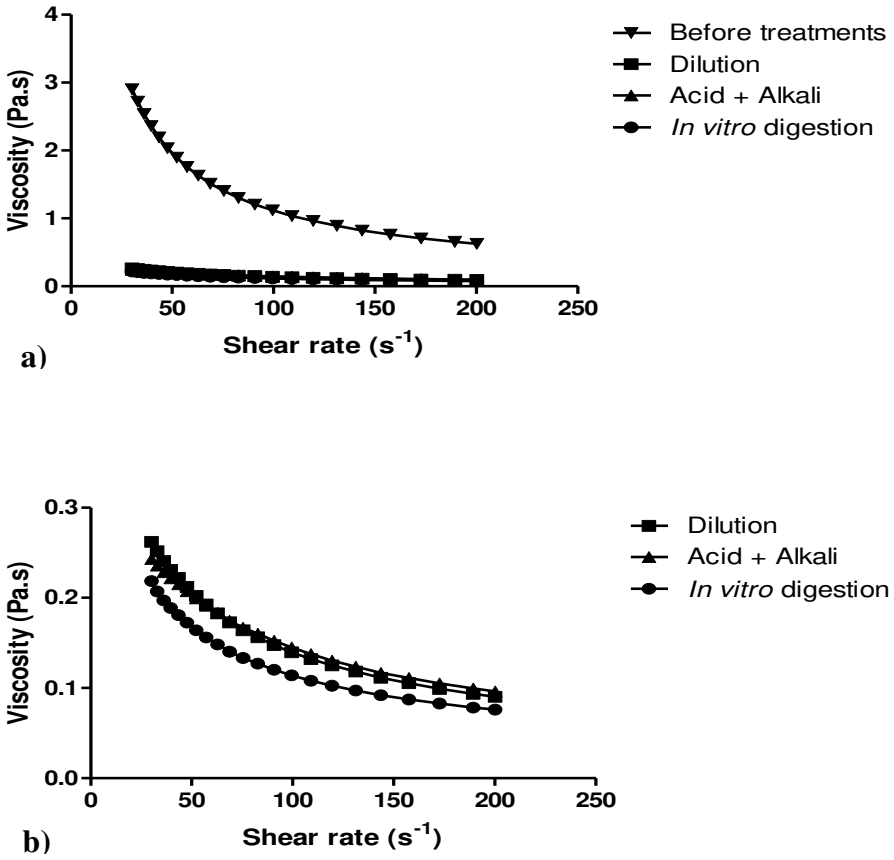


Figure 4-14. Comparison of the viscosity profiles of MV fenugreek gum solutions after exposure to simulated digestion, dilution and pH a) with pre-treatment rheology and b) amongst treatments. Data reported as mean measurements.

The effect of introducing fenugreek gum to acid and alkali in the presence and absence of digestive enzymes is insignificant, as shown through the comparison of the viscosity profiles of both treatments to that of simple dilution depicted in Figure 4-14 b. This enforces the proposal that the gastrointestinal secretions produced during digestion reduce fibre viscosity through a dilution effect as there is no dependence on actual pH changes or the presence of hydrolytic enzymes.

The viscosity profiles after subjecting HV fenugreek gum to each condition is plotted in Figure 4-15.

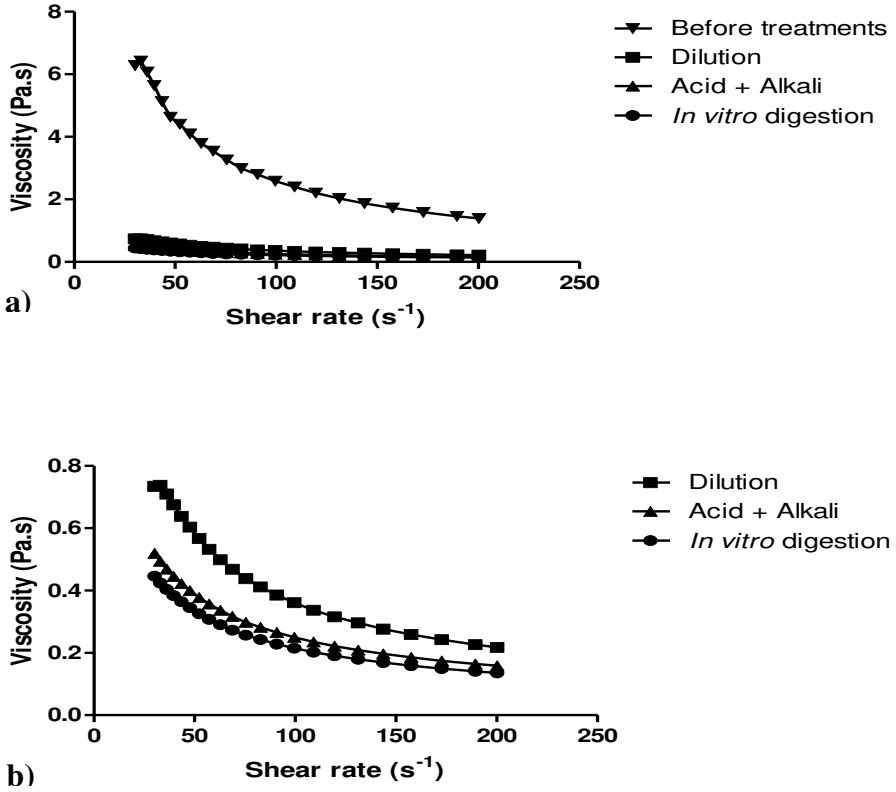


Figure 4-15. Comparison of the viscosity profiles of HV fenugreek gum solutions after exposure to simulated digestion, dilution and pH a) with pre-treatment rheology and b) amongst treatments. Data reported as mean measurements.

Despite the fact that diluting the HV samples led to the greatest reduction in viscosity, alterations in pH seem to enhance the losses attributed by the dilutions, as shown in Figure 4-15. The viscosity values of the solutions after exposure to each treatment are shown in Table 4-7

Table 4-7. Viscosity values calculated as a % of the initial viscosity after LV, MV, and HV fenugreek gum solutions were subjected to simulated digestion, dilution and pH extremes (acid and alkali). Values were calculated at 100 s^{-1}.

Treatment	[1]Remaining viscosity (%)		
	LV	MV	HV
Dilution	11.9[a]	12.5[a]	14.0[b]
Acid + Alkali	12.6[a]	12.9[a]	9.6[a]
In vitro digestion	12.9[a]	10.2[a]	8.3[a]

[1]Data reported as mean values.
Different superscript letters (a,b) within a column indicate significant difference between means ($p<0.05$).

There are no statistical differences to report between the effects of dilutions and the other 2 simulated conditions, on the viscosity of LV or MV fenugreek gum solutions ($p>0.05$). However, analogous to the findings of the guar gum experiments, HV fenugreek gum seems to be susceptible to pH as there is a greater reduction in viscosity when the sample is exposed to either the *in vitro* model, or the acid and alkali conditions ($p<0.05$). There is no difference between the latter 2 treatments ($p>0.05$). This observation may be explained by looking at the conformation of fenugreek gum. When placed in solution, it forms a compact structure, which is held together via hyperentanglements of the

galactose side chains (Wu et al., 2011), as discussed in Section 2.7.3. Once exposed to extreme pH, the formation and subsequent stability of these hyperentanglements is reduced. Another study showed that the addition of increasing concentrations of sodium hydroxide, and therefore pH, led to greater reductions in intrinsic viscosity (Doyle et al., 2009). The researchers of this study proposed that the addition of alkali ionizes the hydroxyl groups, resulting in electrostatic repulsions that subsequently inhibit intermolecular associations, leading to a reduction in both molecular weight and viscosity (Doyle et al, 2009; Goycoolea et al, 1995).

4.3.4. Effect of dilution, pH, and *in vitro* digestion on the viscosity of xanthan gum

Changes in viscosity after simulating each treatment, in LV, MV, and HV solutions are plotted in Figures 4-16, 4-17 and 4-18, respectively.

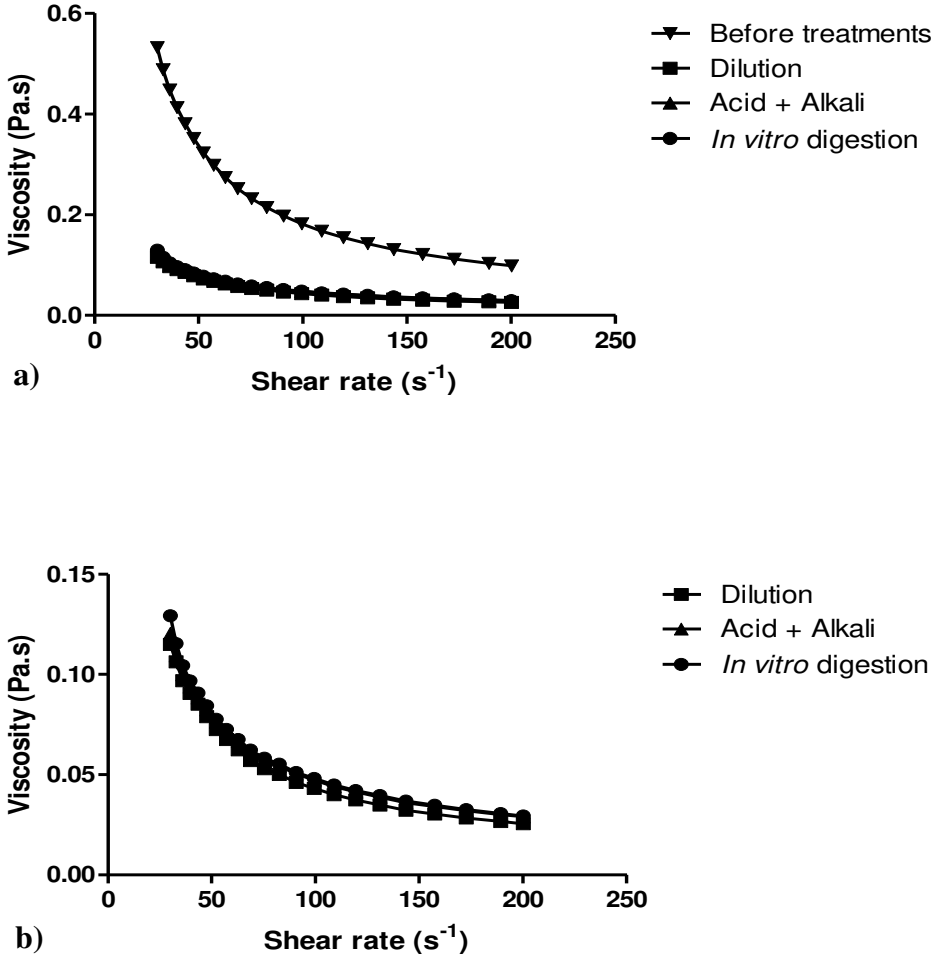


Figure 4-16. Comparison of the viscosity profiles of LV xanthan gum solutions after exposure to simulated digestion, dilution and pH a) with pre-treatment rheology and b) amongst treatments. Data reported as mean measurements.

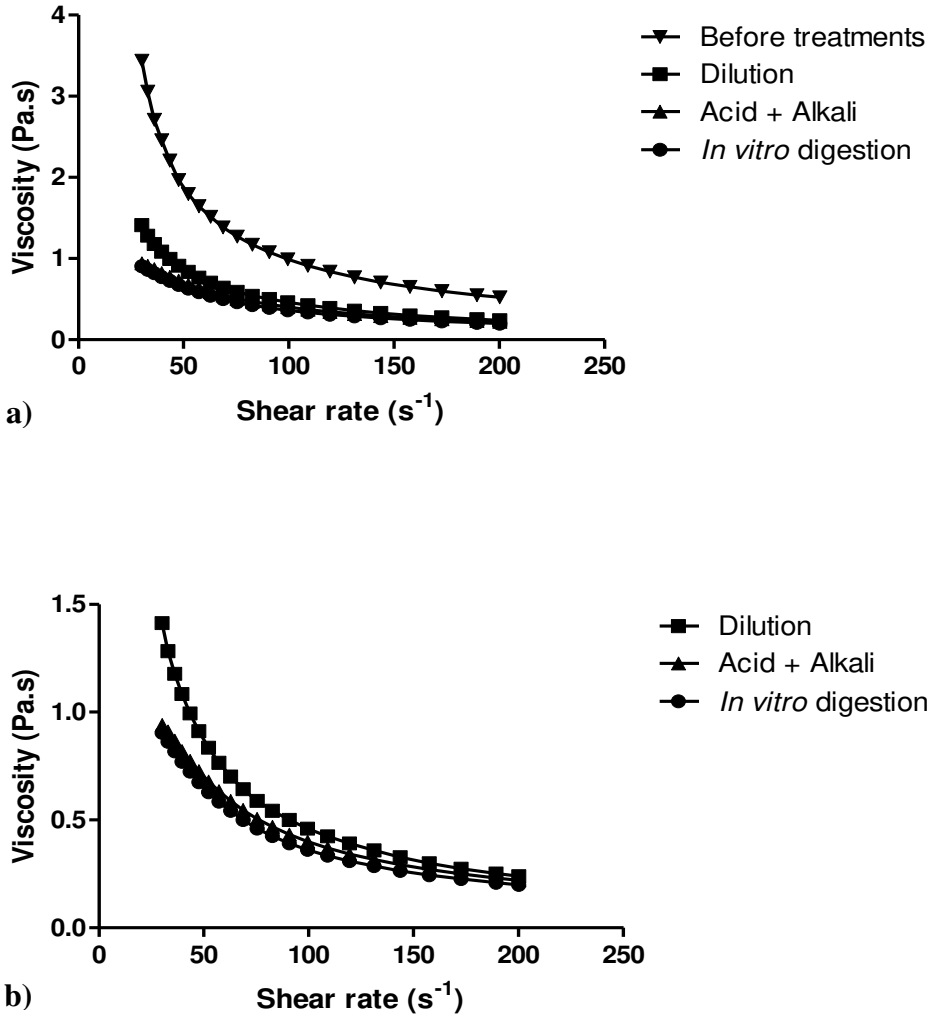


Figure 4-17. Comparison of the viscosity profiles of MV xanthan gum solutions after exposure to simulated digestion, dilution and pH a) with pre-treatment rheology and b) amongst treatments. Data reported as mean measurements.

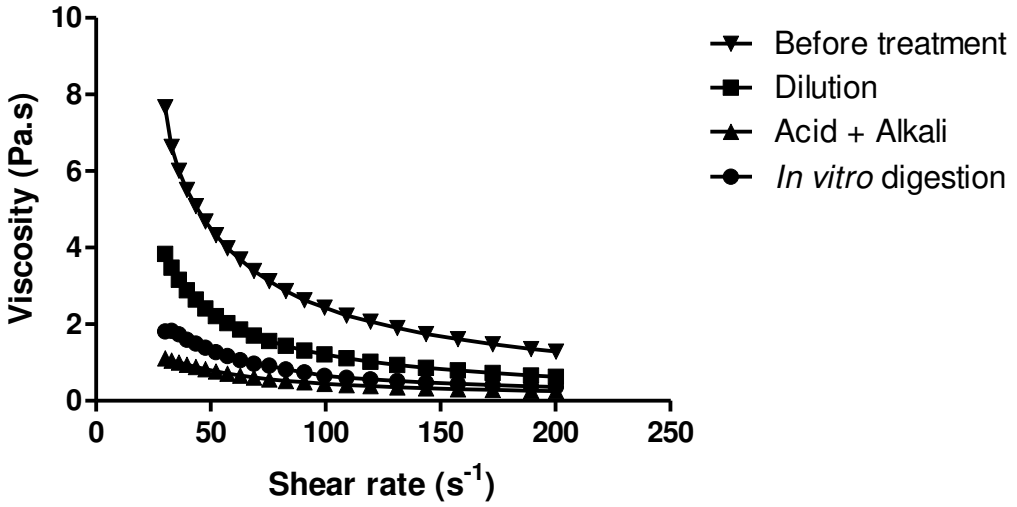


Figure 4-18. Comparison of the viscosity profiles of HV xanthan gum solutions after exposure to simulated digestion, dilution and pH, with pre-treatment rheology. Data reported as mean measurements.

Reductions in viscosity after applying the 3 treatments are apparent at all concentration levels, as shown in Figures 4-16 to 4-18. Viscosity losses of all 3 xanthan gum solutions are considerably lower in comparison to the galactomannans. A study looking at the effects of low pH on the structural properties of various gums including guar gum and xanthan gum, showed that the latter was more resistant to structural changes when placed in an acidic medium, allowing for it to be an ideal ingredient for food products with low pH values, such as yoghurt drinks and salad dressings (Wang et al., 2000). However, in conjunction with the earlier findings, dilutions contribute the greatest influence on structural degradation. Subsequently, the viscosity retained after exposing LV, MV, and HV solutions to the simulated conditions were calculated and are presented in Table 4-8.

Table 4-8. Viscosity values calculated as a % of the initial viscosity after LV, MV, and HV xanthan gum solutions were subjected to simulated digestion, dilution and pH extremes (acid and alkali). Values were calculated at 100 s^{-1}.

Treatment	[1]Remaining viscosity (%)		
	LV	MV	HV
Dilution	23.7[a]	46.5[a]	49.6[b]
Acid + Alkali	26.1[a]	40.0[a]	18.4[a]
In vitro digestion	26.5[a]	36.5[a]	26.8[a,b]

[1]Data reported as mean values.
Different superscript letters (a,b) within a column indicate significant difference between means ($p<0.05$).

For the HV samples, pH further influenced solution rheology as the group that was exposed to dilutions retained nearly 50% more of its structural integrity in comparison to the other 2 treatments ($p<0.05$), as shown in Table 4-8, although this difference was negated at shear rates > 100 s^{-1}. There are no significant differences to report between the effects exerted by the *in vitro* digestion model and those of pH alone on the HV group ($p>0.05$). Other studies have reported that in the presence of acidic solutions, xanthan gum shows a reduction in viscosity. In particular, hydrochloric acid, which was utilized in the present study led to a more accelerated hydrolysis of the polysaccharide in comparison to other reagents that were used, such as acetic and phosphoric acid (Sworn & Monsanto, 2000). Another study explained how the stability of xanthan gum rheology spans over a wide pH range and that solution viscosity only decreased below pH of 3.0, which is considered to be an extreme value and in actuality is higher than the gastric pH simulated in the present study. However, viscosity was recoverable when the solution was neutralised (Sworn, 2010). Similar to this, the results of another study that was done

focusing on analyzing the properties of xanthan gum showed slight losses in solution viscosity at pH<3, however at higher shear rates there were no observed differences between the pH-treated solutions and the native xanthan gum that was prepared. The researchers of the study noted losses in pyruvic acid acetyl groups at low pH. Subsequently, they believe these losses to be the cause of the observed partial reductions in viscosity at lower shear rates (Garcia-Ochoa et al., 2000). In conjunction, other researchers studying the effects of low pH on xanthan gum rheology have also reported decreases in viscosity in acidic environments at low shear rates, thereby creating a more flexible chain due to the suppression of electrostatic repulsions between xanthan side chains (Sworn, 2011). This may help explain why in the present study at higher concentrations pH has a negative effect on viscosity. Despite slight sensitivities to low pH, researchers reported that the acid stability with time is excellent, allowing for its supplementation in foods with low pH, as mentioned earlier (Sworn, 2011). Furthermore, this stability is highlighted in the present study through the ability of xanthan gum to retain viscosity to a much greater degree in comparison to the other polysaccharides. In a study done looking at the alkali effect (pH 6.5) on solution rheology of gum Arabic, flaxseed gum, guar gum, locust bean gum and xanthan gum, researchers reported that the apparent viscosity at 50 and 100 s^{-1} of xanthan gum remained significantly higher than the rest (Mazza & Biliaderis, 1989). The negligible influence of hydrolytic enzymes may be explained by the resilient structure of xanthan gum, as described in Section 2.7.4. The side chains wrap around the β- 1, 4 backbone, thereby providing a barrier to enzyme access and preventing depolmyerisation. This helical conformation, which was also discussed in Section 4.2, may explain why xanthan gum is able to retain more of its

viscous character in comparison to the other hydrocolloids after exposure to all 3 treatments. Furthermore, enzyme resistance of xanthan gum is apparent in many food systems such as pineapple products and spice mixes where xanthan gum is added as an emulsifying agent (Sworn & Monsanto, 2000). It is also noticeable in starch-based systems, the effects of which will be proposed for further research in Chapter 5.

4.3.5. Effect of dilution, pH, and *in vitro* digestion on the viscosity of gum Arabic

The effect that the 3 treatments had on viscosity of LV, MV, and HV gum Arabic solutions are shown in Figures 4-19, 4-20 and 4-21, respectively.

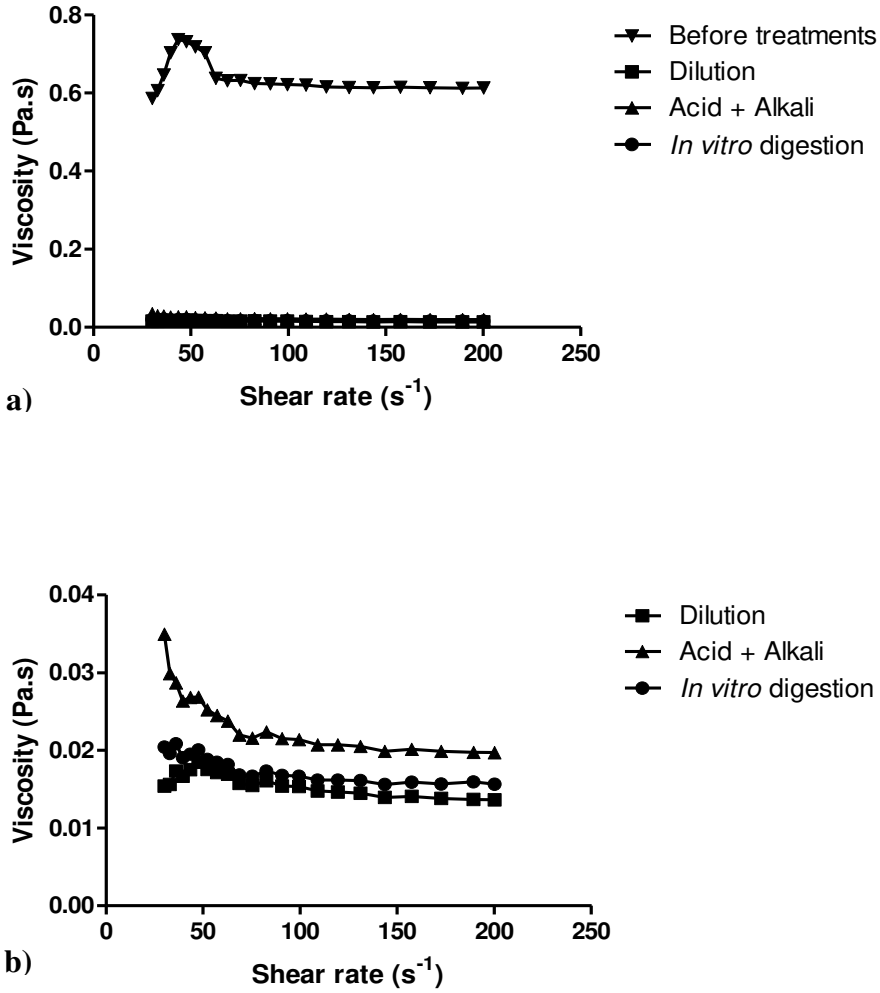


Figure 4-19. Comparison of the viscosity profiles of LV gum Arabic solutions after exposure to simulated digestion, dilution and pH, a) with pre-treatment rheology and b) amongst treatments. Data reported as mean measurements.

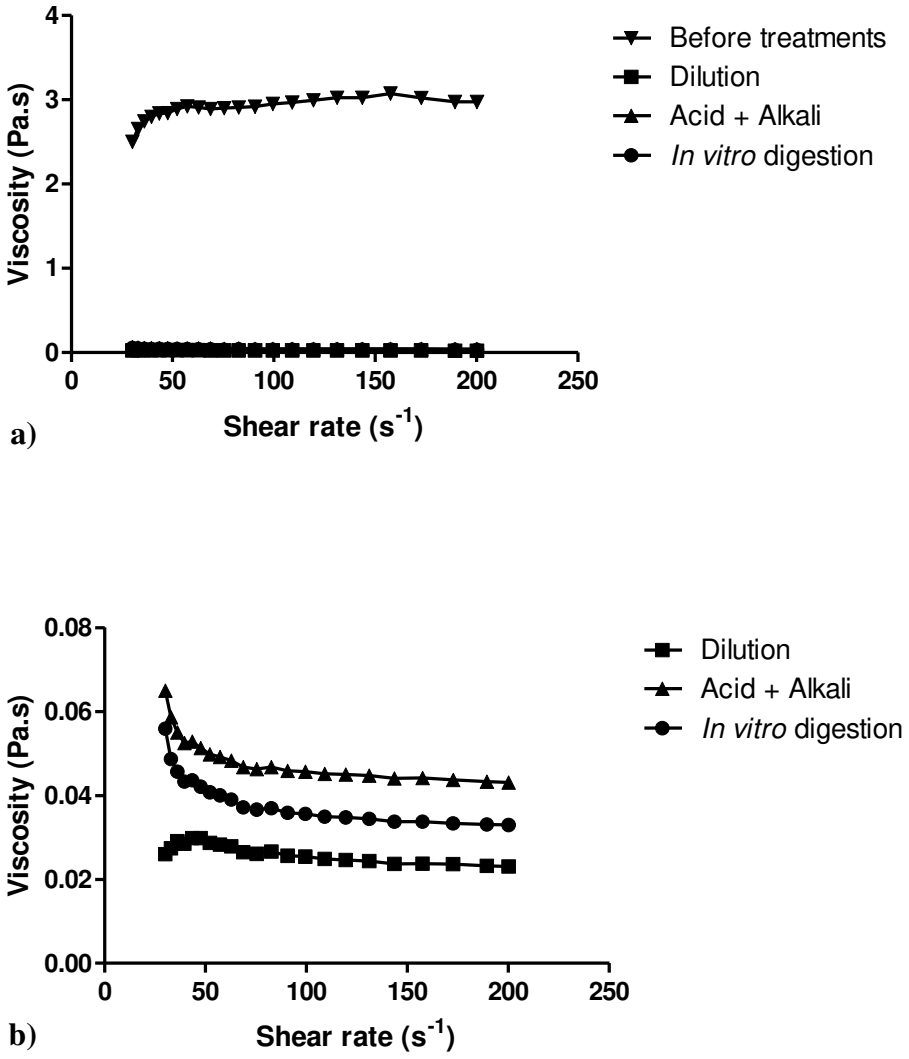


Figure 4-20. Comparison of the viscosity profiles of MV gum Arabic solutions after exposure to simulated digestion, dilution and pH, a) with pre-treatment rheology and b) amongst treatments. Data reported as mean measurements.

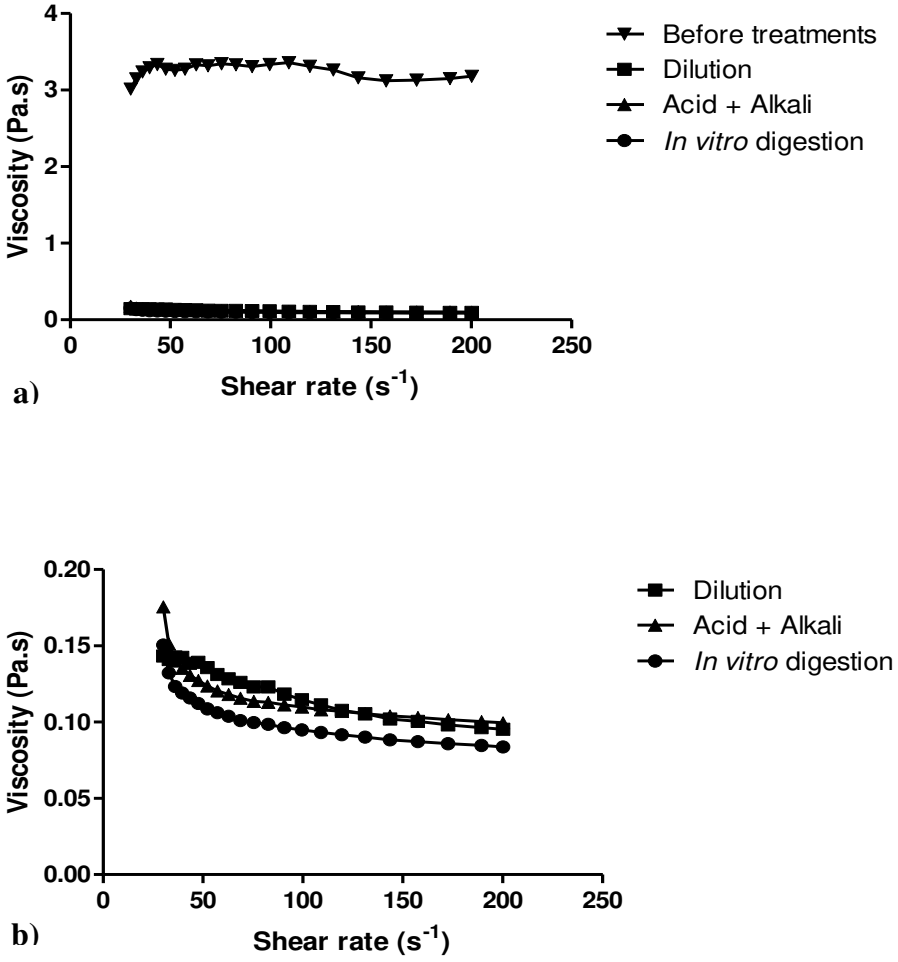


Figure 4-21. Comparison of the viscosity profiles of HV gum Arabic solutions after exposure to simulated digestion, dilution and pH, a) with pre-treatment rheology and b) amongst treatments. Data reported as mean measurements.

After exposing the 3 gum Arabic solutions to the prescribed treatments, a substantial loss in viscosity is obtained, as shown in the above Figures. *In vitro* digestion, dilution, and the presence of acid and alkali all seem to differ in their effects on solution viscosity shown in Figures 4-19 to 4-21. Moreover, the ability to retain viscosity is diminished and this is shown through the analysis of the complex behaviour of gum Arabic, whereby

viscosity data after the 3 treatments were taken and compared to that of the initial viscosity, as shown in Table 4-9.

Table 4-9. Viscosity values calculated as a % of the initial viscosity after LV, MV, and HV gum Arabic solutions were subjected to simulated digestion, dilution and pH extremes (acid and alkali). Values were calculated at 100 s^{-1}.

Treatment	[1]Remaining viscosity (%)		
	LV	MV	HV
Dilution	2.5^{a}	0.86^{a}	3.4^{a}
Acid + Alkali	3.4^{b}	1.5^{b}	3.3^{a}
In vitro digestion	2.7^{a}	$1.2^{a,b}$	2.8^{a}

[1]Data reported as mean values.
Different superscript letters (a,b) within a column indicate significant difference between means ($p<0.05$).

Table 4-9 demonstrates the substantial effect that dilutions have on the viscosity losses of gum Arabic. Dilutions that were present in all the solutions led to the greatest reduction in viscosity when compared to the effects of the other 2 treatments ($p<0.05$). Moreover, the differences between in the *in vitro* digestion model and the presence of acid and alkali in the system also differed ($p<0.05$), as the former, which introduced enzymes and bile into the system, had a greater impact on lowering solution viscosity in comparison to pH changes in their absence.. For the HV group, *in vitro* digestion seemed to add to the effect of simple dilutions as the viscosity profile is slightly more depressed, as shown in Figure 4-21 b and through the values presented in Table 4-9, although no statistically significant difference was reported. In comparison to the previous hydrocolloids, gum Arabic appears to be the least resilient, where greater than 95% of viscosity losses were shown.

Another important finding is that gum Arabic, which previously appeared to be a Newtonian fluid in Section 4.1, is now showing non-Newtonian pseudoplastic behaviour whereby the viscosity is decreasing with increasing shear rate (data not shown). This is consistent with previous reports that demonstrated a greater dependence on shear rate at lower concentrations (Li et al., 2009).

Here the effect of *in vitro* digestion may be associated with a fraction of gum Arabic that is said to contain >60% proteinaceous material (Randall & Williams, 1988). Gum Arabic is often described using the 'wattle blossom model' and this suggestion arises from the fact that the highest molecular weight fraction contains the majority of the protein, which acts as the backbone that forms the AGP complex (Thevenet, 2010), as discussed in Section 2.7.8. Another study mentions how gum Arabic is said to contain various polysaccharide-protein complexes (McClements, 2004) and protein moieties (Paeschke & Aimutis, 2011; Pasquier et al, 1996). Although there are not many studies done evaluating the effect(s) of *in vitro* or *in vivo* digestion on gum Arabic rheology, researchers have demonstrated that the high molecular weight fraction consisting of the large amount of protein was able to be enzymically degraded (Randall & Williams, 1988) irrevocably leading to a loss in viscosity. Moreover, a study demonstrated a decrease in the apparent viscosity of gum Arabic at low pH, across a shear rate range from 0 to 100 s^{-1}, although this was not shown here. Furthermore, the viscosity profile in that study was much lower in comparison to guar gum (Pasquier et al., 1996) and this is consistent with the findings shown here through the significant viscosity losses of gum Arabic solutions.

4.3.6. Effect of dilution, pH, and *in vitro* digestion on the viscosity of psyllium

The viscosity profiles of the 3 psyllium solutions following the different treatments are plotted in Figures 4-22, 4-23 and 4-24.

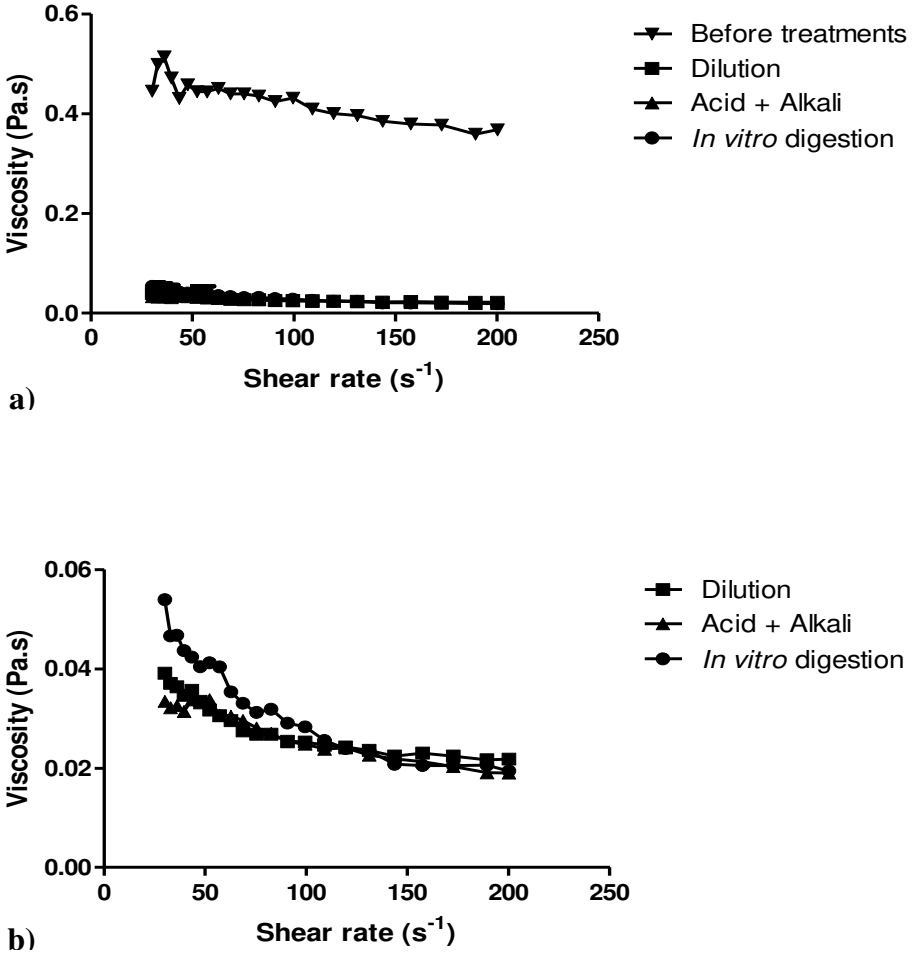


Figure 4-22. Comparison of the viscosity profiles of LV psyllium solutions after exposure to simulated digestion, dilution and pH, a) with pre-treatment rheology and b) amongst treatments. Data reported as mean measurements.

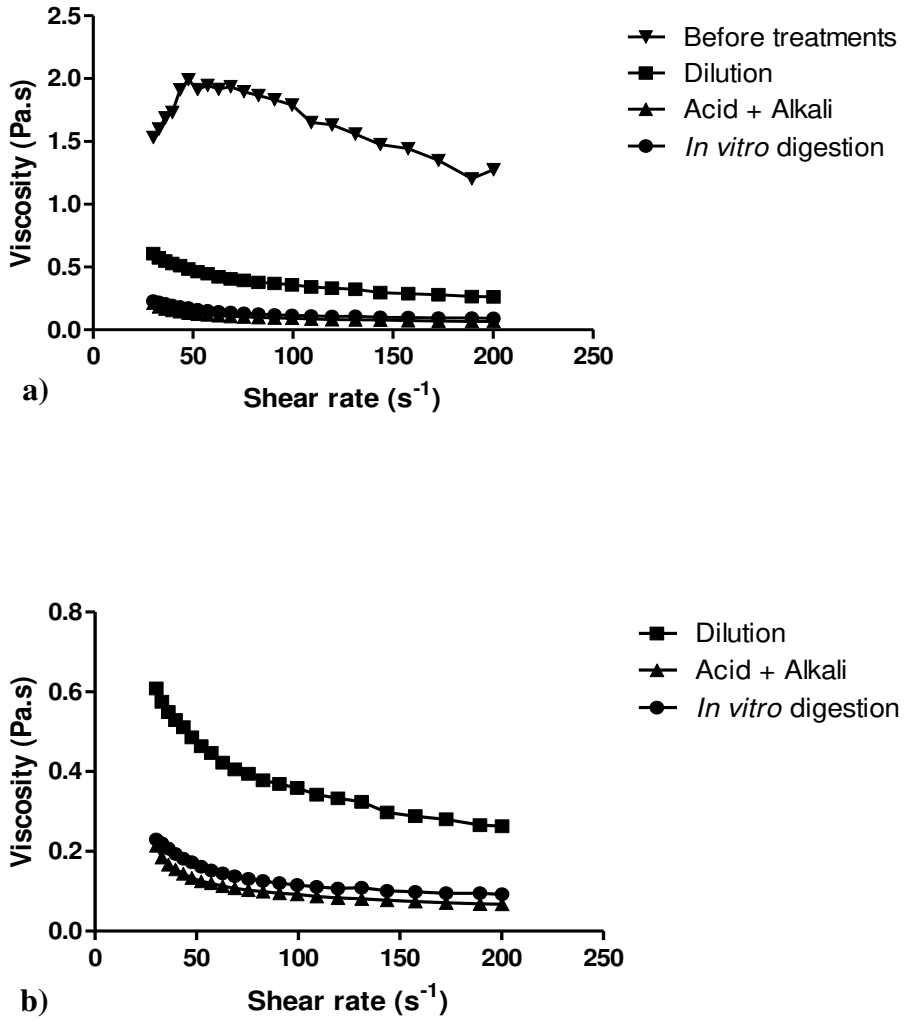


Figure 4-23. Comparison of the viscosity profiles of MV psyllium solutions after exposure to simulated digestion, dilution and pH, a) with pre-treatment rheology and b) amongst treatments. Data reported as mean measurements.

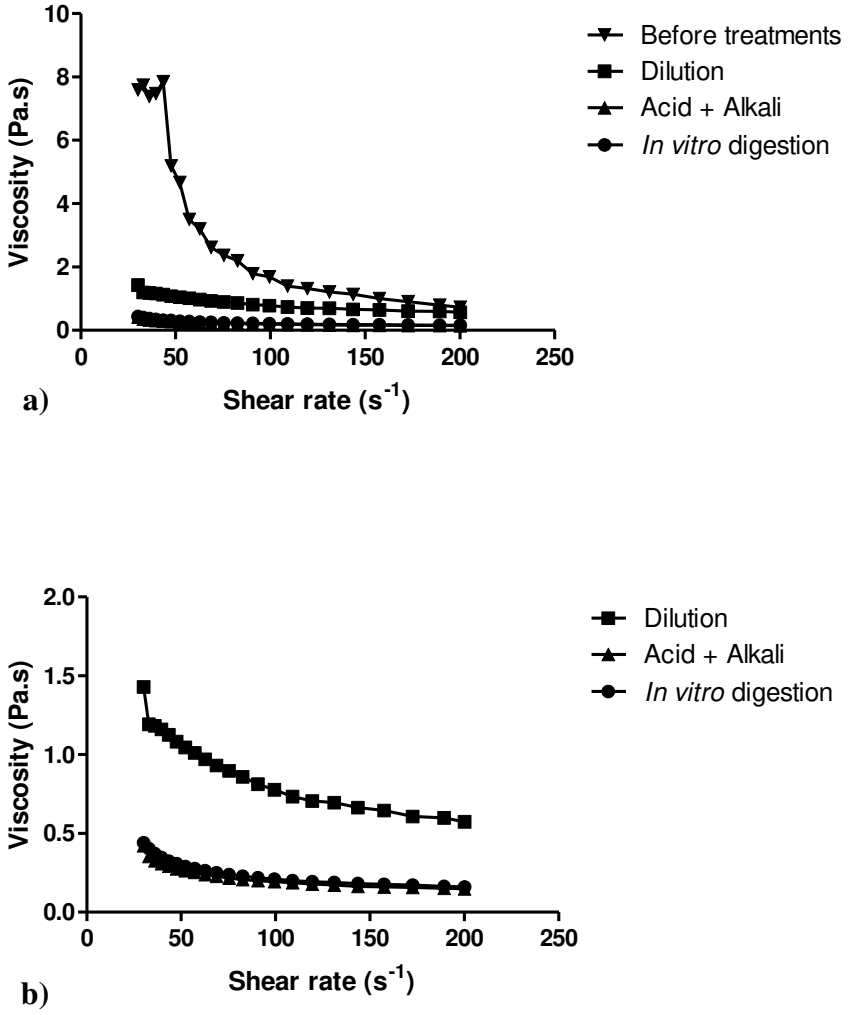


Figure 4-24. Comparison of the viscosity profiles of HV psyllium solutions after exposure to simulated digestion, dilution and pH, a) with pre-treatment rheology and b) amongst treatments. Data reported as mean measurements.

Following each of the 3 treatments, the viscosity of psyllium fibre was strongly reduced, as shown in Figures 4-22 to 4-24. Therefore, a dilution effect is responsible for the reductions in viscosity at all concentration levels. However, for the MV and HV groups pH seemed to play a vital role as the viscosity curve was further depressed, as shown in

Figures 4-23 and 4-24. Calculations of the % viscosity remaining are presented in Table 4-10.

Table 4-10. Viscosity values calculated as a % of the initial viscosity after LV, MV, and HV psyllium solutions were subjected to simulated digestion, dilution and pH extremes (acid and alkali). Values were calculated at 100 s^{-1}.

Treatment	[1]Remaining viscosity (%)		
	LV	MV	HV
Dilution	5.9[a]	20[b]	46.1[b]
Acid + Alkali	5.8[a]	5.2[a]	11.5[a]
In vitro digestion	6.6[a]	6.5[a]	12.5[a]

[1]Data reported as mean values.
Different superscript letters (a,b) within a column indicate significant difference between means ($p<0.05$).

Table 4-10 demonstrates the sizeable difference between the effect of dilution and pH on the viscosity remaining after the conditions were applied to the MV and HV groups ($p<0.05$). There are no differences between *in vitro* digestion and simulated pH ($p>0.05$). As with the other hydrocolloids studied, the presence of water seems to affect the network strength and subsequent solution viscosity. Due to the fact that all 3 simulated conditions expose the hydrocolloid solutions to equal volumes of secretion, as outlined in Section 3.3.2, the high susceptibility to physical breakdown as water is being introduced in the system is clear, as shown through the depression of the viscosity curves illustrated in Figures 4-22 to 4-24. However, the unique conformation of psyllium allows for an additive effect to be attributed to viscosity reduction by changes in pH, which are present throughout digestion. As discussed in Section 4.2, psyllium fibre has been shown to

undergo depolymerisation upon increasing the alkalinity of the solution. Subsequently, the researchers of this study discovered fewer junction zones as a result of a decrease in intermolecular interactions, leading to a reduction in solution viscosity and gel elasticity at both low and high pH (Farahnkay et al., 2010). Moreover, scanning electron micrographs of psyllium, at variable pH, showed changes in polymer surface morphology, allowing for a porous structure with a small pore-size distribution at higher pH in comparison to what is found at lower pH values. They explain this phenomenon by looking at electrostatic repulsions between chains, which are induced by the ionization of carboxyl groups at high pH. They propose this may also be affecting solution rheology. In their results and conclusions, the researchers indicated that the maximum functional properties for psyllium were reported to be at a pH of 4 and 7 (Farahnaky et al, 2010), inadvertently showing that extreme acidic and alkaline conditions affect structural, rheological and functional parameters.

4.3.7. Effect of dilution, pH, and *in vitro* digestion on the viscosity of flaxseed gum

The changes in viscosity of the LV, MV and HV flaxseed gums after in vitro digestion, dilution and pH changes were applied are shown in Figures 4-25, 4-26 and 4-27.

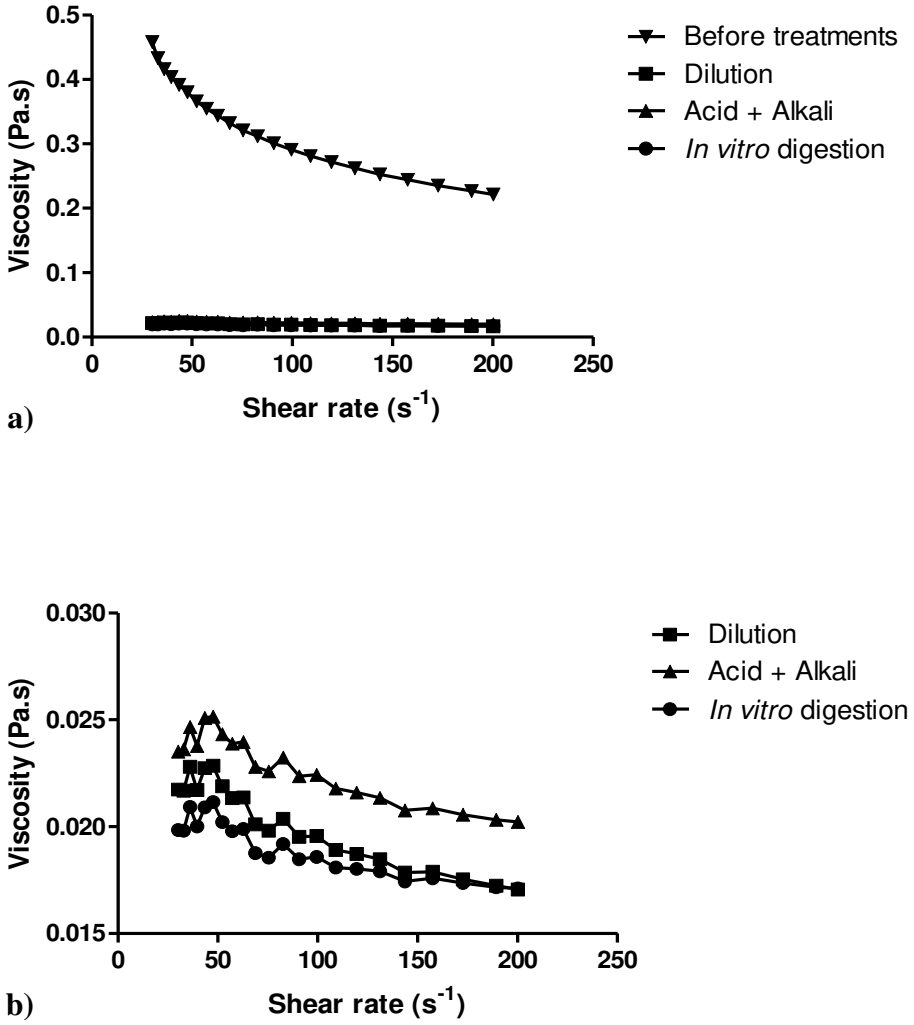


Figure 4-25. Comparison of the viscosity profiles of LV flaxseed solutions after exposure to simulated digestion, dilution and pH, a) with pre-treatment rheology and b) amongst treatments. Data reported as mean measurements.

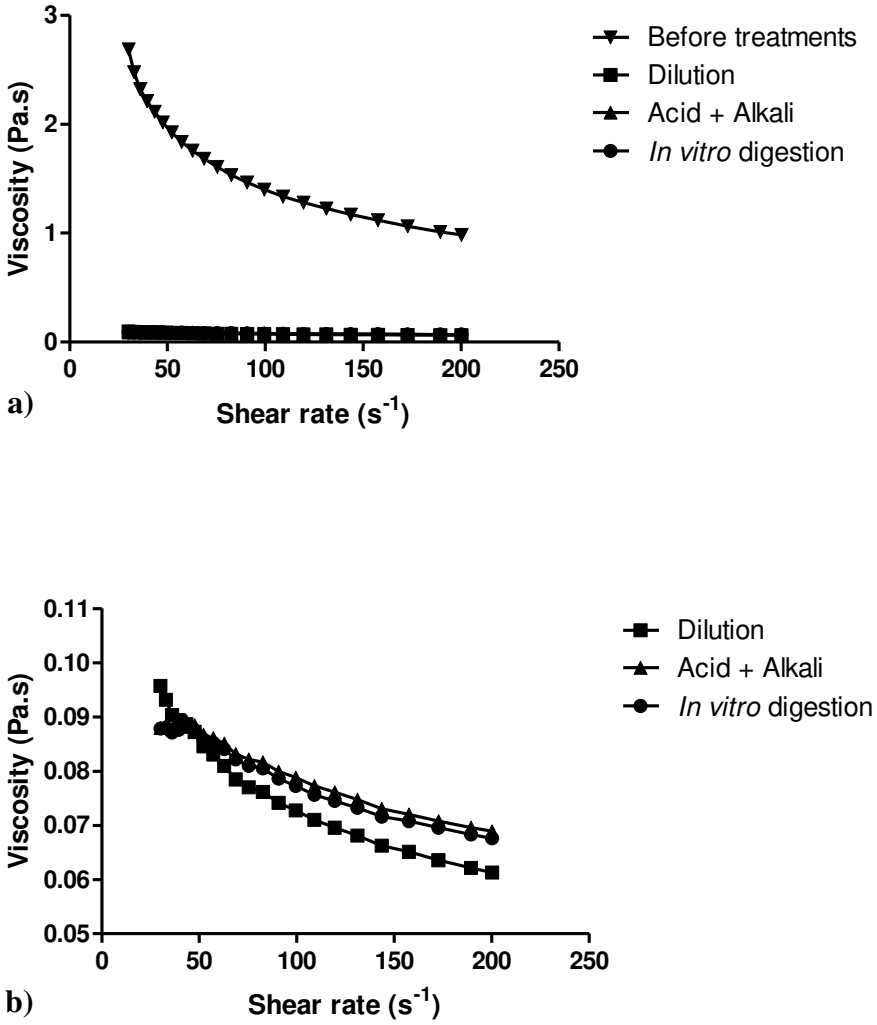


Figure 4-26. Comparison of the viscosity profiles of MV flaxseed solutions after exposure to simulated digestion, dilution and pH, a) with pre-treatment rheology and b) amongst treatments. Data reported as mean measurements.

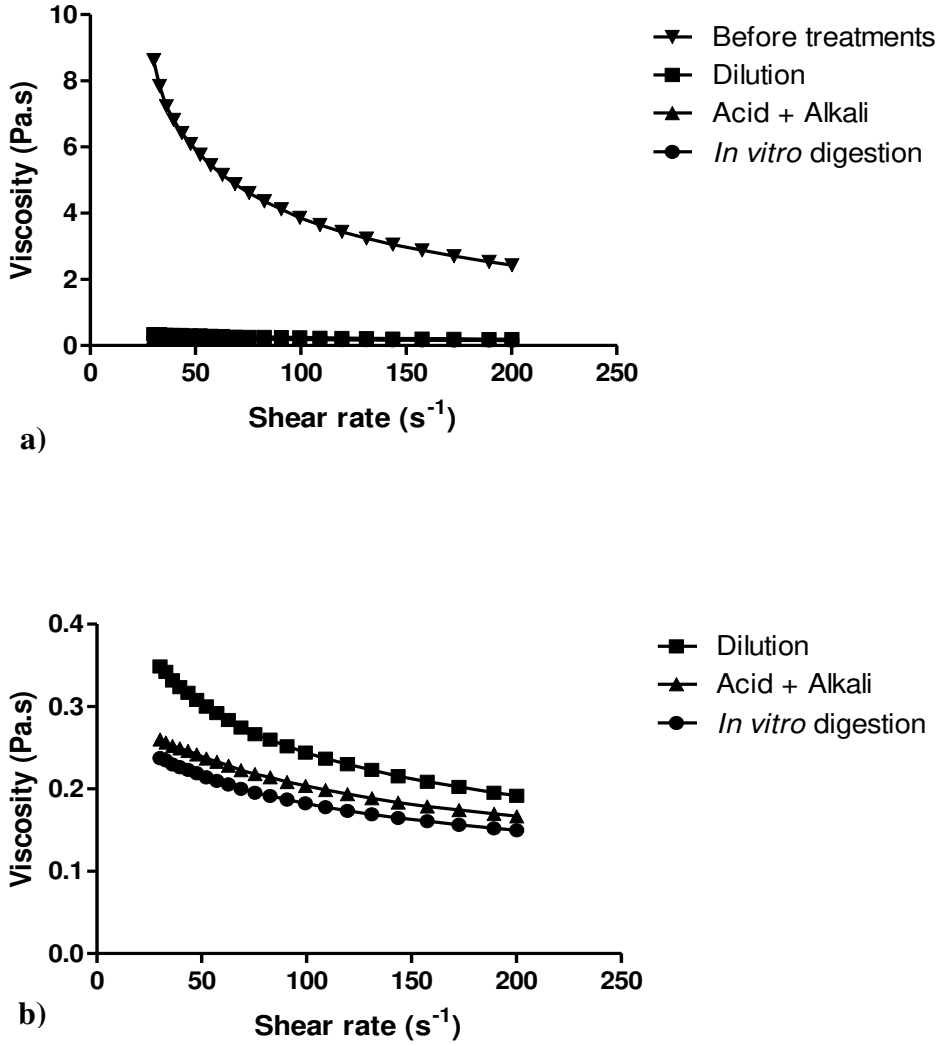


Figure 4-27. Comparison of the viscosity profiles of HV flaxseed solutions after exposure to simulated digestion, dilution and pH, a) with pre-treatment rheology and b) amongst treatments. Data reported as mean measurements.

Figures 4-25 to 4-27 illustrate the significant drop in flaxseed gum viscosity following the 3 treatments. It is evident that a dilution effect creates significant drop in viscosity as there is no difference between the dilution group and the groups exposed to acid and alkali in the presence or absence of digestive enzymes. Researchers have reported the

strong affinity that flaxseed mucilage has for water. They indicated that through continuous hydration, changes in intermolecular binding occur, which influences both polysaccharide-polysaccharide interactions as well as polysaccharide-water binding. Subsequently, this lead to a reduction in apparent viscosity when they studied the rheological properties of flax samples at low shear rates (Wu et al., 2010). In the present study, as the concentration of flaxseed gum increased, so did the effect that pH exerts on solution rheology, as shown in Figure 4-27. A numerical comparison of the effects of each treatment is presented in Table 4-11.

Table 4-11. Viscosity values calculated as a % of the initial viscosity after LV, MV, and HV flaxseed gum solutions were subjected to simulated digestion, dilution and pH extremes (acid and alkali). Values were calculated at 100 s^{-1}.

Treatment	[1]Remaining viscosity (%)		
	LV	MV	HV
Dilution	6.7[a]	5.2[a]	6.3[a]
Acid + Alkali	7.7[a]	5.6[a]	5.3[a]
In vitro digestion	6.4[a]	5.5[a]	4.7[a]

[1]Data reported as mean values.
The same superscript letters within a column indicate no significant difference between means ($p>0.05$).

For the HV groups, *in vitro* digestion and pH simulation led to further depression of the viscosity profile of flaxseed gum following the 4 hr treatments ($p<0.05$), although this effect was negated at shear rates > 75 s^{-1}. Exposing flaxseed gum to acid and alkali environments evidently leads to structural degradation and loss in viscosity at lower shear rates. This is consistent with another study, which showed that changes in pH created

considerable reductions in the viscosity of flaxseed mucilage solutions. The researchers of this study indicated that a pH of 2.0 led to greater than 50% reductions in apparent viscosity at 100 s^{-1}. They discussed that minimal intermolecular interactions are occurring in acidic and alkali environments, thereby reducing the structural features responsible for the viscous properties of flaxseed gum (Mazza & Biliaderis, 1989). Although previous studies have shown that the viscosity of flaxseed mucilage decreases with increasing hydrolyzing time and enzyme loading amount (Wu et al., 2010), this was not seen here as there was no significant difference between the effects of pH in the absence and in the presence of enzymes/bile ($p>0.05$). The researchers of the study included two commercially available forms of pectinase enzymes to hydrolyze the material, which may help explain the dissimilarity in the findings.

4.3.8. Effect of dilution, pH, and *in vitro* digestion on the viscosity of SSPS

The treated viscosity profiles of the LV, MV and HV SSPS solutions are shown in Figures 4-28, 4-29 and 4-30.

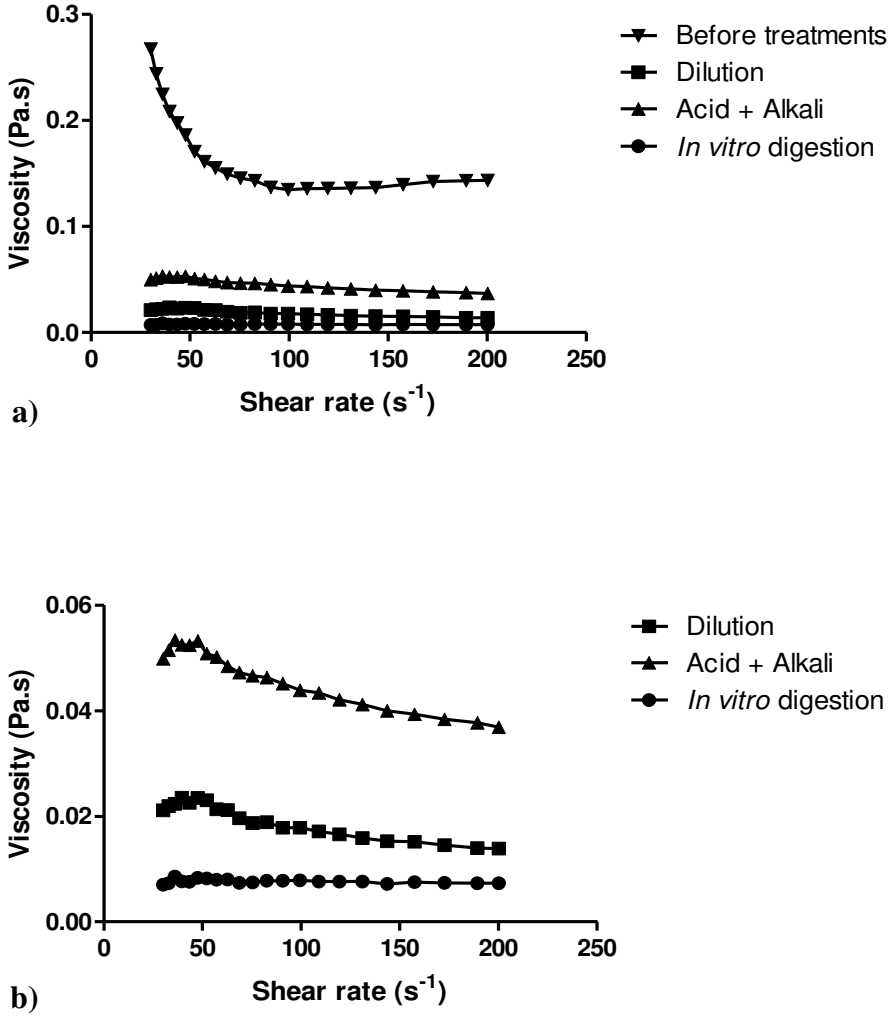


Figure 4-28. Comparison of the viscosity profiles of LV SSPS solutions after exposure to simulated digestion, dilution and pH, a) with pre-treatment rheology and b) amongst treatments. Data reported as mean measurements.

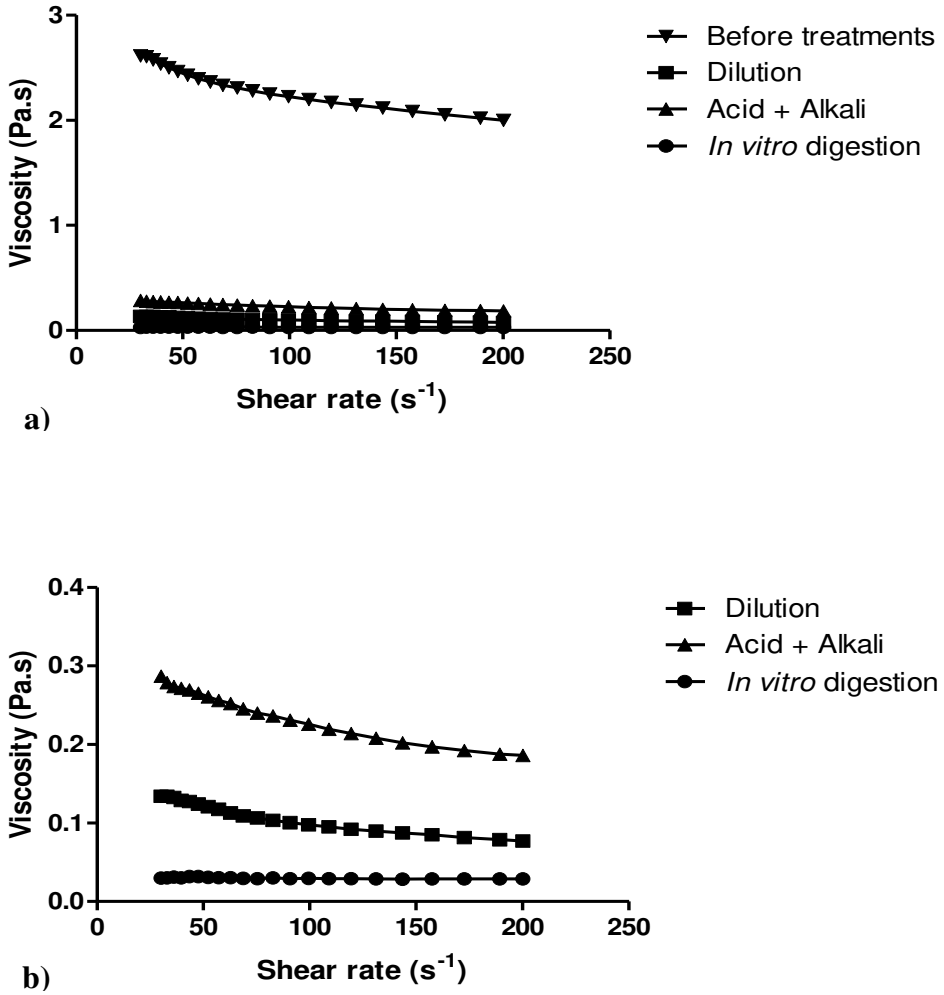


Figure 4-29. Comparison of the viscosity profiles of MV SSPS solutions after exposure to simulated digestion, dilution and pH, a) with pre-treatment rheology and b) amongst treatments. Data reported as mean measurements.

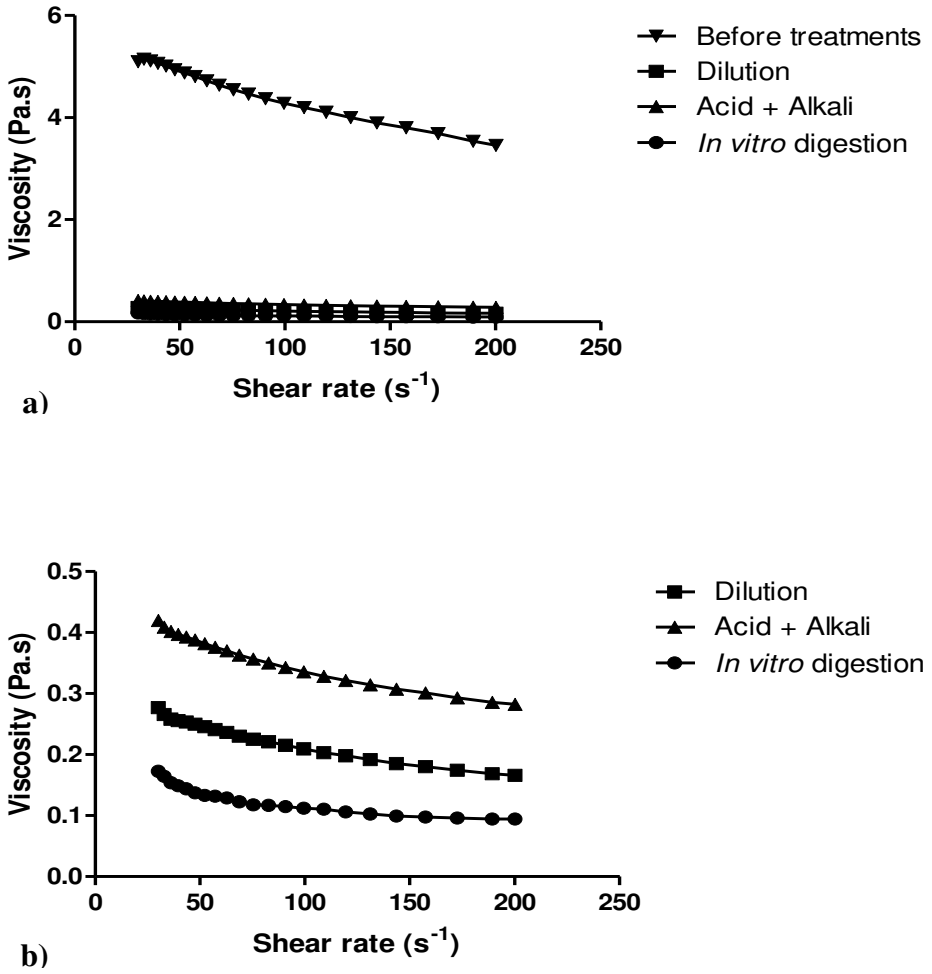


Figure 4-30. Comparison of the viscosity profiles of HV SSPS solutions after exposure to simulated digestion, dilution and pH, a) with pre-treatment rheology and b) amongst treatments. Data reported as mean measurements.

The drop in viscosity of SSPS after applying the 3 treatments is apparent at all concentration levels, as shown in Figures 4-28 to 4-30. The majority of the reduction is attributed by dilutions affecting the structural integrity of the samples, as the affinity for water leads to continuous hydration thereby changing intermolecular binding, which is consistent with what is shown in the previous hydrocolloids. However, the application of the *in vitro* digestion model to SSPS solutions seems to be a key contributor to further

depressing the viscosity curves of the 3 solutions. The extent of viscosity reduction is shown in Table 4-12.

Table 4-12. Viscosity values calculated as a % of the initial viscosity after LV, MV, and HV SSPS solutions were subjected to simulated digestion, dilution and pH extremes (acid and alkali). Values were calculated at 100 s^{-1}.

Treatment	[1]Remaining viscosity (%)		
	LV	MV	HV
Dilution	13.3[b]	4.4[b]	4.9[a]
Acid + Alkali	32.6[c]	10.1[c]	7.8[a]
In vitro digestion	5.8[a]	1.3[a]	2.6[a]

[1]Data reported as mean values.
Different superscript letters (a,b,c) within a column indicate significant difference between means ($p<0.05$).

After 4 hr, the effects of *in vitro* digestion led to further viscosity reductions in comparison to the other 2 treatments ($p<0.05$), as shown in Table 4-12. Interestingly, viscosity seems to be unaffected by the presence of acid and alkali in the system. In fact, the viscosity is slightly enhanced when the pH changes are induced in the absence of digestive enzymes, as shown in Table 4-12. This increase in viscosity was reported in another study that examined the potential use of soy residue in high-calorie emulsions following simulated peptic and tryptic digestion. The researchers of this study reported that acidification (pH 2.0) of the soy-containing emulsions led to a formation of large aggregations and entrapment of oil droplets, consequently the viscosity of these emulsions increased. However, as the digestion proceeded to include the effect of trypsin at a neutral pH (7.0), the viscosity decreased. Nonetheless, after the 2-step process the

soy-emulsion managed to retain more of its viscosity in comparison to the emulsion prepared in its absence (Nakornpanom, 2010). Although this study did not work on isolated SSPS, it still highlights the ability for soy polysaccharides, which are present in the residue, to resist pH changes during digestion. Other studies have also reported on the stability of SSPS to alterations in pH (Maeda, 2000). This may help explain why it is commonly used as a stabilizer in acidified milk beverages (Nakamura et al., 2003). Furthermore, SSPS has been shown to stabilize oil-in-water emulsions in both acid and alkali conditions (Nakamura et al., 2004). This stabilisation is achieved by the protein fraction that allows the carbohydrate to anchor itself to the oil/water interface (Nakamura et al., 2004).

In the present study, it is shown that the presence of digestive enzymes and bile add to the degrading effect of dilutions by further lowering the viscosity of SSPS, as shown in Table 4-12. Correspondingly, studies have been conducted where researchers were able to digest the protein fraction and branched polysaccharide portions of SSPS by using a variety of different enzymes in order to assess the emulsifying activity of SSPS. Although this study used selective hydrolysis to allow measurement, it nevertheless describes the ability of enzymes to degrade SSPS in solution (Nakamura et al., 2006). Another study was done that developed an *in vitro* digestion model containing pepsin at a pH of 2.0 and trypsin at a pH of 7.0 in the presence of bile salts, creating conditions that were present in the current study, as outlined in Section 3.3. Although the study did not have a rheological approach, it nevertheless highlights the ability of enzymes to digest fractions of SSPS. The results indicated that 15 min pepsin digestion, and 60 min trypsin digestion in the presence of bile led to considerable reductions in emulsion stability,

affecting both the oil phase and the sediment phase heights (Fafaungwithayakul, 2011). A third study was done that measured the viscosity of oil-in-water emulsions containing soy residue. Despite the fact that the addition of soy increased the viscosity of the reconstituted emulsions, the results indicated that as simulated digestion proceeded, hydrolysis led to a substantial reduction in viscosity of the soy-containing digesta (Nakornpanom, 2010).

Through literature reports and the results of the present study it is apparent that the conditions in the gastrointestinal tract have an immense effect on the physicochemical structure of SSPS, or any hydrocolloid acting as a source of dietary fibre, leading to considerable reductions in viscosity. Ultimately, this creates heterogeneity in their ability to retain viscosity, which should alter the capability of these substrates to promote technological functionality, such as stability, thickening and viscosity, in food systems and physiological functionality *in vivo*. Further research on this topic will be proposed in Chapter 5.

4.4. Study strengths and limitations

There are a number of strengths associated with this work. This includes the fact that changes in viscosity were studied after *in vitro* digestion. Often the physiological response induced by dietary fibre consumption, such as reduced postprandial glycemia, is explained by the authors as a result of a viscous effect. A test food is supplemented with a viscous dietary fibre and the subsequent physiological response is monitored, concluding that the addition of dietary fibre to a diet leads to health benefits attributed to the viscosity therein. No reports are made that attempt to explain why some sources that contain high viscosity profiles are sometimes less functional than those that possess slightly weaker viscosities at the same concentration. More importantly the exact mechanism by which the viscosity effect is being promoted in the body is yet to be ascertained. As discussed in Chapter 2, viscosity can impart a range of responses in the GI tract during digestion. Moreover, these studies do not take into account the structural changes that are caused by the digestive process, which indisputably leads to reductions in viscosity, as demonstrated by the results in this study. Also, the magnitude of these reductions varies depending on the unique structure of each dietary fibre. This leads to the second strength that is the inclusion of a range of hydrocolloids, each possessing unique physicochemical characteristics. This allowed for a closer determination of how these properties influence viscosity, both in solution and after *in vitro* digestion, bringing the research one step closer to determining how structure and function are related. However, incorporating a complete food matrix throughout the investigations would elicit a more realistic view of how dietary fibre acts in the GI tract, as it is normally consumed as part of a healthy well-balanced diet.

A third strength of the study is that it took into account different viscosity profiles of each of the hydrocolloids. Instead of using the same concentration, by deliberately matching the apparent viscosities of the solutions at 50 to 100 s^{-1}, changes in their flow behaviour after exposure to the treatments could not be attributed to differences in viscosity prior to the 3 simulations. Moreover, assuming viscosity is the important factor, this could provide insight as to which dietary fibres are more biologically functional by observing their flow behaviour, which is comparable due to their equal viscosities before applying any simulated condition(s). However, further research is warranted to evaluate the potential of viscous dietary fibres to act as functional ingredients in foods delivering health benefits.

Furthermore, this study conducted a thorough characterization of the study treatment rheology across a broad range of shear rates, which is not the case in some other studies (Dikeman & Fahey, 2006). Viscosity is dependent on shear rate and therefore there is variability when observing viscosities at different shear rates. This can also lead to justified viscosity comparisons among studies that include identical shear rates.

Some limitations of the study are associated with the difficulty of mimicking human digestive conditions using an *in vitro* model. Namely, the motility patterns that are present in the stomach and small intestine, which are important for digestion, are difficult to simulate in a shaking waterbath. Moreover, the secretions that are produced during digestion are done so in a progressive manor. In our setup, additions of both gastric and small intestinal fluids were incorporated in a single event. Other factors such as the presence of hormones and incorporation of the cephalic phase of digestion (Johnston,

1991) are difficult to mimic using an *in vitro* model and were therefore excluded from our setup.

5. Conclusion and future directions

Locust bean gum, guar gum, fenugreek gum, xanthan gum, gum Arabic, psyllium, flaxseed gum and SSPS all showed significant viscosity reductions when exposed to the conditions of dilutions, acid and alkali, and the *in vitro* digestion model. For the most part, there was no difference between the 3 treatments therefore highlighting that a dilution effect is the cause of lowering fibre viscosity, with minimal to no effect being attributed to pH changes or the action of digestive enzymes. Depending on the hydrocolloid studied, the severity of the observed effects on solution viscosity varied.

The effect that *in vitro* digestion had on solution viscosity was more pronounced in hydrocolloids that contained a greater protein fraction. Gum Arabic and SSPS both showed greater reductions in viscosity after subjecting them to acid and alkali in the presence of hydrolytic enzymes and bile. As discussed in Section 2.5.1, Woolnough et al. (2008) discussed how the action of pepsin in their study was only noticeable when applying it to pastas, as they are known to contain proteinaceous material. Subsequently, the amount of sugar that was released from this group was greater than the pasta group that was not exposed to pepsin. Consequently, this could imply that providing a more complete food matrix through the addition of protein, fat, or starch, and applying it to the *in vitro* digestion model could lead to different responses in the rheological properties of

dietary fibre in comparison to the isolated hydrocolloid solutions in the absence of any other nutrients.

Upon comparison of all the substrates studied, xanthan gum demonstrated the greatest ability to resist structural changes after simulating the 3 conditions, as it was able to maintain viscosity more so than all others. Non-linear regression analysis confirmed the findings as the consistency index (K) of xanthan gum at all 3 concentration levels was considerably higher than the rest. Guar gum also managed to preserve measurable viscosity after applying the 3 conditions. The reduction in viscosity of gum Arabic, flaxseed gum, and SSPS, were substantial. Consequently, this could imply that xanthan gum and some galactomannans may be more biologically functional in terms of modulating certain physiological responses, such as postprandial glycemia or cholesterol reductions, in comparison to the low-viscosity fibres that showed considerable losses in structural integrity.

The present study demonstrated that viscosity is greatly affected by the conditions present in the GI tract, generating extensive reductions in all hydrocolloids studied. Moreover, the extent of these losses is influenced by the structural properties possessed by each of the fibres. As an extension, it could be assumed that the viscosity profile that is prevalent in the small intestine is the one that should be used to characterize a structure-function relationship of any soluble dietary fibre. This leads to the proposal for future research. Having characterized the flow behaviour of different hydrocolloids both before and after *in vitro* digestion, it would be essential to tie the findings in with a physiological response, such as glucose mobility as measured by adding starch.

With the incorporation of starch, a salivary stage is necessary in order to correctly mimic the digestive steps *in vivo*. Briefly, by incorporating controlled enzymic hydrolysis in a 3-step *in vitro* digestion protocol, and a glucose oxidase kit, which has been used in previous studies (Englyst et al, 1992; Regand et al., 2011) it is possible to evaluate the rate of starch digestion and subsequent glucose absorption. Moreover, through the addition of different dietary fibres that were used in the present study it should be possible to determine the effect, if any, that the more resilient fibres, showing greater viscosity after simulated digestion, have on attenuating glucose mobility. In addition to rheology, other analytical techniques such as scanning electron microscopy and fluorescence recovery after photobleaching (FRAP) (Perry et al., 2006) could allow for further evaluation, not only on the structural changes that are occurring in the samples but more importantly the effect that these changes have on the rate of diffusion of nutrients.

References

Ali, B. H., Ziada, A., & Blunden, G. (2009). Biological effects of gum arabic: A review of some recent research. *Food and Chemical Toxicology, 47*(1), 1-8.

Allen, A., & Flemström, G. (2005). Gastroduodenal mucus bicarbonate barrier: Protection against acid and pepsin. *American Journal of Physiology - Cell Physiology, 288*(1), C1-C19.

American Dietetic Association. Position of the American Dietetic Association: Health Implications of Dietary Fiber. *Journal of the American Dietetic Association*. 2008; 108:1716-1731.

Benini, L., Castellani, G., Brighenti, F., Heaton, K. W., Brentegani, M. T., Casiraghi, M. C., et al. (1995). Gastric emptying of a solid meal is accelerated by the removal of dietary fibre naturally present in food. *Gut, 36*(6), 825-830.

Blackwood, A. D., Salter, J., Dettmar, P. W., & Chaplin, M. F. (2000). Dietary fibre, physicochemical properties and their relationship to health. *The Journal of the Royal Society for the Promotion of Health, 120*(4), 242-247.

Borwankar RP. Food Texture and Rheology: A Tutorial Review. *Journal of Food Engineering*. 1992; 16:1-6.

Brennan, C. S., & Tudorica, C. M. (2008). Evaluation of potential mechanisms by which dietary fibre additions reduce the predicted glycaemic index of fresh pastas. *International Journal of Food Science & Technology, 43*(12), 2151-2162.

Brighenti, F., Pellegrini, N., Casiraghi, M. C., & Testolin, G. (1995). In vitro studies to predict physiological effects of dietary fibre. *European Journal of Clinical Nutrition, 49 Suppl 3*, S81-8.

Brownlee, I.A. (2011). The physiological roles of dietary fibre. *Food Hydrocolloids, 25*(2), 238-250.

Canadian Diabetes Association (CDA). The prevalence and costs of diabetes. Clinical Practice Guidelines. 2009.

Chang EB, Sitrin MD & Dennis BD. Gastrointestinal, Hepatobiliary, and Nutritional Physiology. PE: Pennsylvania: Lippincott-Raven Publishers. 1996

Charalambous G, & Doxastakis G. Food Emulsifiers: Chemistry, Technology, Functional Properties and Applications. The Netherlands, Amsterdam: Elsevier Science Publishers. 1989

Chawla, R., & Patil, G. R. (2010). Soluble dietary fiber. *Comprehensive Reviews in Food Science and Food Safety, 9*(2), 178-196.

Codex Alimentarius Commission, ALINORM 10/33/26, Report of the 31st Session of the Codex Committee on Nutrition and Foods for Special Dietary Uses, Düsseldorf, Germany, 2 - 6 November 2009 [ftp://ftp.fao.org/codex/Alinorm10/al33_26e.pdf]

Cui, W., & Mazza, G. (1996). Physicochemical characteristics of flaxseed gum. *Food Research International, 29*(3-4), 397-402.

Cui, W., Mazza, G., & Biliaderis, C. G. (1994). Chemical structure, molecular size distributions, and rheological properties of flaxseed gum. *Journal of Agricultural and Food Chemistry, 42*(9), 1891-1895.

Cummings, J. H., Bingham, S. A., Heaton, K. W., & Eastwood, M. A. (1992). Fecal weight, colon cancer risk, and dietary intake of nonstarch polysaccharides (dietary fiber). *Gastroenterology, 103*(6), 1783-1789.

Cummings, J. H., & Stephen, A. M. (2007). Carbohydrate terminology and classification. *European Journal of Clinical Nutrition, 61 Suppl 1*, S5-18.

Darmodaran S, Parkin KL & Fennema OW. Fennema's Food Chemistry (Fourth Edition). NY, New York: CRC Press. 2008

Dea, I. C. M., Morris, E. R., Rees, D. A., Welsh, E. J., Barnes, H. A., & Price, J. (1977). Associations of like and unlike polysaccharides: Mechanism and specificity in galactomannans, interacting bacterial polysaccharides, and related systems. *Carbohydrate Research, 57*(0), 249-272.

Dea, I. C. M., & Morrison, A. Chemistry and interactions of seed galactomannans. *Advances in carbohydrate chemistry and biochemistry* (pp. 241-312) Academic Press.

Dikeman, C. L., Murphy, M. R., & Fahey, G. C.,Jr. (2006). Dietary fibers affect viscosity of solutions and simulated human gastric and small intestinal digesta. *The Journal of Nutrition, 136*(4), 913-919.

Doyle, J. P., Lyons, G., & Morris, E. R. (2009). New proposals on "hyperentanglement" of galactomannans: Solution viscosity of fenugreek gum under neutral and alkaline conditions. *Food Hydrocolloids, 23*(6), 1501-1510.

Edwards, C., Blackburn, N., Craigen, L., Davison, P., Tomlin, J., Sugden, K., et al. (1987). Viscosity of food gums determined in vitro related to their hypoglycemic actions. *The American Journal of Clinical Nutrition, 46*(1), 72-77.

Edwin R., M. (1990). Shear-thinning of 'random coil' polysaccharides: Characterisation by two parameters from a simple linear plot. *Carbohydrate Polymers, 13*(1), 85-96.

Ehrlein, H., & Stockmann, A. (1998). Absorption of nutrients is only slightly reduced by supplementing enteral formulas with viscous fiber in miniature pigs. *The Journal of Nutrition, 128*(12), 2446-2455.

Ellis, P. R., Apling, E. C., Leeds, A. R., & Bolster, N. R. (1981). Guar bread: Acceptability and efficacy combined. studies on blood glucose, serum insulin and satiety in normal subjects. *The British Journal of Nutrition, 46*(2), 267-276.

Englyst, H. N., Kingman, S. M., & Cummings, J. H. (1992). Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition, 46 Suppl 2*, S33-50.

Eriksson, K., & Lindgärde, F. (1991). Prevention of type 2 (non-insulin-dependent) diabetes mellitus by diet and physical exercise the 6-year malmö feasibility study. *Diabetologia, 34*(12), 891-898.

Fafaungwithayakul, N., Hongsprabhas, P., & Hongsprabhas, P. (2011). Effect of soy soluble polysaccharide on the stability of soy-stabilised emulsions during in vitro protein digestion. *Food Biophysics, 6*(3), 407-415.

Farahnaky, A., Askari, H., Majzoobi, M., & Mesbahi, G. (2010). The impact of concentration, temperature and pH on dynamic rheology of psyllium gels. *Journal of Food Engineering, 100*(2), 294-301.

Ferrua, M. J., & Singh, R. P. (2010). Modeling the fluid dynamics in a human stomach to gain insight of food digestion. *Journal of Food Science, 75*(7), R151-62.

Fischer, M. H., Yu, N., Gray, G. R., Ralph, J., Anderson, L., & Marlett, J. A. (2004). The gel-forming polysaccharide of psyllium husk (plantago ovata forsk). *Carbohydrate Research, 339*(11), 2009-2017.

Foster-Powell, K., Holt, S. H., & Brand-Miller, J. C. (2002). International table of glycemic index and glycemic load values: 2002. *The American Journal of Clinical Nutrition, 76*(1), 5-56.

Garcia-Ochoa, F., & Casas, J. A. (1992). Viscosity of locust bean (ceratonia siliqua) gum solutions. *Journal of the Science of Food and Agriculture, 59*(1), 97-100.

García-Ochoa, F., Santos, V. E., Casas, J. A., & Gómez, E. (2000). Xanthan gum: Production, recovery, and properties. *Biotechnology Advances, 18*(7), 549-579.

Goswami, A. Production of galactomannan products by enzymatic reaction of guar splits. World Intellectual Property Organisation WO1999/004027. 1999

Granfeldt, Y., Bjorck, I., & Hagander, B. (1991). On the importance of processing conditions, product thickness and egg addition for the glycaemic and hormonal responses

to pasta: A comparison with bread made from 'pasta ingredients'. *European Journal of Clinical Nutrition, 45*(10), 489-499.

Guyton, A., Hall, J., 2005. Textbook of medical physiology, Eleventh ed. Saunders, London.

Hannan, J. M., Ali, L., Rokeya, B., Khaleque, J., Akhter, M., Flatt, P. R., et al. (2007). Soluble dietary fibre fraction of trigonella foenum-graecum (fenugreek) seed improves glucose homeostasis in animal models of type 1 and type 2 diabetes by delaying carbohydrate digestion and absorption, and enhancing insulin action. *The British Journal of Nutrition, 97*(3), 514-521.

Health Canada. It's Your health—Type 2 Diabetes. [http://www.hc-sc.gc.ca/hl-vs/alt_formats/pacrb-dgapcr/pdf/iyh-vsv/diseases-maladies/diabete-eng.pdf]. Last updated: December 2006. Last accessed on: May 22, 2011

Health Canada. Proposed Policy: Definition and Energy Value for Dietary Fibre—2010. [http://www.hc-sc.gc.ca/fn-an/alt_formats/pdf/consult/policy-Definition-politique-eng.pdf]. Lasted updated: December 2010. Last accessed on: July 28 2011.

Heath, G., Wilson, R., Smith, J., & Leonard, B. (1991). Community-based exercise and weight control: Diabetes risk reduction and glycemic control in zuni indians. *The American Journal of Clinical Nutrition, 53*(6), 1642S-1646S.

Hipsley, E. H. (1953). Dietary "fibre" and pregnancy toxaemia. *British Medical Journal, 2*(4833), 420-422.

Hur, S. J., Lim, B. O., Decker, E. A., & McClements, D. J. (2011). In vitro human digestion models for food applications. *Food Chemistry, 125*(1), 1-12.

Institute of Medicine of the National Academies (IOM). Proposed definition of dietary fiber. [http://www.iom.edu/Activities/Nutrition/DietaryFiberDef.aspx].
Last updated: October 2009. Last accessed on: May 22 2011.

Jenkins, D. J., Josse, R. G., Jenkins, A. L., Wolever, T. M., & Vuksan, V. (1995). Implications of altering the rate of carbohydrate absorption from the gastrointestinal tract. *Clinical and Investigative Medicine.Medecine Clinique Et Experimentale, 18*(4), 296-302.

Jenkins, D. J., Wolever, T. M., Leeds, A. R., Gassull, M. A., Haisman, P., Dilawari, J., et al. (1978). Dietary fibres, fibre analogues, and glucose tolerance: Importance of viscosity. *British Medical Journal, 1*(6124), 1392-1394.

Jenkins, D. J., Wolever, T. M., Taylor, R. H., Barker, H., Fielden, H., Baldwin, J. M., et al. (1981). Glycemic index of foods: A physiological basis for carbohydrate exchange. *The American Journal of Clinical Nutrition, 34*(3), 362-366.

Jenkins, D. J. A., Jenkins, A. L., Wolever, T. M. S., Collier, G. R., Rao, A. V., & Thompson, L. U. (1987). Starchy foods and fiber: Reduced rate of digestion and improved carbohydrate metabolism. *Scand J Gastroenterol, 22*, 132-141.

Jenkins, D. J. A., Leeds, A. R., Gassull, M. A., Cochet, B., & Alberti, K. G. M. M. (1977). Decrease in postprandial insulin and glucose concentrations by guar and pectin. *Annals of Internal Medicine, 86*(1), 20-23.

Jenkins, D. J., Kendall, C. W., Axelsen, M., Augustin, L. S., & Vuksan, V. (2000). Viscous and nonviscous fibres, nonabsorbable and low glycaemic index carbohydrates, blood lipids and coronary heart disease. *Current Opinion in Lipidology, 11*(1), 49-56.

Johnson LR. Gastrointestinal Physiology. Missouri, St. Louis: Mosby Year Book. 1991

K.T., R. (2011). The physiological and rheological effects of foods supplemented with guar gum. *Food Research International, 44*(5), 1109-1114.

Kajimoto, Y., & Kaneto, H. (2004). Role of oxidative stress in pancreatic beta-cell dysfunction. *Annals of the New York Academy of Sciences, 1011*, 168-176.

Knowler, W. C., Barrett-Connor, E., Fowler, S. E., Hamman, R. F., Lachin, J. M., Walker, E. A., et al. (2002). Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *The New England Journal of Medicine, 346*(6), 393-403.

Kwiatek, M. A., Menne, D., Steingoetter, A., Goetze, O., Forras-Kaufman, Z., Kaufman, E., et al. (2009). Effect of meal volume and calorie load on postprandial gastric function and emptying: Studies under physiological conditions by combined fiber-optic pressure measurement and MRI. *American Journal of Physiology - Gastrointestinal and Liver Physiology, 297*(5), G894-G901.

Li, X., Fang, Y., Al-Assaf, S., Phillips, G. O., Nishinari, K., & Zhang, H. (2009). Rheological study of gum arabic solutions: Interpretation based on molecular self-association. *Food Hydrocolloids, 23*(8), 2394-2402.

Ludwig, D. S., Majzoub, J. A., Al-Zahrani, A., Dallal, G. E., Blanco, I., & Roberts, S. B. (1999). High glycemic index foods, overeating, and obesity. *Pediatrics, 103*(3), e26-e26.

Maeda H. Soluble soybean polysaccharide (From Phillips' & Williams' Handbook of Hydrocolloids). NY, New York: CRC Press. 2000

Madar, Z., Abel, R., Samish, S., & Arad, J. (1988). Glucose-lowering effect of fenugreek in non-insulin dependent diabetics. *European Journal of Clinical Nutrition, 42*(1), 51-54.

Malaki Nik, A. (2011; 2011). Interfacial design of protein-stabilized emulsions for optimal delivery of lipophilic nutrients. (University of Guelph (Canada), University of Guelph (Canada)). *ProQuest Dissertations and Theses,*

Marciani, L., Gowland, P. A., Spiller, R. C., Manoj, P., Moore, R. J., Young, P., et al. (2000). Gastric response to increased meal viscosity assessed by echo-planar magnetic resonance imaging in humans. *The Journal of Nutrition, 130*(1), 122-127.

Marciani, L., Gowland, P. A., Spiller, R. C., Manoj, P., Moore, R. J., Young, P., et al. (2001). Effect of meal viscosity and nutrients on satiety, intragastric dilution, and emptying assessed by MRI. *American Journal of Physiology - Gastrointestinal and Liver Physiology, 280*(6), G1227-G1233.

Mathern, J. R., Raatz, S. K., Thomas, W., & Slavin, J. L. (2009). Effect of fenugreek fiber on satiety, blood glucose and insulin response and energy intake in obese subjects. *Phytotherapy Research : PTR, 23*(11), 1543-1548.

Mazza, G., & Biliaderis, C. G. (1989). Functional properties of flax seed mucilage. *Journal of Food Science, 54*(5), 1302-1305.

McCleary BV & Prosky L. Advanced Dietary Fibre Technology. Ames, Iowa: Blackwell Science Ltd. 2001

Michael J., B. (2009). Inositol trisphosphate and calcium signalling mechanisms. *Biochimica Et Biophysica Acta (BBA) - Molecular Cell Research, 1793*(6), 933-940.

Morris, D. H. (2001). Essential nutrients and other functional compounds in flaxseed. *Nutrition Today, 36*(3)

Na Nakornpanom, N., Hongsprabhas, P., & Hongsprabhas, P. (2010). Effect of soy residue (okara) on in vitro protein digestibility and oil release in high-calorie emulsion stabilized by heated mixed proteins. *Food Research International, 43*(1), 26-32.

Na, N., Hongsprabhas Pranithi, & Hongsprabhas Parichat. (2010). *Effect of soy residue (okara) on in vitro protein digestibility and oil release in high-calorie emulsion stabilized by heated mixed proteins*. Kidlington, ROYAUME-UNI: Elsevier.

Nakamura Akihiro, Maeda Hirokazu, & Corredig Milena. (2006). *Emulsifying properties of enzyme-digested soybean soluble polysaccharide*. Oxford, ROYAUME-UNI: Elsevier.

Nakamura, A., Furuta, H., Kato, M., Maeda, H., & Nagamatsu, Y. (2003). Effect of soybean soluble polysaccharides on the stability of milk protein under acidic conditions. *Food Hydrocolloids, 17*(3), 333-343.

Nakamura, A., Yoshida, R., Maeda, H., Furuta, H., & Corredig, M. (2004). Study of the role of the carbohydrate and protein moieties of soy soluble polysaccharides in their emulsifying properties. *Journal of Agricultural and Food Chemistry, 52*(17), 5506-5512.

National Institute of Diabetes and Digestive and Kidney Diseases. National Diabetes Information Clearinghouse—Diagnosis of diabetes.

[http://diabetes.niddk.nih.gov/dm/pubs/diagnosis/#pre-diabetes]. Last updated: October 2008. Last accessed on: May 30 2011.

Nicoletti, C. (2000). Unsolved mysteries of intestinal M cells. *Gut, 47*(5), 735-739.

Nilsson, A., Ostman, E., Preston, T., & Bjorck, I. (2008). Effects of GI vs content of cereal fibre of the evening meal on glucose tolerance at a subsequent standardized breakfast. *European Journal of Clinical Nutrition, 62*(6), 712-720.

Oomen, A. G., Hack, A., Minekus, M., Zeijdner, E., Cornelis, C., Schoeters, G., et al. (2002). Comparison of five in vitro digestion models to study the bioaccessibility of soil contaminants. *Environmental Science & Technology, 36*(15), 3326-3334.

Oomen, A. G., Rompelberg, C. J. M., Bruil, M. A., Dobbe, C. J. G., Pereboom, D. P. K. H., & Sips, A. J. A. M. (2003). Development of an *in vitro* digestion model for estimating the bioaccessibility of soil contaminants. *Archives of Environmental Contamination and Toxicology, 44*(3), 0281-0287.

Ou, S., Kwok, K., Li, Y., & Fu, L. (2001). In vitro study of possible role of dietary fiber in lowering postprandial serum glucose. *Journal of Agricultural and Food Chemistry, 49*(2), 1026-1029.

Paeschke TM & Aimutis WR. Nondigestible Carbohydrates and Digestive Health. Iowa, Ames: Wiley-Blackwell Publishing Ltd. 2011

Pan, X., Li, G., Hu, Y., Wang, J., Yang, W., An, Z., et al. (1997). Effects of diet and exercise in preventing NIDDM in people with impaired glucose tolerance: The da qing IGT and diabetes study. *Diabetes Care, 20*(4), 537-544.

Pasquier, B., Armand, M., Castelain, C., Guillon, F., Borel, P., Lafont, H., et al. (1996). Emulsification and lipolysis of triacylglycerols are altered by viscous soluble dietary fibres in acidic gastric medium in vitro. *The Biochemical Journal, 314 (Pt 1)*(Pt 1), 269-275.

Perry, P. A., Fitzgerald, M. A., & Gilbert, R. G. (2006). Fluorescence recovery after photobleaching as a probe of diffusion in starch systems. *Biomacromolecules, 7*(2), 521-530.

Phillips GO, & Williams PA. Handbook of Hydrocolloids. NY, New York: CRC Press. 2000

Public Health Agency of Canada. How To Prevent Type 2 Diabetes. [http://www.phacaspc.gc.ca/cd-mc/diabetes-diabete/prevent-prevenir-eng.php]. Last updated: November 14th 2008. Last accessed on: May 22, 2011

Rajendra P., B. (1992). Food texture and rheology: A tutorial review. *Journal of Food Engineering, 16*(1-2), 1-16.

Randall, R. C., Phillips, G. O., & Williams, P. A. (1988). The role of the proteinaceous component on the emulsifying properties of gum arabic. *Food Hydrocolloids, 2*(2), 131-140.

Redard, C., Davis, P., & Schneeman, B. (1990). Dietary fiber and gender: Effect on postprandial lipemia. *The American Journal of Clinical Nutrition, 52*(5), 837-845.

Regand, A., Chowdhury, Z., Tosh, S. M., Wolever, T. M. S., & Wood, P. (2011). The molecular weight, solubility and viscosity of oat beta-glucan affect human glycemic response by modifying starch digestibility. *Food Chemistry, 129*(2), 297-304.

Regand, A., Tosh, S. M., Wolever, T. M. S., & Wood, P. J. (2009). Physicochemical properties of β-glucan in differently processed oat foods influence glycemic response. *Journal of Agricultural and Food Chemistry, 57*(19), 8831-8838.

Rodríguez-Morán, M., Guerrero-Romero, F., & Lazcano-Burciaga, G. (1998). Lipid- and glucose-lowering efficacy of plantago psyllium in type II diabetes. *Journal of Diabetes and its Complications, 12*(5), 273-278.

Ross, A. H., Eastwood, M. A., Brydon, W. G., Anderson, J. R., & Anderson, D. M. (1983). A study of the effects of dietary gum arabic in humans. *The American Journal of Clinical Nutrition, 37*(3), 368-375.

Saghir, S., Iqbal, M. S., Hussain, M. A., Koschella, A., & Heinze, T. (2008). Structure characterization and carboxymethylation of arabinoxylan isolated from ispaghula (plantago ovata) seed husk. *Carbohydrate Polymers, 74*(2), 309-317.

Saura-Calixto, F., Garcia-Alonso, A., Goni, I., & Bravo, L. (2000). In vitro determination of the indigestible fraction in foods: An alternative to dietary fiber analysis. *Journal of Agricultural and Food Chemistry, 48*(8), 3342-3347.

Sayar, S., Jannink, J., & White, P. J. (2006). In vitro bile acid binding activity within flour fractions from oat lines with typical and high β-glucan amounts. *Journal of Agricultural and Food Chemistry, 54*(14), 5142-5148.

Sharma, R. D., Raghuram, T. C., & Rao, N. S. (1990). Effect of fenugreek seeds on blood glucose and serum lipids in type I diabetes. *European Journal of Clinical Nutrition, 44*(4), 301-306.

Slavin, J., & Jacobs, D. R. (2010). Dietary fiber: All fibers are not alike. In T. Wilson, G. A. Bray, N. J. Temple & M. B. Struble (Eds.), (pp. 13-24) Humana Press.

Southgate, D. A. T. (1969). Determination of carbohydrates in foods. I.—Available carbohydrate. *Journal of the Science of Food and Agriculture, 20*(6), 326-330.

Srinivasan, K. (2006). Fenugreek (trigonella foenum-graecum): A review of health beneficial physiological effects. *Food Reviews International, 22*(2), 203-224.

Statistics Canada. Diabetes—2010.
[http://www.statcan.gc.ca/pub/82-229-x/2009001/status/dia-eng.htm#hg].
Last updated: January 2010. Last accessed on: May 10 2011

Steffe, J.F. Rheological Methods in Food Process Engineering, second edition (second printing). MI, East Lansing: Freeman Press.1996

Streppel, M. T., Arends, L. R., van 't Veer, P., Grobbee, D. E., & Geleijnse, J. M. (2005). Dietary fiber and blood pressure: A meta-analysis of randomized placebo-controlled trials. *Arch Intern Med, 165*(2), 150-156.

Swartz, WE. Reduces viscosity, low ash modified guar and process for producing same, US Patent 6,048,563. 2000

Sworn, G. (2011). Xanthan gum – functionality and application. *Practical food rheology* (pp. 85-112) Wiley-Blackwell.

Sworn G. Xanthan Gum (From Imeson A's Food Stabilisers, Thickeners and Gelling Agents). Ames, Iowa: Wiley-Blackwell Publishing Ltd. 2010

Sworn G, & Monsanto. Gum Xanthan (From Phillips' & Williams' Handbook of Hydrocolloids). NY, New York: CRC Press. 2000

Tarté, R. (2009). Meat-derived protein ingredients. In R. Tarté (Ed.), *Ingredients in meat products* (pp. 145-171) Springer New York.

Tester, R. F., & Sommerville, M. D. (2003). The effects of non-starch polysaccharides on the extent of gelatinisation, swelling and α-amylase hydrolysis of maize and wheat starches. *Food Hydrocolloids, 17*(1), 41-54.

Thakur, G., Mitra, A., Pal, K., & Rousseau, D. (2009). Effect of flaxseed gum on reduction of blood glucose and cholesterol in type 2 diabetic patients. *Int J Food Sci Nutr, 60*, 126-136.

Tharakan, A., Norton, I. T., Fryer, P. J., & Bakalis, S. (2010). Mass transfer and nutrient absorption in a simulated model of small intestine. *Journal of Food Science, 75*(6), E339-46.

Thevenet F. Acacia Gum (From Imeson A's Food Stabilisers, Thickeners and Gelling Agents). Ames, Iowa: Wiley-Blackwell Publishing Ltd. 2010

Topping, D. L., & Clifton, P. M. (2001). Short-chain fatty acids and human colonic function: Roles of resistant starch and nonstarch polysaccharides. *Physiological Reviews, 81*(3), 1031-1064.

Tsai, A., Mott, E., Owen, G., Bennick, M., Lo, G., & Steinke, F. (1983). Effects of soy polysaccharide on gastrointestinal functions, nutrient balance, steroid excretions, glucose tolerance, serum lipids, and other parameters in humans. *The American Journal of Clinical Nutrition, 38*(4), 504-511.

Tsai, A., Vinik, A., Lasichak, A., & Lo, G. (1987). Effects of soy polysaccharide on postprandial plasma glucose, insulin, glucagon, pancreatic polypeptide, somatostatin, and triglyceride in obese diabetic patients. *The American Journal of Clinical Nutrition, 45*(3), 596-601.

Wadood, A., Wadood, N., & Shah, S. A. (1989). Effects of acacia arabica and caralluma edulis on blood glucose levels of normal and alloxan diabetic rabbits. *JPMA.the Journal of the Pakistan Medical Association, 39*(8), 208-212.

Wang, Q., Ellis, P. R., & Ross-Murphy, S. B. (2000). The stability of guar gum in an aqueous system under acidic conditions. *Food Hydrocolloids, 14*(2), 129-134.

Wapnir, R., Wingertzahn, M., Moyse, J., & Teichberg, S. (1997). Gum arabic promotes rat jejunal sodium and water absorption from oral rehydration solutions in two models of diarrhea. *Gastroenterology, 112*(6), 1979-1985.

Weijman, I., Ros, W. J. G., Rutten, G. E. H. M., Schaufeli, W. B., Schabracq, M. J., & Winnubst, J. A. M. (2005). The role of work-related and personal factors in diabetes self-management. Patient Education and Counseling, 59(1), 87-96.

Weisz,J., & Fillion N. (1997). *Effect of cooking and storage on the amount and molecular weight of(1→3)(1→4)-β-D-glucan extracted from oat products by an in vitro digestion system*. St. Paul, MN, ETATS-UNIS: American Association of Cereal Chemists.

Whitney E & Rolfes SR. Digestion and Absorption of Carbohydrates. Understanding Nutrition. (Howe E, Feldman E, eds.). CA, Belmont: Thomson Wadsworth. 2005

Wielinga W. Seed Gums (From Imeson A's Food Stabilisers, Thickeners and Gelling Agents). Ames, Iowa: Wiley-Blackwell Publishing Ltd. 2010

Wielinga W. & Maehall AG. Galactomannans (From Phillips' & Williams' Handbook of Hydrocolloids). NY, New York: CRC Press. 2000

Willett, W., Manson, J., & Liu, S. (2002). Glycemic index, glycemic load, and risk of type 2 diabetes. *The American Journal of Clinical Nutrition, 76*(1), 274S-280S.

Wilson, T. A., Behr, S. R., & Nicolosi, R. J. (1998). Addition of guar gum and soy protein increases the efficacy of the american heart association (AHA) step I cholesterol-lowering diet without reducing high density lipoprotein cholesterol levels in non-human primates. *The Journal of Nutrition, 128*(9), 1429-1433.

Wolever, T. M., & Jenkins, D. J. (1986). The use of the glycemic index in predicting the blood glucose response to mixed meals. *The American Journal of Clinical Nutrition, 43*(1), 167-172.

Wood, P. J., Braaten, J. T., Scott, F. W., Riedel, K. D., Wolynetz, M. S., & Collins, M. W. (1994). Effect of dose and modification of viscous properties of oat gum on plasma glucose and insulin following an oral glucose load. *The British Journal of Nutrition, 72*(5), 731-743.

Woolnough, J. W., Monro, J. A., Brennan, C. S., & Bird, A. R. (2008). Simulating human carbohydrate digestion in vitro: A review of methods and the need for standardisation. *International Journal of Food Science & Technology, 43*(12), 2245-2256.

Wu, M., Li, D., Wang, L., Zhou, Y., & Mao, Z. (2010). Rheological property of extruded and enzyme treated flaxseed mucilage. *Carbohydrate Polymers, 80*(2), 460-466.

Wu, Y., Li, W., Cui, W., Eskin, N. A. M., & Goff, H. D. (2012). A molecular modeling approach to understand conformation–functionality relationships of galactomannans with different mannose/galactose ratios. *Food Hydrocolloids, 26*(2), 359-364.

Yamatoya, K., Kuwano, K., & Suzuki, J. (1997). Effects of hydrolyzed guar gum on cholesterol and glucose in humans. *Food Hydrocolloids, 11*(2), 239-242.