Investigations of Mechanisms Involved in Postprandial Glycemia Attenuation with Dietary Fiber Consumption

by

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INVESTIGATIONS OF MECHANISMS INVOLVED IN POSTPRANDIAL GLYCEMIA ATTENUATION WITH DIETARY FIBER CONSUMPTION

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Earlier studies have shown that dietary fibre (DF) can attenuate glycemic response through its ability to alter luminal viscosity and could therefore help minimize the risk of developing Type 2 diabetes (T2D). The main objective of the present work was to gain more understanding of mechanisms involved in attenuation of postprandial glycemic and insulinemic responses as a result of DF intake. Four different DF types, including yellow mustard mucilage, soluble flaxseed gum, fenugreek gum and oat gum, were used in the present study. Concentrations of each DF type that resulted in apparent viscosities (at 60 s\(^{-1}\)) close to apparent viscosities of oat β-glucan were determined. Besides that, the effect of DF on amylolysis and maltose transport in simulated small intestinal conditions at controlled shear rate was studied. Furthermore, a human clinical trial was conducted to investigate the effect of DF on postprandial glycemic and insulinemic responses. Participants consumed high maltose syrup-based and starch-based treatments supplemented with each type of DF at concentrations matching their viscosity (18.54 mPa.s at 60 s\(^{-1}\)) as measured in simulated small intestinal conditions.

Concentrations of DFs that resulted in similar apparent viscosities in the simulated small intestinal conditions were different for each DF type. Nonetheless, the presence of DF at
different concentrations, but matched for simulated intestinal viscosity, resulted in similar
progress of amylolysis \textit{in vitro}. Maltose transport, measured \textit{in vitro}, was not affected by the DF
at concentrations expected to be in the small intestinal lumen after consumption of the
Corresponding pudding treatments. Supplementation of puddings with each type of DF at
different concentrations but matched for post-digestion viscosity attenuated postprandial blood
glucose and plasma insulin peak concentrations and plasma paracetamol concentration to a
similar extent, which was significantly lower compared to the control. It was concluded that
improvement of blood biochemical profile observed in the present study was due to the ability of
DF to alter digesta viscosity through mechanisms including the delay of gastric emptying and to
some degree reduction of amylolysis progress.
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DF - Dietary fibre
T2D - Type 2 Diabetes
YMM - Yellow mustard mucilage
SFG - Soluble flaxseed gum
FG - Fenugreek gum
OG - Oat gum
YMM-ETH - Ethanol-precipitated yellow mustard mucilage
SFG-ETH - Ethanol-precipitated soluble flaxseed gum
FG-WE - Water-extracted fenugreek gum
EFSA - European Food Safety Authority
eq. – Equivalent(s) of the European Food Safety Authority (2011) glycemia control health claim
for cereal β-glucan
DNS - Dinitrosalicylic acid colorimetric method
LOS - Logarithm of slope
Cmax - Peak concentrations
BMI - Body mass index
CANRISK - Canadian Diabetes Risk Assessment Questionnaire
Mw - Weight average molecular weight
Mn - Number average molecular weight
[η] - Intrinsic viscosity
Rg - Radius of gyration
P1 - Polydispersity index
ηrel - Relative viscosities
ηsp - Specific viscosities
kH - Huggins constant
kK - Kraemer constant
η0 - Zero-shear rate viscosity
η∞ - Infinite shear rate viscosity
c* - Critical overlap concentration
c*[η] - Coil-overlap parameter
RESEARCH OBJECTIVES

The overall goal of the present work was to gain more understanding of mechanisms involved in attenuation of postprandial glycemic responses as a result of water-soluble dietary fiber (DF) consumption. The following were the objectives of the present work to explore the goal.

1) To compare rheological properties of different types of DF in simulated small intestinal conditions, and to determine concentrations of each DF that would result in apparent viscosities close to those of cereal β-glucan in simulated small intestinal conditions.

2) To investigate the effect of viscosity altered with different types of DF on the kinetics of amylolysis in simulated small intestinal conditions.

3) To investigate the effect of viscosity altered with different types of DF on maltose transport in simulated small intestinal conditions.

4) To investigate the viscous properties and stability of different types of DF in simulated gastric conditions.

5) To gain more understanding of mechanisms involved in attenuation of postprandial glycemic and insulinemic responses due to DF consumption through interpretation of results of the in vitro experiments (rheological properties in simulated gastric and small intestinal conditions, kinetics of amylolysis in simulated small intestinal conditions and maltose transport in simulated small intestinal conditions) and human clinical trial.
RESEARCH HYPOTHESIS

Some hypothesized major outcomes of the present work are mentioned below.

1) Concentrations of DFs that represent equivalent apparent viscosities in simulated small intestinal conditions would be different, due to heterogeneity in the molecular architecture of each DF type.

2) If the progress of amylolysis is affected by diffusion of enzyme and/or mixing efficiency of digesta, then presence of DFs at concentrations that were matched for post-digestion viscosity would restrict amylolysis to similar extent.

3) According to literature, DF can restrict transport of sugars, therefore in the present work, it was also expected that DFs, at the range of concentrations expected in the human small intestine, would also slow maltose transport.

4) Polysaccharides are known to degrade in the acidic environment, therefore, in the present study, it was expected that DFs would undergo acidic hydrolysis in conditions expected in the human stomach.

5) According to literature, DF attenuates postprandial glycemic and insulinemic responses, which is believed to be due to the ability of DF to alter luminal viscosity. Thus, it was expected that in the present clinical trial supplementation of pudding treatments with DFs at concentrations matched for post-digestion viscosity would lead to 1) attenuation of blood glucose and plasma insulin responses compared to the control pudding and 2) similar blood glucose and plasma insulin responses between DF-containing treatments.
CHAPTER 1. LITERATURE REVIEW

Abstract

Carbohydrates are the important source of energy in the average human diet. Some digestible carbohydrates come in the form of polysaccharides such as starches and disaccharides including sucrose, lactose and maltose which are hydrolyzed by specific glycosidases into monosaccharides in the human gastrointestinal tract. Monosaccharides are absorbed through small intestine and released into the bloodstream. Diabetes mellitus is a chronic disease, associated with inability of the body to metabolize glucose, resulting in abnormally high blood glucose levels. The Type 2 Diabetes (T2D) is believed to relate to insulin resistance in target tissues resulting in reduction of glucose uptake and failure of beta cells in the pancreas to maintain sufficient insulin to compensate for this resistance. There is a clear correlation between prolonged consumption of meals resulting in hyperglycemia and increase of insulin demand and development of T2D diabetes. Food high in dietary fiber (DF) has shown ability to attenuate glycemic responses and thus is recommended to the general public to reduce risk of T2D. For subjects with diabetes, DF benefits by reducing frequency of hyperglycemic episodes. It has been proposed that ability of DF to reduce plasma glucose relates to its potential to alter rheological properties of digesta. Many hydrocolloids of carbohydrate nature that potentially have physiological benefits, such as the attenuation of postprandial glycemic responses, can be extracted from agricultural products grown in Canada. The exact mechanism of glycemic response attenuation by viscous DF is unclear. There are three main proposed mechanisms described in the literature, namely, delay of glucose diffusion in the small intestine, reduction of digestive enzyme activity in the small intestine, and delay of gastric emptying.
1.1. Carbohydrate digestion, absorption and metabolism

Carbohydrates can be classified based on the number of monosaccharides they contain. Monosaccharides such as glucose, fructose, and galactose, also called simple sugars, are the least complex carbohydrates. Alternatively, disaccharides and oligosaccharides are composed of 2 and 3-10 monosaccharides joined together, respectively, and polysaccharides consist of long chains of simple sugars (>>10).

Carbohydrates may be classified as digestible, featuring those that can be digested by enzymes present in upper gastrointestinal tract such as sucrose and starch, or those that can be absorbed without digestion, including glucose, and indigestible including inulin, pectin and cellulose (discussed in detail in section 1.5) that are not digested. The most common digestible carbohydrates in the human diet are di-, oligo- and polysaccharides, which are broken down into simple sugars as a result of chemical, mechanical, and enzymatic processes that begin in the oral cavity and continue to the stomach and the small intestine. Simple sugars are less common in human diet, however fructose and glucose can be found in certain fruits, honey and processed food (Gropper, Smith & Groff, 2005a).

Starch is a digestible polysaccharide composed of glucose units and it is the main source of energy for humans. In the body, starch is hydrolyzed by amylolytic enzymes into glucose units. Digestion of starch begins in the oral cavity where chewed food is lubricated by saliva that contains α-amylase, an enzyme that cleaves the α-1,4-glycosidic bonds of starch molecule. Then, the bolus (mixture of food and saliva) travels to the stomach through the esophagus. Starch hydrolysis continues in the stomach until acidity of the stomach reaches the point when the salivary α-amylase becomes inactivated. At that point, starch is already partly hydrolyzed into maltose, dextrins and short-chain polysaccharides. Some consumed foods may contain small
oligosaccharides or disaccharides wherein digestion of these carbohydrates does not occur in the mouth or stomach.

Further, chyme (digesta expelled from the stomach) is forced through the pylorus into the first part of the small intestine called the duodenum. Entrance of digesta into the duodenum triggers the release of gastrointestinal hormones that regulate further digestion process. One of these hormones is cholecystokinin (CCK), a peptide-hormone that stimulates pancreatic secretion and bile release from the gall bladder. Other hormones such as secrin, gastric inhibitory peptide, and motilin, which coordinate digestion in the small intestine, are also released (Chater, Wilcox, Pearson and Brownlee, 2015). Motilin is a hormone discharged by epithelium of the small intestine that plays an impotent role in migrating motor complex. The release of motilin increases the force of peristaltic wave that initiates within the esophagus and passes through the whole gastrointestinal tract.

Bicarbonate-containing pancreatic secretions in the duodenum elevate pH of the acidic chyme back to values close to neutral and starch digestion resumes by α-amylase discharged into the duodenum from the pancreas. Contractions in the small intestine, called segmentation, help to ensure sufficient mixing of digestive juices and luminal contents. Products of starch hydrolysis by α-amylases, mostly maltose, maltotriose, maltotetraose and α-limit dextrins, are brought into contact with the intestinal brush border containing specific glucosidases that hydrolyze the products further to simple sugars (Robyt, 2008). Some of the specific glucosidases include maltase, sucrase, lactase and isomaltase. The latter catalyzes the cleavage of α 1-6 bond, a branch-point of α-limit dextrins.

The presence of glucose in the small intestine motivates enteroendocrine cells in the mucosa to release glucagonlike peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide
(GIP). Both of them activate the release of insulin (a hormone that plays a main role in glucose uptake from circulation into liver, muscle and adipose cells), while GLP-1 also reduces gastric emptying. Liberated monosaccharides are transported across the brush border and basolateral membrane of the enterocyte with aid of transport proteins. Galactose and glucose are transported with sodium dependent glucose cotransporter-1 (SGLT1) proteins, while glucose transporter-5 (GLUT5) proteins transport fructose.

Absorbed sugars are carried directly to the liver through the portal vein (Zheng & Berthoud, 2008). Glucose is the favored carbohydrate for storage and metabolism, therefore absorbed fructose and galactose are usually converted to glucose by the liver. As a result of the absorption of sugar into circulation, postprandial blood glucose levels rise, which signals to beta cells in the pancreas to release insulin that brings blood glucose levels back to normal.

The uptake of glucose by the cell requires crossing the cell plasma membrane, which occurs with help of glucose transporters (GLUT). Insulin-regulated glucose transporter-4 (GLUT4) proteins are primarily found in adipose tissues and muscle. Insulin binds to receptors on cell membranes and activates GLUT4 that assists glucose in passing into these cells. GLUT1 and GLUT2 are also glucose transporters that are not influenced by insulin and have less significant impact on glucose disposal from the blood. GLUT2 promotes entrance of glucose into liver cells and GLUT1 plays a role in glucose access into liver, muscle and adipose cells.

The glucose uptake by these cells is dependent on the blood glucose level. Initial rises in blood glucose concentration increases the rate of uptake, but this uptake declines as transport proteins become saturated. One of the main functions of the liver is to maintain normal blood glucose level, which the body regulates through hormones. Depending on body’s energy demand, cellular glucose can be catabolized for energy through glycolysis and the Krebs cycle or
stored in the form of glycogen. Glycogen is a polysaccharide that is the primary storage form of glucose in animal cells of the muscles and liver. When the blood glucose level is high, the liver can reduce the level through conversion of glucose into glycogen, a pathway called glycogenesis. A drop in blood glucose concentration below homeostatic level results in lower insulin secretion and an increased release of the hormone glucagon. Glucagon works opposite to insulin as it increases glucose concentration in the bloodstream back to normal levels through the metabolic pathway called glycogenolysis, where glycogen is converted back into glucose (Gropper et al. 2005a).

1.2. Diabetes mellitus

After the consumption of a meal, some carbohydrates get digested and absorbed in the small intestine leading to a rise of glucose levels in blood. That glucose is used as the main source of energy for cells in most living organisms. The normal blood glucose concentration in healthy human is maintained between 70-150 mg/dL (Weir, Bonner-Weir, & Sharma, 2012). Usually, the increase of blood glucose signals β-cells in the pancreas to produce hormone insulin. Insulin controls the uptake of glucose by cells, removal of glucose excess from the blood and converting it into glycogen. There is a correlation between insulin secretion and the concentration of glucose in blood. In healthy subjects consumption of higher amounts of easy digestible carbohydrates results in higher insulin production (Gropper et al., 2005a).

Diabetes (derived from Greek words dia which means “through”, and bainein which means “go”) is a term used to characterize a group of disorders associated with increased urine production. Diabetes “mellitus” (derived from Latin word mel which means “honey”) or also known as sugar diabetes, is a chronic disease which is linked with the inability of the body to
metabolize glucose, thus resulting in abnormally high blood glucose levels (hyperglycemia).

Depending on the mechanism, diabetes is divided into two types:

- Type 1, insulin-dependent type, which results from autoimmune destruction of insulin-producing β-cells in the pancreas,

- Type 2, (T2D) non-insulin-dependent type (accounting for 80-90% all reported diabetes cases),

which is usually associated with the following two processes:

1. insulin resistance in target tissues, resulting in reduced glucose uptake. In the muscle cells, insulin resistance is associated with the inadequate activity of transporters to translocate glucose to the plasma membrane (Gropper et al., 2005a) and,

2. failure of β-cells in the pancreas to maintain sufficient amount of insulin to compensate for this resistance (Salmeron et al., 1997). Insulin insufficiency is especially pronounced during the first phase, whereas the overall insulin secretion could be unaffected (Gerich, 1996). Long term high plasma glucose results in malfunctioning of pancreas β-cells, which are not able to maintain sufficient amounts of insulin and eventually leads to state of diabetes. It is still not clear if this defect is the result of the loss of beta cells function due to exhaustion resulting in insulin oversecretion, or glucose toxicity to beta cells due to elevated blood glucose levels.

The condition that is the precursor to T2D, is called prediabetes or Impaired Glucose Tolerance (IGT). It is characterized by elevated, however still lower than observed in diabetic patients, fasting blood glucose levels (between 6.1 and 6.9 mmol/L, according to Canadian Diabetes Association, 2008). As a rule, individuals with IGT have higher risk of developing T2D in the future (Ur, 2008).

The differences in plasma insulin and glucose responses between healthy, IGT and T2D subjects during 2h after oral ingestion of glucose can be seen in Fig. 1.1. Compared to healthy
humans, the insulin response is reduced during early stage in IGT subjects and even more reduced in T2D patients. It can be also seen that as the glucose tolerance deteriorates (healthy→IGT→T2D), the level of plasma glucose after oral glucose admission becomes higher (Gerich, 1996).

Chronic abnormally high blood glucose levels cause damage of tissues in the body, and result in a variety of serious complications including: blindness, kidney disease, nerve damage and coronary events. Complications from diabetes account for more than 60% of all nontraumatic amputations. Additionally, individuals suffering from diabetes have a 2-6 fold higher risk of developing cardiovascular disease and twice higher mortality risk than those without diabetes (Skyler, 2012). These complications and risks can be minimized through reducing hyperglycemic episodes.

Figure 1.1. Plasma insulin and glucose responses in healthy (○), IGT (■) and T2D (●) subjects during 2h after oral ingestion of glucose. © Gerich JE (1996). Pathogenesis and treatment of type 2 (noninsulin-dependent) diabetes mellitus (NIDDM). Hormone and Metabolic Research. 28, p. 405, figure 2.
Today, more than 3 million Canadians are diagnosed with diabetes (Canadian Diabetes Association, 2013). Around 8.3% of the Ontario population was diagnosed with diabetes in 2010, and that number is expected to reach 11.9% by 2020. As a result, the economic burden of diabetes in the province will rise from $4.9 to $7.0 billion from 2010 to 2020 (Canadian Diabetes Association, 2012).

1.3. Diet and blood glucose

Studies suggest that T2D, unlike type 1 diabetes, can be prevented or at least delayed with medication or even with nonpharmacological lifestyle interventions. Lindstrom et al. (2003) and Tuomilehto et al. (2001) reported that adding moderate-intensity physical exercises and reduction of fat and calories in the diet prevented development of diabetes in 58% of IGT participants. Diets low in calories and fats and high in dietary fiber (DF) are also prescribed to individuals with diabetes to improve glycemic control (Gougeon, Aylard, Nichol, Quinn, & Whitham, 2008). There is a clear correlation between prolonged consumption of meals resulting in hyperglycemia and T2D development (Willett, Manson, & Liu, 2002).

Different foods containing the same amounts of available (for digestion) carbohydrates can result in different postprandial glycemic responses. Factors such as particle size, nature, processing conditions and food matrices can impact carbohydrate digestion and metabolism. Thus information on the glycemic responses to various foods could be beneficial especially to diabetic patients who try to minimize hyperglycemia, but also to healthy subjects such as athletes who find fast energy recovery useful. The concept of glycemic index (GI) was introduced to assess glycemic responses of different foods with a given amount of available carbohydrates (Jenkins et al., 1981; Wolever et al., 1991). Some suggest that GI represents the quality of the
Evaluation of GI of a particular food is based on comparing its glycemic response with glycemic response of a standard, usually dextrose or white bread. Tested food and standard are used in amounts that provide 50g of available carbohydrates per tested portion. Glycemic responses of healthy subject are obtained by taking blood samples every 15-30 min usually during 2h following 12h fasting. The experiment is longer (3h) for diabetic patients due to their tendency to maintain prolonged high blood glucose levels. The observed responses for tested food and standard are plotted against time to obtain glycemic response curves and the areas under the curves (AUC) are calculated. The GI is expressed as a percentage ratio of test food AUC to standard AUC (Equation 1.1) (Wolever et al., 1991).

\[
GI = \frac{AUC_{test\ food}}{AUC_{standard}} \times 100\%
\]  

(Equation 1.1)

Wolever et al. (1991) mentions GI of some common foods (Table 1.1). A more detailed list ( > 750 foods) of glycemic indexes has been published by Foster-Powell, Holt, & Brand-Miller

Table 1.1. Glycemic index of foods.


<table>
<thead>
<tr>
<th>Food</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole-meal bread</td>
<td>100 ± 5</td>
</tr>
<tr>
<td>White spaghetti</td>
<td>67 ± 16</td>
</tr>
<tr>
<td>White rice</td>
<td>77 ± 12</td>
</tr>
<tr>
<td>Corn</td>
<td>80 ± 11</td>
</tr>
<tr>
<td>Corn flakes</td>
<td>121 ± 13</td>
</tr>
<tr>
<td>Oatmeal</td>
<td>89 ± 10</td>
</tr>
<tr>
<td>Potato</td>
<td>98 ± 26</td>
</tr>
<tr>
<td>Kidney beans</td>
<td>38 ± 9</td>
</tr>
<tr>
<td>Apple</td>
<td>52 ± 5</td>
</tr>
<tr>
<td>Bannana</td>
<td>84 ± 14</td>
</tr>
<tr>
<td>Orange</td>
<td>59 ± 14</td>
</tr>
</tbody>
</table>
It has been suggested that making GI information available to consumers can be beneficial. In Australia, food product labels already contain information on their GI values (Foster-Powell et al., 2002; Sluijs et al., 2013).

Canadian Diabetes Association divides food into three groups depending on its GI:

- High-GI (GI ≥ 70) food, which contains easily digestible and rapidly absorbed carbohydrates, which result in high plasma glucose. These types of foods are usually characterized with a fast jump in blood glucose concentration, resulting in a high peak and following rapid decline. The overall plasma glucose concentration during 2h test is also higher after the consumption of high-GI foods (Sluijs et al., 2013). Some examples of high-GI foods include white bread, corn flakes, bran flakes and short-grain rice.

- Low-GI (GI ≤ 55) food, which contains slowly digested and absorbed carbohydrates resulting in a gradual increase and then gradual decrease of blood glucose concentration. The blood glucose peak and total blood glucose during 2h after consumption of low-GI food are lower than after consumption of high-GI food. Some examples of low-GI foods include: 100% stone ground whole wheat bread, barley, pasta/noodles, sweet potato, yam and various legumes.

- Medium-GI (56 ≤ GI ≥ 69) food, which results in intermediate blood glucose concentration. Some examples of medium-GI foods include: whole wheat bread, quick oats, basmati and brown rice, potato, sweet corn and popcorn.

The GI represents only the glycemic responses of a food portion containing 50g of available carbohydrates. However, the amount of available carbohydrates and thus glycemic responses of food varies with food portion size. The glycemic load (GL) concept takes into account the amount of available carbohydrate in particular portion of test food. GL can be calculated by multiplying GI of the test food by the amount of available carbohydrates in the given dose, and
dividing by 100. Thus GL represents both the quality and quantity of carbohydrates in the given food of particular size (Salmeron et al., 1997). Knowing the GL of particular food can help to predict the postprandial glycemic outcome.

Consumption of high GI food has been associated with development of insulin resistance and T2D. Evidence suggests that replacing high GI carbohydrates with low GI carbohydrates can lower the risk of T2D development in healthy subjects and improve glycemic control in T2D patients. The influence of diet enriched with glucose, amylose and amylopectin (which represent fast, medium and slow digestible carbohydrates respectively) on the development of insulin resistance was investigated by Higgins et al. (1996). Rats fed with glucose and amylose developed insulin resistance much faster (glucose rich diet for 8 weeks), than amylopectin-fed rats (12-26 weeks). This observation suggests that diet containing easily digestible carbohydrates promotes insulin resistance and T2D development.

Numerous studies suggest that high DF diets reduce risks of several diseases, including T2D. In the large-scale study by Salmeron et al. (1997), 65173 women between 40-65 years of age were examined for correlation between risk of T2D and their diet. A positive correlation between diabetes and the glycemic load and inverse association between risk of diabetes and cereal fibre intake were found. The combination of a high cereal fibre intake and low glycemic load further reduced the risk of diabetes (Fig. 1.2).

The fact that DF can delay sugar absorption and help to better control blood sugar levels has been supported by many other studies. As a result, various diabetes associations in United States of America, Great Britain, Canada, Europe, India, Japan and South Africa recommend that people with diabetes incorporate DF into their diet (Anderson, Randles, Kendall, & Jenkins (2004). Based on their fixed-effects meta-analyses Anderson et al., 2004 recommend that the diet
of diabetic individuals should provide 25-50g of DF per day.

1.4. Dietary fiber

Dietary fiber is a nondigestable carbohydrate, the structural constituent of the edible parts of plants, and therefore it has been a part of the human diet for centuries. Nevertheless, its significance was understood only recently. Today DF is recognized as a vital part of the human diet due to its physiological functions and health benefits (Chawla & Patil, 2010).

A precise definition of DF as well as methods for measuring the amount of DF have been under discussion for a long time. The struggle arises mainly from the difficulty to combine in one definition both chemical composition and physiological benefits of DF (Prosky, 2008). Health Canada has also proposed its version of definition, which states as follows:

![Figure 1.2. Relationship between relative risk of T2D, glycemic load and cereal dietary fiber intake.](image_url)

"Dietary fibre consists of:

1. carbohydrates with a DP of 3 or more that naturally occur in foods of plant origin and that are not digested and absorbed by the small intestine; and
2. accepted novel fibres.

Novel fibres are ingredients manufactured to be sources of dietary fibre and consist of carbohydrates with a DP of 3 or more that are not digested and absorbed by the small intestine. They are synthetically produced or are obtained from natural sources which have no history of safe use as dietary fibre or which have been processed so as to modify the properties of the fibre contained therein. Accepted novel fibres have at least one physiological effect demonstrated by generally accepted scientific evidence." (Health Canada, 2012).

The most universally accepted definition of DF has been adopted in 2009 by the CODEX Alimentarius Commission and states as follows: “Dietary fibre denotes carbohydrate polymers (1) with 10 or more monomeric units (2), which are not hydrolysed by the endogenous enzymes in the small intestine of humans and belong to the following categories:

- Edible carbohydrate polymers naturally occurring in the food consumed.
- Carbohydrate polymers, which have been obtained from food raw material by physical, enzymatic or chemical means and which have been shown to have a physiological benefit to health, as demonstrated by generally accepted scientific evidence to competent authorities.
- Synthetic carbohydrate polymers that have been shown to have a physiological benefit to health, as demonstrated by generally accepted scientific evidence to competent authorities.

Notes:

(1) Includes also lignin and other compounds if quantified by AOAC 991.43.
(2) Decision on whether to include carbohydrates with a degree of polymerization from DP 3 to 9 should be left to national authorities”.

DF, depending on its solubility in water, can be divided into soluble and insoluble. Both types have different molecular characteristics and different physiological effects. Insoluble fibres are known to affect colon health because of their ability to expand fecal bulk, which decreases transit time in the colon. Insoluble fibres include cellulose, some hemicellulose, lignin, cutin, suberin, chitin, chitosan and resistant starches. Soluble DF include inulin, fructooligosaccharides, gums and pectins. Some soluble DF can create viscous solutions (guar, pectin, oat gum and psyllium) known to lower serum cholesterol and blood glucose levels and thus are known as cardiovascular health promoting DF (Dikeman & Fahey 2006; Malkki, 2004).

1.5. Soluble dietary fiber from agricultural products grown in Canada

Cell wall and seed coat of many crops grown in massive amounts in Canada are treated as agricultural by-products and often used as animal feed or even discarded. Often these by-products are rich in polysaccharides that can resist hydrolysis by digestive enzymes and create viscous solutions and gels. Thus those polysaccharides potentially can act as a soluble DF that are left unutilized. Those materials need to be examined to verify their ability to bring physiological benefits. Perhaps some of them can act as DF and can be employed in the food industry. Several agricultural sources of such polysaccharides of Canadian origin are listed below.

1.5.1. Yellow mustard mucilage

Canada is the largest producer of mustard seed in the world. Three most common
mustard types include: yellow (*Sinapis alba*), brown, and oriental (*Brassica juncea*). Mustard seed becomes sticky when wetted due to presence of “mucilage” deposited on the surface of the bran. In the seasoning industry only endosperm of the mustard seed is used, while bran is considered to be a by-product. All mustard varieties contain mucilage, however interest has been driven mostly to yellow mustard mucilage due to its unique functional properties (emulsification, water binding and textural control) and higher yield.

Yellow mustard mucilage (YMM) can be readily extract with water from whole seed (yield ~5%) and from the mustard bran (yield 15-25%). Mucilage contains neutral (47%) and acidic pectic-like polysaccharide (63%). The neutral polysaccharide is composed of (1→4)-linked $\beta$-D-glucosyl backbone where some hydroxyl groups at C2, 3 and 6 are substituted by ethyl and propyl ether groups. Ethyl groups introduce structural irregularity to the cellulose-like backbone chain promoting its solubility (Fig. 1.3). Neutral polysaccharide is the major component responsible for the shear-thinning behavior of YMM. The acidic (pectic-like) polysaccharide is composed of disaccharide backbone repeating units of $\rightarrow$2)-$\alpha$-L-Rham-$\rightarrow$4)-$\alpha$-D-GalpA-$(1\rightarrow$. Oligosaccharide branches are attached to the 4 position of the 2-linked rhamnose residue. The substitution ratio (substituted/unsubstituted) of the O-4 position 2-linked $\alpha$-L-rhamnose is 2:1. The side chains are composed of a terminal 4-O-Me-$\beta$-D-GlcA attached to the 4 position $\alpha$-L-Rhamp in the backbone mainly through 6-linked $\beta$-D-Galp (Cui, Eskin, Wu, & Ding, 2006).

Aqueous solutions of YMM have distinct shear-thinning behavior even at low concentrations resembling the rheological behavior of xanthan gum solutions. Viscosity of the soluble mucilage is affected by the pH of the solution and presence of NaCl. Apparent viscosity (at 93s$^{-1}$) is lowest in the pH range between 4.0 and 7.0. Apparent viscosity of water-soluble YMM increases with increase of NaCl concentration (Cui, 2001a).
Figure 1.3. Molecular structure of (A) neutral fraction where R1 and R2 are ethyl and propyl groups and (B) pectic fraction from the water-soluble fraction of yellow mustard mucilage. © Cui, S. W., Eskin, M. A., Wu, Y., & Ding, S. (2006). Synergisms between yellow mustard mucilage and galactomannans and applications in food products—A mini review. Advances in Colloid and Interface Science, 128, p.25. Fig. 1. Structures of a β-D-glucan (A, R1 and R2 are ethyl and propyl groups) and a pectic polysaccharide (B) from the water-soluble fraction of yellow mustard mucilage.

1.5.2. Soluble flaxseed gum

Canada is also a leader in production and export of oil-type flaxseed. Flaxseed contains mucilage layer which is deposited in the outermost layer of the seed coat. Upon addition of water, mucilage hydrates rapidly and becomes solubilized. Mucilage has been extracted from whole flaxseed (yield 5-9%), flaxseed hull (yield 18%) and flaxseed meal, the primary side-product of the oil crushing industry, (yield 9%). However, the latter one can be highly contaminated with protein (up to 29%).

Water-soluble part of flaxseed mucilage, or also called in the literature “soluble flaxseed gum” (SFG), contains neutral high-molecular weight arabinoxylan 75% and acidic low-molecular weight pectin-like rhamnogalacturonan 25%. The neutral polysaccharides or
arabinoxylans are composed of linear β-(1→4)-xylopyranose backbone substituted with arabinofuranose side chains attached via α-(1→3) and/or α-(1→2) linkages. The neutral fraction is mainly responsible for the high viscosity and weak gellike properties of flaxseed gum. The acidic fraction contains a backbone consisting of rhamnogalacturonan-I which may be interrupted by small amounts of homorhamnan or homogalacturonan (Fig. 1.4) (Cui, 2001b; Qian, Cui, Nikiforuk, & Goff, 2012a).

Rheological properties of SFG aqueous solutions are affected by the pH. The apparent viscosity of SFG solution is the highest in the pH region between 6.0-9.0. The viscosity declines steadily with reduction of pH from 6.0 to 2.0. Increase of NaCl concentration up to 0.1M have been shown to reduce apparent viscosity of SFG solution.

1.5.3. Fenugreek gum

Fenugreek is an annual legume plant mainly grown in warm climates of Mediterranean Europe and South Asia, where it is used for human and animal consumption. Currently fenugreek is being cultivated in Western Canada. The storage polysaccharide of fenugreek seed endosperm is a galactomannan. The galactomannan can be used by plant as a nutrient during seed germination and it also prevents seed from drying by holding moisture.

Locust bean and guar gums are main sources of galactomannans for industry, while fenugreek galactomannan is less common. Commercially, fenugreek gum (FG) is extracted with water or dilute alkali from the endosperm or ground whole seed. From various reports the yield can be from 13.6 to 38%, depending on cultivar and extraction procedure (Prajapati, 2013).

The galactomannans are polysaccharides composed of linear (1-4)-D-mannan backbone with varying amounts of single D-galactose units attached to the main backbone by (1-6)-glycosidic
Figure 1.4. Proposed molecular structure of acidic fraction of water-soluble flaxseed gum.
© Qian, K. Y., Cui, S. W., Nikiforuk, J., & Goff, H. D. (2012). Structural elucidation of rhamnogalacturonans from flaxseed hulls. Carbohydrate Research, 362, p.54. Fig. 7. Proposed repeating unit of the acidic fraction gum. (HR, RG-I and HG refer to homorhamnan, rhamnogalacturonan-I and homogalacturonan, respectively. The locations of HR, RG-I and HG are interchangeable; \((m+n)/(n+i) \approx 1.5\). The substitution rate of R1 is \(\approx 54\%\). R1 is mostly monosaccharide (\(\alpha/\beta\) -D-Galp-(1\-, \(\alpha\)-L-Fucp-(1\- or \(\beta\)-D-Xylp-(1\-). R1 may also occasionally be a longer side chain with more than two residues beginning with \(-4\)-\(\alpha\)-GalpA-(1\- or \(-2\)-\(\alpha\)-L-Rhap-(1\-, wherein the side-chain structure may be similar to part of the main chain.)

bonds (Fig. 1.5). Galactomannans from different sources differ from each other by the mannose/galactose ratio. Presence of galactose side groups disturb molecular regularity of galactomannans reducing their interchain associations. Therefore, water solubility of galactomannans improves with increase of the degree of galactosyl substitution. FG is a galactomannan with the highest degree of galactosyl substitution, where nearly all mannose molecules in the backbone are substituted with a single galactose unit. That makes FG readily dissolved in water. Like many other random coil polysaccharides, aqueous solutions of fenugreek gum exhibits shear-thinning behavior at higher concentrations (Brummer, Cui, & Wang, 2003).

1.5.4. Cereal β-glucan

Cereal β-glucan is the main constituent of starchy endosperm cell walls and the aleurone layer of cereals such as oats, barley, wheat, and rye (Miller & Fulcher, 1994; Wood, 2007). The polysaccharide is also found in the cell walls of baker’s yeast, bacteria, some fungi and algae but
with glucose units joined by primarily by (1-3) linkages and occasional (1-6) branch points. Both types can bring health benefits. The concentration of β-glucan varies depending on the type of cereal. Skendi, Biliaderis, Lazaridou, & Izydorczyk (2003) reports the following β-glucan content of most common cereals as, barley 3-11%, oats 3-7%, rye 1–2% and wheat 1%. The content of β-glucan is also cultivar-depend (Miller & Fulcher, 1994).

Cereal β-glucan is an unbranched homopolysaccharide composed of β-D-glucopyranosyl units joined by (1-3) and (1-4)-β-D-linkages (Fig. 1.6). The β-glucan molecule can be subdivided into three types of structural blocks including cellotriosyl, cellotetraosyl and cellulose-like fragment, that are composed of three, four or 5-28 β-D-glucopyranosyl units joined by (1-4) linkages respectively. These structural blocks are connected to each other by single (1-3) linkages. Blocks are present in the molecule in a random fashion (Izydorczyk & Dexter, 2008).

Cellotriosyl and cellotetraosyl are two main fractions which account for 90-95 % of the cereal β-glucan molecule. The cellotriosyl/cellotetraosyl ratio is unique for each type of cereal and considered to be a fingerprint of β-glucan molecular structure. Lazaridou & Biliaderis (2007)
reports the following cellotriosyl/cellotetraosyl ratios for cereals, wheat (3.0-4.5), barley (1.8-3.5), rye (1.9-3.0) and oats (1.5-2.3). The ratio also can depend of the location of β-glucan in the kernel. In barley and oats, the ratio is higher in aleurone tissues than in starchy endosperm (Izydorczyk, Jacobs, & Dexter, 2003; Wood, Weisz, & Blackwell, 1994a). Presence of (1-3)-β-bonds bring irregularity to molecular structure of the polysaccharide, therefore water-solubility of cereal β-glucan improves with increase in cellotriosyl/cellotetraosyl ratio.

It is considered that the key mechanism by which β-glucan alters glycemic response is related to viscosity of β-glucan aqueous solution (Jenkins et al., 1978; Wood, 2010 and Wood et al., 1994b). Therefore, molecular weight of β-glucan is a critical factor for its bioactivity. The molecular weight values of cereal β-glucan can be as high as 3100 x 10^3 g/mol (Lazaridou & Biliaderis, 2007). However, the genetics and growing environment of the plant as well storage and processing conditions can affect the molecular weight of β-glucan.
1.6. Soluble dietary fiber and glycemia control

Many clinical trials have shown evidence that consumption of some soluble DF of various origins (flaxseed, fenugreek, soybean, pectin, guar gum, sugar-beet, seaweed, yellow mustard and cereals) results in reduction of blood glucose levels.

Study of Thakur, Mitra, Pal, & Rousseau (2009) showed that flaxseed gum can improve blood biochemical profile of patients (120) with T2D. Patients were consuming meals that included bread supplemented with flaxseed gum during 3 months. The control group was consuming same diet but without flaxseed gum. The study showed that, after three months, the fasting blood sugar concentration was significantly lower in treatment group (136 ± 7 mg/dl) than in control group (154 ± 8 mg/dl).

There is evidence that presence of fenugreek gum in the diet can bring physiological benefits. Study of Sharma, Raghuram, & Sudhakar Rao, (1990) showed that defatted fenugreek seed powder (gum 19.2% (w/v)) can alter serum lipid profile and blood glucose levels in diabetic (type 1) patients. Addition of the powder (100g/day) to the diet of patients during 10 days significantly reduced fasting blood glucose as well as total and LDL cholesterol compared to control group.

The impact of soy polysaccharide on plasma glucose of T2D patients was investigated by Tsai, Vinik, Lasichak, & Lo (1987). Patients were divided into two groups: one group consumed control meal, which included 10 g soy polysaccharide enriched noodles, and second group consumed the same meal but without soy polysaccharide. Researchers found that soy polysaccharide supplemented meal significantly reduced postprandial hyperglycemia during 240 min test.
Schwartz et al. (1988) demonstrated the effect of 4 week high apple pectin diet (20 g/day) on plasma glucose. Pectin ingestion significantly decreased the incremental area under the glucose tolerance curve from 34.8 ± 3 mmol/L (observed in T2D patients being on low-fiber diet) to 27.9 ± 3.2 mmol/L (observed in T2D patients on high-fiber diet). Supplementation of diet with pectin resulted also in significant delay of gastric emptying.

Morgan, Tredger, Wright, & Marks (1990) studied the impact of different types of DF on the physiological changes in six healthy subjects. The significant improvement of glucose tolerance with supplementation of meal with guar and sugar-beet fiber (mixture of pectin and insoluble polysaccharide) was observed. Particularly, the peak arterialized glucose levels were 7.26, 8.07 and 9.55 mmol/L for guar-gum, sugar-beet fiber and control meals accordingly. The glucose AUCs were also significantly reduced with both fibers compared to control.

Harden, Richardson, Dettmar, Corfe, & Paxman (2012) tried to manipulate the post-ingestion characteristics of meal and resulting blood glucose levels outcomes by designing a drink formulated with sodium alginate and calcium carbonate. They proposed that after ingestion of the meal with test drink, calcium gets liberated in the acidic environment of the stomach and cross-links with the sodium alginate, resulting in gel formation. According to authors, formation of solid gel particles in the stomach can reduce nutrient uptake and thus result in more controllable glycemia. Indeed, treatment meal resulted in significant reduction of blood glucose peak (14%) and AUC (52%) levels compared to CaCO₃-free control.

In the study of Jenkins et al. (1987) white bread was supplemented with mustard fibre (10g per 50g available carbohydrates) extracted from yellow mustard bran with water. The supplementation resulted in attenuation of glycemic responses in healthy subjects and diabetic patients. The mean GI of mustard fibre-enriched bread was significantly reduced (GI=82)
compared to control bread (GI=100).

The impact of yellow mustard bran on the blood glucose AUC reduction was investigated by Lett, Thondre, & Rosenthal (2013). The mean blood glucose concentrations at 15, 30 and 90 min after ingestion of soup supplemented with 5g yellow mustard bran were significantly lower than after consumption of control soup. Authors hypothesize that observed effect was caused by the presence of soluble DF in yellow mustard bran.

Wheat arabinoxylan also showed potential to decrease blood glucose levels in fourteen subjects during six-week dietary intervention study (Garcia et al., 2007). Consumption of arabinoxylan supplemented bread rolls (15 g) resulted in significant lowering of postprandial glucose and insulin responses. Wheat arabinoxylan can also benefit T2D patients. Lu, Walker, Muir, O’Dea (2004) showed that consumption of diet containing 15 g/day of wheat arabinoxylan during 5 weeks significantly lowered the fasting plasma glucose and insulin levels in diabetic subject.

A large body of published literature supports the statement that cereal β-glucan attenuates postprandial glycemic responses. Tappy, Gugolz, & Wursch (1996) investigated the effect of extruded cereals with three different contents of oat bran on the glycemic response. They found that increase of β-glucan content resulted in a linear reduction of blood glucose. Jenkins, Jenkins, Zdravkovic, Würsch, & Vuksan (2002) found that supplementation of food products with β-glucan resulted in significant decrease of their GI values. On average, every additional gram of β-glucan in the food caused 3.8 unit reduction in a food’s GI. Thondre & Henry (2009) investigated the impact of β-glucan content in flatbread on the postprandial glycemia. Authors found that bread with 4.1 % and 7.8 % of β-glucan reduced the glycemic index by 43 and 47 %, respectively. In the study of Juvonen et al. (2009) human subjects consumed sucrose-containing
beverages supplemented with either high or low molecular weight oat β-glucan. The weight concentration of β-glucan was constant in both beverages. Results showed that the beverage with high-molecular weight β-glucan resulted in significantly lower postprandial glucose and insulin responses than the low-molecular weight β-glucan beverage. Authors also found that gastric emptying was accelerated with reduction of molecular weight of β-glucan.

These studies showed that viscous DF of various botanical nature is capable to attenuate postprandial glycemic responses. However, the exact mechanisms of postprandial blood glucose attenuation after ingestion of meals enriched with soluble DF are not fully understood. It has been however proposed that ability of soluble DF to reduce plasma glucose relates to its capacity to create viscous solution (Ellis, Roberts, Low, & Morgan, 1995; Jenkins et al., 1978 and Wood et al., 1994b).

1.7. Glycemia control health claim for cereal β-glucan

Based on the scientific opinion provided by the panel on Dietetic Products, Nutrition and Allergies, in 2011 the European Food Safety Authority (EFSA) officially recognized the ability of cereal β-glucan to reduce postprandial glycemic responses (European Food Safety Authority, 2011). The opinion of the Panel was based on the studies of Behall et al. (2005), Holm et al. (1992), Juntunen et al. (2002), Liljeberg et al. (1996), Östman et al. (2006) and Yokoyama et al. (1997). Particularly, in randomized cross-over human intervention studies of Liljeberg et al. (1996) and Östman et al. (2006), a significant reduction postprandial glycemic and insulinemic responses after consumption of the test meals that contained β-glucan (4.6-14g per 30g of available carbohydrates) compared to test meal in healthy humans was observed. In the study by Juntunen et al. (2002) postprandial insulinemic responses for test meal (5.4 g of β-glucan per 50g
of available carbohydrate) were significantly reduced compared to control while the effect on postprandial glycemic responses was not significant. Holm et al. (1992) and Yokoyama et al. (1997) studied the effect of supplementation of pasta with cereal β-glucan on postprandial glycemic and insulinemic responses in healthy humans. Consumption of pasta enriched with β-glucan (3.6g per 30g of available carbohydrates) significantly reduced both postprandial responses (Yokoyama et al., 1997). In the study of Holm et al. (1992) supplementation of pasta with β-glucan (6%) did not affect postprandial glycemic responses but significantly attenuated postprandial insulinemic responses. In the work of Behall et al. (2005) human subjects consumed oat and barley β-glucan containing treatments (1.8 and 6.5g/30g available carbohydrates, respectively). Both treatments significantly reduced peak glucose and insulin concentrations and area under the curve for glucose responses compared to control. The attenuations were significantly lower for barley test foods than for oat test foods, which could be due to the difference between amounts of β-glucan used in two treatments. Based on these evidence panel concluded that supplementation of a meal with 4 g of cereal β-glucan per each 30 g of available carbohydrates can help to attenuate postprandial glycemic responses.

1.8. Relationship between viscosity of dietary fiber and lowering postprandial glycemia

Several studies suggest that meals enriched with soluble DF can reduce postprandial glycemia as a result of their ability to create viscous solutions. Jenkins et al. (1978) found that 50 g glucose containing drinks supplemented with 14.5 g various DF including: guar, pectin, gum tragacanth and methylcellulose significantly reduce mean peak in blood glucose. That reduction was positively correlated with the viscosities of the DFs. Conversely, hydrolyzed guar gum resulted in lower viscosity drink and reduced ability to attenuate blood glucose.
The effect of viscosity of cereal \( \beta \)-glucan solution on the glycemic response was investigated by Wood et al. (1994b). Particularly, the effect of \( \beta \)-glucan concentration and molecular weight was investigated. Subjects consumed drinks fortified with cereal \( \beta \)-glucan rich “oat gum” (the production process described by Wood, Weisz, Fedec, & Burrows (1989). A negative linear relationship between glucose response and viscosity of the drink was found, and a similar trend was observed for insulin response. The viscosity of solution usually depends on the concentration and molecular weight of solute. Thus, the same level of viscosity can be obtained through adjusting of one or both of these factors. In consequence, the reduction in plasma glucose and insulin levels can be achieved by the consumption of low molecular weight oat \( \beta \)-glucan at high doses or by high molecular weight at lower doses (Wood et al., 1994b).

There is evidence that intake of viscous DF results in glycemia reduction due to alteration of gastric and/or small intestinal luminal viscosity. Guerin et al. (2001) showed that some DF can increase apparent viscosity (at 45 s\(^{-1}\)) of gastric contents in pigs. Cherbut, Albina, Champ, Doublier, & Lecannu (1990) found that besides enhancing apparent viscosity (at 10 s\(^{-1}\)) of gastric contents, guar gum also increased viscosity of duodenal and jejunal digesta in pigs. In both studies it was found that DF, which was more successful in enhancing luminal viscosity, was also more effective in increasing transit time in the upper GI tract, particularly gastric retention time and retention time between mouth and the ileal cannula in works of Guerin et al. (2001) and Cherbut (1990) respectively (one of the mechanisms believed to be involved in glycemia attenuation). Roberts, Smith, & Low (1990a,b) also found that supplementation of meal with guar gum increased zero-shear viscosity of jejunal digesta in pigs. A similar result was found by Ellis et al. (1995), who additionally showed a negative correlation between zero-shear viscosity of jejunal digesta and glycemic responses in pigs.
1.9. Mechanism involved in attenuation of postprandial plasma glucose after DF intake

Even though the relationship between viscosity and ability to reduce blood glucose has been generally accepted, the exact impact of viscosity is unclear. It is unclear what stage of digestion (gastric or small intestinal) is influenced by viscosity. It is also unclear how viscosity can affect digestive enzymes and hormones produced by the body during digestion or how viscosity can affect motor activity of gastrointestinal tract. Three most popular proposed mechanisms are: 1) delayed gastric emptying, 2) delayed diffusion of hydrolyzed starch fragments or glucose in the small intestine, and 3) reduced digestive enzyme activity in the small intestine (Dikeman & Fahey, 2006).

After consumption, food undergoes physical disintegration due to contractions in the stomach. The most severe disintegration occurs in the antrum portion of the stomach, where food becomes partly liquefied (chyme) as a result of high frequency contractions. Later, chyme gets emptied by peristaltic movements from the antrum through pylorus into the duodenum. The rate of gastric emptying is coordinated by the neural and hormonal feedback of the receptors in the duodenum. The gastrointestinal tract reacts by adjusting the pressure on the digesta in the fundus of the stomach and regulating duodenal and antral contractions (Gropper, Smith, & Groff, 2005b).

The rate of emptying can vary from 2 to 10 mL per minute and is influenced by the physical properties, composition, volume and osmolality of the chyme. Thus, the level of nutrients delivered (including available carbohydrates) from stomach to the small intestine for digestion and absorption can also vary significantly, depending on the gastric emptying. Therefore, it is expected that alterations of gastric emptying rate would affect glycemic response (Gropper et al., 2005b; Ma, Rayner, Jones, & Horowitz, 2009). In fact, Gonlachanvit et al. (2003) showed that
postprandial glycemic response in diabetic patients can be controlled by pharmacological alteration of gastric emptying. In that study, the intake of gastric emptying accelerating erythromycin resulted in higher postprandial plasma glucose concentrations. Contrarily, morphine, which delays gastric emptying, caused lower plasma glucose concentrations.

The infusion of 25% glucose solution directly into the duodenum of human participants at different rates 1, 2 and 4 kcal/min, simulated different gastric emptying rates (Pilichiewicz et al., 2007). The increase of glucose infusion rate resulted in higher glucose and insulin responses in blood. The experiment showed that gastric emptying rates have direct influence on the postprandial glycemic response.

There are several other studies that support the hypothesis that the delay of gastric emptying after consumption of meal enriched with viscous DF is a main mechanism involved in attenuation of blood glucose. Holt, Carter, Heading, Prescott, & Tothill (1979) studied the mechanisms involved in attenuation of glycemic responses and suggested that viscous DF effects attenuation of glycemic response by altering gastric emptying rates. The study involved seven healthy volunteers and a man who had total gastrectomy (full stomach removed). The consumption of orange juice (400 mL) supplemented with pectin (10 g) significantly slowed gastric emptying in volunteers, when compared to control. The gastric emptying half time ($T_{1/2}$) (when half of the consumed meal has been emptied from the stomach) was longer for meal supplemented with DF ($49.9 \pm 15.2$ min) when compared to control ($23.1 \pm 5.5$ min). Pectin supplemented juice also significantly attenuated postprandial blood glucose concentration in volunteers. However, it did not alter blood glucose in a patient after total gastrectomy.

Similarly, in the study by Leclere et al. (1994) the effect of viscous DF ingestion on gastric emptying and attenuation of glycemic responses was investigated. In this study the test meals
were prepared using ether high viscosity or low viscosity guar gum at concentration 5.6%. Meals were administrated either orally, or pre-digested (with pepsin for 30 min, diluted to 9% and neutralized) and infused directly into duodenum using feeding tube. The postprandial insulin concentration was lower after oral consumption of glucose containing meal supplemented with high viscosity guar gum, than after consumption of control glucose containing meal (without gum). When the same guar gum meal was pre-digested and administered directly into the duodenum, the reduction of insulin concentration was not observed. Thus authors concluded that the delay in diffusion rate in the duodenum as a result of chyme viscosity does not have significant impact on the glycemic response. Instead gastric emptying seems to be the main mechanism.

The results from the already mentioned study with the apple pectin diet (Schwartz et al., 1988) suggest that pectin ingestion decreases plasma glucose concentration and increases T\(\text{t}_{1/2}\). Particularly, the T\(\text{t}_{1/2}\) was prolonged from 83.4 ±6.8 min to 119.3 ± 14.4 after pectin was administered. When high DF diet was discontinued and replaced by low DF diet the gastric emptying went back to faster rates.

The delay of gastric emptying as a result of meal enhancement with viscous DF was also observed in other studies in following paragraphs. However, none of these studies included postprandial blood glucose measurements. Darwiche, Björgell, & Almer (2003) demonstrated that gastric emptying rate was delayed after consumption of 300 g rice pudding containing 58% carbohydrate supplemented with 6g Nestargel (high in locust been gum commercial product). The gastric emptying rate for period from 15 to 90 min after ingestion of meal was expressed as the percentage of antral cross-sectional area reduction measured by ultrasound. The result showed that the gastric emptying rate was slowed to 54% with addition of DF compared to 63% in
Marciani et al. (2001) described echo-planar magnetic resonance imaging technique as suitable for measuring gastrointestinal volumes with high precision and thus useful in the noninvasive determination of secretions and emptying rates in the gastrointestinal tract. In this study slower (4.7 ± 0.3 ml/min) gastric emptying rate was observed after consumption of dextrose containing meals with high viscosity locust bean gum compared to low viscosity gum (6.1 ± 0.6 ml/min). Additionally, the gastric secretion volumes at 60 min were found to be higher (75 ± 13 mL) after ingestion of dextrose meals supplemented with high viscosity gum, than after ingestion of low viscosity gum (51 ± 23 ml). The addition of nutrients including lipids and carbohydrates into the meal formulation maintained the same trends of gastric emptying and secretion but the values were higher than just for the dextrose containing meal.

Effect of locust bean gum concentration (0.35 g/100mL and 0.45 g/100mL) on the gastric emptying of thirty-nine infants with regurgitation episodes was studied by Miyazawa, Tomomasa, Kaneko, & Morikawa (2006). The gastric emptying as determined by measuring antral cross-section areas ultrasonographically was significantly reduced at 60, 90, 120 and 150 min after injection of milk-based formula supplemented with locust bean gum at concentration 0.45 g/100mL. Formula supplemented with gum at concentration 0.35 g/100mL significantly delayed gastric emptying, but only for 60 min. Authors concluded that gastric emptying in infants was reduced due to the increase of meal viscosity.

The postprandial motor activity in the small bowel after consumption of glucose drink or solid meal with and without 5 g of guar gum was studied by Schonfeld, Evans, & Wingate (1997) using catheters supplemented with pressure sensors. Supplementation with guar gum prolonged the motor activity in the small intestine after consumption of both glucose drink (from
123 ± 19 min to 199 ± 24 min) and solid meal (from 310 ± 92 min to 419 ± 22 min). Authors suggested that prolonged motor activity was a result of slower gastric emptying.

Gastric emptying controls the delivery of carbohydrates to the small intestine and thus has a direct impact on the postprandial glycemic responses. Many studies showed that viscous DF can delay gastric emptying where it was suggested that attenuation of postprandial glycemic responses after ingestion of viscous DF can be a result of the gastric emptying delay.

However, some researchers argue that gastric emptying is not the main mechanism related to attenuation of blood glucose and insulin levels after ingestion of viscous DF. Other mechanisms associated with the intestinal viscosity can also affect the blood glucose and insulin levels. In several studies the effect of viscous DF (guar gum (Rydning, Berstad, Berstad, & Hertzenberg, 1985; van Nieuwenhoven, Kovacs, Brummer, Westerterp-Plantenga, & Brouns, 2001) and alginate (Hoad et al., 2004) on the gastric emptying rate was not found.

The effect of several polysaccharides including xanthan, locust bean and guar gums on gastric emptying and lowering postprandial glucose and insulin levels in sixteen volunteers were tested by Edwards et al. (1987). Volunteers consumed a 250 mL drink containing 50 g glucose and 2.5 g of the polysaccharide. Additionally, to determine gastric emptying $^{99m}$Tc sulphur colloid tracer was added to the drink. The combination of xanthan gum and locust bean gum had the strongest impact on gastric emptying delay, however, that formulation did not result in greater differences in glucose or insulin levels than other gums. The xanthan gum alone did not show any effect on the reduction of gastric emptying rate however it was successful in reducing the plasma glucose and insulin. Furthermore, locust bean gum accelerated gastric emptying rate, at the same time resulting in attenuation of postprandial plasma glucose and insulin levels. These results suggest that mechanisms other than gastric emptying could be involved in attenuation in
blood glucose or insulin levels after ingestion of viscous DF. Blackburn, Holgate, & Read (1984), Jarjis, Blackburn, Redfern, & Read (1984), Hlebowicz, Darwiche, Björgell, & Almér (2008) and Sandhu, El Samahi, Mena, Dooley, & Valenzuela (1987) also did not find a correlation between gastric emptying rate and postprandial blood glucose levels after consumption of meals supplemented with various hydrocolloids.

The relationship between absorption rates of nutrients (carbohydrate, protein, fat and energy) and guar gum concentration in the meal was studied by Ehrlein & Stockmann (1998). The low and high caloric density test meals with guar gum at five different concentrations (0-4.4 g/L) were perfused directly into the jejunum of four miniature pigs, thus avoiding gastric phase. It was found that nutrient absorption declined with an increase of gum concentration. That decline is believed to be related to the increased viscosity in the small intestine.

In the work by Meyer, Gu, Jehn, & Taylor (1988), the impact of 0.2 mol/L glucose solution with and without one of the hydrocolloids (pectin 33 g/L, polyethylene glycol (PEG) 400 g/L or guar gum 11g/L) on blood glucose and gastric emptying rates in dogs was investigated. Each dog used in the experiment had a cannula in the second portion of the duodenum and sewn tubing beyond the cannula. On the day of the experiment, catheter was inserted and its balloon created a tight seal. The set up was organized in such way that chyme emptied from the stomach could be removed and immediately exchanged for a different solution and infused into the duodenum at the same rate. The impact of various combinations of arrangements 1. stomach-glucose, duodenum-glucose, 2. stomach-glucose, duodenum-hydrocolloid, 3. stomach-hydrocolloid, duodenum-glucose and 4. stomach-hydrocolloid, duodenum-hydrocolloid were investigated. It was found that blood glucose and gastric emptying rates were lower if hydrocolloid solution was present in both intestine and stomach at the same time. Study indicates that intestinal viscosity
also plays an important role in the glycemic response. These studies suggest that mechanism(s) other than delay of gastric emptying can be involved in attenuation of glycemic responses.

Several *in vitro* studies showed that presence of viscous DF in the small intestinal lumen can 1) delay diffusion of glucose (Jenkins, Jenkins, Wolever, Taylor, & Ghafari, 1986; Kwong, Wolever, Brummer, & Tosh, 2013; Lecumberri et al., 2007; Ou, Kwok, Li, & Fu, 2001), thus DF can slow transport of hydrolyzed starch fragments and glucose for further digestion and absorption at the absorptive surfaces, and 2) reduce activity of digestive enzymes in the small intestine, (Shelat et al., 2010; Shelat, Vilaplana, Nicholson, Gidley, & Gilbert, 2011; Ou et al., 2001) thus slowing the rate of carbohydrate hydrolysis. Those processes can potentially result in attenuation of glycemic responses. The activity of digestive enzymes would also depend on the diffusion rate of these enzymes to the substrate in the lumen. Shelat et al. (2010) and Shelat et al. (2011) showed that viscous DF can restrict diffusion of particles with a size similar to α-amylase. Thus the presence of viscous DF in intestinal lumen can reduce rates of starch hydrolysis by pancreatic amylase due to the reduction of its activity. The more deliberate literature review for last two mechanisms that can be evolved in attenuation of glycemic responses due to DF consumption, delayed diffusion of hydrolyzed starch fragments in the small intestine and reduced digestive enzyme activity in the small intestine, can be found in introductions for Chapter 4 and Chapter 5 respectively.

### 1.10. Overview of *in vitro* digestion techniques

*In vitro* digestion is often used in the research fields such as food science, animal science and pharmacology as it has several advantages over *in vivo* studies. The benefits of *in vitro* digestion include lower cost, the absence of ethical limitations, possibility to use toxic materials and high
control over the experiment. However, *in vitro* models often have limitations and cannot fully simulate all complex processes occurring in a real gastrointestinal tract, and thus cannot guarantee predictive values (Venema, Havenaa, & Minekus, 2009).

Typically, *in vitro* digestion techniques fall into two categories: static models, where conditions of digestion remain fixed and do not mimic dynamic gradual processes occurring *in vivo* such as, various intensity peristaltic movements, pH changes, absorption of products of digestion and water, gastric and intestinal secretions and gastrointestinal transit including gastric emptying; and dynamic models, which try to represent these processes (Parada & Aguilera, 2007).

First static *in vitro* digestion methods were proposed by Southgate (1969), Englyst, Wiggins, & Cummings (1982) and Jenkins et al. (1982). The interest to *in vitro* digestion started to expand in the beginning of 1990s, as a result, large variety of methods sometimes with significant differences were reported (Woolnough, Monro, Brennan, & Bird, 2008). Methodology of many static models used for carbohydrate digestion is usually based on 3-stage digestion:

1. Oral stage. Mechanically disrupted food is mixed with a simulated salivary fluid containing $\alpha$-amylase, the sample is incubated for 3-15 min at pH 5.0-6.9. Actual chewing of food samples by volunteers was used in some models instead of simulated oral phase.

2. Gastric phase. Simulated gastric fluid containing pepsin is added to the digesta after 1$^{st}$ stage bringing pH down to values 1.1-2.8 and sample is allowed to incubate for 30-180 min at 37 °C. Pepsin is used to hydrolyze protein in the food samples that can restrict susceptibility of starch to amylolysis.

3. Duodenal phase. Simulated intestinal fluid containing pancreatin, amyloglucosidase and sometimes invertase and bile fluids are added to digesta after 2$^{nd}$ stage bringing pH to values 6.3-
7.8, and digesta is incubated for additional 60-360 min at 37°C. Concentration of sugars liberated during starch digestion is often determined using colorimetric methods such as glucose oxidase-peroxidase method that allows to determine glucose concentration or methods that are used to determine total reducing sugar content such as dinitrosalicylic acid method (Hollebeeck, Borlon, Schneider, Larondelle, & Rogez, 2013).

Typically, static models do not represent dynamic physiological processes occurring in the gastrointestinal tract during digestion. To improve in vitro-in vivo correlation, more biorelevant dynamic models were developed to simulate rapidly changing environment in the human gut lumen (Minekus, Marteau, & Havenaar, 1995; McAllister, 2010). Below is a list of some dynamic models reported in the literature.

A two compartmental artificial stomach duodenal model (ASD) has been used in several studies to test dissolution processes of drug products. The model utilizes peristaltic pumps controlled by computer to represent dynamic gastric and duodenal secretions and gastric emptying. The gastric reservoir receives flux of simulated gastric fluid at a controlled rate. Contents of the gastric reservoir are simultaneously emptied at the certain rate into the duodenal compartment (Carino, Sperry, & Hawley, 2006; Castela-Papin et al., 1999).

Kong & Singh (2010) introduced human gastric simulator (HGS) that represents closely breakdown and disintegration of semi-solid food in the stomach by applying mechanical forces that mimic realistic continuous peristaltic waves. The model also simulates biological gastric secretions and emptying. First, to simulate oral digestion, a food sample is mixed with simulated saliva and allowed to rest for 2 min. After the oral phase, sample is placed into the latex chamber maintained at 37°C and preloaded with 50 mL simulated gastric juice (pepsin 1 g, gastric mucin 1.5 g, NaCl 8.775 g in 1 L distilled water) to mimic the presence of gastric juices during fasting.
as it is observed in vivo. At the same time, the gastric juice secretion is resumed at the rate 2.5 mL/min. Gastric emptying is mimicked by taking 45 mL of digesta every 15 min, matching the emptying rate of 3 mL/min. Authors concluded that HGS can be a useful tool to study processes occurring in the human stomach.

The TIM system developed by TNO nutrition and research institute is the most sophisticated multicompartmental model in which many dynamic physiological digestion processes are taken into account (Minekus et al., 1995). It contains gastric, duodenum, jejunum and ileum compartments. Each compartment has a form of a tube with flexible walls inside and glass surface. The movement of digesta through the tube is achieved by creating peristaltic motions by controlling the water pressure between flexible walls and glass surface. The digesta is transferred from one compartment to another though pipes using peristaltic pumps. The jejunum and ileal compartments are connected with hollow-fiber devices, which allow absorbing water and products of digestion. The gastric and ileal delivery is controlled by computer managed peristaltic pumps programmed to follow Elashoff, Reedy, & Meyer (1982) formula (Equation 1.2).

\[ f = 1 - 2^{-\left(\frac{T}{T_{1/2}}\right)^{\beta}} \]  
(Equation 1.2)

where \( f \) is a fraction of a meal delivered, \( T \) time, \( T_{1/2} \) half-time delivery and \( \beta \) parameter that describes the shape of the curve. The pH in the gastric compartment is controlled by a computer using 1M HCl or water to follow values previously predetermined in vivo. In duodenal compartment pH 6.4 is maintained by injecting either 1M NaHCO\(_3\) or water. Pepsin, lipase, bile, pancreatic, jejunal, ileal fluids are secreted at controlled rates in appropriate compartments. The digestion of carbohydrate, protein and fat using the first version of the model TIM-1 has been validated (Venema et al., 2009).
TIM system is believed to represent many physiological processes occurring during digestion \textit{in vivo} more closely than other models. However, its high cost may make that option not viable to many laboratories.

1.11. Conclusions

Some soluble dietary fibre can attenuate glycemic responses and thus are recommended to people with impaired glucose tolerance to reduce the risk of type 2 diabetes and to diabetic patients to decrease the number of hyperglycemic events. Many hydrocolloids that can have physiological benefits, including attenuation of postprandial glycemic responses, can be extracted from Canadian agricultural products or even from by-products. Unfortunately, some of these sources of dietary fibre are left unutilized. The possibility of these hydrocolloids to bring health benefits needs to be investigated.

Attenuation of plasma glucose relates to the ability of a soluble dietary fibre to create a viscous solution. However, the exact mechanism of glycemic response attenuation by the viscous dietary fibre is unclear (Dikeman & Fahey, 2006). Some believe that the mechanism is related to the alteration of rheological properties of digesta in the small intestine (Ellis et al., 1995). As a result, diffusion of glucose becomes limited (Jenkins et al., 1986) and the activity of digestive enzymes slows down (Hansen & Schulz, 1982). Others believe that delay of gastric emptying is the major route (Schwartz et al., 1988). Literature supporting both hypotheses can be found.

Understanding more about mechanism involved in attenuation of glycemic responses can help to design foods that reduce blood glucose more effectively. For instance, it is known that rheological properties of some hydrocolloids depend on pH. Thus some hydrocolloids at the same concentration can be more viscous at lower pH (such as pH of the stomach), and some at
higher pH (such as pH of the small intestine). Understanding which phase of digestion, gastric or intestinal, plays major role in postprandial glucose attenuation would help to choose right hydrocolloid for application.

The interest in dietary fibre is growing as the quantity of scientific evidence supporting its physiological benefits is increasing. Additionally, the deteriorating health of humankind and ageing baby boomer generation imposes the need for the development of strategies for treatment of serious disorders such as cardio-vascular diseases and diabetes. The knowledge about dietary fibre is thus of great importance since it could help to mitigate and/or solve these problems.
CHAPTER 2. RHEOLOGICAL BEHAVIOR OF DIETARY FIBRE IN SIMULATED SMALL INTESTINAL CONDITIONS

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Abstract

Rheological properties of water-soluble dietary fibre (DF), including yellow mustard mucilage (YMM), soluble flaxseed gum (SFG), fenugreek gum (FG) and oat gum (OG) were studied following a simulated gastric and small intestinal digestion. Experimentally obtained steady shear flow curves of FG, SFG and OG were fitted to the Cross model. Based on the values of the Huggins constant, small intestinal fluid at 37°C is a good solvent for SFG and FG and a theta solvent for OG. The degree of space occupancy at critical overlap concentration ($c^*[\eta]$) for OG and FG were lower than expected from “normal” concentration-dependency. This departure was attributed to intermolecular associations of these DF in simulated small intestinal conditions. Apparent viscosities (at 60 s\textsuperscript{-1}) of simulated small intestinal digesta containing oat β-glucan at concentrations expected in the small intestine after consumption of a meal that meets from 0.5 to 3 times the value of the European Food Safety Authority (2011) health claim on reduction of postprandial glycemic responses were determined and were used as benchmarks. Concentrations of YMM, SFG and FG that resulted in apparent viscosities close to the benchmark apparent
viscosities in simulated small intestinal conditions were found. In the majority of cases, these concentrations were different for each DF.

2.1. Introduction

Diabetes mellitus is a chronic disease, arising from the inability of the body to effectively metabolize glucose, thus resulting in abnormally high postprandial plasma glucose levels. Chronic high blood glucose levels damage body tissues, causing a variety of serious complications. As a result, individuals suffering from diabetes have twice higher mortality risk than those without diabetes (Skyler, 2012). World Health Organization projects that the number of deaths from diabetes will increase more than 50% in the next 10 years and by 2030 diabetes will become the 7th leading cause of death in the world (World Health Organization, 2015). Studies suggest that Type 2 diabetes, which accounts for up to 90% all diabetes cases, can be prevented or at least delayed with lifestyle interventions, such as reduction of consumption of meals resulting in hyperglycemia (Lindstrom et al., 2003; Tuomilehto et al., 2001; Willett et al., 2002). Research evidence has also shown that supplementation of a meal with some types of DF can attenuate glycemic responses. Thus diets rich in DF are an important management strategy for these with Type 2 diabetes and can potentially reduce risk of becoming diabetic (Salmeron et al., 1997).

DF can be divided into water soluble and insoluble fractions. Both types have different molecular characteristics and physiological effects on gastrointestinal function. Some soluble DF (guar, pectin, oat gum and psyllium) can develop viscous solutions (viscous DF) and these have the potential to lower serum cholesterol and plasma glucose levels (Dikeman & Fahey, 2006; Malkki, 2004).
It has been proposed that the ability of soluble DF to reduce plasma glucose relates to its capacity to create a viscous solution (Jenkins et al., 1978; Wood et al., 1994). There is evidence that intake of viscous DF results in glycemia reduction due to alteration of gastrointestinal luminal viscosity (Ellis et al., 1995; Roberts et al., 1989; Roberts et al., 1990). Even though the relationship between luminal viscosity and ability of DF to attenuate plasma glucose has been generally accepted, the exact mechanism of that attenuation is unclear (Dikeman & Fahey, 2006).

Agricultural by-products of many crops grown in abundance in North America contain polysaccharides that can resist hydrolysis by digestive enzymes and create viscous solutions. Thus those polysaccharides potentially can act as viscous DF. For instance, bran of yellow mustard seed contains “mucilage” deposited on its surface. When wetted “mucilage” forms a viscous mass. The mucilage is composed of a neutral (1→4)-linked β-D-glucan (47%) and an acidic pectic-like polysaccharide (53%). The neutral polysaccharide contains a (1→4)-linked β-D-glucosyl backbone where some hydroxyl groups at C2, 3 and 6 are substituted by ethyl and propyl ether groups. Ethyl groups introduce structural irregularity to the cellulose-like backbone chain promoting its solubility. The neutral polysaccharide is mainly responsible for the high viscosity of the mucilage in aqueous solution (Cui, 2000).

The surface of the flaxseed hull also contains mucilage that can be easily extracted with water. The mucilage is composed of neutral high-molecular weight arabinoxylanan (75%) and acidic low-molecular weight pectin-like rhamnogalacturonan (25%). The neutral polysaccharides are composed of a linear β-(1-4)-xylopyranose backbone substituted with arabinofuranose side chains attached via α-(1-3) and/or α-(1-2) linkages. The neutral fraction is mainly responsible for the high viscosity and weak gel like properties of flaxseed gum (Qian, Cui, Wu, & Goff, 2012b).
Fenugreek is an annual legume plant mainly grown in warm climates of Mediterranean Europe and South Asia, where it is used for human and animal consumption. Currently fenugreek is being cultivated in Western Canada. The storage polysaccharide of fenugreek seed endosperm is a galactomannan. Nearly all mannose molecules in the backbone of the fenugreek gum are substituted with a single galactose unit. Similar to many other random coil polysaccharides, fenugreek gum exhibits shear-thinning behavior at higher concentrations (Brummer et al., 2003).

Cereal β-glucan is an unbranched homopolysaccharide composed of β-D-glucopyranosyl units joined by (1-3) and (1-4)-β-D-linkages. The β-glucan molecule can be subdivided into three types of structural blocks including cellotriosyl, cellotetraosyl and cellulose-like fragment, that are composed of three, four or 5-28 β-D-glucopyranosyl units joined by (1-4) linkages respectively. These structural blocks are connected to each other by single (1-3) linkages. Blocks are present in the molecule in a random fashion (Izydorczyk & Dexter, 2008). Cereal β-glucan is the main element of starchy endosperm cell walls and the aleurone layer of many cereals (Miller & Fulcher, 1994). A large body of published study supports the opinion that cereal β-glucan attenuates postprandial glycaemic responses (some of the recent publications include Brummer, Duss, Wolever, & Tosh (2012); Juvonen et al. (2011); Kwong et al. (2013)). Additionally, in 2011 the European Food Safety Authority (EFSA) officially recognized the ability of cereal β-glucan to reduce postprandial glycemic responses.

The rheological properties of various non-starch polysaccharides in solution, mostly in water at 20-25°C, have been studied extensively. Nevertheless, conditions specific to the human digestive tract, including ionic strength, presence of divalent cations, pH and temperature, can affect the conformation of polysaccharide molecules and interactions between them. Thus, the
present work focuses on rheological properties of four types of DF in conditions similar to human small intestine.

The objectives of the present study were: to compare rheological properties of four different types of DF at various concentrations in simulated small intestinal conditions; and to determine concentrations of each DF that would result in apparent viscosities close to those of cereal β-glucan in simulated small intestinal conditions and physiological shear rate.

2.2. Materials and methods

2.2.1. Extraction of yellow mustard mucilage and soluble flaxseed gum

Yellow mustard mucilage (YMM) and soluble flaxseed gum (SFG) were extracted from yellow mustard bran (G.S. Dunn, Hamilton, Canada) and flaxseed hull (Natunola Health Inc., Winchester, Canada) respectively. Mustard bran and flaxseed hull were soaked in cold tap water (1:20 (Weber, Taillie, & Stauffer, 1974) and 1:12 respectively) at room temperature during 15 h. Spent bran and hull were removed from the extract by filtering through a sieve. The obtained extract was centrifuged (10,000 g, 10°C, 60 min). A thin formed layer on the top was discarded and resulting supernatant was freeze-dried and further soaked in food grade ethanol solution (68% v/v for yellow mustard and 71% v/v for flaxseed extracts) during 15 h at room temperature. Extracts were recovered from ethanol and air-dried during 72 h at room temperature. Yellow mustard mucilage (YMM-ETH) and soluble flaxseed gum (SFG-ETH) were used as a starting material for purified yellow mustard mucilage (YMM) and purified soluble flaxseed gum (SFG).

To prepare YMM and SFG, YMM-ETH and SFG-ETH were dissolved in phosphate buffer (pH 7.5, 50 mM) supplemented with NaN₃ (0.02% w/v) during 5h at room temperature with
constant stirring. Further, protein in samples was hydrolyzed with protease from *Streptomyces griseus* (Sigma-Aldrich, P-8811) (enzyme/protein = 1/10) using shaking waterbath set at 37°C during 36 h. Then, solutions were centrifuged (4500 g, 22°C, 30 min) and the resulting supernatant was dialyzed (MWCO: 6-8,000, Spectrum Laboratories, Inc., Rancho Dominguez, United States) against distilled water at room temperature during 72 h and after that centrifuged again (4500 g, 22°C, 30 min). The volume of the supernatant was reduced to 1/3 of original volume using a vacuum evaporator at 80°C. Further, gums were precipitated with ethanol (final concentration 68% v/v for YMM and 71% v/v for SFG) and the resulting mixture was centrifuged (2000 g, 20 min, 22°C). The precipitate was air-dried at room temperature during 48 h, dissolved in distilled water (5 h, 22°C) and finally freeze-dried resulting in YMM and SFG.

### 2.2.2. Extraction of oat gum

The extraction procedure of high β-glucan oat gum (OG) was slightly modified from the dual-enzyme procedure described in the work of Lazaridou, Biliaderis, Micha-Screttas, & Steele (2004). Oat bran concentrate (OBC) (Viterra, Portage la Prairie, Canada) with concentration of β-glucan ≈ 20 % was used as a starting material. First, endogenous enzymes in OBC were deactivated in reflux 82% v/v ethanol solution at 85°C during 2 h. Deactivated OBC was precipitated using centrifugation (5000 g, 15 min, 22°C), washed three times with 95% v/v ethanol solution and air-dried at room temperature during 72 h. Further, OBC was extracted with distilled water (1:30) at 52°C during 2 h of constant stirring, following centrifugation (4000 g, 15 min, 22°C). Starch in the supernatant was hydrolyzed with 1% v/v preheated (95°C, 30 min) thermostable α-amylase (Termamyl 120 L, Novozymes A/S, Bagsvaerd, Denmark) at 95°C, pH 4.5 during 3 h. After that, the mixture was centrifuged again (4000 g, 15 min, 22°C) and the
resulting supernatant was supplemented with NaN₃ (0.02% w/v). Further, protein was hydrolyzed by incubating the mixture with protease (19 mg/100 mL extract) from Streptomyces griseus during 36 h in the shaking waterbath set at 37°C. The pH of the solution was maintained at value 7.5 with 1N NaOH throughout the incubation. Then, the mixture was centrifuged (4000 g, 15 min, 22°C) and resulting supernatant was dialyzed during 72 h against distilled water. The volume of the solution was reduced to 1/3 of the original volume using vacuum evaporation at 80°C. Gum from the solution was precipitated with ethanol (final concentration 71% v/v) following centrifugation (2000 g, 20 min, 22°C). The precipitate was air-dried at room temperature during 48h, dissolved in distilled water (90°C, 2 h) at 1% w/v and finally freeze-dried. The cereal β-glucan content in OG was determined according to AACC 32-23.

2.2.3. Extraction of fenugreek gum

CANAFEN® Gum (Emerald Seed products Ltd., Avonlea, Canada) was used as a starting material for fenugreek gum extraction. CANAFEN® Gum was stirred with cold tap water (1:200) at room temperature during 15 h. The mixture was centrifuged (10,000 g, 22°C, 60 min) and the resulting supernatant was freeze-dried. Water-extracted fenugreek gum (FG-WE) was used as a starting material for purified fenugreek gum (FG).

To prepare FG, FG-WE was dissolved (90°C, 1 h) in distilled water at 0.8% w/v. The gum from the resulting solution was precipitated with ethanol (final concentration 64% v/v) following centrifugation (2000 g, 20 min, 22°C). The precipitate was air-dried at room temperature during 48h and then dissolved in distilled water (3 h, 90°C) at 0.8% w/v. The FG was obtained by freeze-drying the resulting solution.
2.2.4. Chemical composition analysis

The total sugar content was determined using phenol-sulfuric acid assay (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The absorbance was read at 490nm. Glucose standard solutions were used to construct a calibration curve. Protein was determined using LECO FP-528 Nitrogen/Protein analyzer (LECO Corporation, Saint Joseph, United States) as described in AACC 46-30.01 method, nitrogen to protein conversion factor 6.25 was used. Total ash was determined according to AACC 08-01.01 method. The m-hydroxyphenyl colorimetric method was used to determine the uronic acid content of YMM and SFG (Blumenkrantz & Asboe-Hansen, 1973). Glucuronic acid and galacturonic acid (1:1) solutions were used as standards. Each sample was analyzed in triplicate.

2.2.5. Neutral monosaccharide composition analysis

The neutral monosaccharides analyses were conducted as described in the work of Ding et al. (2014) using high performance anion exchange chromatography system (HPAEC) coupled with a pulse amperometric detector (PAD) (Dionex-5500, Dionex Corporation, California, United States). In short, DF were hydrolyzed with H₂SO₄ at 100°C for 2h, and then diluted with Milli-Q water. Samples were filtered and injected into HPAEC for analyses. Arabinose, fucose, galactose, glucose, mannose, rhamnose and xylose standards were used.

2.2.6. High-performance size exclusion chromatography

Weight average molecular weight (Mw), number average molecular weight (Mn), intrinsic viscosity [η], radius of gyration (Rg) and polydispersity index (PI) of DF were determined using high performance size exclusion chromatography (HPSEC) system (Shimadzu Scientific...
Instruments Inc., Maryland, United States). HPSEC was equipped with three columns in series: two of AquaGel PAA-M and PolyAnalytik PAA-203 (Polyanalytik Canada, London, Canada), and multiple detectors including: a UV detector (Viscotek 2600, Malvern Instruments Ltd, Malvern, United Kingdom), a refractive index (RI) detector, a differential pressure viscometer, a right angle laser light scattering detector and a low angle laser light scattering detector (Viscotek TDA 305, Malvern Instruments Ltd, Malvern, United Kingdom). The columns, viscometer and RI detector were operating at 40 °C. Detectors were calibrated using pullulan standards (JM Science Inc., New York, United States). DF were dissolved at 1 g L\(^{-1}\) in mobile phase (0.1 M NaNO\(_3\) and 0.005 M NaN\(_3\)), SFG at 80 °C during 1 h (Qian et al., 2012), FG at 90 °C during 3 h (Youssef, Wang, Cui, & Barbut, 2009) and OG at 90 °C during 2 h (Wang, Wood, Huang, & Cui, 2003) with constant magnetic stirring. DF solutions were filtered (0.45 µm, Millipore, Fisher Scientific) and 100 µL of sample was injected into the system for analyses. The eluent flow rate was 0.6 mL min\(^{-1}\). The dn/dc value of 0.146 mL g\(^{-1}\) was used. TriSEC software of Viscotek was used for data acquisition. YMM solution could not be filtered effectively and thus was not analyzed.

2.2.7. Preparation of simulated small intestinal digesta

The amount of DF required to obtain a specific concentration of DF in the final solution (25 mL) was divided into two equal parts by weight. Each part was dissolved in 10 mL of distilled water during 1 h at 80 °C, 3 h at 90 °C, 2 h at 80 °C and 2 h at 90 °C for SFG, FG, YMM and OG, respectively, with constant magnetic stirring (Qian et al., 2012; Wu, Cui, Eskin, & Goff, 2009; Wang et al., 2003; Youssef et al., 2009). Solutions of DF were first subjected to the gastric phase of the simulated digestion. The pH of the contents of one tube was adjusted to pH 4.0 and the pH
of the contents of the other tube was adjusted to pH 1.8 with HCl solution, thus representing some pH dynamics of postprandial stomach observed in vivo (Kalantzi et al., 2006 a; Malagelada, Go, & Summerskill, 1979; Malagelada, Longstreth, Summerskill, & Go, 1976). Tubes were incubated in the water bath at 37 °C at constant stirring during 60 min.

To prepare simulated small intestinal digesta, contents of both tubes were combined and supplemented with NaCl and CaCl$_2$. The pH of the resulting mixture was adjusted to 6.5 with NaOH solution, close to the value observed in human small intestine (Kalantzi et al., 2006 b; Persson et al., 2005). Finally, the volume of the solution was brought to 25 mL with distilled water. The concentrations of NaCl and CaCl$_2$ in the final solution was 150 mM and 10 mM respectively, thus representing physiological ionic strength and natural level of calcium in the human small intestine in the fed state (McClements & Li, 2010).

2.2.8. Apparent viscosity determination

Steady shear measurements of DF solutions were carried out on a ARES rheometer (TA instruments, New Castle, United States) operating at 37°C. The rheometer was equipped with concentric cylinder geometry (inner diameter 32 mm, outer diameter 34 mm). The sample (9 mL) was left to rest for 15 min before each measurement. Measurements were performed at shear rate ranging from 0.01 to 1000 s$^{-1}$.

2.2.9. Intrinsic viscosity determination

The intrinsic viscosities [$\eta$] of the DF in simulated small intestinal conditions were determined as follows. Relative ($\eta_{rel}$) and specific ($\eta_{sp}$) viscosities were found using the following relationships: $\eta_{rel} = \eta/\eta_s$ and $\eta_{sp} = \eta_{rel} - 1$, where $\eta$ and $\eta_s$ are viscosities of DF solution
and solvent respectively. Viscosities were determined using glass capillary viscometer (Cannon #50, Cannon Instrument Company, State College, United States) operating in the constant temperature bath (Cannon CT-500 series II, State College, United States) set at 37°C. The range of DF concentrations met the following requirement: $1.2 \leq \eta_{rel} \leq 2$ (Doyle, Lyons, & Morris, 2009). Values of $\eta_{sp}/c$ and $\ln \eta_{rel}/c$ were plotted against concentration and were extrapolated further to infinite dilution. The extrapolations were following Huggins (equation 1) and Kraemer (equation 2) relationships.

$$\eta_{sp}/c = [\eta] + k_H [\eta]^2 c \quad (1)$$

$$\ln \eta_{rel}/c = [\eta] + k_K [\eta]^2 c \quad (2)$$

where $k_H$ and $k_K$ are Huggins and Kraemer constants respectively. The value of $[\eta]$ was obtained as the average of these two intercepts. Slope of the Huggins fitting, which equals to $(k_H)[\eta]^2$, was used to obtain $k_H$ values. Values of $[\eta]$ and $k_H$ for each DF were determined in duplicate. The mean values and standard deviations are reported.

### 2.3. Results

#### 2.3.1. Chemical composition and yield

The total sugar, protein, ash and uronic acid (for YMM and SFG) contents and yield of water-extracted, ethanol-treated and purified DF are shown in Table 2.1. The CANAFEN® Gum was originally high in soluble dietary fibre (>75%, according to the producer), thus it resulted in high yield (>80%) of FG-WE. Ethanol treatment helped to remove a large portion of protein from YMM-WE and FG-WE and almost all ash from FG-WE. The protein content of “mustard mucilage” also extracted from yellow mustard bran in the work of Weber et al. (1974) (based on reported nitrogen content 2.38 %) was comparable to values of YMM-ETH extracted in the
present study (10.55% ± 0.05). Qian et al. (2012b) reports the protein content of “soluble flaxseed gum” (before protein hydrolysis) also extracted from flaxseed hull to be 11.8% ± 1.0, which is again similar to protein content of SFG-ETH (12.21% ± 0.06). At the same time, treatment with ethanol resulted in reduction of yield. Yield of YMM-ETH extracted in the present study (4.65%) was lower than yield values reported by Weber et al. (1974) (minimum 15%). Perhaps, higher yield value in the work of Weber et al. (1974) can be attributed to having a bran defatting step in the extraction procedure. However, yield of “soluble flaxseed gum” (9.7%) reported by Qian et al. (2012b) was comparable to yield of SFG-ETH (6.77%).

Extensive purification substantially reduced the presence of contaminants in YMM-ETH, SFG-ETH and OG (< 4% protein and ash). Thus protein hydrolysis with protease from Streptomyces griseus in combination with dialysis used in the present study seemed to be effective in removing protein from these DF. Cui, Eskin, & Biliaderis (1993a) and Cui, Mazza, & Biliaderis (1994) showed that dialysis can considerably reduce ash content of yellow mustard mucilage and flaxseed gum. In the present work, dialysis was also effective in removing the ash fraction. The protein and ash contents of FG were less than 1%, thus protein hydrolysis and dialysis steps were omitted for FG. The β-glucan content of OG obtained in the present study was 87.39% ± 0.78. Lazaridou et al. (2004) and Lazaridou, Biliaderis, & Izydorczyk (2003), who also used dual-enzyme digestion for extraction of oat gum from oat and oat concentrates, also obtained high β-glucan material (> 93%).

Uronic acid content (Table 2.1) and neutral monosaccharide composition (Table 2.2) of YMM were comparable to values of “water soluble fraction of yellow mustard mucilage” reported by Cui et al. (1993a) (total neutral monosaccharide composition was taken as 100%). However, small amounts of xylose reported by Cui et al. (1993a) were not detected in YMM.
Table 2.1. Chemical composition and yield of water-extracted (WE), ethanol-treated (ETH) and purified yellow mustard mucilage (YMM), soluble flaxseed gum (SFG), fenugreek gum (FG) and oat gum (OG). Concentrations reported on a dry weight basis.

<table>
<thead>
<tr>
<th></th>
<th>Total sugar (%)</th>
<th>Uronic acid (%)</th>
<th>Protein (%)</th>
<th>Ash (%)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>YMM-WE</td>
<td>61.77 ± 0.17</td>
<td>18.25 ± 0.03</td>
<td>14.07 ± 0.22</td>
<td>11.21</td>
<td></td>
</tr>
<tr>
<td>SFG-WE</td>
<td>65.63 ± 3.59</td>
<td>11.94 ± 0.02</td>
<td>15.72 ± 0.00</td>
<td>8.87</td>
<td></td>
</tr>
<tr>
<td>FG-WE</td>
<td>91.77 ± 2.18</td>
<td>2.18 ± 0.03</td>
<td>10.67 ± 0.14</td>
<td>82.29</td>
<td></td>
</tr>
<tr>
<td>YMM-ETH</td>
<td>68.31 ± 2.04</td>
<td>10.55 ± 0.05</td>
<td>14.31 ± 0.39</td>
<td>4.65</td>
<td></td>
</tr>
<tr>
<td>SFG-ETH</td>
<td>67.59 ± 1.55</td>
<td>12.21 ± 0.06</td>
<td>13.00 ± 0.47</td>
<td>6.77</td>
<td></td>
</tr>
<tr>
<td>FG</td>
<td>99.90 ± 1.47</td>
<td>0.95 ± 0.07</td>
<td>0.13 ± 0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>YMM</td>
<td>92.72 ± 5.34</td>
<td>19.04 ± 0.28</td>
<td>3.48 ± 0.08</td>
<td>2.18 ± 0.18</td>
<td></td>
</tr>
<tr>
<td>SFG</td>
<td>91.46 ± 5.01</td>
<td>27.42 ± 0.77</td>
<td>1.93 ± 0.04</td>
<td>3.63 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>OG</td>
<td>93.56 ± 1.88</td>
<td>1.37 ± 0.14</td>
<td>1.59 ± 0.07</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Uronic acid content and neutral monosaccharide composition of SFG were also comparable to “soluble flaxseed gum” and “dialyzed flaxseed gum” reported by Qian et al. (2012b) and Cui et al. (1994) respectively. As would be expected, FG was composed of galactose and mannose units at almost equal proportions. Analyses showed that OG consists of only glucose units, thus again confirming high purity of the gum.

### 2.3.2. Molecular weight

The HPSEC analysis revealed that extracted in the present study SFG contains two distinct peaks. Qian et al. (2012b) reported that “soluble flaxseed gum” extracted in their study was also composed of two fractions: first, neutral high-molecular weight arabinoxylanan and second, acidic low-molecular weight pectin-like rhamnogalacturonan. However, peak molecular weights of these two fractions in the work of Qian et al. (2012) were lower (1550 kDa and 253 kDa) than values of SFG (1840 ± 33.94 kDa and 454 ± 9.19 kDa) extracted in the present study.
Molecular weight of cereal β-glucan is an important parameter that controls development of luminal viscosity and thus health-promoting benefits of that DF (Tosh, Brummer, Wolever, & Wood, 2008). According to some reports molecular weight of oat β-glucan can be as high as 3100 x 10^3 g/mol (Lazaridou & Biliaderis, 2007). Thus, molecular weight of β-glucan in OG extracted in the present study (Table 2.3) was relatively low, but at the same time it was in the range of molecular weight values (200-800 kDa) observed in β-glucan-enriched baked goods (Aman, Rimsten, & Andersson, 2004; Frank, Sundberg, Kamal-Eldin, Vessby, & Aman, 2004). Additionally, studies of Brummer et al. (2012) and Kwong et al. (2013) showed that a meal containing oat β-glucan with molecular weight as low as 326,000 g/mol and 145,000 g/mol respectively, can significantly attenuate glycemic responses compared to control meal. The Mw of FG (1,241 ± 40.31 kDa) extracted in the present study was comparable to the value (1,418 kDa) of fenugreek gum extracted by Brummer et al. (2003).

### 2.3.3. Rheological properties of DF in simulated small intestinal conditions

The steady shear flow curves of DF solutions at different concentrations in simulated small intestinal conditions can be seen in Fig. 2.1. Experimentally obtained data was fit to the Cross
Table 2.3. Weight-average molecular weight (Mw), number average molecular weight (Mn), intrinsic viscosity ([η]), radius of gyration (Rg) and polydispersity index (Mn/Mw) of oat gum (OG) and fenugreek gum (FG), obtained with high performance size exclusion chromatography.

<table>
<thead>
<tr>
<th></th>
<th>Mw (kDa)</th>
<th>Mn (kDa)</th>
<th>[η] (dL/g)</th>
<th>Rg (nm)</th>
<th>Mn/Mw</th>
</tr>
</thead>
<tbody>
<tr>
<td>OG</td>
<td>225 ± 7.78</td>
<td>108 ± 10.61</td>
<td>2.17 ± 0.01</td>
<td>20.5 ± 0.32</td>
<td>2.09 ± 0.13</td>
</tr>
<tr>
<td>FG</td>
<td>1241 ± 40.31</td>
<td>678 ± 3.33</td>
<td>9.54 ± 0.42</td>
<td>96.04 ± 0.06</td>
<td>1.83 ± 0.05</td>
</tr>
</tbody>
</table>

model (Eq 3) by means of least-squares curve fitting (Cross, 1965) (Fig. 2.1).

\[ \eta = \eta_\infty + \frac{\eta_0 - \eta_\infty}{1 + (\tau \gamma)^n} \]  

(3)

where \( \eta \) is the experimentally obtained apparent viscosity (Pa s) at shear rate \( \gamma \) (s\(^{-1}\)), \( \eta_0 \) and \( \eta_\infty \) are extrapolated zero-shear rate and infinite shear rate viscosity (Pa s) respectively. The \( \tau \) is time constant (s), the \( 1/\tau \) equals the shear rate at which \( \eta = (\eta_0 - \eta_\infty)/2 \) and \( n \) is a dimensionless exponent that describes the intensity of the shear-thinning behavior (Smith et al., 2014). Table 2.4. reports Cross parameters obtained at several different concentrations. Fits of the Cross model and experimentally obtained steady shear flow curves for SFG, FG and OG were in good agreement (\( R^2 \sim 0.9667 \), Table 2.4). However, attempts to obtain satisfactory Cross model fittings for YMM solutions have failed in the present study (\( R^2 \ll 0.90 \)).

At lower concentrations DF solutions exhibited Newtonian-like behavior, when viscosity was constant for the whole range of shear rates. In such dilute regime, individual polymer chains move independently through the solvent. At higher concentrations, shear-thinning behavior was observed for all four DF solutions. The intensity of the shear-thinning behavior was becoming more pronounced (increase of \( n \) parameter, Table 2.4) with increase of DF concentration. In the so-called semi-dilute regime, polymer chains get entangled as they are forced to overlap and interpenetrate. The disruption and formation of these entanglements is balanced at low shear

53
Figure 2.1. The steady shear flow curves of yellow mustard mucilage (YMM) at concentration range from 0.02% to 1.38% (A), soluble flaxseed gum (SFG) at concentration range from 0.12% to 2.50% (B), fenugreek gum (FG) at concentration range from 0.06% to 1.00% (C) and oat gum (OG) at concentration range from 0.27% to 3.78% (D) in simulated small intestinal conditions. Experimentally obtained values (○) and Cross model fittings (…).

rates, ensuing constant viscosity (\(\eta_0\)). Disruption of entanglements predominates at higher shear rates resulting in shear-thinning. Increase of polymer concentration in solution results in an increase in the degree of chain overlapping. Thus structural breakdown of formed entanglements becomes more shear dependent as the concentration of polymer growth (Gorret, Renard, Famelart, Maubois, & Doublier, 2003). Therefore, shear rate at which transition from Newtonian to shear-thinning behavior occurs shifts to lower values (increase of \(\tau\) parameter, Table 2.4) with
Table 2.4. Parameters of Cross model for soluble flaxseed gum (SFG), fenugreek gum (FG) and oat gum (OG) at five different concentrations in simulated small intestinal conditions.

<table>
<thead>
<tr>
<th>c (%)</th>
<th>( \eta_0 ) (Pa s)</th>
<th>( \tau ) (s)</th>
<th>( n )</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.00</td>
<td>0.436</td>
<td>0.1000</td>
<td>0.55</td>
<td>0.9974</td>
</tr>
<tr>
<td>1.60</td>
<td>0.099</td>
<td>0.0493</td>
<td>0.49</td>
<td>0.9913</td>
</tr>
<tr>
<td>SFG</td>
<td>1.10</td>
<td>0.034</td>
<td>0.0374</td>
<td>0.42</td>
</tr>
<tr>
<td>0.80</td>
<td>0.020</td>
<td>0.0371</td>
<td>0.39</td>
<td>0.9934</td>
</tr>
<tr>
<td>0.60</td>
<td>0.010</td>
<td>0.0179</td>
<td>0.38</td>
<td>0.9995</td>
</tr>
<tr>
<td></td>
<td>0.80</td>
<td>0.551</td>
<td>0.1611</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td>0.60</td>
<td>0.110</td>
<td>0.1033</td>
<td>0.57</td>
</tr>
<tr>
<td>FG</td>
<td>0.45</td>
<td>0.051</td>
<td>0.0467</td>
<td>0.54</td>
</tr>
<tr>
<td>0.28</td>
<td>0.008</td>
<td>0.0382</td>
<td>0.42</td>
<td>0.9986</td>
</tr>
<tr>
<td>0.20</td>
<td>0.005</td>
<td>0.0182</td>
<td>0.41</td>
<td>0.9929</td>
</tr>
<tr>
<td></td>
<td>3.80</td>
<td>0.645</td>
<td>0.0148</td>
<td>0.64</td>
</tr>
<tr>
<td></td>
<td>3.50</td>
<td>0.391</td>
<td>0.0147</td>
<td>0.61</td>
</tr>
<tr>
<td>OG</td>
<td>3.00</td>
<td>0.169</td>
<td>0.0105</td>
<td>0.56</td>
</tr>
<tr>
<td>2.70</td>
<td>0.128</td>
<td>0.0097</td>
<td>0.55</td>
<td>0.9878</td>
</tr>
<tr>
<td>2.16</td>
<td>0.047</td>
<td>0.0058</td>
<td>0.50</td>
<td>0.9700</td>
</tr>
</tbody>
</table>

increase of DF concentration.

The OG solutions exhibited slight shear-thinning behavior only at relatively high concentrations (\( c > 1.3\% \)). Minor shear-thinning behavior of oat \( \beta \)-glucan aqueous solutions, with Mw (270 kDa) similar to Mw of \( \beta \)-glucan in OG extracted in present study, at 20 °C was also observed by Skendi et al. (2003). At the same time, YMM exhibited shear-thinning behavior even at concentrations below 0.1%. Pronounced shear thinning behavior of “yellow mustard mucilage water-soluble fraction” has also been reported previously by Cui et al. (1993a) and Cui, Eskin, & Biliaderis, (1993b). Wu et al. (2009) also observed dramatic (at least four decades) reduction of apparent viscosity of 1% aqueous solution of “non-pectic polysaccharides from yellow mustard mucilage” with increase of shear rate from 0.001 to 1000 s\(^{-1}\) at 25°C.

Generally, solutions of polymers with higher hydrodynamic volume result in higher apparent viscosity than solutions of polymers with lower hydrodynamic volume at equivalent
concentrations. Based on that, we would expect that hydrodynamic volume of the DF studied would increase in the following order: OG→SFG→FG→YMM.

The Huggins and Kraemer plots for four types of DF in simulated small intestinal conditions obtained with capillary viscometer are shown in Fig. 2.2 and derived $[\eta]$ for SFG, FG and OG are presented in Table 2.5. The values of $[\eta]$ increased in the following order: OG→YMM→SFG→FG. That is in agreement with speculations from the previous paragraph based on hydrodynamic volume of OG, SFG and FG. However, $[\eta]$ of YMM was probably underestimated. Use of capillary viscometry can result in underestimation of $[\eta]$, especially for polymers with highly shear-thinning behavior (Robinson, Ross-Murphy, & Morris, 1982).
Table 2.5. Intrinsic viscosity $[\eta]$, Huggins constant $k_H$ (obtained with glass capillary viscometer), critical overlap concentration $c^*$, coil-overlap parameter $c^*[\eta]$, lower and upper slopes for soluble flaxseed gum (SFG), fenugreek gum (FG) and oat gum (OG) in simulated small intestinal conditions.

<table>
<thead>
<tr>
<th></th>
<th>$[\eta]$ (dL/g)</th>
<th>$k_H$</th>
<th>$c^*$ (%)</th>
<th>$c^*[\eta]$</th>
<th>Lower Slope</th>
<th>Upper Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFG</td>
<td>5.35 ± 0.019</td>
<td>0.282 ± 0.010</td>
<td>0.77</td>
<td>4.12</td>
<td>1.35</td>
<td>3.91</td>
</tr>
<tr>
<td>FG</td>
<td>10.03 ± 0.126</td>
<td>0.466 ± 0.032</td>
<td>0.31</td>
<td>3.11</td>
<td>1.37</td>
<td>4.68</td>
</tr>
<tr>
<td>OG</td>
<td>2.19 ± 0.007</td>
<td>0.604 ± 0.019</td>
<td>1.34</td>
<td>2.94</td>
<td>1.39</td>
<td>4.38</td>
</tr>
</tbody>
</table>

The $[\eta]$ of SFG in the present work was higher ($5.37 \pm 0.019$ dL/g) than the value for “soluble flaxseed gum” ($4.46 \pm 0.034$ dL/g) reported by Qian et al. (2012b), even though the measurement in their work was performed at lower temperature ($25^\circ$C) than in the present study ($37^\circ$C). However, peak molecular weights of both fractions of “soluble flaxseed gum” were also lower than molecular weights of SFG. The values of $[\eta]$ for FG in the present work determined with a glass capillary viscometer in simulated small intestinal conditions and with HPSEC in mobile phase operating at $40^\circ$C were close, $10.03 \pm 0.126$ dL/g and $9.54 \pm 0.420$ dL/g respectively (Table 2.3 and Table 2.5). Brummer et al. (2003) reported similar value of $[\eta]$ ($9.61$ dL/g) for fenugreek gum obtained by HPSEC operating at $40^\circ$C. Nevertheless, $[\eta]$ for fenugreek gum in water at $20^\circ$C reported by Doyle et al. (2009) was $16$ dL/g. That study showed reduction of fenugreek gum $[\eta]$ to $12.8$ dL/g when a solution of the polysaccharide was prepared in $0.1M$ NaOH. The reduction is believed to be due to ionization of hydroxyl groups resulting in inhibition of intermolecular association. That work also showed that supplementation of fenugreek gum solution in $0.1M$ NaOH with NaCl (final concentration $0.9$ M) reduced its $[\eta]$ even further ($9.8$ dL/g). The authors proposed that the observed reduction was due to suppression of intramolecular electrostatic repulsions. The value of $[\eta]$ of OG in simulated small intestinal
conditions found using capillary viscometer (2.19 ± 0.007 dL/g) was also close to the value obtained by HPSEC (2.17 ± 0.010 dL/g) operating at 40°C (Table 2.3).

Values of the Huggins constant ($k_H$), a parameter which characterizes solute-solvent interaction, for SFG, FG and OG in simulated small intestinal conditions obtained with capillary viscometer are shown in Table 2.5. Values increase in the following order: SFG→FG→OG. Generally, $k_H$ of highly hydrophilic polymers in aqueous solutions is between 0.3 and 0.7 (Durand, 2007). The solvent quality deteriorates with increase of $k_H$ value. Particularly, for a good solvent $k_H$ is below 0.5, for a poor solvent it is large than 0.7 and for a theta solvent it is in the range between 0.5 and 0.7 (Sakai, 1968). Thus, simulated small intestinal fluid at 37°C is a good solvent for SFG and FG. However, $k_H$ value for OG was lower and belonged to the range for theta solvent. Value of $k_H$ for oat β-glucan (0.68 ± 0.05) in water at 25°C reported by Wang, Wood, Cui, & Ross-Murphy (2001) was also in the range for theta solvent. According to the authors, lower $k_H$ was attributed to tendency of oat β-glucan to form aggregates in aqueous solutions.

Double logarithmic plots of zero shear specific viscosity $\eta_{sp0}$ (eq. 1) ($\eta_0$ was obtained through Cross model fittings), as a function of DF concentration in simulated small intestinal conditions for SFG, FG and OG are shown in Fig 2.3. The change in power-law slope was observed for each DF type above the specific overlap concentration ($c^*$). The change in slope with increase of polymer concentration is typical for many “random coil” polysaccharides. The change indicates the transition from the dilute to the semi-dilute regime. Derived $c^*$ values for SFG, FG and OG in simulated small intestinal conditions are shown in Table 2.5. As would be expected, based on [$\eta$], values of $c^*$ increased in the following order: FG→SFG→OG.
Figure. 2.3. Concentration dependence of “zero shear” specific viscosity for soluble flaxseed gum (SFG) (▲), fenugreek gum (FG) (■) and oat gum (OG) (●) in simulated small intestinal conditions.

The dimensionless product of polymer concentration and its intrinsic viscosity ($c\eta$) is denoted as degree of space occupancy. Morris, Cutler, Ross-Murphy, Rees, & Price (1981) showed that when $\eta_{sp0}$ is plotted against $c\eta$ on a double-logarithmic plot, data superimposes closely on one another, regardless of polysaccharide, resulting in a single master curve with two power-law slopes ($\approx 1.4$ and $\approx 3.3$). The change in slope of master curve occurs at $c^*\eta \approx 4$.

Double-logarithmic plots of $\eta_{sp0}$ against $c\eta$ for SFG, FG and OG are presented in Fig 2.4. The slopes and values of degree of space occupancy at which transition from dilute to semi-dilute regime occurs ($c^*\eta$) are summarized in Table 2.5. As can be seen, data that represents the dilute regime was in a good agreement between three types of DF. The lower slopes of SFG, FG and OG were close to the value ($\approx 1.4$) proposed by Morris et al. (1981) for polysaccharides solutions at $c < c^*$. However, the parts of the graphs that describe semi-dilute regime had
Figure 2.4. Variation of “zero-shear” specific viscosity with degree of space occupancy (c[η]) for soluble flaxseed gum (SFG) (▲), fenugreek gum (FG) (■) and oat gum (OG) (●) in simulated small intestinal conditions.


differences. Particularly, values of upper slope and values of c*[η] were different. Upper slope of SFG had the lowest value (3.91) among the three types of DF, following by OG (4.38) and FG (4.68). The c*[η] value for SFG (4.12) was close to value (≈ 4) proposed by Morris et al. (1981). However, c*[η] values for OG and FG were smaller (2.94 and 3.11 respectively).

The value of the upper slope of fenugreek gum in water at 20°C reported by Doyle et al. (2009) was also steeper (≈ 4.2) than the upper slope ≈ 3.3 for “normal” concentration-dependence for disordered polysaccharide coils proposed by Morris et al. (1981). A steeper upper slope is an indicator of self-association of polymers as their concentration in the solution grows (Doyle et al., 2009; Morris et al., 1981). Doyle et al. (2009) also reports lower c*[η] values (≈ 2.8) for fenugreek gum. Lower c*[η] values (≈ 2.5) for locust bean gum and guar gum were found by Morris et al. (1981). Departure from the “normal” concentration-dependence of
galactomannans in these reports was attributed to the formation of associations, “hyperentanglements”, between polymer chains with increase of concentration, resulting in growth of average molecular weight and thus viscosity. It was initially proposed that these “hyperentanglements” take place between unsubstituted regions of the galactomannan backbone. However, Doyle et al. (2009) showed that these associations do not necessarily occur between unsubstituted regions.

Double-logarithmic plots of $\eta_{sp0}$ against $c[\eta]$ for cereal $\beta$-glucan have been constructed in numerous publications. Studies of Agbenorhevi, Kontogiorgos, Kirby, Morris, & Tosh (2011); Doublier & Wood (1995); Irakli, Biliaderis, Izydorczyk, & Papadoyannis, (2004); Lazaridou et al. (2003); Lazaridou et al. (2004); Skendi et al. (2003) and Vaikousi, Biliaderis, & Izydorczyk, (2004) report lower and upper slopes values to be $1.03 \pm 0.08$ and $3.84 \pm 0.27$ respectively (averages and standard deviations of these seven studies) for oat and barley $\beta$-glucan. However, Bohm & Kulicke (1999) reported a larger value (5.18) for upper slope for barley $\beta$-glucan, measured at 25°C.

The degree of space occupancy at overlap concentration for cereal $\beta$-glucan has also been reported previously. Studies of Agbenorhevi et al. (2011); Doublier & Wood (1995); Irakli et al. (2004); Lazaridou et al. (2003); Lazaridou et al. (2004) and Vaikousi et al. (2004) report values of $c^*[\eta] \leq 0.75$ and $c^{**}[\eta] \leq 3.2$. However Skendi et al. (2003) reported higher values, $c^*[\eta] = 1.11-1.25$ and $c^{**}[\eta] = 7.75-10.05$ for oat $\beta$-glucan.

Numerous reports have suggested that molecules of cereal $\beta$-glucan form aggregates in aqueous solutions. The aggregation is believed to be due to the formation of hydrogen bonding between molecules of the polysaccharide. Junction zones are formed mostly between cellotriosyl fragments. However, hydrogen bonding between cellulose-like fragments of molecules also takes
place. Thus β-glucan with higher trisaccharide-to-tetrasaccharide ratio has higher tendency for intermolecular associations. Li, Cui, Wang, & Yada (2011) showed that reduction of molecular weight of β-glucan can promote aggregation further, perhaps due to increase of diffusivity of polysaccharide molecules.

2.3.4. Apparent viscosities of DF at physiological shear rate

According to EFSA (2011), supplementation of a meal with 4 g of cereal β-glucan per each 30 g of available carbohydrates can help to attenuate postprandial glycemic responses. Thus, if a meal is 500 mL and contains 50 g of available carbohydrates (amount usually used in human clinical studies, where postprandial glycemic responses are studied) then to meet guidelines of the health claim, the concentration of cereal β-glucan in the meal should be 1.33% (w/v).

According to Borgstrom, Dahlqvist, Lundh, & Sjovall (1957), 500 mL meal gets diluted minimum 3 fold, by the time it reaches the duodenum. Based on that, the highest expected concentration of β-glucan in the duodenum after consumption of a meal that meets requirements of EFSA (2011) would be 0.44 % (w/v) (1 health claim equivalent).

Some of the proposed mechanisms that could be involved in attenuation of postprandial glycemic responses after consumption of soluble dietary fibre are related to its ability to alter luminal viscosity of small intestine (Dikeman & Fahey, 2006). Thus, apparent viscosities (at 60 s⁻¹) of OG at concentrations expected to be in the small intestine after consuming a meal containing from 0.5 to 3 times health claim equivalents (based on β-glucan concentration) in simulated small intestinal conditions were measured and were used as benchmarks for YMM, SFG and FG. Concentrations of YMM, SFG and FG that result in apparent viscosity at 60 s⁻¹ in simulated small intestinal conditions close (± 5%) to benchmarks are shown in Table 2.6. The
Table 2.6. Apparent viscosities at 60 s$^{-1}$ of simulated small intestinal digesta after consumption of oat β-glucan (OG) at concentrations that represent from 0.5 to 3.5 EFSA (2011) health claim equivalents, and concentrations of flaxseed gum (SFG), yellow mustard mucilage (YMM) and fenugreek gum (FG) that result in equivalent apparent viscosities in simulated small intestinal digesta.

<table>
<thead>
<tr>
<th>Health Claim Equivalent</th>
<th>η (mPa.s)</th>
<th>Concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OG</td>
<td>SFG</td>
</tr>
<tr>
<td>3.0</td>
<td>18.54 ± 0.07</td>
<td>1.62</td>
</tr>
<tr>
<td>2.5</td>
<td>11.77 ± 0.05</td>
<td>1.35</td>
</tr>
<tr>
<td>2.0</td>
<td>7.34 ± 0.02</td>
<td>1.08</td>
</tr>
<tr>
<td>1.5</td>
<td>4.19 ± 0.04</td>
<td>0.81</td>
</tr>
<tr>
<td>1.0</td>
<td>2.33 ± 0.03</td>
<td>0.54</td>
</tr>
<tr>
<td>0.5</td>
<td>1.28 ± 0.00</td>
<td>0.27</td>
</tr>
</tbody>
</table>

The shear rate (60 s$^{-1}$) chosen in the present study lies in the range of shear rates used in literature where viscosities of small intestinal digesta were compared (Dikeman & Fahey, 2006; Fabek, Messerschmidt, Bruilport, & Goff, 2014; Hardacre, Yap, Lentle, & Monro, 2015; Tharakan, 2009).

In the majority of the cases, concentrations of each type of DF at which they resulted in similar apparent viscosities were different. Particularly, concentrations of OG were the highest when compared with concentrations of all other types of DF for the whole range of health claim equivalents. Among all DF types, FG resulted in high apparent viscosities in the vicinity of 60s$^{-1}$ at relatively low concentrations (Fig. 2.1). As a consequence, concentrations that represent equivalent apparent viscosities were the lowest for FG for the whole range of health claim equivalents, followed by YMM and SFG (Table 2.6). Concentrations of YMM that represent health claim equivalents < 1.5 were lower than concentrations of SFG. However, concentrations of YMM that represent health claim equivalents > 1.5 were higher than concentrations of SFG.
2.4. Conclusions

Yellow mustard mucilage and soluble flaxseed gum can be easily extracted with water from yellow mustard bran and flaxseed hull. Treatment of these water-extracted materials with ethanol and further air-drying produced a semi-purified polysaccharide. Additional steps included protein hydrolysis with protease from *Streptomyces griseus*, dialysis and ethanol precipitation can be used to achieve purified versions of these DF. Monosaccharide composition of extracted DF was similar to previously reported.

The steady shear flow curves of FG, SFG and OG were in good agreement with fits of the Cross model. Solutions of four types of DF in simulated small intestinal conditions exhibited distinct rheological properties. Particularly, SFG, FG and OG showed different \( [\eta] \) and \( c^* \) values. Based on the \( k_H \), small intestinal fluid at 37°C is a good solvent for SFG and FG and theta solvent for OG. Double logarithmic plots of \( \eta_{sp0} \) against \( c[\eta] \) revealed that coil overlap of OG and FG occurred at concentrations lower than would be expected from “normal” concentration-dependency, suggesting that associations between molecules of these DF occurs in simulated small intestinal conditions.

Apparent viscosities (at 60 s\(^{-1}\)) of simulated small intestinal digesta containing oat \( \beta \)-glucan at concentrations expected in the small intestine after consumption of a meal that meets from 0.5 to 3 times the EFSA (2011) health claim were determined and were used as benchmarks. Concentrations of YMM, SFG and FG that resulted in apparent viscosities close to these benchmarks were found. In the majority of cases, these concentrations were different for each DF type. Based on that, assuming that luminal viscosity affects postprandial glycemic responses, dietary recommendation for each dietary fibre would have to be specified. Apparent viscosities of YMM, SFG and FG solutions in simulated small intestinal conditions similar to apparent
viscosities of OG could be achieved by varying concentrations of these three types of DF. Thus, if luminal viscosity is predictive of postprandial glycemic responses, then YMM, SFG and FG potentially can attenuate postprandial glycemia at appropriate concentrations.

The composition of simulated small intestinal digesta prepared in the study was simplistic and represented only some basic conditions specific to the human digestive tract, including ionic strength, presence of divalent cations, pH and temperature. The occurrence of other compounds that are found in the small intestinal lumen in vivo (bile salts, mucin, etc.) potentially can also affect rheological properties of digesta supplemented with these types of DF.

Acknowledgments

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CHAPTER 3. IMPACT OF DIETARY FIBRE ON IN VITRO DIGESTIBILITY OF MODIFIED TAPIOCA STARCH: VISCOSITY EFFECT

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Abstract

In the current work, amylolysis of modified tapioca starch in simulated small intestinal conditions was studied in the presence of each of four dietary fibre types including yellow mustard mucilage, soluble flaxseed gum, fenugreek gum or oat gum. Each fibre was used at concentrations that were matched across the four fibre types for post-digestion viscosity. Due to molecular heterogeneity these concentrations were different for each fibre. The progress of amylolysis was studied by measuring the decline of digesta apparent viscosity over time at constant shear rate 60 s\textsuperscript{-1}. Expressing these data as a percentage of initial viscosity, and plotting it as a natural logarithm against time transformed the data to a linear form with a slope denoted as a viscosity decay constant ($k_v$). Additionally, for some of the digesta samples progress of amylolysis was assessed by evaluating increases in reducing sugar concentration. Based on the $k_v$ values, the progress of amylolysis was reduced as the concentration of each fibre and therefore viscosity of digesta increased. However, the influence to hinder amylolysis was diminishing with increase of digesta viscosity. The progress of amylolysis was similar when each fibre was present at a concentration to match for post-digestion viscosity. Measurements of changes in
reducing sugar content also confirmed these findings. Therefore, it was concluded that to alter
amylolysis to a similar extent fibres have to be present at amounts to result in similar post-
digestion viscosity even though their concentrations may not match.

3.1. Introduction

Native starch is a polysaccharide produced by green plants as an energy store. In nature
starch occurs in the form of granules with semi-crystalline structure. Most starch granules are
composed of two polymers of D-glucose linked to one another through glycosidic bonds: the
linear amyllose and branched amylopectin. Starch consumed by humans has often undergone
thermal treatment in the presence of water, up to an extent when irreversible loss of the granular
structure occurs, referred to as gelatinization. Native granular starches are also often physically
and chemically modified to obtain “modified food starches" that are used to formulate food
products with specific textural qualities.

Starch is an important energy source for humans. Digestion of starch begins in the mouth by
salivary $\alpha$-amylase and continues in the stomach until gastric pH is lowered below $\sim$ 4.0 to
inactivate the enzyme. When chyme reaches the small intestine, its pH is elevated ($\sim$ 6.5) and
starch digestion resumes by pancreatic $\alpha$-amylase and possibly by a portion of salivary $\alpha$-
amylase that stays intact after passing through the stomach (Fried, Abramson, & Meyer, 1987;
Kalantzi et al., 2006; Persson et al., 2005). $\alpha$-amylases hydrolyze starch in a multiple attack
action pattern when several glycosidic bonds are cleaved after the first random attack before
dissociation of the enzyme-substrate complex (Bijttebier, Goesaert, & Delcour, 2008; Gray,
1992). Studies have shown that human salivary and porcine pancreatic $\alpha$-amylases hydrolyze
starch mostly to maltose, maltotriose, maltotetraose and $\alpha$-limit dextrins (Robyt, 2008). These
products are further hydrolyzed at the intestinal brush border rather than in the lumen by specific glycosidases to produce single glucose units as a final product of starch digestion. Glucose is then transported across intestinal mucosal cells into the blood (Gray, 1992).

Several in vivo studies reported differences in postprandial glycemic and/or insulinemic responses after consumption of meals containing equal amounts of starch (Byrnes, Miller, & Denyer, 1995; Ek, Wang, Copeland, & Brand-Miller, 2014; Ells, Seal, Kettlitz, Bal, & Mathers, 2005; Englyst, Englyst, Hudson, Cole, & Cummings, 1999; Goñi, Garcia-Alonso, & Saura-Calixto, 1997; O'dea, Snow, & Nestel, 1981; Sasaki, Sotome, & Okadome 2015). These differences were related to the kinetics of amylolysis, as established by authors in in vitro experiments. Therefore, knowledge on digestion kinetics of various starches and methods of its manipulation can help to develop a dietary strategy to improve glycemic control, a factor that is believed to be important in the reduction of risk for Type 2 diabetes (Willett, Manson, & Liu, 2002).

Several features such as the botanical nature of starch, the type and extent of physicochemical modification, and the presence of protein and lipids can affect the kinetics of amylolysis (Varatharajan et al., 2011; Wang & Copeland, 2013; Zhang, Dhital, & Gidley, 2015a). Numerous in vitro studies revealed that addition of water-soluble dietary fibre (DF) can also alter the progress of amylolysis (Aravind, Sissons, & Fellows (2012), Bordoloi, Singh, & Kaur (2012), Dartois, Singh, Kaur, & Singh (2010); Dhital, Dolan, Stokes, & Gidley (2014), Fabek & Goff (2015), Hardacre, Yap, Lentle, & Monro (2015), Koh, Kasapis, Lim, & Foo (2009), Sasaki at al. (2015), Slaughter, Ellis, Jackson, & Butterworth (2002), Symons & Brennan (2004) and Thondre, Monro, Mishra, & Henry (2010)). In fact, several in vivo studies showed that supplementation of starch-containing meals with DF can attenuate glycemic responses
(Chillo, Ranawana, Pratt, & Henry, 2011; Ellis, Roberts, Low, & Morgan, 1995; Lan-Pidhainy, Brummer, Tosh, Wolever, & Wood, 2007; Lu, Walker, Muir, & O’Dea, 2004; Thakur, Mitra, Pal, & Rousseau, 2009; Thondre & Henry, 2009; Tosh, Brummer, Wolever, & Wood, 2008; Wood, Braaten, Scott, Riedel, & Poste, 1990). However, a correlation between the effect of DF on the kinetics of amylolysis and glycemic responses was not investigated in these studies. Moreover, in 2011 the European Food Safety Authority (EFSA) officially recognized the ability of cereal β-glucan (a type of DF) to reduce postprandial glycaemic responses.

Some DF including many polysaccharides such as arabinoxylans, galactomannans, pectins, and β-glucans, create thick solutions when mixed with water. The extent of thickening depends on molecular weight, concentration, chemical composition and conformation of a polysaccharide. Tosh et al. (2008) showed that there is a correlation between the ability of cereal β-glucan to enhance the viscosity of aqueous solutions and glycemic responses after consumption of a starch-containing meal. Particularly, there is evidence that DF results in attenuation of glycemic responses through its ability to alter luminal viscosity (Ellis et al., 1995). However, the exact mechanism by which DF causes this attenuation is unclear. Three most popular proposed mechanisms are: 1) delayed gastric emptying, 2) reduced digestive enzyme activity and 3) reduced rate of nutrient absorption (Dikeman & Fahey, 2006).

Several studies showed that DF can reduce the activity of pancreatic digestive enzymes including α-amylase (Hansen & Schulz, 1982; Ikegami et al., 1990; Isaksson, Lundquist, & Ihse, 1982). This reduction can be simply a result of reduced diffusion of the enzyme to starch substrate due to increased viscosity of lumen (Shelat et al., 2010). Therefore, if viscosity is the only factor that governs kinetics of amylolysis in DF-containing systems then DF of different biological nature would restrict amylolysis to a similar extent, as long as DF is present at
concentrations to maintain a minimal level of viscosity.

Nevertheless, alternative mechanisms for the inhibition of amylolysis in the presence of DF have been proposed. Slaughter et al. (2002) observed that guar gum has an inhibitory effect on \( \alpha \)-amylase. These authors concluded that the inhibition was due to the absorption of the enzyme to the galactomannan rather than due to enzyme diffusion impairment. Sasaki et al. (2015) established that interaction between xanthan gum and amylopectin occurs, perhaps through hydrogen bonding. These authors suggested that the interaction could result in inhibition of starch hydrolysis observed in the study.

The objective of the present study was to investigate the effect of four different DF types on digestion kinetics of modified tapioca starch in simulated small intestinal conditions at a shear rate equivalent to physiological. Each type of DF was chosen to produce equal viscosity at very different concentrations (due to the variances in botanical source of the DF). The progress of amylolysis was studied indirectly, by measuring the decline of digesta apparent viscosity over time. Additionally, changes in digesta reducing sugar concentration for some of the digesta samples were determined.

3.2. Materials and methods

3.2.1. Extraction of dietary fibre

DF including yellow mustard mucilage (YMM), soluble flaxseed gum (SFG), fenugreek gum (FG) and oat gum (OG) were extracted as described in Chapter 2. Briefly, YMM and SFG were extracted from the yellow mustard bran (G.S. Dunn, Hamilton, Canada) and flaxseed hull (Natunola Health Inc., Winchester, Canada) respectively with water. Both types of DF were purified using steps including protein hydrolysis, dialysis against distilled water and ethanol
precipitation. FG was extracted by suspending CANAFEN® Gum (Emerald Seed products Ltd., Avonlea, Canada) in water followed by centrifugation. Gum was precipitated with ethanol from the supernatant. OG was extracted from oat bran concentrate (Viterra, Portage la Prairie, Canada) using a dual-enzyme procedure. Chemical and monosaccharide compositions, as well as the rheological behavior of these four DF types, are described in Repin et al. (2016).

3.2.2. Establishing dietary fiber concentrations for in vitro digestion

Hypothetically, if a meal of 500 mL contains 50 g of available carbohydrates, and if this meal is diluted 3 fold by the time it reaches the small intestine, the expected concentration of β-glucan in the small intestine after consumption of a meal that meets requirements of EFSA (2011) health claim on reduction of postprandial glycemic responses would be 0.44 % (w/v) (1 health claim equivalent (eq.)). Concentrations of YMM, SFG, and FG that under simulated small intestinal conditions represent from 0.5 to 3 apparent viscosity (at 60 s\(^{-1}\)) eq. of EFSA (2011) glycemia control health claims for cereal β-glucan were obtained as described in detail by Repin et al., 2016. Briefly, apparent viscosities (at 60 s\(^{-1}\)) of starch-free simulated small intestinal digesta samples supplemented with OG at several concentrations were measured and were used as benchmarks. Particularly, samples were supplemented with OG at amounts to result in concentrations of β-glucan expected in the small intestine after consumption of a meal that meets from 0.5 to 3 times the value of the EFSA (2011) health claim. Concentrations of YMM, SFG and FG that resulted in apparent viscosities (at 60 s\(^{-1}\)) close to the benchmark apparent viscosities in simulated small intestinal conditions were found (Table 3.1.). Shear rate equal to 60 s\(^{-1}\) was representing physiological shear rate experienced by digesta in the small intestine and lies in the range of shear rates used in literature where viscosities of small intestinal digesta were compared
(Dikeman & Fahey, 2006; Fabek, Messerschmidt, Bruilport, & Goff, 2014; Hardacre et al., 2015; Tharakan, 2009).

3.2.3. Preparation of simulated small intestinal digesta

Simulated small intestinal digesta was prepared similarly as described by Repin et al. (2016). The amount of DF required to obtain a specific concentration of DF in the final solution (25 mL) was divided into two equal parts by weight. Each part was dissolved in 10 mL of distilled water during 1 h at 80 °C, 3 h at 90 °C, 2 h at 80 °C and 2 h at 90 °C for SFG, FG, YMM and OG, respectively, with constant magnetic stirring (Qian, Cui, Wu, & Goff, 2012; Wu, Cui, Eskin, & Goff, 2009; Wang, Wood, Huang, & Cui, 2003; Youssef, Wang, Cui, & Barbut, 2009). Solutions of DF were first subjected to the gastric phase of the simulated digestion. The pH of the contents of one tube was adjusted to pH 4.0 and the pH of the contents of the other tube was adjusted to pH 1.8 with HCl solution, thus representing some pH dynamics of postprandial stomach observed in vivo. Tubes were incubated in the water bath at 37 °C at constant stirring during 60 min. Contents of both tubes were then combined together and supplemented with NaCl and CaCl₂, to prepare simulated small intestinal digesta. Modified (gelatinized) tapioca starch (TEXTRA PLUS®, Ingredion, Mississauga, Canada) (1 g) was dissolved in the sample using magnetic stirrer at 60 C° during 30 min. The pH of the resulting mixture was adjusted to value 6.5 with NaOH solution. The volume of the sample was brought to 25mL with distilled water. Concentrations of NaCl and CaCl₂ in the final solution were 150 mM and 10 mM respectively. Each digesta sample (except control digesta) was supplemented with one of the four DF types at one of the three different concentrations that represented 0.5, 1 or 3 apparent viscosity (at 60 s⁻¹) eq. of the EFSA (2011) health claim for cereal β-glucan (Table 1).
Table 3.1. Concentrations of yellow mustard mucilage (YMM), soluble flaxseed gum (SFG), fenugreek gum (FG) and oat gum (OG) that represent 0.5, 1, 2 and 3 apparent viscosity (at 60 s\(^{-1}\)) equivalents of the European Food Safety Authority (2011) glycemia control health claim for cereal β-glucan.

<table>
<thead>
<tr>
<th>Health Claim Equivalent</th>
<th>Concentration (% (w/v))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>YMM</td>
</tr>
<tr>
<td>3.0</td>
<td>1.15</td>
</tr>
<tr>
<td>2.0</td>
<td>0.61</td>
</tr>
<tr>
<td>1.0</td>
<td>0.19</td>
</tr>
<tr>
<td>0.5</td>
<td>0.07</td>
</tr>
</tbody>
</table>

3.2.4. Measurements of digesta apparent viscosity changes during in vitro digestion

In vitro digestion was carried out in a rheometer (TA 2000, TA instruments, New Castle, United States) equipped with cup and bob geometry (inner diameter 30 mm, outer diameter 28 mm) at 37°C. Digesta (8mL) was placed into the cup containing pancreatin from porcine pancreas (MP Biomedicals, Santa Ana, United States). Pancreatin was added at an amount to obtain α-amylase activity in the final mixture equal to 0.18 units/mL. The α-amylase activity of pancreatin was assessed using 3,5-dinitrosalicylic acid method similar to described previously (Ohdan et al., 1999). One unit of the enzyme activity was defined as the amount of enzyme that liberates 1 µmol of reducing sugar (calculated as glucose) from soluble starch per minute at 37°C and pH 5.5.

Brookfield viscosity standard # 5 (4.7mPa.s at 25°C) (1mL) (Brookfield AMETEK, Inc., Middleboro, United States) was added on the top of the sample to prevent formation of a film on the surface that was affecting measurements. Digesta and pancreatin were premixed during 0.5 min at a constant shear rate of 60 s\(^{-1}\) before a recording of measurements began, denoted as 0 min. The constant shear rate of 60 s\(^{-1}\) was maintained during the entire experiment and digesta
apparent viscosity was recorded every minute. Our preliminary experiments have shown that pH of the digesta does not deviate from the value adjusted initially (6.5), at least during 2h of digestion. Mean values and standard deviations of three measurements are reported. When digestion was carried out to collect samples for reducing sugar analyses, viscosity standard was omitted.

3.2.5. Determination of reducing sugar content

Digesta samples (1mL) were withdrawn from the cup of the rheometer at the required time points and added to 4mL of absolute ethanol to stop enzymatic activity. Each load of the digesta to rheometer was used to obtained one sample at a specific time point. Remaining starch was precipitated from the solutions by centrifugation (15 min, room temp., 5000 x g). Resulting supernatants were collected in clean tubes and were stored at -20°C until analysis.

Main products of starch hydrolysis by α-amylases include maltose, maltotriose, maltotetraose and α-limit dextrins. Therefore, methods that test for the presence of free carbonyl group, and thus sensitive to reducing sugars are often used to study digestion of starch by α-amylase. In the present study, the dinitrosalicylic acid (DNS) colorimetric method described by (Miller, 1959) was used to determine a concentration of reducing sugars liberated from starch over the course of digestion.

Briefly, 3mL of DNS reagent (1% (w/v) dinitrosalicilic acid, 0.2% (w/v) phenol, 1% (w/v) sodium hydroxide and 0.05% (w/v) sodium sulfate) was added to each tube containing 3mL of a sample. Tubes were covered and placed in the water bath at 100°C for 15 min. Subsequently, Rochelle salt (40% (w/v) potassium sodium tartrate solution) (1mL) was added to each tube before placing them into the ice bath for 10 min. The absorbance of the samples was read using a
spectrophotometer (Beckman DU 7400, Beckman Coulter, Fullerton, United States) at 575 nm. A series of maltose standards were also analyzed to obtain a standard curve which was used to express changes of reducing sugar content in digesta samples as maltose equivalents (main product of starch hydrolysis by α-amylase) (Butterworth, Warren, Grassby, Patel, & Ellis (2012) & Zhang et al., 2015b).

3.2.6. Statistical analysis

Statistical analysis was performed using GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, United States). Univariate analysis of variance (ANOVA) followed by a post hoc Tukey test were conducted for mean group comparison of the digesta viscosity decay constant values. The significance level was set at p < 0.05.

3.3. Results

3.3.1. Changes of digesta apparent viscosity

Apparent viscosity of control digesta (starch only) at 0 min was 30.5 ± 0.7 mPa.s. Increasing concentration of each of the four types of DF resulted in a higher digesta apparent viscosity (Fig. 3.1), which was attributed to an increase in the degree of overlapping between polymers resulting in enhancement of structure stabilization. Initial apparent viscosities (0 min) of starch-containing digesta supplemented with each of the four types of DF at concentrations resulting in similar number of eq. in most of the cases were close to each other. Values of initial apparent viscosity of digesta supplemented with DF at concentrations that represent 0.5 and 1 viscosity eq. of oat β-glucan were 39.02 ± 1.93 and 46.70 ± 3.86 mPa.s respectively. However, the initial apparent viscosity of digesta supplemented with OG at concentration representing 3 eq. was higher.
(138.00 mPa.s.) than the initial apparent viscosity of digesta samples supplemented with YMM, SFG, and FG at concentrations representing 3 eq. (116.07 ± 5.51 mPa.s) (Fig. 3.1C). This difference could be a result of some interaction that is peculiar to β-glucan/starch system but does not occur between starch and other three DF types, though the nature of the interaction was not investigated in the present work.

Apparent viscosities of starch-containing digesta samples decreased considerably during the first 25 min of digestion in the presence of pancreatin. Viscosity reduction profiles of digesta samples supplemented with each of the four DF types at concentrations representing the same number of eq. were close to each other despite differences in the DF type and concentration. Even though initial apparent viscosity of digesta supplemented with OG at concentration representing 3 eq. was higher than the initial apparent viscosity of digesta samples supplemented with YMM, SFG, and FG, also at 3 eq., the viscosity reduction profiles of all four digesta samples were approaching as the digestion progressed and finally merged at the 25th minute.

Conversely, the presence of pancreatin did not affect the apparent viscosity of starch-free digesta samples supplemented with each of the four DF types at concentrations representing 3 eq. (Fig. 3.1 C). The decrease of the apparent viscosity of control digesta occurred more rapidly than in any other sample. However, in the absence of pancreatin control digesta maintained constant viscosity (data not shown), thus, it was concluded that the decline in digesta apparent viscosity was a result of a conversion of viscous starch into low viscosity products of amylolysis by α-amylase. Decline of starch-containing digesta apparent viscosity during in vitro digestion has been also observed by Bordoloi et al. (2012); Dartois et al. (2010); Hardacre et al. (2015) and Hardacre, Lentle, Yap, & Monro (2016) and was also attributed to amylolysis by α-amylase.
Figure 3.1. Changes in apparent viscosity of digesta samples during *in vitro* digestion. (A) Control and samples with DF at concentrations representing 0.5 apparent viscosity (at 60 s⁻¹) equivalent (eq.) of EFSA (2011) glycemia control health claim for cereal β-glucan, (B) control and samples with DF at concentrations representing 1 eq. and (C) control and samples with DF at concentrations representing 3 eq., (●) control starch-containing digesta, (♦) starch-containing digesta with yellow mustard mucilage, (■) starch-containing digesta with soluble flaxseed gum, (▲) starch-containing digesta with fenugreek gum, (▼) starch-containing digesta with oat gum, (○) starch free digesta with yellow mustard mucilage, (□) starch free digesta with soluble flaxseed gum, (△) starch free digesta with fenugreek gum and (▽) starch free digesta with oat gum.
In the works of Hardacre et al. (2015) and Hardacre et al. (2016) apparent viscosity of starch-containing digesta samples declined during the first 20 min of digestion until a plateau was reached. In the present study viscosity reduction occurred on a similar time scale. Changes in apparent viscosity of all starch-containing digesta samples reached a plateau (apparent viscosity changes < 5%) during the first 25 min of digestion. The apparent viscosity of starch-free digesta samples supplemented with each type of DF at concentrations representing 3 eq., were matching the apparent viscosity of corresponding starch-containing digesta samples in the plateau region (Fig 3.1 C). That result suggests that by the time the plateau was reached, starch was already hydrolyzed to an extent that it does not contribute to the viscous character of the sample. Thus, only the DF was mainly responsible for a relatively high apparent viscosity of the digesta at the plateau region.

Data presented in Fig. 3.1 was converted so that the apparent viscosity of each digesta at the plateau region was taken as 0%, and initial apparent viscosity of digesta was taken as 100%. The apparent viscosity of digesta between these two points was presented as a percentage of initial viscosity (Fig. 3.2). Hardacre et al. (2015), who also expressed decline of digesta apparent viscosity as a percentage of initial viscosity, showed that the presence of some types of DF can slow progress of digesta viscosity decline compared to control. In the present study, supplementation of digesta with each type of DF also slowed the decline of its viscosity compared to control and progress of that decline was reduced with an increase of DF concentration. As a consequence, an increase of concentration of each DF type resulted in an extension of the time point when viscosity plateau was reached.

Rate of amylolysis decreases with time as the concentration of substrate falls over the course of the reaction (Butterworth et al., 2012; Goñi et al., 1997). Hardacre et al. (2015) and Hardacre
et al. (2016) showed that digesta apparent viscosity expressed as a percentage of initial viscosity declines exponentially with time and can be described with the following exponential decay form.

\[ V_t = 100e^{-kt} \]  

(1)

Figure. 3.2. Changes in percentage of initial viscosity of control digesta (●) and digesta samples supplemented with yellow mustard mucilage (A), soluble flaxseed gum (B), fenugreek gum (C) and oat gum (D) at concentrations representing 0.5 apparent viscosity (at 60 s⁻¹) equivalent (eq.) of EFSA (2011) glycemia control health claim for cereal β-glucan (●), 1 eq. (■) and 3 eq. (▲) during in vitro digestion.
where term 100 represents a percentage of initial viscosity taken as 100%, \( V \) is a percentage of initial viscosity at time point \( t \) min (%), and \( k_v \) is a viscosity decay constant (\( \text{min}^{-1} \)). Therefore, taking the natural logarithm (ln) of values representing the percentage of initial viscosity and plotting them against time transforms the data to a form that can be described with a straight line, with slope equal to \( k_v \). Thus, decline of apparent viscosity of each digesta sample can be characterized with a single number (\( k_v \)).

Data presented in Fig. 3.2 was transformed to ln (percentage of initial viscosity) and plotted against time (Fig. 3.3). All resulting plots could be described well with a straight line (\( R^2 < 0.98 \)). Values of \( k_v \) for each of the digesta samples derived from the slopes of the corresponding plots are summarized in Table 3.2. An increase of the concentration of each of the four types of DF resulted in a reduction of \( k_v \) values, which suggests that progress of amylolysis slows down as the concentration of the DF in digesta increases.

Similar \( k_v \) values for the digesta samples that contained DF at concentrations representing a same number of eq. were obtained, even though the concentrations and botanical source of each DF were different. Digesta samples supplemented with DF at concentrations representing 3 eq. were not an exception, even though initial viscosity of digesta supplemented with OG at concentration representing 3 eq. was higher, nevertheless \( k_v \) values for all four digesta samples were not significantly different.

OG and FG used in the present study were at the two extreme concentrations. The difference between concentrations of these two DF types representing the same number of eq. were at least 3 fold (Table 3.1), however, even for these two types of DF, similar \( k_v \) values were obtained, despite such large differences in concentrations. That result suggests that the presence of each type of DF at a concentration to match for apparent viscosity in small intestinal conditions
Figure 3.3 Changes in percentage of initial viscosity (presented as natural logarithm) of control digesta (●) and digesta samples supplemented with yellow mustard mucilage (A), soluble flaxseed gum (B), fenugreek gum (C) and oat gum (D) at concentrations representing 0.5 apparent viscosity (at 60 s⁻¹) equivalent (eq.) of EFSA (2011) glycemia control health claim for cereal β-glucan (●), 1 eq. (■) and 3 eq. (▲) during in vitro digestion. Lines represent best fit straight line to experimental data of each samples.
Table 3.2. Values of digesta viscosity decay constant ($k_v$) for control digesta and digesta samples supplemented with yellow mustard mucilage (YMM), soluble flaxseed gum (SFG), fenugreek gum (FG) and oat gum (OG) at concentrations representing 0.5, 1 and 3 apparent viscosity (at 60 s$^{-1}$) equivalents of the European Food Safety Authority (2011) glycemia control health claim for cereal β-glucan. Means shearing a common superscript letter are not significantly different (p>0.05).

<table>
<thead>
<tr>
<th>Health Claim Equivalent</th>
<th>YMM</th>
<th>SFG</th>
<th>FG</th>
<th>OG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$k_v$ (min$^{-1}$)</td>
<td>$k_v$ (min$^{-1}$)</td>
<td>$k_v$ (min$^{-1}$)</td>
<td>$k_v$ (min$^{-1}$)</td>
</tr>
<tr>
<td>3.0</td>
<td>0.166$^a$ ± 0.0098</td>
<td>0.151$^a$ ± 0.0008</td>
<td>0.160$^a$ ± 0.0092</td>
<td>0.150$^a$ ± 0.0021</td>
</tr>
<tr>
<td>1.0</td>
<td>0.212$^b$ ± 0.0115</td>
<td>0.192$^b$ ± 0.0051</td>
<td>0.206$^b$ ± 0.0090</td>
<td>0.196$^b$ ± 0.0057</td>
</tr>
<tr>
<td>0.5</td>
<td>0.281$^c$ ± 0.0049</td>
<td>0.278$^c$ ± 0.0201</td>
<td>0.275$^c$ ± 0.0025</td>
<td>0.262$^c$ ± 0.0004</td>
</tr>
<tr>
<td>Control</td>
<td>0.362 ± 0.0279</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

reduced the progress of amylolysis to a similar extent. Therefore, the evolution of amylolysis was not affected by the botanical source of DF but was rather affected by viscosity in the small intestinal condition.

In the work of Slaughter et al., (2002) it has been proposed that inhibition of α-amylase can occur from absorption of the enzyme to guar galactomannan. Galactomannan (FG) was also one of the studied DF types in our work, however it was not more effective in hindering amylolysis then the other DF types.

The $k_v$ values for the control digesta and the digesta samples supplemented with SFG at concentrations representing from 0.5 to 3 eq. were plotted against values of apparent viscosity of starch-free digesta samples with SFG at corresponding concentrations (Fig. 3.4), thus Fig. 3.4 represents the effect of digesta apparent viscosity due to DF addition on the progress of starch digestion. The increase of digesta apparent viscosity resulted in a $k_v$ reduction, however, the reduction was more pronounced in a region where DF concentrations were ≤ 1eq. and less noticeable in a region where DF concentrations were > 1eq., even though concentrations of DF that represent ≤ 1eq. are below the critical overlap concentration and concentrations of DF that represent 3eq. are above the critical overlap concentration (Repin et al., 2016). Almost 3.3 times
Figure 3.4. The relationship between digesta viscosity decay constant \((k)\) and digesta apparent viscosity (starch free) for control digesta and digesta samples supplemented with soluble flaxseed gum at concentrations representing 0.5, 1, 2 and 3 apparent viscosity (at 60 s\(^{-1}\)) equivalents of EFSA (2011) glycemia control health claim for cereal \(\beta\)-glucan.

The increase in viscosity (0.71 mPa.s versus 2.33 mPa.s for control and SFG at concentration representing 1eq.) reduced \(k\) by 1.9 times. More dramatic viscosity increase, 26.1 times (18.54 mPa.s for SFG at concentration representing 3eq.) caused a reduction of \(k\) only by 2.4 times when compared to control digesta.

In the work of Dhital et al. (2014), DF delayed amylolysis (determined through changes of the glucose concentration of digesta with time) when digestion was carried out at both steady-state (no mixing) and constant mixing (200 and 750 rpm) conditions. However, the amylolysis hindering effect of DF was minimized when digestion was carried out at constant mixing. These authors also showed that an increase of DF concentration from 1% to 2% (w/v), which resulted
in an increase of digesta apparent viscosity compared to control by 10 and 100 times respectively, was not much more effective in the reduction of starch digestion progress, a finding that is similar to results obtained in the present study.

In the study of Ellis et al. (1995), supplementation of a starch-containing meal with guar gum increased the viscosity of jejunal digesta in pigs and reduced amount of glucose absorbed (determined based on veno-arterial differences of plasma glucose). The relationship between the amount of glucose absorbed and viscosity of jejunal digesta was not linear. Authors concluded that lower digesta viscosity was associated with a proportionally greater reduction of glucose absorption than digesta of higher viscosity. Again, this finding is similar to results obtained in the present study and study of Dhital et al. (2014).

Therefore, usage of DF at higher concentrations may not be much more effective in restricting amylolysis in the small intestine. Probably, as a consequence, glycemic responses also would not be attenuated much more noticeably at higher DF concentrations. At the same time, higher DF concentrations can cause undesirable overtexturization as well as increased cost of a food product.

3.3.2. Changes of digesta reducing sugar content

Changes of reducing sugar content during in vitro digestion were studied to compare to findings obtained by measurements of the digesta apparent viscosity decline. Digestograms of the control digesta and the digesta samples supplemented with FG at concentrations representing 1 and 3 eq. and digesta supplemented with OG at concentration representing 3 eq. are shown in
Figure 3.5. Digestograms of control digesta (●) and digesta samples supplemented with fenugreek gum at concentrations representing 1 apparent viscosity (at 60 s⁻¹) equivalent (eq.) of EFSA (2011) glycemia control health claim for cereal β-glucan (▲) and 3 eq. (△) and digesta supplemented with oat gum at concentration representing 3 eq. (▼). The figure represents changes of reducing sugar concentration expressed in maltose equivalents.

Fig. 3.5. The OG and FG were chosen as these two types of DF were used at two opposite extreme concentrations to represent a similar number of eq. (Table 3.1).

In all four samples, digestion was progressing during at least 100 min. However, a decline of digesta apparent viscosity of the corresponding samples did not exceed 25 min (Fig. 3.2). Hardacre et al. (2015) reported similar findings, in their study apparent viscosity of digesta samples reached a plateau during the first 20 min of digestion and reducing sugar concentration analyses revealed that only 30% of starch had been hydrolyzed at that time point. That suggests that when a viscosity plateau is reached, starch molecules in digesta are already too small to notably contribute to its rheological characteristics even though amylolysis progresses well beyond that point.
Several investigators reported that changes in concentration of products of amylolysis can be fitted to the following first-order equation

\[ C_t = C_\infty (1 - e^{-kt}) \]  

(2)

where \( C_t \) and \( C_\infty \) are concentrations of the products at time \( t \) and at the end of the reaction respectively and \( k \) is digestion rate constant (Butterworth et al., 2012; Goñi et al., 1997). Taking derivative from Eq.2 results in

\[ \frac{dC}{dt} = C_\infty ke^{-kt} \]  

(3)

Eq. 3 can be rearranged to the following form

\[ \ln \left( \frac{dC}{dt} \right) = \ln(C_\infty k) - kt \]  

(4)

thus plot of \( \ln(dC/dt) \) (logarithm of slope (LOS)) against \( t \) is of linear form with slope equal to \(-k\) and \( y\)-intercept equal to \( \ln(C_\infty k) \). LOS plots have been used by Butterworth et al. (2012), Edwards, Warren, Milligan, Butterworth, & Ellis (2014) and Zhang et al. (2015b) to determine kinetic parameters of amylolysis.

To construct LOS plots, data that represents changes of reducing sugar concentration of four different digesta samples was treated as described by Butterworth et al. (2012) (Fig. 3.6). LOS-treated data could be described reasonably well with a single straight line. For all samples \( R^2 > 0.90 \), the exception was FG at concentration representing 3eq. (\( R^2 = 0.84 \)). Therefore, digestion of each sample can be characterized with a single rate constant, which is typical for hydrothermally-processed starches (Edwards et al., 2014). Derived values of \( k \) and \( C_\infty \) for four digesta samples are summarized in Table 3.3.

Findings obtained through measurements of changes in reducing sugar concentration were similar to results that were attained with measurements of the progress of digesta apparent
Figure 3.6. The logarithm of slope (LOS) plots obtained for control digesta (A) and digesta samples supplemented with fenugreek gum at concentrations representing 1 apparent viscosity (at 60 s\(^{-1}\)) equivalent (eq.) of EFSA (2011) glycemia control health claim for cereal β-glucan (B) and 3 eq. (C) and digesta supplemented with oat gum at concentration representing 3 eq. (D).

viscosity decline. The \( k \) value was largest for control digesta, suggesting that amylolysis was progressing faster in control digesta among all other samples. Supplementation of digesta with FG and OG hindered the amylolysis progress, \( k \) values of DF-containing digesta samples were
Table 3.3. Values of digestion rate constant \((k)\) and concentration of the product of digestion at the end of the reaction \((C_\infty)\) (expressed in maltose equivalents), for control digesta and digesta samples supplemented with fenugreek gum (FG) at concentrations representing 1 apparent viscosity (at 60 s\(^{-1}\)) equivalent (eq.) of the European Food Safety Authority (2011) glycemia control health claim for cereal β-glucan and 3 eq. and digesta supplemented with oat gum (OG) at concentration representing 3 eq. estimated from the logarithm of slope analysis.

<table>
<thead>
<tr>
<th>Digesta</th>
<th>(k) (min(^{-1}))</th>
<th>(C_\infty) (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.030</td>
<td>11.37</td>
</tr>
<tr>
<td>FG 1 eq.</td>
<td>0.017</td>
<td>11.10</td>
</tr>
<tr>
<td>FG 3 eq.</td>
<td>0.010</td>
<td>11.33</td>
</tr>
<tr>
<td>OG 3 eq.</td>
<td>0.011</td>
<td>9.88</td>
</tr>
</tbody>
</table>

smaller than the value of \(k\) for control. The increase of FG concentration resulted in a reduction of \(k\) suggesting an increase of hindrance of amylolysis as the concentration of DF increases.

Additionally, values of \(k\) obtained for digesta samples supplemented with FG and OG at concentrations representing 3eq. were close to each other, thus again advocating that the progress of amylolysis was not affected by the botanical source of these DF, but rather affected by viscosity in the small intestinal conditions due to DF addition.

Plotting values of \(k\) and \(k_v\), obtained for four different digesta samples, against each other showed that there is a linear relationship between these values (Fig. 3.7). That result shows that measurements of changes in digesta apparent viscosity over time can be a suitable on-line method to study the kinetics of amylolysis.

LOS analyses of Butterworth et al. (2012), Edwards et al. (2014) and Zhang et al. (2015b) showed that various starches and starch-containing products can be digested at a different velocity and to a different extent, based on \(k\) and \(C_\infty\). In the present study, an addition of each type of DF reduced values of \(k\) compared to control, however, values of \(C_\infty\) for all four digesta samples were relatively close to each other (Table 3.3). Therefore, suggesting that the addition of
Figure 3.7. The relationship between digesta viscosity decay constant \(k_v\) and digestion rate constant \(k\) obtained for control digesta (Control) and digesta samples supplemented with fenugreek gum at concentrations representing 1 apparent viscosity (at 60 s\(^{-1}\)) equivalent (eq.) of EFSA (2011) glycemia control health claim for cereal \(\beta\)-glucan (FG 1eq.) and 3 eq. (FG 3eq.) and digesta supplemented with oat gum at concentration representing 3 eq (OG 3eq).

FG and OG affected the amylolysis velocity, yet, the effect of these two DF types on the extent of amylolysis was less noticeable. Nonetheless, \(C_\infty\) value for sample supplemented with OG at concentration representing 3 eq. was lower (9.88 mM) comparing to values of other three samples (11.27 ± 0.14 mM), suggesting that there could be some specific effect of OG on the extent of starch digestion which does not occur on addition of FG.

3.4 Conclusions

Supplementation of digesta with DF reduced the progress of both digesta apparent viscosity decline and changes in digesta reducing sugar content during simulated small intestinal digestion.
due to hindered amylolysis. Delayed amylolysis in the small intestine is one of the mechanisms that can be involved in attenuation of glycemic responses observed \textit{in vivo} after addition of DF to starch-containing meals. Consequently, YMM, SFG, FG and OG potentially can improve glycemic control, in fact, \textit{in vivo} studies have shown that supplementation of starch-containing meal with mustard fibre (Jenkins et al., 1987), flaxseed gum (Thakur et al., 2009), defatted fenugreek seed powder (gum 19.2% (w/v)) (Sharma, Raghuram, & Sudhakar Rao, 1990) and oat bran (\(\beta\)-glucan ~ 22\% (w/v)) (Tosh et al., 2008) attenuate glycemic response compared to control. Thus, based on our results, hypothesis that reduced glycemic responses observed after supplementation of starch-containing meals with DF is an outcome of hindered amylolysis in the small intestine cannot be rejected.

The progress of both digesta apparent viscosity decline and changes in digesta reducing sugar content were lessening with an increase of the DF concentration. When each DF type was present at a concentration to match for apparent viscosity in simulated small intestinal conditions, the progress of both digesta apparent viscosity decline and changes in digesta reducing sugar content were reduced to a similar extent, even though the concentrations of each fibre were different. Therefore, it can be concluded that to alter amylolysis to a similar extent fibres have to be present at amounts to result in similar post-digestion viscosity even though their concentrations may not match. Thus, it seems reasonable to further conclude that, the hindrance of amylolysis due to addition of YMM, SFG, FG and OG was a result of reduced diffusion of enzyme and/or substrate and/or restricted mixing efficiency rather than due to absorption of enzyme and/or substrate to DF. Consequently, assuming that progress of amylolysis affects glycemic responses, the recommendation of quantity for each type of DF for blood glucose control would have to be specified based on the ability of DF to alter physical properties of
aqueous solutions.

The relationship between digesta viscosity and viscosity decay constant was not linear. Reduction of digesta viscosity decline was more pronounced in the region where FG concentrations were $\leq 1$ eq. and less noticeable in the region where FG concentration were $> 1$ eq.. With this in mind, supplementation of digesta with the DF at lower concentrations was more effective (relative to an ability to enhance digesta viscosity) at hindering the amylolysis progress, than digesta with the DF at higher concentrations.

Measurements of changes in digesta reducing sugar content revealed findings similar to the results obtained through analysis of the progress of digesta apparent viscosity decline. Additionally, a linear correlation between rate constants of changes in digesta reducing sugar content and digesta viscosity decline constants was found.

**Acknowledgments**

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CHAPTER 4. EFFECT OF DIETARY FIBRE ON MALTOSE TRANSPORT IN SIMULATED SMALL INTESTINAL CONDITIONS

Abstract

The hindrance of sugar transport in the small intestinal lumen is one of the proposed mechanisms that can be involved in attenuation of postprandial glycemic responses after consumption of dietary fibre. In order to examine the mechanism, the transport of maltose was studied \textit{in vitro} in the presence of various dietary fibre types (yellow mustard mucilage, soluble flaxseed gum, fenugreek gum and oat gum). The transport was evaluated using a diffusion cell with controlled shear rate. The presence of each dietary fibre type at concentration representing 3 times apparent viscosity (at 60 s$^{-1}$) equivalent of EFSA (2011) glycemia control health claim for cereal $\beta$-glucan, increased apparent viscosity of simulated small intestinal digesta 27 fold compared to control digesta. Nonetheless, that increase in viscosity was not effective in reduction of maltose transport. The amount of transported maltose was reduced slightly only when a concentration of fenugreek gum was $\geq 1\%$, which corresponds to an apparent viscosity of digesta at 60 s$^{-1}$ $\geq 0.25$ Pa.s. ($\geq 350$ fold higher than control). Taking into account dilution of the meal due to the secretions in the gastrointestinal tract during digestion, it seems that concentration of dietary fibre in the meal has to be excessively high in order to impendiment maltose transport in the small intestine. Therefore, we propose that hindrance of sugar transport in the small intestine would not be the primary mechanism involved in attenuation of glycemic responses as a result of dietary fibre consumption.
4.1. Introduction

In the human gastrointestinal tract starch undergoes hydrolysis first by salivary and later by pancreatic $\alpha$-amylases. Then products of $\alpha$-amylase hydrolysis, mostly maltose, move to the small intestinal brush border where they are hydrolyzed further by specific glycosidases to produce single glucose units which are then transported across intestinal mucosal cells into the blood. Several in vitro studies have shown that some types of dietary fibre (DF) can hinder diffusion of sugars in simulated small intestinal conditions through altering the viscosity of the solution. Thus, potentially DF can reduce diffusion of sugars from intestinal lumen to the brush-border of the small intestine and that can result in a delay of sugar absorption and as a consequence, it can potentially lead to attenuation of glycemic responses.

Jenkins et al. (1986) examined the effect of guar gum, tragacanth, pectin and methyl cellulose on glucose diffusion. In their study, dialysis tube was filled with solution containing glucose alone or glucose and one of the studied DF type. The tube with sample was placed into the reservoir with distilled water. For all studied samples the concentration of the glucose in the reservoir was increasing with time due to the glucose diffusion from sample through the dialysis tube. The increase for control was occurring faster than for DF-supplemented samples. It was concluded that DF restricted glucose diffusion through alteration of solution viscosity.

Edwards, Johnson & Read (1988) studied the effect of guar gum on the transport of glucose from dialysis tube under different mixing conditions that mimic intestinal contractions. The tube filled with the sample containing glucose and tied at each end experienced intestinal contractions simulated by moving cylindrical paddles. The concentration of glucose in the outside fluid was determined every 15 min. Authors found that the glucose in the outside fluid was reduced when guar gum was present in the sample. Increasing the rate of contractions from 36 to 72
contractions/min increased the glucose concentration in the outside fluid for the control sample. However, when guar gum was present in the sample the effect was not observed.

Srichamroen, Thomson, Field, & Basu (2009) examined an effect of fenugreek galactomannan on glucose uptake by segments of jejunum and ileum obtained from rats. Intestinal segments were incubated in the solution containing radioactive-labeled glucose and galactomannan at different concentrations during 6 min at 37°C. Researchers found that increase of galactomannan concentration reduced glucose uptake. The reduction was also proportional to apparent viscosity (1.29 s⁻¹) of the solution at 37°C. It was concluded that reduction of glucose uptake by intestinal fragments was a result of restricted glucose diffusion in the presence of galactomannan.

Dhital et al. (2014) studied the effect of concentration of barley β-glucan (0%, 1%, and 3%) and mixing conditions (0, 200 and 750 rpm) on glucose diffusion. Sample with DF and glucose was placed in dialysis tube containing magnetic stirrer inside. It was found that the mass transfer coefficient was reduced with an increase of DF concentration. Though, the coefficient was increased with mixing speed.

Thus there is evidence that enhanced digesta viscosity can be a factor in attenuation of glycemic responses after ingestion of meals containing viscous DF. On the other hand, it needs to be acknowledged that in vivo, meal undergoes substantial progressive dilutions due to gastrointestinal secretions. Borgstrom et al. (1957) revealed that 500 ml test meal containing oil, glucose, lactose and milk proteins gets diluted 3-5 fold by the time it reaches duodenum, and the concentration keeps dropping as digesta moves further down from duodenum. Such dilutions can cause substantial reduction of DF concentration in the small intestine, resulting in a great decrease of digesta viscosity (Fabek et al., 2014). Thus, it is uncertain if such diluted solutions can alter sugar transport in the small intestine.
The aim of the present work was to study the effect of different types of DF, including yellow mustard mucilage (YMM), soluble flaxseed gum (SFG), fenugreek gum (FG) and oat gum (OG), on maltose transport in simulated small intestinal conditions.

4.2. Materials and methods

4.2.1. Diffusion device

The diffusion device was composed of diffusion cell and TA 2000 rheometer (TA Instruments, New Castle, United States) equipped with peltier (without cup) and bob (28 mm) (Fig. 4.1.). The custom designed diffusion cell was made of one end-closed cylindrical stainless steel tube with inner diameter 30 mm (Fig. 4.2). Therefore, the inner diameter of the tube was the same as an inner diameter of a cup supplied by the rheometer manufacturer. The top part of the tube was fixed on the plastic stand. The tube had a circle opening on the side (diameter 16.75 mm). A membrane, rehydrated in distilled water (24 °C, 10 min), was placed on the side of the cell in such way that it was fully covering the side opening. The membrane was a dialysis tube (MWCO: 6-8,000, Spectrum Laboratories, Inc., Rancho Dominguez, United States) cut and

![Fig. 4.1. Picture of diffusion device.](image1)

![Fig. 4.2. Picture of diffusion cell.](image2)
unfolded to one layer. Rubber gasket and metal plate with openings (diameter 16.75 mm) were placed on the side of the cell in such a way that their openings were fully matching the opening on the side of the cell. Two stainless steel clamps were used to hold the membrane, gasket and plate.

Assembled cell was placed into the peltier and was fixed in place with two screws. Peltier with the installed cell was mounted on the TA 2000 rheometer. The rheometer software settings and calibration were exactly the same as for rheometer equipped with cup and bob geometry with inner and outer diameters 30 mm and 28 mm respectively.

4.2.2. Preparation of simulated small intestinal digesta

YMM, SFG, FG and OG used in the experiment were extracted as described in Chapter 2. Concentrations of each DF that under simulated small intestinal conditions represent 3 apparent viscosity (at 60 s⁻¹) eq. of EFSA (2011) glycemia control health claims for cereal β-glucan were obtained as described in detail in Chapter 2.

Simulated small intestinal digesta was prepared similarly as described in Chapter 2. Briefly, the required amount of one of the four hydrocolloids was divided into two equal by weight parts. Each part was dissolved in a separate tube containing 10 mL of distilled water. First, solutions of hydrocolloids underwent gastric phase. The pH of contents of one tube was adjusted to pH 4.0 and pH of contents of the other tube was adjusted to pH 1.8 with HCl solution. Tubes were incubated at 37 ºC at constant stirring during 60 min. Further, to prepare simulated small intestinal digesta, contents of both tubes were combined together and supplemented with NaCl and CaCl₂. Maltose (Sigma-Aldrich) (1 g) was dissolved in the sample using magnetic stirrer at 60 C° during 30 min. The pH of the mixture was adjusted to value 6.5 with NaOH solution. The volume of the sample
was brought to 25 mL with distilled water. Concentrations of NaCl and CaCl$_2$ in the final solution were 150 mM and 10 mM respectively.

4.2.3. Loading the diffusion device

A simulated intestinal fluid (144 mL) (solution of NaCl and CaCl$_2$ at concentrations 150 mM and 10 mM respectively in distilled water) was loaded into recipient part of the cell and the digesta sample (9 mL) was loaded into the inner part of the cell. The volume of the added simulated intestinal fluid was calculated to have matched levels of fluid in the inner and recipient (when bob is submerged into the sample) parts of the cell. A preliminary experiment showed that volume of the digesta in the inner part of the cell was constant during the entire experiment, therefore confirming the validity of volume calculations.

The experiment was carried out at a constant shear rate (60 s$^{-1}$) and temperature (37°C). Distilled water (1.41 mL) was added to the recipient part of the cell every 30 min to compensate for evaporated water (established in the preliminary experiment). The fluid in the recipient part of the cell was constantly agitated with air supplied by an air pump. Aliquot samples (3 mL) were withdrawn from the recipient part of the cell at required time points and immediately replaced with 3 mL of simulated intestinal fluid.

4.2.4. Determination of maltose concentration

Dinitrosalicylic acid (DNS) colorimetric method was used to determine the concentration of maltose in samples as described in materials and methods section of Chapter 3. Series of maltose standards were also analyzed to obtain a standard curve which was used to obtain maltose concentrations in samples.
4.2.5. Apparent viscosity determination

Steady shear measurements of digesta samples were carried out on a rheometer (TA 2000, TA Instruments, New Castle, United States) operating at 37°C. The rheometer was equipped either with cup and bob (inner diameter 30 mm, outer diameter 28 mm) or cone and plate (60 mm, 1.59º) geometry. Measurements of apparent viscosities at 60 s\(^{-1}\) of digesta samples were done in triplicates, mean values and standard deviations are reported.

4.2.6. Statistical analysis

Statistical analysis was performed using GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, United States). Univariate analysis of variance (ANOVA) followed by a post hoc Tukey test were conducted for mean group comparison of the slope of maltose concentration change values. The significance level was set at \(p < 0.05\).

4.3. Results and discussions

The increase of maltose concentration in the recipient part for control digesta and digesta samples supplemented with different types of DF during 7 h can be seen in Fig. 4.3. The increase of maltose concentration in the receiving part was associated with transport of maltose from the simulated intestinal digesta, located inside the cell, into the recipient part through membrane driven by the concentration gradient. The system reaches equilibrium when a concentration of solute on both sides of the membrane are equilibrated. Based on the amount of maltose used and a total volume of solution (inner plus recipient part) that concentration equals to 2.35 g/L. In contrast, the mean concentration of maltose at the end of the 7\(^{th}\) hour of the experiment for control digesta was only 0.54 g/L, suggesting that at that point of time system was still far from
In the work of Dhital et al. (2014) around 80% of glucose was transported into the outer chamber during 5 h, in contrast only around 20% of maltose was transported during 7 h in our model. The difference could be explained by the distinctions in the area through which permeate could pass (~35 cm² versus ~9 cm² in the works of Dhital et al. (2014) and in the present study respectively).

For all samples, an increase of maltose concentration could be described with a straight line ($r^2 > 0.98$). Thus, we can assume that mass flux for all digesta samples was constant during at least 7 h of the experiment. Therefore, slopes of the lines can be used to compare changes of maltose concentration between samples.

The apparent viscosity at 60 s⁻¹ of the digesta samples supplemented with each DF type at the concentration to represent 3 eq. was ~ 0.019 Pa.s, compared to only ~ 0.0007 Pa.s for control digesta. Therefore, potentially DF-containing digesta could restrict diffusion of sugars through an increase of digesta viscosity. Conversely, slopes for digesta samples supplemented with all four types of DF at concentration representing 3 eq. were not significantly different from the slope of control digesta. Thus, based on the model used in the present study, we can conclude that none of the DF types used here at concentrations representing 3 eq. would restrict maltose transport in the small intestine.

The effect of FG concentration on the progress of maltose diffusion can be seen in Fig. 4.4. The increase of FG concentration from 0.43% (represents 3 eq.) up to 0.8% did not have a significant effect on maltose diffusion compared to control sample. However, slopes of maltose diffusion for digesta samples containing FG at 1% and 1.3% were significantly lower than the value of the slope for control digesta. The reduction is believed to be due to the increase of
Figure 4.3. Changes of maltose concentration with time in the recipient side for control digesta (●) and digesta samples supplemented with YMM 1.15% (◆), SFG 0.93% (■), FG 0.43% (▲) and BG 1.62% (○).

viscosity of the digesta with an increase of FG concentration that reduced the rate of maltose diffusion.

Obtained values of the slopes of maltose concentration changes were plotted against apparent viscosities of digesta samples supplemented with FG at corresponding concentrations (Fig. 4.5). The increase of apparent viscosity of digesta from 0.0007 Pa.s up to 0.156 Pa.s (for control digesta and digesta sample with FG 0.8% respectively) did not reduce maltose transport significantly compared to control. Further increase of the digesta apparent viscosity (≥ 0.045 Pa.s) was effective in maltose transport reduction. In the work of Dhital et al. (2014) addition of cereal β-glucan at 1% and 2% increased the apparent viscosity of sample by 10 and 100 fold, compared to control, however, that increase in viscosity reduced glucose mass transfer
Figure 4.4. Changes of maltose concentration with time in the recipient side for control digesta (♦) and digesta samples supplemented with fenugreek gum at various concentrations 0.43% (▲), 0.60% (●), 0.80% (■), 1.00% (×) and 1.3% (○).

coefficient only by a factor of 1.5 and 2.5. That result is in agreement with findings of the present study where a small reduction in maltose transport was obtained only with the large increase of digesta apparent viscosity.

4.4. Conclusions

This work represents in vitro studies on maltose transport in simulated small intestinal conditions in the presence of DF using diffusion cell where a shear rate of simulated digesta was controlled. None of the studied DF types, including YMM, SFG, FG or OG at concentrations representing 3 eq. were effective in reduction of maltose diffusion. The effect of a wide range of
Figure 4.5. Effect of digesta apparent viscosity at 60 s$^{-1}$ (achieved by manipulating fenugreek gum concentration) on the slope of maltose concentration change.

FG concentrations on maltose diffusion was also investigated. Results showed that maltose diffusion was reduced when FG was present in digesta at concentration $\geq 1\%$ (w/v). Digesta with FG at 1% (w/v) had an apparent viscosity at 60 s$^{-1}$ almost 350 fold higher than control digesta, nevertheless such significant increase in digesta apparent viscosity reduced slope of maltose concentration change from 0.077±0.003 to only 0.0674±0.003.

Borgstrom et al. (1957) showed that meal is diluted minimum 3 fold, by the time it reaches duodenum. Based on that and results of this experiment, in order to reduce transport of maltose in the small intestine concentration of the FG in the meal has to be at least 3%. According to our experience, it would be rather challenging to formulate food products with any of the studied DF types at concentrations higher than concentrations that represent 3 eq. Thus, based on our findings, which correlate with results obtained by Dhital et al. (2014), it seems that delay of
sugar transport in the small intestine would not be the primary mechanism involved in attenuation of postprandial blood glucose after consumption of DF observed *in vivo*.

In the present study, the effect of DF on the maltose transport from the bulk of simulated digesta was investigated. Nonetheless, besides alteration of physical properties of luminal bulk there is evidence that DF can also change permeability of mucous layer that is located on the mucosal surface of the small intestine, therefore affecting mass transfer of nutrients through it (Mackie, Rigby, Harvey, & Bajka, 2016a and Mackie et al., 2016b). Thus, if YMM, SFG, FG and OG can alter permeability of the mucous layer (not investigated in the present study) at concentrations that represent $\leq 3$ eq., that can potentially lead to attenuation of postprandial glycemic responses.
CHAPTER 5. VISCOUS PROPERTIES AND STABILITY OF DIETARY FIBRE IN SIMULATED GASTRIC CONDITIONS

Abstract

In the present work behavior of modified tapioca starch and three types of dietary fibre including yellow mustard mucilage, soluble flaxseed gum and fenugreek gum in simulated gastric conditions at three different values of shear rate 60, 120 and 240 s\(^{-1}\) and two pH values 1.8 and 4.0 were studied. Even at pH 1.8, the lowest value of pH used in the study, simulated gastric digesta samples supplemented with modified tapioca starch, yellow mustard mucilage and soluble flaxseed gum maintained constant values of apparent viscosity during 120 min at 37 °C, thus degradation of these polysaccharides in a human stomach would not be expected. The apparent viscosity of FG-containing digesta was declining during the course of the experiment, which could be due to dissociation of galactomannan complexes or/and degradation of galactomannan in an acidic environment. Values of the apparent viscosity of digesta samples containing yellow mustard mucilage and soluble flaxseed gum were lower at pH 1.8 than at pH 4.0 probably due to conformational changes of pectic-like polysaccharides of yellow mustard mucilage and soluble flaxseed gum on pH lowering. In the majority of the cases, simulated gastric digesta samples had values of apparent viscosities different from each other.

5.1. Introduction

Water-soluble dietary fibre (DF), when incorporated in a diet, can bring physiological benefits, including attenuation of postprandial blood glucose levels (Jenkins et al., 1987; Sharma et al., 1990; Thakur et al., 2009; Thondre & Henry, 2009). These benefits are associated with the rheological behavior of DF in the gastro-intestinal lumen, however, the exact mechanism of that
attenuation is unclear (Dikeman, & Fahey, 2006).

Studies of Holt et al. (1979), Leclere et al. (1994) and Schwartz et al. (1988) showed evidence that DF intake can lead to attenuation of plasma glucose by controlling gastric emptying. In fact, several studies have indicated that DF can prolong transit time in the upper portion of the gut due to the delayed gastric emptying (Darwiche et al., 2003; Miyazawa et al., 2006; Schonfeld et al., 1997; Bergmann et al., 1992; Wilmshurst & Crawley, 1980; Di Lorenzo, Williams, Hajnal, & Valenzuela (1988) and Marciani et al., 2001). It was shown that delay in gastric emptying after DF intake is related to the luminal viscosity. Study of Marciani et al. (2001) demonstrated that addition of high-viscosity locust bean gum to the meals delayed gastric emptying compared to low-viscosity locust bean gum containing meals. Thus, water-soluble DF can have a direct impact on the glycemic responses through delay of gastric emptying that is affected by the rheological behavior of DF in the gastro-intestinal lumen. Therefore, it would be helpful to obtain knowledge on the rheological properties of DF in conditions specific to the human gastric lumen that includes factors such as ionic strength, pH, temperature and presence of divalent cations that can greatly affect physical properties of DF solution.

It also has to be considered that DF can be subjected to degradation in the acidic environment of the stomach. The combination of factors such as elevated temperatures, extensive physical treatment and high acidic environment can cause polysaccharide depolymerization. Foster (1967) observed that when pH of guar and locust bean gum solutions (1% w/v) were adjusted to pH 4.0, the viscosity of these solutions was reduced after incubation during 1 h at 75°C, however the neutralization of this solution prevented the viscosity reduction. Wang, Ellis, & Ross-Murphy (2000) showed that based on changes in viscosity, acidic degradation of guar galactomannan is an outcome of a combination of two factors, pH of the solution and temperature. These authors
concluded that at $4.0 \geq \text{pH} > 2.0$ and temperature $< 50^\circ\text{C}$, any significant acidic degradation of guar galactomannan would not likely occur. The decrease of the pH in combination with an increase in the temperature caused acidic degradation of polysaccharide that followed random scission process.

The pH of the gastric contents increases instantly after ingestion of meal from value $< 2.0$ (at fasted state) as a result of buffering stomach acidic environment, then it gradually decreases due to secretion of gastric juices as shown in Fig. 5.1. Therefore, the pH of the postprandial stomach during the first hour is about 5.0 - 3.0 and between 1.5 and 2.0 during the 2nd hour (Malagelada et al., 1976; Rydning, Nesland, & Berstad, 1984). Therefore, it would not be expected for any significant acidic degradation of polysaccharides in the human stomach to occur during the first hour postprandially. It is possible however for some acidic degradation to take place at higher acidic environment (pH $\sim 1.8$) during the second hour.

![Figure 5.1. Postprandial changes of pH in the human stomach.](image)

Several researchers have been investigating the stability of polysaccharides in simulated gastric conditions. Capron, Yvon, & Muller (1996) studied the stability of carrageenan in the artificial automated stomach where dynamic processes such as gradual gastric emptying and pH change were represented. Only trivial reduction of carrageenan molecular weight was observed under most severe gastric conditions. Wang et al. (2000) studied the effect of low pH and elevated temperature on the stability of guar galactomannan. Only slight degradation, based on viscosity reduction, of polysaccharide was observed by the end of the 4th hour of incubation at 37°C and pH 1.5 and no degradation at pH 2.0. These authors concluded that only insignificant degradation of guar galactomannan can occur in the gastric conditions. Johansson et al. (2006) did not observe degradation of cereal β-glucan in the simulated gastric environment (pH 1.0, 37°C) either during 12 h period.

The objective of the present study was to investigate the rheological behavior and stability of water-soluble DF, including yellow mustard mucilage (YMM), soluble flaxseed gum (SFG), fenugreek gum (FG) and modified tapioca starch, in simulated gastric conditions.

5.3. Materials and methods

5.3.1. Preparation of simulated gastric digesta

In Chapter 2, concentrations of YMM, SFG, and FG that under simulated small intestinal conditions represent from 0.5 to 3 apparent viscosity (at 60 s⁻¹) equivalent (eq.) of EFSA (2011) glycemia control health claims for cereal β-glucan were obtained (Table 2.6). These concentrations were calculated based on the assumption that meal is diluted 3 fold by the time it reaches the small intestine. Thus, if we assume that semi-solid meal is diluted 2 fold in the stomach, concentrations of SFG, YMM and FG that represent 3 eq. in the gastric digesta would
be 1.40%, 1.73% and 0.65% (w/v) respectively (Minekus et al. 2014). Based on the amount of
the modified tapioca starch (TEXTRA PLUS®, Ingredion, Mississauga, Canada) used in starch-
containing treatments in clinical trial carried out in present work (Chapter 6) and assuming that
meal is diluted two-fold in the stomach, concentration of the starch in the stomach would be
expected to be 3.76% (w/v).

To prepare simulated gastric digesta, first the required amount of DF or starch was
dissolved in distilled water during 1 h at 80 ºC, 3 h at 90 ºC, 2 h at 80 ºC, 2 h at 90 ºC and 0.5 h at 60 ºC for
SFG, FG, YMM, OG and tapioca starch respectively, with constant magnetic stirring (Qian et al.,
2012b; Wu et al., 2009; Wang et al., 2003) and then supplemented with NaCl and CaCl₂. The pH
of the mixture was adjusted to value either to 4.0 or 1.8 with HCl solutions at different
concentrations to represent mean pH of the first or second hour of the postprandial human
stomach and a final volume of the sample was brought to 25 mL with distilled water (Malagelada
et al., 1976). Concentrations of NaCl and CaCl₂ in the final solution were 36.1 mM and 0.075
mM, representing physiological concentrations of these salts in the stomach (Minekus et al.,
2014).

5.3.2. Measurements of gastric digesta apparent viscosity

Measurements of gastric digesta apparent viscosity were carried out on the rheometer (TA
2000, TA Instruments, New Castle, United States) equipped with cup and bob geometry (inner
diameter 30 mm, outer diameter 28 mm) at 37°C. Simulated gastric digesta (8 mL) was placed
into the cup of the rheometer. Brookfield viscosity standard # 5 (4.7 mPa.s at 25°C) (1mL)
(Brookfield AMETEK, Inc., Middleboro, United States) was added on the top of the sample to
prevent water evaporation. A constant shear rate (60, 120 or 240 s⁻¹) was maintained during
entire experiment (120 min) (Curt & Pringle, 1969). Values of the apparent viscosity of digesta were recorded every 5 min. Each sample was analyzed in duplicate, the mean value and standard deviations are reported. At the end of the experiment, sample was transferred into the clean tube and its pH was adjusted to 7.0 with 1N NaOH solution. Finally, the sample was frozen and then freeze-dried. Preliminary analysis showed that pH values of all simulated gastric digesta samples were constant during the experiment.

5.3.3. Statistical analysis

Statistical analysis was performed using GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, United States). Univariate analysis of variance (ANOVA) followed by a post hoc Tukey test were conducted for mean group comparison of the apparent viscosities of simulated gastric digesta. The significance level was set at p < 0.05.

5.4. Results and discussion

Changes of apparent viscosities of simulated gastric digesta samples supplemented with each type of DF or tapioca starch at two pH values and three different shear rates relevant to human postprandial stomach are presented in Fig. 5.2. Simulated gastric digesta samples supplemented with YMM, SFG and starch maintained constant values of apparent viscosity during entire experiment (120 min). In contrast, apparent viscosity values of FG-containing digesta samples were declining during the course of the experiment. Particularly, at 0 min digesta samples with FG at both pH values had the same apparent viscosity at equivalent shear rates (~ 0.081, ~ 0.061 and ~ 0.044 Pa.s at 60, 120 and 240 s\(^{-1}\) respectively) followed by gradual decline with time. The progress of the decline was pH-dependent and occurred more rapidly at pH 1.8, at least 60% of
initial (0 min) apparent viscosity reduction during 120 min, than at pH 4.0, at least 20% apparent viscosity reduction at all three shear rates. Besides measurements of changes in apparent viscosity at pH 1.8 and 4.0 additional measurement for FG-containing digesta sample was done at pH 6.5 and 120s⁻¹. The apparent viscosity of digesta sample with FG was constant (0.064 Pa.s) at pH 6.5 and was slightly higher (6.4%) than the apparent viscosity of this sample at 0 min at pH 1.8 and pH 4.0. This is not likely to be an effect of galactomannan degradation, as the time was too short for degradation to occur. Changes of apparent viscosity of digesta samples supplemented with FG at all three pH values can be seen in Fig. 5.3. The progress of apparent viscosity decline occurred faster with pH reduction.

Polysaccharides can undergo hydrolysis in an acidic environment, as a result reduction in viscosity of polysaccharide solution would be expected. In the present study, apparent viscosities of YMM, SFG and starch containing simulated gastric digesta samples maintained constant viscosity during entire 120 min, even at the lowest pH value used in the present study (pH 1.8). Therefore, it can be concluded, those polysaccharides contained in YMM, SFG and starch did not depolymerize at studied pH values and therefore they would not be expected to degrade in a human stomach.

Doyle et al. (2009) have proposed that in neutral environment associations (‘‘hyperentanglements’’) are formed between chains of fenugreek galactomannans in aqueous solutions (in Chapter 2 of the current work presence of associations between molecules of galactomannan of FG was also confirmed). In their study, the number of these associations grew with an increase of polymer concentration, resulting in an increase of average molecular weight and thus the solution viscosity. When galactomannan was dissolved in an alkali at the same concentration, reduction in solution viscosity was observed, which was practically totally
Figure 5.2. Changes of apparent viscosity of simulated gastric digesta supplemented with yellow mustard mucilage 1.73% (w/v) (♦), soluble flaxseed gum 1.40% (w/v) (▲), fenugreek gum 0.65% (○) and modified tapioca starch 3.76% (w/v) (●) at three different constant shear rates 60 s\(^{-1}\) (A, B), 120 s\(^{-1}\) (C, D) and 240 s\(^{-1}\) (E, F) and two pH values 1.8 (A, C and E) and 4.0 (B, D and F).
reversed on neutralization. The reduced viscosity in alkali solution is believed to be due to ionization of hydroxyl groups resulting in inhibition of intermolecular association.

As mentioned, the apparent viscosity of FG-containing gastric digesta at pH 1.8 and 4.0 declined with time. Potentially, the decline can be a result of gradual dissociation of galactomannan complexes in an acidic environment under constant agitation. The dissociation could occur due to reduced number of hydrogen bonds between molecules of polysaccharide as a result of weak protonation of hydroxyl groups at lower pH values. Another possible mechanism is degradation of fenugreek galactomannan in an acidic environment. However, acid hydrolysis of galactomannan would not be expected at pH values as high as 4.0. Wang et al. (2000) studied the effect of pH and temperature on the stability of guar galactomannan. Slight degradation, based on viscosity reduction, of the polysaccharide was observed by the end of the 4th hour of incubation at 37°C at pH 1.5 and no degradation at pH 2.0, suggesting that only insignificant degradation of polysaccharide can occur in the gastrointestinal tract.

Figure 5.3. Changes of apparent viscosity of simulated gastric digesta with fenugreek gum at 0.65% (w/v) during constant shear rate 120 s⁻¹ and three different pH values: 1.8 (▲), 4.0 (◆) and 6.5 (●).
The apparent viscosity of starch was not affected notably by pH (Fig. 5.4). In contrast, apparent viscosities of digesta samples supplemented with YMM and SFG showed great pH dependence. Particularly, values of apparent viscosities of both DF types were higher at pH 4.0 than at pH 1.8 at all three shear rates. The conformation of polysaccharides, especially the ones that contain anionic groups, can be affected by the pH of an environment. Lowering pH below \( \text{pK}_a \) value can suppress ionization of carboxylic acid groups resulting in the less extended conformation of the molecule and as a consequence reduction of solution viscosity is observed.

Both YMM and SFG contain pectic-like polysaccharide fractions, that represent 53% and 25% respectively (Cui, 2000; Qian et al., 2012). Therefore, it is probable that reduction of apparent viscosity of digesta samples with YMM and SFG at lower pH could be a result of altered conformation of pectic-like polysaccharide fractions of these DF types at pH 1.8.

The opposite effect of pH reduction on apparent viscosity at 92.32 s\(^{-1}\) of “water soluble yellow mustard mucilage fractions” aqueous solution was reported Cui et al. (1993a). In their

![Figure 5.4. Apparent viscosities (mean values of 120 min) of simulated gastric digesta with yellow mustard mucilage 1.73% (w/v) (diamonds), soluble flaxseed gum 1.40% (w/v) (triangles) and modified tapioca starch 3.76% (w/v) (circles) at pH 1.8 (closed symbols) and pH 4.0 (open symbols) at three different shear rates.](image-url)
study, apparent viscosity of hydrocolloid at 22 °C increased with reduction of pH from 4.0 to 2.0. The pH reduction could remove electrostatic repulsion between pectic-like polysaccharides, therefore, promoting their self-association and as result higher viscosity.

In the semi-dilute regime, polymer chains of many polysaccharides get entangled as they are forced to overlap and interpenetrate. The disruption and formation of these entanglements are balanced at low shear rates, ensuring constant viscosity. Disruption of entanglements predominates at higher shear rates resulting in shear-thinning. In Chapter 2 we showed that at concentrations above specific overlap concentrations, shear-thinning behavior was observed for simulated small intestinal digesta samples supplemented with all four DF types at a shear rate ranging from 0.01 to 1000 s⁻¹. Simulated small intestinal digesta samples supplemented with each of the four DF types at concentrations representing 3 eq. exhibited shear-thinning behavior (Fig. 2.1.). In the current model, concentrations of DF that represent 3 eq. in the gastric digesta are higher than concentrations of the same DF types that represent 3 eq. in the small intestinal digesta (1/2 versus 1/3 meal dilution factor). Thus shear-thinning of simulated gastric digesta samples with each of the four DF types at concentrations representing 3 eq. would be expected.

The apparent viscosity of gastric digesta with YMM at both pH values was affected greatly (~ 2.5 fold reduction) by an increase of shear rate from 60 to 240 s⁻¹. Similar to this finding, dramatic reduction of apparent viscosity of YMM solution in simulated small intestinal conditions was observed (Chapter 2). Distinct shear thinning behavior of “yellow mustard mucilage water soluble fraction” has also been reported by Cui et al. (1993a) and Cui et al., (1993b). Wu et al. (2009) also observed intense reduction of apparent viscosity of 1% aqueous solution of “non-pectic polysaccharides from yellow mustard mucilage” with an increase of shear rate from 0.001 to 1000 s⁻¹ at 25°C.
The reduction of apparent viscosity of gastric digesta with SFG at pH 4.0 was only observed when shear rate was increased from 120 to 240 s\(^{-1}\) when the same sample exhibited only slight shear-dependency at pH 1.8 with a shear rate increase from 60 to 240 s\(^{-1}\). Reduced degree of chain overlapping and thus lower apparent viscosity and less shear-thinning behavior would be expected when conformation of a pectic-like polysaccharide is less extended at pH 1.8. Starch containing sample exhibited only slight shear-thinning behavior at both pH values.

Concentrations of DF at which they result in the same number of EFSA (2011) health claim equivalents, including 3 eq., have matching apparent viscosities in simulated small intestinal conditions at 60 s\(^{-1}\). However, in the present chapter we found that apparent viscosities of simulated gastric digesta samples supplemented with YMM and SFG at concentrations representing 3 eq. were significantly different in a majority of the studied conditions, except for two cases, 1\(^{st}\) when pH 4.0 at 60 s\(^{-1}\) and 2\(^{nd}\) when pH 1.8 at 120 s\(^{-1}\). The largest difference in apparent viscosity values between simulated gastric digesta samples containing YMM and SFG was almost 2 fold at pH 4.0 and 240 s\(^{-1}\). At the same time, the difference between simulated gastric digesta without DF (water at 37 °C) and digesta with a lowest apparent viscosity in the present study (YMM at pH 1.8 and 240 s\(^{-1}\)) would be around 25 fold. It is difficult to compare apparent viscosity of FG-containing gastric digesta as its values were changing with time.

### 5.5. Conclusions

Solutions of YMM, SFG and starch maintained constant values of apparent viscosity at conditions expected to be in the human stomach. Therefore, we concluded that polysaccharides containing YMM, SFG and starch would not be expected to degrade to a significant extent in a human stomach. In contrast, apparent viscosity values of FG-containing digesta samples at pH
values 4.0 and 1.8 were declining steadily during the course of the experiment. The progress of apparent viscosity decline was pH-dependent, the decline occurred faster with reduction of pH. The decline could be a result of gradual dissociation of fenugreek galactomannan complexes in the acidic environment on constant agitation or/and degradation of fenugreek galactomannan in the acidic environment. Though, acid hydrolysis of galactomannan would not be expected at pH values as high as 4.0

The values of apparent viscosity of digesta samples supplemented with YMM and SFG at pH 1.8 were lower than values at pH 4.0. Perhaps the reduction was related to the transition of pectic-like polysaccharides of YMM and SFG to less extended conformation as electrostatic repulsion between segments of these molecules lessened on pH lowering. As expected, values of apparent viscosity of all gastric digesta samples were affected by the increase of shear rate from 60 to 240 s⁻¹, when YMM exhibited the most intense shear-thinning behavior. In the majority of the cases, significant differences between values of apparent viscosities were observed between YMM and SFG containing gastric digesta samples, despite that both DF solutions in simulated small intestinal conditions had an equal apparent viscosity at 60 s⁻¹ (Chapter 2). Several studies suggest that gastric emptying can be affected by the viscosity of digesta in the stomach. However, no information is currently available on what levels of apparent viscosity would affect the gastric emptying rate. Therefore, differences in apparent viscosity found between simulated gastric digesta samples with different types of DF may not translate into differences in rates of gastric emptying in vivo.
CHAPTER 6. MECHANISMS INVOLVED IN POSTPRANDIAL GLYCEMIA AND INSULINEMIA ATTENUATION AS A RESULT OF DIETARY FIBER CONSUMPTION: CORRELATION BETWEEN IN VITRO AND IN VIVO STUDIES

Abstract

This study investigates mechanisms involved in postprandial glycemic and insulinemic responses after intake of dietary fibre, using results of the in vitro experiments and postprandial glycemic and insulinemic responses in humans. In addition, to in vitro experiments, including rheological properties of simulated gastric and small intestinal conditions, kinetics of amylolysis in simulated small intestinal conditions and maltose transport in simulated small intestinal conditions (Chapters 2-5), a human clinical trial was conducted to investigate the effect of three dietary fibre types, yellow mustard mucilage, soluble flaxseed gum and fenugreek gum, on postprandial glycemic and insulinemic responses. Adults (n=15) at risk for type 2 diabetes consumed pudding treatments supplemented with one of three types of dietary fibre at a concentration that would match 3 times apparent viscosity (at 60 s⁻¹) equivalent of European Food Safety Authority (2011) glycemia control health claim for cereal β-glucan as measured in simulated small intestinal conditions. Treatments also differed by the type of available carbohydrates used, high maltose syrup and modified tapioca starch.

The human clinical trial showed that pudding treatments supplemented with each of the three types of dietary fibre significantly lowered peak blood glucose and plasma insulin concentrations and paracetamol concentration 1h AUC values compared to the control pudding but values were not different among the dietary fibre-containing treatments, despite their different concentrations. The mean values of peak blood glucose and plasma insulin concentrations obtained for high maltose corn syrup-based treatments were generally higher than mean values for modified tapioca starch-based treatments but these differences were not statistically different.
Therefore, results of this study showed that attenuation of postprandial glycemic and insulinemic responses was independent of the botanical source of dietary fibre but was rather affected by the ability of DF to alter physical properties of aqueous solutions. Additionally, evidence was found that observed attenuations could be due to the delay of gastric emptying and to some degree due to a hindrance of amylolysis.

6.1. Introduction

Diabetes mellitus is a chronic disease, which is linked with an inability of the body to properly metabolize glucose, thus resulting in abnormally high blood glucose levels. Depending on the mechanism diabetes is divided into two types; Type 2 (T2D) accounts for 80-90% all diabetes cases. Chronic, abnormally high blood glucose levels damage body tissues, causing a variety of serious complications. As a result, individuals suffering from diabetes have twice the mortality risk than those without diabetes (Skyler, 2012). The incidence of T2D is dramatically increasing worldwide. For instance, in Canada, around 8.3% of Ontario population was diagnosed with diabetes in 2010, and that number is expected to reach 11.9% by 2020. As a result, the economic burden of diabetes in the province will rise from $4.9 to $7.0 billion from 2010 to 2020 (Canadian Diabetes Association, 2012).

Studies suggest that T2D can be prevented or at least delayed with medication or even with nonpharmacological lifestyle interventions, such as limited consumption of meals resulting in hyperglycemia (Lindstrom et al., 2003; Tuomilehto et al., 2001; Willett et al., 2002). The research evidence has shown that consumption of some types of dietary fibre (DF) attenuates glycemic responses. Thus diets high in DF are often prescribed to individuals with diabetes to improve glycemic control (Gougeon et al., 2008; Anderson et al., 2004). Diets rich in DF also
can reduce risks of several diseases, including T2D (Salmeron et al., 1997).

DF can be divided into soluble and insoluble depending on its solubility in water. Both types have different molecular characteristics and physiological effects. Some soluble DF (guar, pectin, oat gum and psyllium) can develop viscous solutions (viscous DF) and have the potential to lower serum cholesterol and blood glucose levels (Malkki, 2004; Dikeman & Fahey 2006). Many in vivo studies have shown evidence that consumption of viscous DF of various origins including flaxseed (Thakur et al., 2009), soybean (Tsai et al., 1987), apple (Schwartz et al., 1988), guar gum (Morgan et al., 1990), sugar-beet (Morgan et al., 1990), seaweed (Harden et al., 2012), yellow mustard (Jenkins et al. (1987) and cereals (Garcia et al., 2007; Lu et al., 2004; Tappy et al., 1996; Thondre & Henry, 2009), results in attenuation of blood glucose levels.

It has been proposed that the ability of soluble DF to reduce blood glucose relates to its capacity to create a viscous solution. Jenkins et al. (1978) found that glucose-containing drinks supplemented with various DF reduce peak concentration (Cmax) and 1h area under the curve (AUC) blood glucose and generally reduced serum insulin concentrations. That reduction was positively correlated with the viscosities induced by the DF. Wood et al. (1994) found a negative relationship between Cmax and 2h AUC blood glucose and plasma insulin values and viscosity of DF-enriched drinks. There is evidence that intake of viscous DF results in glycemia reduction due to alterations of gastric and/or small intestinal luminal viscosity. Guerin et al. (2001) showed that some DF can increase the apparent viscosity (at 45 s⁻¹) of gastric contents in pigs. Cherbut et al. (1990) found that besides enhancing apparent viscosity (at 10 s⁻¹) of gastric contents, guar gum also increased the viscosity of duodenal and jejunal digesta in pigs. In both studies, it was found that DF which enhanced luminal viscosity to a greater degree was also more effective in increasing transit time in upper GI tract (one of the mechanisms believed to be involved in
glycemia attenuation). Roberts et al. (1990a) and Roberts et al. (1990b) also found that supplementation of the meal with guar gum increased the viscosity of jejunal digesta in pigs. A similar result was found by Ellis et al. (1995), who additionally showed a negative correlation between the viscosity of jejunal digesta and glycemic responses in pigs.

Even though the relationship between luminal viscosity and ability to attenuate blood glucose after DF intake has been generally accepted, the exact mechanism of that attenuation is unclear. Three popular proposed mechanisms are: 1) delayed gastric emptying, 2) delayed transport of hydrolyzed starch fragments or glucose in the small intestine and 3) reduced digestive enzyme activity in the small intestine (Dikeman & Fahey, 2006).

After consumption, food undergoes disintegration in the stomach, where it becomes partly liquefied (chyme). Further, chyme gets emptied from the antrum through the pylorus into the duodenum. The rate of gastric emptying is coordinated through neurohormonal feedback mechanisms (Brownlee, 2011). Thus, the amount of nutrients including available carbohydrates delivered to the small intestine for further digestion and absorption can also vary, depending on the gastric emptying rate. Therefore, alterations of gastric emptying rate can potentially affect glycemic response (Gropper et al., 2005; Ma et al., 2009; Pilichiewicz et al., 2007). DF generally accelerates overall gut transit time (Brownlee, 2011). Nevertheless, there is evidence to suggest that transit time in the upper portion of the gut can be prolonged with DF intake due to the delayed gastric emptying (Darwiche et al., 2003; Miyazawa et al., 2006; Schonfeld et al., 1997; Bergmann et al., 1992; Wilmshurst & Crawley, 1980; Di Lorenzo et al., 1988; Marciani et al., 2001). It was shown that delay in gastric emptying after DF intake is related to the luminal viscosity. In vivo study of Marciani et al. (2001) demonstrated that addition of high-viscosity locust bean gum to meals (both with and without nutrients) delayed gastric emptying compared
to low-viscosity locust bean gum-containing meals. Therefore, viscous DF can have a direct impact on the glycemic responses through delay of gastric emptying. In fact, studies involving blood glucose measurements in humans by Holt et al. (1979), Leclere et al. (1994) and Schwartz et al. (1988), provided evidence that delay in gastric emptying can lead to attenuation of blood glucose after viscous DF intake.

At the same time, in other studies the effect of viscous DF (guar gum (Rydning et al., 1985; Van Nieuwenhoven et al., 2001) and alginate (Hoad et al., 2004)) on the gastric emptying rate was not found. Also, Blackburn et al. (1984), Edwards et al. (1987), Jarjis et al. (1984), Hlebowicz et al. (2008), Sandhu et al. (1987) did not find correlation between gastric emptying rate and postprandial blood glucose levels after consumption of meals supplemented with various hydrocolloids (xanthan, locust bean, guar gums, oat β-glucan, pectin). In the in vivo work of Ehrlein & Stockmann (1998), a mixture of nutrients was perfused directly into the jejunum of miniature pigs, thus avoiding gastric phase. It was found that addition of guar gum to the mixture reduced absorption rates of the nutrients. These studies suggest that mechanism(s) other than delay of gastric emptying can be involved in attenuation of glycemic responses.

Several in vitro studies showed that presence of viscous DF in the small intestinal lumen can 1) delay diffusion of glucose (Jenkins et al., 1986, Kwong et al., 2013; Lecumberri et al., 2007; Ou et al., 2001), thus DF could slow transport of hydrolyzed starch fragments and glucose for further digestion and absorption at the absorptive surfaces, and 2) reduce activity of digestive enzymes in the small intestine, (Shelat et al., 2010; Shelat et al., 2011; Ou et al., 2001; Hansen & Schulz, 1982; Ikekami et al., 1990; Isaksson, Lundquist, & Ihse, 1982) therefore slowing the rate of starch hydrolysis. Those processes can potentially result in attenuation of glycemic responses. Understanding mechanisms involved in attenuation of postprandial glycemic and insulinemic
responses can help to design foods that reduce these responses more effectively.

The objectives of the present work were 1) to investigate the effect of three types of DF, including yellow mustard mucilage, soluble flaxseed gum and fenugreek gum at concentrations matching small intestinal luminal viscosity, established in *in vitro* experiments, on postprandial glycemic and insulinemic responses in humans, 2) investigate the effect of degree of polymerization of available carbohydrates (high maltose syrup and modified tapioca starch) present in the meal on postprandial glycemic and insulinemic responses, 3) draw parallels between results of postprandial biochemical blood profile and *in vitro* experiments (rheological properties in simulated gastric and small intestinal conditions, kinetics of amylolysis in simulated small intestinal conditions and maltose transport in simulated small intestinal conditions) to gain more understanding of mechanisms involved in attenuation of postprandial glycemic and insulinemic responses as a results of dietary fiber intake.

### 6.2. Materials and methods

#### 6.2.1. Extraction of dietary fibre

Dietary fibres, including yellow mustard mucilage, soluble flaxseed gum, fenugreek gum and oat gum were extracted from yellow mustard bran (G.S. Dunn, Hamilton, Canada), flaxseed hull (Natunola Health Inc., Winchester, Canada), CANAFEN® Gum (Emerald Seed Products Ltd., Avonlea, Canada) and oat bran concentrate (Viterra, Portage la Prairie, Canada) respectively, as described in Chapter 2. Water-extracted fenugreek gum (FG-WE) and ethanol-precipitated yellow mustard mucilage (YMM-ETH) and soluble flaxseed gum (SFG-ETH) were used in clinical trial. Purified versions of these DF types (FG, YMM and SFG) and OG, were used in the *in vitro* experiments. Results of compositional analyses of these DF types, are shown in Tables
2.1 and 2.2.

6.2.2. Preparation of treatments for clinical trial

Treatments of the present study included control glucose (50 g) beverage (Trutol®, model 401272P, Fisher Scientific Company, Waltham, United States) with water (combined total volume 500 mL) and eight pudding treatments. Two lines of pudding treatments differed by the source of available carbohydrates, including syrup-based (high maltose corn syrup, Ingredion Canada Incorporated, Cardinal, Canada) and starch-based (modified tapioca starch, TEXTRA®PLUS, Ingredion, Brampton, Canada) were tested. Maltose is the main product of starch hydrolysis by α-amylase, therefore differences in the postprandial biochemical profile of blood between corresponding syrup-based and starch-based treatments would be expected to be related to the action of α-amylase.

Each line of these treatments included four puddings, a control pudding treatment (without extracted DF) and three pudding treatments supplemented with one of the extracted DF type (FG-WE, YMM-ETH or SFG-ETH).

Concentrations of YMM, SFG and FG at which they result in equivalent values of apparent viscosity at 60 s\(^{-1}\) in simulated small intestinal conditions presented in Table 2.6 were obtained based on the assumption that meal is diluted 3 fold by the time it reaches the small intestine (Borgstrom et al., 1957). According to this approach, concentrations of these DF types in the meal should be three times higher. Also, YMM, SFG and FG were purified versions of YMM-ETH, SFG-ETH and FG-WE respectively (Chapter 2, Table 2.6). Therefore, differences in the amounts of contaminants such as protein and ash were considered when concentrations of YMM-ETH, SFG-ETH and FG-WE for formulations of pudding treatments were calculated.
Each DF type was used to represent 3 times the apparent viscosity (at 60 s\(^{-1}\)) equivalent of EFSA (2011) glycemia control health claim for cereal β-glucan.

To prepare the puddings, first one of the DF types was left to hydrate in 500 mL of water with constant agitation with electrical mixer during 12 h at 24 ºC. Then, to achieve complete solubilisation of DF, solution was warmed up to 80 ºC for 2 h, 80 ºC for 1 h, and 90 ºC for 3 h at constant stirring for YMM-ETH, SFG-ETH and FG-WE respectively (Wang et al., 2003; Qian, 2012b; Wu et al., 2009). Control pudding treatments did not contain extracted DF, therefore the above steps were omitted for these treatments.

YMM-ETH, SFG-ETH and FG-WE contained different levels of protein (Table 2.2) and were used in treatments at various concentrations. Therefore, soy protein isolate (Soy Protein Isolate, Now Foods, Bloomingdale, United States) was added to each DF solution at different amounts to compensate for protein differences and to have an equal total protein content for all pudding treatments. Added soy protein isolate together with gelatin (9.80 g, Knox Kraft Foods, Northfield, United States) was dissolved in the solution during additional 45 min at 80 ºC and constant mixing. Then, depending on the treatment type (syrup-based or starch-based) each mixture was supplemented either with syrup or starch, which were dissolved in the solution (60 ºC, 30 min) together with chocolate powder (19.09 g, NESQUIK, 33% less sugar, Nestlé, Halifax, Canada). When the mixture was cooled down to room temperature, a slurry of paracetamol (1500 mg, Tylenol Extra Strength Caplets, McNeil Consumer Healthcare, Markham, Canada) in water was added, and the final volume of the mixture was brought to 500 mL with water. Then mixture was stirred for additional 5 min to achieve homogeneity and the contents of the cup were poured into a bowl. The bowl with pudding was placed into the refrigerator (4 ºC) (day 0). The pudding was served to a participant on the morning of day 1 or
morning of day 3. All pudding treatments were 500 mL and contained equal amounts of protein (8.9 g), fat (1.4 g) and available carbohydrates (50 g). The composition of all pudding treatments is presented in Table 6.1. Treatments were supplementation with paracetamol to assess the rate of gastric emptying.

6.2.3. Clinical trial

The randomized, double-blinded, crossover, controlled human study was led by Brittney Kay as described in detail elsewhere (Kay, 2016). Briefly, human subjects (10 males and 5 females, mean age 55.1 year-old) with body mass index (BMI), homeostatic model assessment for insulin resistance (HOMA-IR²) and fasting blood glucose mean values equal to 29.5 kg/m², 1.2 and 5.5 mmol/L respectively at risk for T2D (based on the score of ≥21 on the CANRISK questionnaire) were recruited. The study protocol was approved by University of Guelph Human Research Ethics Board and all participants provided written informed consent. Each participant consumed two control glucose beverage treatments (first and last visits) and eight pudding treatments. Participants reported to the University of Guelph Human Nutraceutical Research Unit in the morning after a 10-12 h fast, with a minimum 5 days washout period between visits. Participants were asked to consume no more than 1 cup of water in the 1 h prior to the study visit. On each visit, a fasting blood sample was obtained by finger-prick and the participant started to consume a treatment (no additional beverages or food besides the actual treatment was provided). Patricians were asked to consume treatment within 10 min. When treatment was consumed a timer was started and further blood samples were collected at 15, 30, 60, 90 and 120 min. Part of the blood sample at each time point was used for glucose analysis (HemoCue® 201, HemoCue®, Ängelholm, Sweden) and the remainder of the sample was centrifuged (2000 g, 21
Table 6.1. Nutritional composition of the control pudding treatments and pudding treatments supplemented with ethanol-precipitated yellow mustard mucilage (YMM-ETH), ethanol-precipitated soluble flaxseed gum (SFG-ETH), and water-extracted fenugreek gum (FG-WE) used in clinical trial.

<table>
<thead>
<tr>
<th></th>
<th>Syrup-based puddings</th>
<th>Starch-based puddings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control YMM-ETH SFG-ETH FG-WE</td>
<td>Control YMM-ETH SFG-ETH FG-WE</td>
</tr>
<tr>
<td>Available carbohydrates (g)</td>
<td>50 50 50 50</td>
<td>50 50 50 50</td>
</tr>
<tr>
<td>Fibre from extracted fibres (g)</td>
<td>0 15.5 11.4 5.9</td>
<td>0 15.5 11.4 5.9</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>8.9 8.9 8.9 8.9</td>
<td>8.9 8.9 8.9 8.9</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>1.4 1.4 1.4 1.4</td>
<td>1.4 1.4 1.4 1.4</td>
</tr>
</tbody>
</table>

°C, 3 min) to obtain blood plasma that was stored at -80 °C until insulin (enzyme-linked immunosorbent assay, Model 80-INSHU-E10.1, ALPCO, Salem, United States) and paracetamol (PARACETAMOL, (Acetaminophen) K8002 assay kit, Cambridge Life Sciences, Ely, United Kingdom) analysis. All measurements were done in duplicate.

6.2.4. Data analysis

The peak concentration (Cmax) and 2h AUC values for postprandial blood glucose and plasma insulin concentrations as well as 1h AUC for paracetamol concentration were determined using GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, United States). All data was examined for normal distribution. The insulin data were not normally distributed and were therefore natural log transformed before statistical analysis. The statistical analysis of postprandial blood glucose and plasma insulin Cmax and 2h AUC values were performed using
the Statistical Analysis Software (Version 9.4, Cary, United States) with significance level set at 
p≤0.05. Values were compared among pudding treatments using ANOVA followed by Tukey’s 
test. The mean group comparison of paracetamol 1h AUC values was performed with GraphPad 
Prism 5.0. using ANOVA followed by a post hoc Tukey test. The significance level was set at p < 0.05.

6.3. Results and discussion

Thorough results of the clinical trial are reported in the work of Kay (2016).

6.3.1. Impact of fibre on postprandial glycemic, insulinenic and paracetamol 
concentrations

Several in vivo studies have shown that supplementation of a meal with water-soluble DF can 
lead to attenuation of postprandial glycemic and insulinenic AUC and Cmax values (Jenkins et 
al. 1978; Wood et al. 1994b). In the present study postprandial blood glucose and plasma insulin 
2h AUC values did not significantly differ among any of the treatments. Conversely, significant 
differences of blood glucose and plasma insulin Cmax values between some pudding treatments 
were found (Fig 6.1 and Fig 6.2). Particularly, supplementation of puddings with each of the 
three types of DF significantly reduced blood glucose and plasma insulin Cmax values compared 
to control, for both syrup-based and starch-based pudding treatments. Specifically, blood glucose 
and plasma insulin mean Cmax values for treatments supplemented with DF were ~13% and ~ 
40% lower than controls in the syrup-based and starch-based pudding treatments, respectively. It 
was also found that blood glucose and plasma insulin Cmax values between syrup-based pudding 
treatments supplemented with each of the three DF types were not significantly different
between each other. A similar finding was observed for the starch-based pudding treatments (Fig. 6.1 and Fig. 6.2).

Gastric emptying can be assessed indirectly using paracetamol absorption test. Particularly, paracetamol concentration AUC values have been shown to be the most reliable parameter for the assessment of gastric emptying (Willems, Quartero, & Numans, 2001). In the present study, paracetamol concentration 1h AUC values showed similar trend observed with postprandial glycemic and insulinemic Cmax values (Fig 6.3). Specifically, both syrup-based and starch-based puddings supplemented with DF had significantly reduced AUC values compared to control. Again AUC values for treatments supplemented with DF were not significantly different between each other. Starch-based treatment with FG-WE was an exception, AUC value for the treatment was not significantly different from control. These results show that supplementation of pudding treatments with each type of DF significantly slows gastric emptying.

As discussed in Chapter 2, all three DF types differed in terms of monosaccharide composition, the number of functional groups, molecular weight and molecular architecture.

Nevertheless, the presence of each of the three DF types in both syrup-based and starch-based pudding treatments significantly attenuated blood glucose and plasma insulin Cmax values and paracetamol concentration AUC values compared to their controls. The concentration of each DF type was chosen to result in equivalent viscous characteristics under simulated small intestinal conditions. Therefore, it can be concluded that attenuation of blood glucose and plasma insulin Cmax values and paracetamol concentration AUC values were independent of the botanical source of DF but were rather affected by the ability of DF to alter physical properties of the solution. A similar finding was observed in Chapter 3, the kinetics of amylolysis in simulated small intestinal conditions were also independent of the source of DF but
Figure 6.1. Mean values of postprandial peak blood glucose concentrations for high maltose syrup-based (open bars) and modified tapioca starch-based (filled bars) pudding treatments, including control treatments and treatments supplemented with ethanol-precipitated yellow mustard mucilage (YMM-ETH), ethanol-precipitated soluble flaxseed gum (SFG-ETH) and water-extracted fenugreek gum (FG-WE) and standard deviations. Data with a superscript * indicate a significant (P<0.05) difference compared to the control pudding treatments.

were rather affected by the viscosity due to DF addition.

Each type of DF was used in pudding treatments at different concentrations, for example, the amount of dietary fibre in the pudding that came from YMM-ETH (15.5 g) was ~2.6 times higher than the amount of DF that originated from FG-WE (5.9 g), still blood glucose and plasma insulin Cmax values and paracetamol concentration 1h AUC values for both treatments were not significantly different (besides the exception of paracetamol concentration 1h AUC value for starch-based treatment with FG-WE). That result suggests that to slows gastric
Figure 6.2. Geometric mean values of postprandial peak plasma insulin concentrations, for high maltose syrup-based (white bars) and modified tapioca starch-based (filled bars) pudding treatments, including control treatments and treatments supplemented with ethanol-precipitated yellow mustard mucilage (YMM-ETH), ethanol-precipitated soluble flaxseed gum (SFG-ETH) and water-extracted fenugreek gum (FG-WE) and 95% confidence intervals. Data with a superscripts * indicate a significant (P<0.05) difference compared to the control pudding treatments.

emptying and attenuate postprandial glycemic and insulinemic responses to similar extent, each DF type should be present at amounts to result in similar viscous properties even though their concentrations may not match.

The dose-response of DF was not investigated in the present work. Previous studies have shown that ability of DF to attenuate postprandial glycemic responses improves with an increase of DF concentration in the meal (Tosh, 2013). Thus, it would be also expected that changes of DF concentration in the treatments from values used in the study would also influence
Figure 6.3. Postprandial paracetamol concentration 1h AUC values, for high maltose syrup-based (white bars) and modified tapioca starch-based (filled bars) pudding treatments, including glucose drink (mean of two), control treatments and treatments supplemented with ethanol-precipitated yellow mustard mucilage (YMM-ETH), ethanol-precipitated soluble flaxseed gum (SFG-ETH) and water-extracted fenugreek gum (FG-WE) and standard deviations. Data with a superscript * indicates a significant (P<0.05) difference compared to the control pudding treatments.

Postprandial blood glucose and plasma insulin Cmax values. Thus, if each DF type would be used at matching concentrations rather than at matching viscosities, perhaps blood glucose and plasma insulin Cmax values between DF-containing would not be similar anymore.
6.3.2. Mechanisms involved in the attenuation of postprandial glycemic and insulinemic responses

Three most popular proposed mechanisms that could be involved in the attenuation of postprandial glycemic responses due to ingestion of water-soluble DF mentioned in the literature are: reduced amylolysis progress due to hindrance of α-amylase activity (mechanism 1), reduced rate of sugar transport from the small intestinal lumen (mechanism 2), and delayed gastric emptying (mechanism 3) (Dikeman & Fahey, 2006). Below, we discuss the possibility of each of these three mechanisms to contribute to attenuation of postprandial blood glucose and plasma insulin concentrations observed in our clinical trial.

6.3.2.1. (Mechanism 1) Effect of dietary fibre amylolysis progress

Available carbohydrates from chocolate powder were contributing close to 30% of total available carbohydrates in each pudding treatment, most of it likely was in form of sugar, the rest of available carbohydrates was either in form of high maltose corn syrup or modified tapioca starch. The blood glucose Cmax mean value obtained for control syrup-based treatment (10.02 mmol/L ± 1.61 (mean and SD)) was higher than mean value for control starch-based treatment (8.95 mmol/L ± 1.35). Besides that, the blood glucose Cmax mean value obtained for syrup-based DF-containing treatments (8.51 mmol/L ± 1.60 (mean and SD of three treatments)) was also higher than mean value for starch-based DF-containing treatments (7.88 mmol/L ± 1.29). Nonetheless, these differences were not significant. The same trend was observed when plasma insulin Cmax mean values between corresponding treatments were compared. The plasma insulin Cmax geometric mean value obtained for control syrup-based treatment (654.11 pmol/L ± 369.63 (geometric mean and 95% confidence intervals)) was higher than value for
control starch-based treatment (484.15 pmol/L ± 192.04). Again the plasma insulin Cmax geometric mean value obtained for syrup-based DF-containing treatments (339.68 pmol/L ± 209.26 (geometric mean and 95% confidence intervals of three treatments)) was also higher than value for starch-based DF-containing treatments (267.13 pmol/L ± 121.19). However, again these differences were not significant. At the same time, pooled blood glucose AUC and Cmax values for all four syrup-based treatments (one control and three DF-enriched treatments) were significantly higher than pooled blood glucose AUC and Cmax values for all four starch-based treatments. However, pooled plasma insulin AUC and Cmax values were not significantly different between treatments with different types of available carbohydrates. Therefore, results of the study showed that degree of polymerization of available carbohydrates used in pudding treatments had an effect on the glycaemic and insulinemic responses, however that effect was not substantial.

Maltose is the major product of starch hydrolysis by α-amylases, thus any considerable digestion of maltose by α-amylases would not be expected, consequently significant digestion of maltose would not likely occur in the human lumen either. In contrast, starch has to be hydrolyzed by the α-amylases first to proceed to further digestion. Thus, it would be expected that consumption of pudding treatments containing more readily digestible maltose would result in higher postprandial glycemic and insulinemic responses than starch-based pudding treatments. Results of the trial showed that the progress of starch hydrolysis in the small intestine perhaps could play a role in these attenuations, however, its effect was not as substantial.

The α-amylase would have minimum effect on the digestion of smaller dextrins, therefore the first proposed mechanism would be applicable predominantly to the food products where the majority of available carbohydrates are in the form of starch. Thus, observed postprandial
attenuations of the blood glucose and plasma insulin Cmax values after ingestion of syrup-based pudding treatments with DF could be a result of either delayed transport of maltose in the small intestine (mechanism 2) and/or delayed gastric emptying (mechanism 3). In addition to these two mechanisms, postprandial glycaemic and insulinemic responses of starch-based pudding treatments potentially could be affected by the rates of starch digestion by $\alpha$-amylase (mechanism 1). In fact, in Chapter 3 of the present thesis, it has been shown that all three DF types, at concentrations expected to be in the small intestine after consuming pudding treatment, can slow starch digestion in simulated small intestinal conditions. Significant difference found between pooled blood glucose AUC and Cmax values for all four syrup-based treatments and corresponding pooled values for all four starch-based treatments could be due to the fact that digestion of maltose occurs faster than the digestion of starch. Nonetheless, the fact that blood glucose and plasma insulin Cmax values between syrup and starch based treatments were not significantly different imposes a conclusion that hindrance of amylolysis that occurred in vivo due to the presence of DF did not have a substantial impact on the blood glucose and plasma insulin Cmax values.

It is possible that digestion of starch in vivo occurs much faster than in in vitro model of the present work, therefore the effect of the hindrance of amylolysis observed in vitro could become insignificant to affect glycemic and insulinemic responses in vivo. Perhaps if pudding treatments would contain more starch than the amount used in the current study, glycemic and insulinemic responses would be dependent more on the digestion kinetics of starch by the $\alpha$-amylase.

It should also be considered that starch in starch-based treatments was hydrolyzed to some extent by salivary $\alpha$-amylase. According to Woolnough, Bird, Monro, & Brennan (2010), up to 43% of the starch in chewed bolus can be hydrolyzed during first 10 min. Besides that, after
Ingestion of food the acidic environment of a stomach is quickly buffered, and gastric pH values can be as high as ~ 5.0 followed by gradual reduction to value close to ~ 3.0 during 1st hour (Malagelada et al., 1976; Rydning et al, 1984). The α-amylase is inactivated only when pH drops below value 3.5, therefore further starch hydrolysis by salivary α-amylase can occur in the stomach during 1st postprandial hour (Fried et al., 1987). Consequently, predigestion of starch by salivary α-amylase would shorten its digestion time in the small intestine and therefore reduce differences of postprandial blood glucose and plasma insulin Cmax values between corresponding syrup-based and starch-based treatments.

6.3.2.2. (Mechanism 2) Effect of dietary fibre on sugars transport

Several in vitro studies showed that water-soluble DF can impede transport of sugars through altering the solution viscosity, implying that transport of sugars from the small intestinal lumen to the intestinal brush border can be also prolonged in the presence of DF and that potentially can affect glycaemic responses (mechanism 2). In Chapter 4 of the current thesis, we showed that supplementation of simulated small intestinal digesta with FG > 1% w/v can reduce maltose transport. However, taking into account secretions in the upper GI tract it would be rather challenging for someone to consume a meal with such high concentration of DF. When YMM, SFG and FG were present in simulated small intestinal digesta at concentrations to represent 3 eq. (the same concentration as in clinical trial) expected to be in the small intestine, the maltose transport was not altered compared to control sample. Thus, based on the results obtained with our model it seems that reduction of sugar transport from the lumen to the small intestinal brush border due to alteration of luminal viscosity by DF would not be the mechanism involved in attenuation of postprandial glycemic and insulinemic responses observed in the
current clinical study.

Sugars from the luminal bulk first have to cross the mucus layer to gain access to the brush border of the small intestine. There is evidence that DF can change properties of the intestinal mucus layer, therefore affecting mass transfer of nutrients through it. Mackie et al. (2016a) showed that permeability of the small intestinal mucus layer was reduced when cereal β-glucan was present in the diet. That can be a result of an additive effect of DF on the reduction of mucus permeability (Mackie et al., 2016b). Also, Brownlee, Havler, Dettmar, Allen, & Pearson (2003) revealed that the thickness of the mucus layer in the gut can be increased when DF is present in the diet. Perhaps this increase could be due to enhanced mucus secretion triggered by DF (Hino et al., 2013). Effect of DF on the mucus layer was not investigated in the present study, therefore it can be one of the potential mechanisms that led to physiological responses observed in the current clinical study.

6.3.2.3. (Mechanism 3) Effect of dietary fibre on gastric emptying

Another mechanism that could be involved in attenuation of postprandial glycemic and insulinemic responses observed in the current clinical study is a delay of gastric emptying. There is evidence that gastric emptying rate can be affected by the presence of DF due to alteration of the luminal viscosity (Darwiche et al., 2003; Miyazawa et al., 2006; Guerin et al., 2001). However, it is not clear at what stage of the digestion DF affects the gastric emptying. Study of Meyer et al. (1988) showed that gastric emptying was reduced more effectively when the viscous polymer was present at the same time in both stomach and small intestine. According to our study design, viscous characteristics of the small intestinal lumen in vivo after consumption of pudding treatments supplemented with each DF type were expected to be similar. Despite that
solutions supplemented with each DF type had similar apparent viscosities in the simulated small intestinal conditions, in the majority of the cases solutions of each DF type at concentrations representing 3 eq. exhibited different values of apparent viscosity in simulated gastric conditions (Fig. 5.4). Nonetheless, differences in values of apparent viscosity between control simulated gastric digesta sample and samples supplemented with each type of DF would be expected to be much larger than variations between DF-containing samples. Also, no information on exact values of luminal apparent viscosity causing the gastric emptying delay is currently available in the literature. Therefore, differences in apparent viscosity found between simulated gastric digesta samples with different types of DF may not translate into differences in rates of gastric emptying in vivo. Besides that, dynamic processes such as dilution that occurs in vivo can greatly affect the rheological characteristics but are not represented in in vitro static digestion models, including the current model.

It would be expected that starch-based pudding treatments would have a higher viscosity in the gastro-intestinal lumen compared to corresponding syrup-based pudding treatments due to an additional contribution of starch. The difference can potentially lead to distinctive gastric emptying rates and perhaps different postprandial glycemic and insulinenic responses as an outcome. However, results of the trial showed that degree of polymerization of available carbohydrates used in pudding treatments could potentially have an influence on the gastric emptying rates that affected blood glucose and plasma insulin Cmax values, though that effect was not substantial. On the other side, the differences between two lines of treatments could be minimized due to amylolysis by salivary α-amylase that could occur during consumption of starch-based treatments. In Chapter 3 of the present thesis, we showed that apparent viscosity of starch solution declines rapidly in the presence of α-amylase in simulated small intestinal
condition. A similar decline of digesta viscosity can occur in the stomach until gastric pH is reduced to values at which α-amylase is inactivated.

Paracetamol concentration AUC values showed similar trend observed with postprandial glycemic and insulinemic Cmax values. Specifically, pudding treatments supplemented with each DF type significantly reduced postprandial glycemic and insulinemic Cmax values and paracetamol concentration AUC values compared to control. The result shows that delay of gastric emptying caused by DF indeed could affect postprandial glycemic and insulinemic responses.

6.4. Conclusions

Results of the current study showed that supplementation of puddings with each of the three types of DF significantly reduced blood glucose and plasma insulin Cmax values compared to controls, for both syrup-based and starch-based pudding treatments. Additionally, despite the differences (in some cases as high as 2.6 fold) in concentrations of each DF type used, blood glucose and plasma insulin Cmax values between syrup-based pudding treatments supplemented with each of the three DF types were not significantly different between each other. Exactly the same finding was observed for starch-based pudding treatments.

The concentration of each DF type for pudding treatments was chosen to result in equivalent viscous characteristics, which was established by in vitro experiments. Therefore, it can be concluded that attenuation of postprandial glycemic and insulinemic responses observed in the present study was independent of the botanical source of DF but was rather affected by the ability of DF to alter physical properties of the solution. That result shows that for attenuation of postprandial glycemic and insulinemic responses, dietary recommendation for each DF type
would have to be specified based on the viscous properties of particular DF in aqueous solutions.

Three most popular proposed mechanisms that could be involved in the attenuation of postprandial glycemic responses due to consumption of water-soluble DF mentioned in the literature are: reduced amylolysis progress due to hindrance of $\alpha$-amylase activity, reduced rate of sugar transport from the small intestinal lumen, and delayed gastric emptying (Dikeman & Fahey, 2006). The involvement of each mechanism in attenuation of postprandial blood glucose and plasma insulin concentrations observed in our clinical trial was discussed.

In the present study, blood glucose and plasma insulin Cmax values obtained for high maltose corn syrup-based treatments were generally higher than mean values for corresponding starch-based treatments, but these differences were not significant. That result indicates that amylolysis progress could play role in attenuation of blood glucose and plasma insulin Cmax values, nonetheless its effect was not substantial. It has been also proposed that water-soluble DF can lead to attenuation of postprandial glycemic and insulinemic responses, due to the hindrance of sugar transport from the small intestinal lumen to the intestinal brush border. Nonetheless, our in vitro experiment with diffusion cell showed that all three types of DF at concentrations expected to be in the small intestine after consumption of pudding treatment would not affect maltose transport in simulated small intestinal conditions. Based on that finding it is more likely that hindrance of sugar transport from the small intestinal luminal bulk to the intestinal brush border was not a mechanism involved in attenuation of postprandial glycemic and insulinemic responses observed in the present study. However, attenuation of postprandial glycemic and insulinemic responses for treatments supplemented with DF could be due to reduction of small intestinal mucus layer permeability.

Generally, puddings supplemented with each DF type significantly reduced paracetamol
AUC values compared to control, indicating that DF reduced gastric emptying. Therefore, showing that gastric emptying correlated with reduction of blood glucose and plasma insulin Cmax values. That indicates that delay of gastric emptying caused by DF could be involved in attenuation of postprandial glycemic and insulinemic responses observed in the present study.
GENERAL CONCLUSIONS AND FUTURE DIRECTIONS

Numerous in vivo studies have shown that supplementation of a meal with water-soluble dietary fibre (DF) can attenuate glycemic response. The exact mechanisms of this attenuation are not well understood, but there is evidence that it is related to the ability of DF to alter luminal viscosity. Three most popular mechanisms proposed in the literature are: delayed gastric emptying, reduced digestive enzyme activity in the small intestine, and delayed transport of hydrolyzed starch fragments in the small intestine. Understanding mechanisms involved in attenuation of postprandial glycemic responses would help to design foods that reduce these responses more effectively and therefore would benefit the diabetic population and the general public.

Some products and by-products of Canadian agricultural industry contain water-soluble polysaccharides that can resist hydrolysis by digestive enzymes in the gastrointestinal tract and create viscous solutions. Thus those polysaccharides potentially can act as a soluble DF. The possibility of these polysaccharides to bring health benefits, including attenuation of postprandial glycemic responses, needs to be investigated. In the current work yellow mustard mucilage and soluble flaxseed gum were extracted from yellow mustard bran and flaxseed hull with water. Water-extracted fenugreek gum (FG-WE) was extracted from a commercial fenugreek product. Treatment of these water-extracted materials with ethanol produced semi-purified polysaccharides (YMM-ETH, SFG-ETH and FG). Additional steps including protein hydrolysis with protease from Streptomyces griseus, dialysis and ethanol precipitation were used to achieve purified versions of these DF types (YMM and SFG). High $\beta$-glucan oat gum (OG) was extracted from oat bran concentrate.

One of the objectives of the present work was to study the rheological properties of these four
types of DF in conditions similar to the human small intestine, including ionic strength, presence of divalent cations, pH and temperature. Experimentally obtained steady shear flow curves of FG, SFG and OG were fitted to the Cross model to obtain values of zero shear viscosity. Constructed double logarithmic plots of zero shear specific viscosity against degree of space occupancy ($c[\eta]$) for OG and FG revealed that associations between molecules of these DF occurs in simulated small intestinal conditions.

A large body of published studies has shown that cereal β-glucan attenuates postprandial glycemic responses. Besides that, according to EFSA (2011), supplementation of a meal with 4 g of cereal β-glucan per each 30 g of available carbohydrates can help to attenuate postprandial glycemic responses. Therefore, in the present study viscous characteristics of oat β-glucan solution in simulated small intestinal condition was used as a standard for YMM, SFG and FG. Apparent viscosities (at 60 s$^{-1}$) of simulated small intestinal digesta containing oat β-glucan at concentrations expected in the small intestine after consumption of a meal that meets from 0.5 to 3 times the EFSA (2011) health claim were determined and were used as benchmarks. Concentrations of YMM, SFG and FG that resulted in apparent viscosities close to these benchmarks were found. These concentrations were different for each DF type, the exception was only YMM and SFG representing 1.5 times EFSA (2011) health claim.

Starch is one of the most important energy sources for humans. In the gastrointestinal tract, long chains of starch are hydrolyzed by salivary and pancreatic $\alpha$-amylases into dextrins which are further hydrolyzed at the intestinal brush border by glycosidases to produce glucose units that are transported into the blood. Numerous studies have shown that supplementation of starch-containing meals with DF can attenuate glycemic responses in vivo, the observed attenuations could be due to alteration of amylolysis kinetics.
In the present work, we studied the effect of four different DF types including YMM, SFG, FG and OG on the digestion kinetics of modified tapioca starch in simulated small intestinal conditions where the concentration of each DF type was chosen to produce equal viscosity. The progress of amylolysis was studied indirectly by measuring the decline of digesta apparent viscosity over time in the presence of pancreatin. Additionally, changes in digesta reducing sugar concentration for some of the digesta samples were determined.

Supplementation of simulated small intestinal digesta with each of the four DF types reduced the progress of both digesta apparent viscosity decline and changes in digesta reducing sugar content during digestion and the progress was decreasing with an increase of the DF concentration. When each DF type was present at a concentration to match for apparent viscosity in simulated small intestinal conditions, the progress of both digesta apparent viscosity decline and changes in digesta reducing sugar content were reduced to a similar extent, even though the concentrations and the botanical source of each fibre were different. Thus, we conclude that amylolysis was independent of the botanical source of DF, but instead was affected by viscosity in the small intestinal conditions due to DF addition. It can be concluded further, that the hindrance of amylolysis due to addition of DF was most likely a result of reduced diffusion of enzyme and/or substrate and/or restricted mixing efficiency rather than due to absorption of enzyme and/or substrate to DF, as proposed in several earlier studies. The relationship between digesta viscosity and viscosity decay constant was not linear, particularly the supplementation of digesta with the DF at lower concentrations was more effective (relative to an ability to enhance digesta viscosity) at hindering the amylolysis progress, than digesta with the DF at higher concentrations.

Several *in vivo* studies have shown that, besides starch-containing meals, DF can attenuate
blood glucose after consumption of meals where available carbohydrates are in the form of sugar. It has been proposed in the literature that observed attenuation could be due to changes in sugar transport from the small intestinal lumen to the brush-border of the small intestine as a result of luminal viscosity alteration. In the present work, the effect of YMM, SFG, FG and OG on maltose transport in simulated small intestinal conditions was studied using a diffusion cell where a shear rate of simulated digesta was controlled. Results showed that none of the studied DF types, at concentrations representing 3 EFSA equivalents, resulted in the reduction of maltose transport. A modest reduction, relative to control, in maltose transport was observed when FG was present in digesta at higher concentrations (≥ 1% w/v). Earlier studies showed that a meal is diluted minimum 3 fold by the time it reaches the duodenum. Considering that, and results of this experiment, in order to reduce transport of maltose in the small intestine, concentrations of DF in the meal have to be so high that it would be rather challenging to formulate and then consume food products with such concentrations. Thus it seems that delay of sugar transport from the small intestinal luminal bulk to the brush-border of the small intestine would not be the primary mechanism involved in attenuation of postprandial glycemic and insulinemic responses after consumption of DF observed in our clinical trial. Nonetheless, alteration of small intestinal mucus layer properties with DF as has been reported earlier by Brownlee et al. (2003), Hino et al. (2013) and Mackie at al. (2016a) (2016b), although not investigated in the present work, could restrict transport of maltose through it. Therefore, it could be useful to investigate the effect of DFs used in the present study on the sugar transport through intestinal mucus layer.

Water-soluble DF can affect glycemic responses through delay of gastric emptying, which is believed to be controlled, in part, by the rheological behavior of DF in the gastro-intestinal
lumen. Besides that, DF can be subjected to degradation in the acidic environment of the stomach. Thus, another objective of the present study was to investigate the rheological behavior and stability of YMM, SFG and FG in simulated gastric conditions.

Simulated gastric digesta samples containing YMM, SFG and modified tapioca starch maintained constant values of apparent viscosity at conditions expected to be in the human stomach, therefore, they would not be expected to degrade to a significant extent in a human stomach. In contrast, the viscosity of digesta samples supplemented with FG at pH values 4.0 and 1.8 declined during the course of the experiment. The decline was pH-dependent, i.e. it occurred faster with reduction of pH. The decline could be a result of dissociation of fenugreek galactomannan complexes or/and degradation of galactomannan in the acidic environment. Though acid hydrolysis of galactomannan would not be expected at pH values as high as 4.0, nonetheless, viscosity reduction of FG-containing digesta was observed at this pH value.

The apparent viscosity of samples supplemented with YMM and SFG at pH 1.8 were lower than values at pH 4.0, perhaps due to conformational changes of molecules that compose these DF. In the majority of the cases, significant differences between values of apparent viscosities were observed between gastric digesta samples containing YMM and SFG, despite that both DF solutions in simulated small intestinal conditions had an equal apparent viscosity at 60 s$^{-1}$. However, it is not clear if these differences would translate into differences in rates of gastric emptying in vivo.

In addition to the in vitro experiments, a human clinical trial was conducted in the present work to investigate the effect of YMM-ETH, SFG-ETH and FG-WE on postprandial glycemic and insulinemic responses. Adults at risk for T2D consumed high maltose syrup-based and modified tapioca starch-based pudding treatments supplemented with one of three types of DF at
concentrations matching their small intestinal viscosity as measured in vitro, specifically at concentrations representing 3 EFSA equivalents.

The human clinical trial showed that puddings supplemented with all three types of DF significantly lowered blood glucose and plasma insulin Cmax values compared to the control puddings. Therefore, this result indicates that yellow mustard mucilage, soluble flaxseed gum and fenugreek gum, that were considered as a novel potential DFs, indeed can attenuate postprandial glycemic and insulinemic responses. Even though more scientific evidence is required to support this finding, our study indicates that these materials potentially can be used as sources of DF in food products to benefit the diabetic population and healthy individuals by reducing the risk of type 2 diabetes. Therefore, perhaps the industry would be interested in developing technology for extraction of these DF types and developing food products with these types of DF.

It was also found, that blood glucose and plasma insulin Cmax values were not significantly different among the DF-containing treatments containing the same type of available carbohydrates, despite the differences in botanical sources of each DF type and concentrations used. Therefore, it can be concluded that attenuation of postprandial glycemic and insulinemic responses observed in the present study were affected by the ability of DF to alter viscous properties of the solution.

A large number of studies has shown that cereal β-glucan attenuates postprandial glycemic and insulinemic responses. Thus, even though cereal β-glucan was not used in our clinical trial, it would make a good positive control to compare postprandial blood glucose and plasma insulin values with other three types of DF.
The health agencies of several countries recommend consuming a generalized amount of DF to maintain health. In the present study, we showed that treatments supplemented with FG and YMM, which had a ~2.6-fold difference in the amount of DF used, attenuated postprandial glycemic and insulinemic responses to a similar extent. That result indicates that recommendation of quantity for glycemic and insulinemic control would have to be specified for each type of DF. Therefore, perhaps the recommended amounts of each DF should be based on the physical properties of particular DF in aqueous solutions. Additionally, perhaps some standardized method that evaluates viscous properties of each DF type could be developed to determine the amount of each DF needed to be in the meal to achieve attenuation of postprandial glycemic responses.

It would be expected that starch-based pudding treatments would be digested slower than maltose based pudding treatments, and perhaps that difference would be reflected in the biochemical profile of blood. In the present clinical trial blood glucose and plasma insulin Cmax mean values obtained for high maltose corn syrup-based treatments were generally higher than mean values for modified tapioca starch-based treatments (comparing between two controls and between two values of pooled DF-containing treatments), but these differences were not statistically significant. Nonetheless, starch in starch-based treatments could be hydrolyzed to some extent before it reached the small intestine, which was not investigated in the present study, that could minimize differences between Cmax values of two lines of treatments. Therefore, an important question to answer would be, how much starch is being hydrolyzed by salivary α-amylase in vivo during the oral phase and in the stomach before it is emptied into the small intestine?

Generally, puddings supplemented with each DF type significantly reduced paracetamol
AUC values compared to control, indicating that DF reduced gastric emptying. That result shows that delay of gastric emptying caused by DF could be involved in attenuation of postprandial glycemic and insulinemic responses observed in the present study.

Summarizing findings of our *in vitro* experiments and clinical trial it can be concluded that alteration of sugar transport from the small intestinal luminal bulk to the intestinal brush border was not a primary mechanism involved in attenuation of postprandial glycemic and insulinemic responses after consumption of DF observed in the present study. Nonetheless, reduction of small intestinal mucous layer permeability, not investigated in the present study, could be involved in observed postprandial attenuations. Also, the progress of starch hydrolysis in the small intestine perhaps could play a role in these attenuations, however, its effect was not as substantial as would be expected. Based on the postprandial changes of plasma paracetamol concentration, delay of gastric emptying caused by DF could be the mechanisms involved in attenuation of postprandial glycemic and insulinemic responses.
STUDY LIMITATIONS

Digestion of food is a complex biological process where several parameters including pH, gastric emptying rate, the amount of secretions, intensity of gastrointestinal motility, occurs simultaneously. Therefore, mimicking digestion process is complicated, especially using static models and does not represent all changes that could have an important effect on the postprandial responses. There are still much unknown about digestion that makes it challenging to simulate. For example, the exact values of shear rate experienced by digesta in the stomach and small intestine are not known. In the present study shear rate equal to 60 s\(^{-1}\) was used to mimic shear rate in the small intestine. This value was chosen as a mean of the range of shear rates (0.005 – 120 s\(^{-1}\)) used by several researches to compare digesta viscosity (Dikeman & Fahey, 2006; Fabek et al., 2014; Hardacre et al., 2015; Tharakan, 2009). However, these researchers have not validated used shear rate values with in vivo. In the small intestine, besides peristalsis digesta also is disturbed by segmentation contractions which occur periodically. Therefore, in vivo digesta in the small intestine would experience a range of shear rates rather than a constant shear rate, how it was represented in our model. Thus, the progress of starch digestion and mass transport of sugars, which are governed by the shear rate, measured in the present study could differ from values expected in vivo.

Besides that, the composition of simulated digesta prepared in the current study was simple and represented only some basic conditions specific to the human digestive tract, including ionic strength, presence of divalent cations, pH and temperature. Other compounds that are found in the GI tract in vivo (bile salts, mucin, etc.) potentially can also affect rheological properties of digesta, progress of amylolysis and sugars transport.

Several parameters of digestion in vivo are regulated by feed-back mechanisms that depend
on chemico-physical properties of a particular food. Therefore, obtaining more information on responses of the GI tract to particular foods *in vivo* would help to mimic these feed-back mechanisms *in vitro* and achieve more biorelevant results.
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