Assessment of gastrointestinal tolerance of three novel type 4 resistant starches in a human intervention study

by

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ABSTRACT

Background: Dietary fibre (DF) offers potential health benefits, yet intake in Canada is half of that recommended. A type of DF known as resistant starch (RS) can replace digestible carbohydrates in flour-based foods and is typically well tolerated, offering potential physiological benefits to the consumer. However, before novel RS can be used to enrich the food supply with DF, an assessment of gastrointestinal (GI) tolerance is essential. The objective of this study was to assess the GI tolerance of three novel variants of RS type 4 (RS4) at increasing doses from 10 to 50 g/day in healthy adults.

Method: In a randomized, double-blind, placebo-controlled, parallel four-arm human trial, 40 participants were assigned to consume either one of three RS4s (derived from either hi-maize, potato, or tapioca starch) or digestible corn starch (placebo). During the four-week dietary intervention, the dose of RS4 was increased weekly from 10 g to 50 g/day. A composite GI tolerability score (sum of individual GI symptoms; score ranged from 0 to 12), bowel movement habits, and perceived satiety were assessed at the end of each study week.

Results: Overall, the average supplementation compliance was high at 98.9%. Composite GI tolerability score was significantly affected by increasing supplementation dose, with moderate yet significant increases in composite score reported at doses ≥35 g/d in all treatments except Potato RS4. No significant difference was detected between RS4 treatments and placebo. A 50g/d dose of Potato RS4 led to looser stool but not the other supplementation. Supplementation with RS4s or placebo did not significantly affect percived satiety reported upon awaking nor 2-hours after a meal relative to baseline. The effect of dose on perceived satiety was only significant at four hours after a meal enriched with Potato RS4 at the 35 g/d dose.

Conclusion: The novel RS4s tested are well tolerated when compared to other RS types and DFs, and no differences were detected compared to digestible corn starch. Therefore they are promising DFs for closing the 'fiber gap', as they can be used up to doses of 50 gram. To optimally use these RS4s to fill the 'fiber gap', additional human intervention studies evaluating physiologically relevant health markers of these novel RS4s are necessary where doses of at least 35 g/day are supplemented. Potato RS4s may be able to increase perceived satiety. However, due to the high inter-individual variation at baseline and the low sample size, further investigation on RS4 effects on satiety would be favorable to reinforce this finding. The findings obtained in this study provide important pilot information for the development of future nutritional studies that aim at closing the "fibre gap".

Keywords: Resistant Starch Type 4; dietary fibre; gastrointestinal symptoms; bowel habits; perceived satiety

Preface

This thesis is an original work by Chen Yang to fulfill an MSc. No part of this thesis has been previously published. The results presented in this thesis are part of the gastrointestinal tolerance study led by the University of Alberta under the leadership of Dr. Jens Walter. Ingredion Inc. provided all the study supplements. Edward Deehan, a PhD student at the University of Alberta, supervised Chen Yang and all aspects of the human trial, including data analyses. Lucila Triador, a research assistant at the University of Alberta, contributed to participant recruitment and participant visits. Nguyen Khoi Nguyen, a PhD student at the University of Alberta, assisted with statistical analyze for the study. Janis Cole, a research coordinator at the University of Alberta, assisted with the funding application. Muneet Sandhu, and Paulina Morales Castillo, undergraduate students at the University of Alberta, assisted with data entry. Brandon Gruber, an undergraduate student at the University of Alberta that was not involved with the analysis of the data, facilitated the double-blinding of the study supplements. This research project received ethics approval from the University of Alberta Health Research Ethics Board – Biomedical Panel (Pro00069884), project name "Gastrointestinal Assessment of Three Novel Resistant Starch". The study was registered on ClinicalTrials.gov: NCT03255603.

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LIST OF ABBREVIATIONS

BMI	Body mass index
BSS	Bristol Stool Score
CHD	Coronary heart disease
DF	Dietary fibre
DRI	Dietary Reference Intakes
DP	Degree of polymerization
RS	Resistant starch
RS2	Resistant starch type 2
RS3	Resistant starch type 3
RS4	Resistant starch type 4
GI	Gastrointestinal
MET	Metabolic Equivalent of Task
TPSS	Total Perceived Stress Score
VAS	Visual analogues scales
SCFA	Short-chain fatty acid
SLIM	Satiety Labeled Intensity Magnitude

Chapter 1. Background

1.1 Dietary fibre

1.1.1 Definition

The definition of dietary fibre (DF) was agreed on by the Codex Committee on Nutrition and Foods for Special Dietary Uses in 2008 and 2009, and the Codex Alimentarius Commission adopted this definition of DF in 2009 for nutrition labelling (De Menezes et al., 2013). The definition and its two footnotes read as follows:

DF is defined as carbohydrate polymers1 with 10 or more monomeric units2, which are not hydrolysed by the endogenous enzymes in the small intestine of humans and belong to the following categories:

1. Edible carbohydrate polymers naturally occurring in the food as consumed.

2. Carbohydrate polymers, which have been obtained from food raw material by physical, enzymatic or chemical means and have been shown to have a physiological benefit to health, as demonstrated by generally accepted scientific evidence to competent authorities.

3. Synthetic carbohydrate polymers, which have been shown to have a physiological benefit to health as demonstrated by generally accepted scientific evidence to competent authorities (Codex Alimentarius Commission, 2009).

Footnote 1: When derived from a plant origin, DF may include fractions of lignin and/or other compounds associated with polysaccharides in the plant cell walls. These compounds also may be measured by certain analytical method(s) for DF.

Footnote 2: Decision on whether to include carbohydrates of 3 to 9 monomeric units should be left up to national authorities (Codex Alimentarius Commission, 2009).

Footnote 1 includes lignin and 'associated compounds' to be a part of the DF complex; footnote 2 allows national authorities the option of including digestion-resistant oligomers with degree of polymerization (DP) three to nine, thus enabling different definitions of DF to be used. Many national authorities include indigestible carbohydrates with a DP of three to nine as DF, including Canada, America, Australia, New Zealand, and Europe (Jones, 2014).

In the DF definition accepted by Health Canada, carbohydrates (DP > 2) extracted from natural sources or synthetically produced that are not digested and absorbed by the small intestine are accepted as a DF. Therefore, the definition includes resistant oligosaccharides, resistant starch, and resistant maltodextrins since they all have health benefits (Health Canada, 2012).

1.1.2 Health benefits of dietary fibre

DF has been considered to have positive effects on type 2 diabetes, coronary heart disease (CHD), some cancers, and obesity (Dahl & Stewart, 2015; Huang et al., 2015; Kaczmarczyk et al., 2012; Mcrae, 2017b, 2017a). In 2010, the United States Food and Drug Administration approved the health claims supporting the role of DF in preventing cancer and CHD (US Food and Drug Administration, 2010). Health Canada also suggested that DF has at least one of the following physiological effects: improves laxation, reduces blood total and/or low-density lipoprotein cholesterol levels, reduces post-prandial blood glucose and/or insulin levels, and provides energy-yielding metabolites through colonic fermentation (Health Canada, 2012). The

laxative effect not only softens hard stools but also shortens colonic transit times, which can be beneficial to health by allowing less protein breakdown and amino acid fermentation to occur as digestive materials move through the large bowel, resulting in less putrefactive substances being produced (Macfarlane & Macfarlane, 2011).

A cohort study of men and women aged 40-65 who were initially free of cardiovascular disease, cancer, and diabetes was conducted in the US. After a 6-year follow-up, women who consumed lower cereal fibre were found to have an increased risk of diabetes (Salmeron et al., 1997). Another cohort study in Finland that followed women and men aged 40-69 who were initially free of diabetes for 10 years showed an inverse association between whole-wheat intake and type 2 diabetes (Montonen et al., 2003). Further, a meta-analysis suggested that DF intake may have an inverse association with the risk of type 2 diabetes (Yao et al., 2014). Moreover, many intervention studies have shown that DF added to a food matrix reduces the post-prandial glycaemic response compared to a low-fibre control (Behall et al., 2006; Solà et al., 2010; Chandalia et al., 2000). For example, one human trial reported that muffins high in β-glucan and resistant starch lowered post-prandial blood glucose and insulin levels more effectively than muffins low in DF (Behall et al., 2006). Similarly, psyllium husk supplementation of 14 g per day for 8 weeks reduced serum insulin levels compared to placebo (Solà et al., 2010). Further, DF can aid in improving glycaemic control and decreases hyperinsulinaemia in patients with type 2 diabetes (Chandalia et al., 2000).

In addition, epidemiological studies have indicated that DF is an important dietary component for the prevention of CHD and total stroke (Mozaffarian et al., 2017; Rimm, 1996; Threapleton et al., 2013; Veronese et al., 2018; Wolk, 1999). Evidence from intervention studies showed that a high-DF intake may be able to reduce systemic low-grade inflammation and lower

plasma lipid concentrations in patients with type 2 diabetes, aiding in the prevention of CHD; however, the results remain inconsistent and are likely dependent on the DF type (Buyken et al., 2014; Chandalia et al., 2000; Johansson-Persson et al., 2014). A systematic review suggested that insoluble fibre and fibre from cereal, fruit and vegetable sources were inversely associated with the risk of CHD (Threapleton et al., 2013).

Moreover, a higher intake of DF is associated with a significantly lower risk of gaining weight and fat (Tucker & Thomas, 2009). One potential mechanism underlying this effect could be that consuming more DF at or above the recommended amount may help enhance satiety, which has been shown to correlate with a reduction in total energy intake and weight loss (Babio et al., 2010; Kristensen & Jensen, 2011; Slavin, 2005). A systematic review concluded that the satiety-enhancing effects of β-glucan, lupin kernel fibre, rye bran, whole grain rye, or a mixed high-fibre diet were supported in more than one publication (Clark & Slavin, 2013). Further evidence in intervention studies suggested that adding 12 g of psyllium husk and cellulose mix to a breakfast preload meal increased the sensation of fullness for overweight/obese women (Burton-freeman et al., 2017), and the consumption of high-fibre bread resulted in higher satiety compared to white bread and high-protein bread (Holt et al., 2001).

1.1.3 Dietary recommendations and the "fibre gap"

In Canada and the US, the Dietary Reference Intakes (DRIs) recommend a consumption of 14 g of total DF per 1,000 kcal. This recommendation is based on the median DF intake level observed in epidemiologic studies to protect against CHD and reduce the risk of type 2 diabetes (Institute of Medicine, 2005). This amount equates to a recommendation of 25 g/day for adult women and 38 g/day for adult men (Trumbo et al., 2002). This recommendation, however, does

not take DF type or gastrointestinal (GI) symptoms into consideration (Grabitske & Slavin, 2009). Over the last decade, substantial public health efforts have aimed to increase DF intake (Lichtenstein et al., 2006); however, the average intake within North America remains at only half of what has been recommended for over the last two decades. The discrepancy between recommended and actual intake in the population is referred to as a "fibre gap" (Deehan & Walter, 2016; Jones, 2014; King et al., 2012).

Importantly, research on the health benefits of DF stemming from human intervention trials remains inconsistent and less convincing than epidemiological studies (Buyken et al., 2014; Davison & Temple, 2018; Deehan & Walter, 2016). Since human digestive physiology has evolved over millions of years in a completely different dietary context that is evident today, with much higher doses of DF, the changes in our diet and lifestyle developed faster than human genetic adaptation (Eaton et al., 1997). This low fibre diet has resulted in a series of chronic diseases at present that our ancestors did not experience, as well as a reduction in microbial species and poor production of the end products with essential physiological and immunological functions due to a low-fibre diet (Sonnenburg & Sonnenburg, 2014); thus, the diet of our Palaeolithic ancestors should be a target for contemporary human nutrition (Deehan & Walter, 2016; Eaton et al., 1997; Jew et al., 2009).

Given these evolutionary considerations, DF recommendations should be reconsidered, and the inconsistent findings in DF intervention trials might be due to insufficient levels that are unable to lead to optimal health effects and maintain microbiome diversity (Deehan & Walter, 2016; Sonnenburg et al., 2014); those human intervention studies that have supplemented DF in doses that mirror what was consumed daily by our ancestors, estimated to be over 100 g per day (Eaton et al., 1997), found that the physiological effects of DF were significant (O'Keefe et al.,

2015; Anderson et al., 1980; Johansson-Persson et al., 2014). One study found that introducing African Americans to a traditional South African diet with a daily dose of 55 g of DF significantly improved markers of colon cancer within 2 weeks (O'Keefe et al., 2015). Another study suggested that a high-DF diet with 34 g of plant-based fibre/1000 kcal prevented hypertriglyceridaemia and improved glucose metabolism compared to diets with less than half of the plant fibre in the high-DF diet in patients with diabetes mellitus (Anderson et al., 1980). Similarly, 48 g/day of DF has been shown to reduce low-grade systemic inflammation, while 30 g/day of DF did not (Johansson-Persson et al., 2014). These results provide evidence to strengthen the idea that the "fibre gap" may be higher than what is suggested in the current recommendations and that the recommendations of ~25 to 38 g/day may be too low for DF to have significant physiological effects. This finding also provides evidence that the inconsistent results in human intervention studies on DF could be due to insufficient supplementation (Makki et al., 2018). Based on the current evidence, the DF intake should be higher than 50 g/day for the general population and in clinical trials to exert its health benefits (O'Keefe, 2018). Thus, use of DF amounts that consistently produce important health effects is important in studies examining these health effects.

Consuming DF in amounts equal to or exceeding those recommended in DRIs with conventional food items is challenging (Deehan & Walter, 2016). The potential barriers include, but are not limited to, limited understanding of the health benefits of DF in the general population, insufficient consumption of vegetables and fruits, absence of knowledge for identifying food high in DF, emphasis of the DF content in whole grain, limited food choices on the market, sensory barriers, higher cost, and gastrointestinal side effects (American Diabetes Association, 2007; Clemens et al., 2012; Mobley et al., 2014). However, a possible way to

overcome these barriers could be to enrich and fortify the food supply, specifically flour-based foods, with DF sources, such as resistant starches (RS), after characterization as a DF (Deehan & Walter, 2016; Makki et al., 2018).

1.1.4 Risks of consuming high doses of dietary fibre at once

Although DF is considered important for health and higher doses should be encouraged, this recommendation is not without risk. DF intake can exacerbate abdominal distension, flatulence, constipation, and diarrhoea, which are influenced by gas and water retention in the bowel (Eswaran et al., 2013).

Many of the health benefits of DF are related to the inability of the digestive enzymes to break down DF into absorbable monosaccharaides (Scheppach et al., 2001) and the subsequent fermentation of unabsorbed carbohydrates in the colon by our GI microbiome (Grabitske & Slavin, 2009). As a result, a higher consumption of DF could increase the rate of fermentation in the colon, which increases the production of gasses, including carbon dioxide, hydrogen, and methane, causing abdominal discomfort, bloating, and flatulence (Livesey, 2001).

In addition, high doses of DF can potentially induce diarrhoea. Insoluble DF stimulates GI mucosal receptors and induces mucus secretion and peristalsis, with large, coarse particles providing greater laxative efficacy than fine, smooth particles (Lewis & Heaton, 1999; Tomlin et al., 1988). Minimally fermented soluble viscous DF has a high water-holding capacity and resists dehydration effects throughout the large bowel, thus resulting in looser stools (McRorie et al., 1998), while well-fermented soluble non-viscous DF and soluble viscous DF increase stool bulk by increasing bacterial cell mass and fermentation by-products after DF consumption (Stephen & Cummings, 1980). Rapid fermentation can happen after abundant DF intake, which may lead to

excess metabolic end products that are produced faster than the absorption rate and temporarily promote diarrhoea by pulling water into the large intestine (Livesey, 2001).

In general, individuals first notice symptoms related to DF fermentation, such as GI rumbling and excessive flatulence, followed by abdominal discomfort or pain; when the capacity of the colonic microbiota to ferment DF has been exceeded, diarrhoea can develop (Marteau & Flourié, 2001).

Factors that permit tolerance to different DF types include how fast the bowel fills during fermentation and its ability to evacuate the excessive gas; thus, the tolerability varies greatly among DFs and amongst individuals (Livesey, 2001). Nevertheless, the symptoms presenting as a consequence of excess DF intake are not considered detrimental to health (Livesey, 2001). Additionally, considering that the GI symptoms could be improved over time with the adaptation of the individuals GI tract and microbiome to the DF (Mego et al., 2017), consumption of 50 g/day of DF would be realistic, especially when more slowly fermented DFs are consumed, for example RS, arabinoxylan, resistant maltodextrin, or acacia gum (Makki et al., 2018). Although some DFs are well tolerated, others are not, for example, inulin is used as a treatment for constipation for its strong laxative and exerts effects at only four grams (Gruenwald et al., 2009). The identification of the upper intake level of different DF types with regard to tolerance is necessary to achieve the maximal health benefits of DF while minimizing the concomitant GI symptoms (Grabitske & Slavin, 2009).

However, high DF diet (50 g/day) is not recommended to all adult population. In patients with GI disorders such as gastroparesis, inflammatory bowel disease, diverticulosis, Crohn's

disease with stenosis of the intestine, low-fibre foods are recommended to avoid intolerances and relapse (Opstelten et al., 2018; Rao et al., 2015; Sadiya, 2012).

1.2 Resistant starch

1.2.1 Definition and structure

RS is defined as an insoluble dietary starch that resists enzymatic digestion in the small intestine and passes to the colon where it is fermented by gut microbiota (Englyst & Cummings, 1985). The main sources of RS in the diet include legumes, breads, cereals, pastas, and starchy vegetables (Murphy et al., 2008).

Factors that affect resistance to the digestion of starch include the size and type of starch granules, associations between starch, other food components (i.e., lipids, proteins, sugars, gums, etc.), and chemical modifications (Birt et al., 2013; Wei et al., 2010).

There are five types of resistant starch:

RS type 1 (RS1) is naturally present within starch granules that are surrounded by a protein matrix and cell wall material, which prevent water penetration during cooking and enzymatic hydrolysis since the granules are inaccessible and not enough moisture is present for the starch to gelatinize or be exposed to amylase in the small intestine (Birt et al., 2013).

RS type 2 (RS2) is a starch protected from digestion due to its crystalline structure. It exists in uncooked potatoes, green bananas, peas and beans, gingko, and high-amylose maize (Birt et al., 2013). The RS2 content of starch is positively associated with the amount of amylose present

(Lockyer & Nugent, 2017) since amylose is digested slowly, whereas amylopectin is digested rapidly due to its helicon structure (Lockyer & Nugent, 2017; Ring et al., 1988).

RS type 3 (RS3) is a retrograded starch. After starchy food is cooked and cooled to near refrigeration temperatures (4–5°C), double helices are formed, and digestive enzymes can no longer hydrolyse the starch. However, when it is warmed up again, it can be hydrolysed (Birt et al., 2013).

RS type 4 (RS4) is a chemically modified starch formed by conversion, substitution or cross-linking (Han & BeMiller, 2007; Kim et al., 2008). These chemical modifications change the structure and composition of starch granules and restrict enzymatic hydrolysis, and a common method is cross-linking with phosphorus (Birt et al., 2013; Raigond et al., 2015).

RS type 5 is a starch that forms a helical-complex structure when amylose and long branch chains of amylopectin interact with complex fatty acids (Ai et al., 2013). The amylose-lipid complex also entangles amylopectin molecules, restricting the swelling of starch granules and enzyme hydrolysis (Seneviratne & Biliaderis, 1991).

Different chemical structure of RS has been shown to supported different groups of colonic bacteria (Martínez et al., 2010). Therefore, the effects of these RS4s on human GI tolerability, perceived satiety and microbiome composition need to be investigated and compared.

1.2.2 Health benefits of resistant starch

RS contributes to the quantity of DF in starches according to the Association of Analytical Communities gravimetric method of DF analysis (AOAC 991.43, 2009.01) and yields physiological beneficial effects that go beyond resistance to digestibility (Bindels et al., 2015; Brouns et al., 2007; Grabitske & Slavin, 2009; McCleary et al., 2012; Prosky et al., 1985). RS is

safe; can legally be sold; is claimed to be a DF in many countries including the US, Australia, and Japan; and is proposed to have prebiotic potential (Bird et al., 2010; Goldring, 2004). Australia's Commonwealth Scientific and Industrial Research Organization recommended that the total intake of resistant starch should be approximately 20 g/day for good health (Fuentes-Zaragoza et al., 2010). The European Food Safety Authority made a health claim for RS2 derived from high-amylose maize, which referred to "reduction of post-prandial glycemic and insulinaemic responses when replacing at least 14% of the digestible starch in baked foods" (European Food Safety Authority, 2011).

Being resistant to digestion, food products with RS have a lower glycaemic index and caloric density (1.6–2.8 kcal/g) than regular digestible starch (4 kcal/g), which is one primary reason for the decrease in post-prandial glycaemic and insulinaemic responses and is essential when treating obesity due to a decrease in energy absorption (Bindels et al., 2015; Lattimer & Haub, 2010; Lockyer & Nugent, 2017). Clinical studies also provide evidence that suggests RS is capable of improving the blood lipid profile, controlling blood glucose levels, and modulating insulin sensitivity in healthy and obese volunteers, as well as in patients with metabolic syndrome and type 2 diabetes (Johnston et al., 2010; Karimi et al., 2016; Maki et al., 2012; Park et al., 2004).

The physiological effects of RS can be partially attributed to its fermentation. In the large intestine, RS can be fermented by the GI microbiota to produce short-chain fatty acids (SCFAs), which consequently lowers the luminal pH (Erickson et al., 2018; Topping & Clifton, 2001). This shifts the GI microbiota composition by enhancing levels of different health-promoting taxa depending on the chemical structure of RS and reducing the abundance of other bacteria related to protein fermentation (Bindels et al., 2017, 2015; Martínez et al., 2010; Paturi et al., 2012). RS

can also be associated with regulation of insulin sensitivity by reducing the excretion of bile acid (Perino et al., 2014; Thomas et al., 2009), as well as provide a protective effect against colon cancer (Bindels et al., 2017; van Munster et al., 1994). Further, RS has also been shown to promote an immunoregulatory effect by reducing proinflammatory cytokines, such as tumour necrosis factor alpha, compared to digestible starch (Peterson et al., 2018).

The majority of clinical research studies on RS have been conducted to evaluate the effects of RS2 and RS3, with fewer clinical studies focusing on RS4 (Stewart et al., 2018). Those that have suggested that RS4 significantly improved blood cholesterol profiles and dyslipidaemia and reduced body fat percentage in patients with metabolic syndrome (Lockyer & Nugent, 2017; Nichenametla et al., 2014). Two studies on two novel RS4s, which are used in this thesis (Maize RS4 and Potato RS4), suggested that RS4, similar to RS2, can significantly reduce post-prandial glucose and insulin responses in healthy adults compared to digestible starch (Stewart & Zimmer, 2017a). RS4 also elicits a greater capacity to attenuate the glucose response and improve plasma insulin levels than RS2 both in humans and mice (Bindels et al., 2017; Haub et al., 2010). However, considering the insufficient number of studies and the differences in the chemical structure of RS4s that are partially due to different chemical modifications, more studies assessing the potential health benefits of RS4 supplementation or enrichment are needed.

1.3 Impact of dietary fibre consumption on gastrointestinal tolerability

1.3.1 Assessment of gastrointestinal tolerability

The GI symptoms caused by DF intake that are often recorded in studies are based on participants' perceptions of flatulence, bloating, distension, loose stools, and increased stool

frequency (Grabitske & Slavin, 2009). No validated assessment scales for the evaluation of GI effects exist, so the results in the literature are difficult to compare (Bonnema et al., 2010).

Recently, clinical studies that assessed the GI tolerance of DF tended to choose symptoms based on previous reviews or those that were commonly reported (Grabitske & Slavin, 2009). Tolerance is commonly measured by asking the participants to score each of the individual GI symptoms that they experienced, including gas, bloating, nausea, flatulence, GI cramping, diarrhoea, constipation, GI rumbling, and abdominal pain, with a scale that rates the perceptive severity of the symptoms (Boler et al., 2011; Bonnema et al., 2010; Briet et al., 1995; Dahl et al., 2014; Fastinger et al., 2008; Maki et al., 2013). Both the individual symptoms chosen and the scales vary depending on the studies (Crincoli et al., 2016; Housez et al., 2012; Maki et al., 2013; Stewart et al., 2010).

GI tolerance measurement also usually includes self-reported bowel movement frequency and consistency. To assess stool consistency, which is also considered a predictor of intestinal transit time, the validated 'Bristol Stool Scale' has been commonly used (Crincoli et al., 2016; François et al., 2014; Maki et al., 2013; Saad et al., 2010). This simple tool is generally well accepted by both study participants and clinic patients (Lewis & Heaton, 1997). On top of frequency and consistency, ease of passage during bowel movement was assessed in some studies (Holscher et al., 2014). In addition, symptoms such as straining during bowel movements and a sensation of incomplete evacuation following a bowel movement (all rated on a scale of 1=none to 4=severe) were assessed in some studies, as they were commonly reported by constipated patients, giving a more direct perception of how DF influences bowel movements (Maki et al., 2013; Saad et al., 2010).

1.3.2 Evidence on gastrointestinal tolerability of resistant starch from human trials

RS has previously been shown to have good GI tolerability (Grabitske & Slavin,2009; Storey et al., 2007). Studies suggested that consuming 25 - 28 g/day of RS is well tolerated with no significant changes in bloating, flatulence, or cramping and had only minimal effects on laxation (Klosterbuer et al., 2013; van Munster et al., 1994). In addition, a study in which participants consumed 0 to 60 g/day RS3 revealed no difference in the incidence of nausea, bloating, borborygmi, or flatulence across doses in healthy adults (Storey et al., 2007). Studies on RS4 showed similar results. A high-amylose corn RS2 and a phosphorylated cross-linked RS4 were used in a study at doses of 33 g/day and resulted in significant yet moderate increase in flatulence, but significant detrimental effects observed on bowel movement, stool consistency, or discomfort (Martínez et al., 2010). Moreover, consumption of 30 g/day of potato-derived RS4 resulted in a significant increase in flatulence; however, average symptoms were minor, suggesting that high levels of RS4 can be incorporated into a typical diet without adverse symptoms (Dahl et al., 2016). Further, a recent study using a high-maize-derived RS4 as used in this thesis showed that 25 g/d, but not 50 g/day, of Maize RS4 was well tolerated in healthy adults (Stewart & Zimmer, 2017). Based on these studies, RS4s are generally tolerated well at approximately 30 g/day.

1.4 Resistant starch consumption and perceived satiety

1.4.1 Evidence of resistant starch affecting satiety from human trials

Consuming a meal high in DF can slow gastric emptying and stimulate appetite-regulating hormones such as GLP-1 and PYY in humans, which lead to increases in perceived satiety (Keenan et al., 2006; Pereira & Ludwig, 2001; Verhoef et al., 2011; Ye et al., 2015). However, the results of studies on the effects of different types of DF on appetite have been inconsistent (Slavin & Green, 2007). Moreover, only a few studies have investigated the effects of a diet with RS on appetite, and the majority of studies used RS2 or RS3 (Bodinham et al., 2010; Zhou et al., 2008).

Raben et al. conducted a crossover study examining the effect of consuming 50 g of potato starch (54% RS) on satiety compared to 50 g of 100% digestible potato starch in ten healthy men who consumed an identical diet for 3 days before the test day (Raben et al., 1994). The starches were mixed in 500 ml of diluted fruit syrup. Although consuming the starch mix that consisted of digestible potato starch resulted in greater feelings of fullness than the one containing RS when both were eaten, the perceived satiety of the RS mix was found to last twice as long post-prandially as that of the digestible starch. Further, a study that supplemented 48 g of RS found no associated effect on subjective appetite measures; however, participants ate less of the weighed food provided within the study unit after the RS treatment (Bodinham et al., 2010). Willis and colleagues reported that eight grams of RS resulted in a significant increase in feelings of fullness 180 minutes after consuming a muffin containing RS in healthy adults (Willis et al., 2009). In contrast, a study in which participants consumed 30 g/day of RS2 and RS3 showed little influence on appetite and food intake (de Roos et al., 1995). Similarly, a study assessing

two novel potato RS4s found that 30 g of either RS4 did not affect satiety responses when compared with the control (Haub et al., 2012). The findings on satiety and RS are inconsistent, and the method of assessment, time of assessment, and both dose and type of RS may have effects; therefore, satiety assessment on novel RS4 products is needed.

1.4.2 Assessment of perceived satiety

There are several terms to use to describe the operations of the appetite system. Satiation or intra-meal satiety leads to the termination of eating; therefore, determining the meal size, also known as intra-meal satiety. Satiety inhibits further eating, declines in hunger, increases in fullness after a meal has finished, also known as post-ingestive satiety or inter-meal satiety. Appetite, hunger, and fullness are also terms to describe the operations of the appetite system (Blundell et al., 2013).

Since the bi-directional visual analogue scales (VASs) were developed in 1966 (Silverstone & Stunkard, 1968), numerous investigators have been using them to evaluate the sensory and perceptual dimensions of human hunger, fullness and desire to eat, among others parameters (Blundell & Burley, 1987; Bodinham et al., 2010; Burton-freeman et al., 2017; Willis et al., 2009). However, the following limitations to their use exist: 1) the labelled points on the scale rarely define equal intervals (Cardello et al., 2005) and 2) categorical scales have a 'central tendency' or 'regression' effect that results in under-use of the end categories (Stevens & Galanter, 1957). Therefore, the results obtained with the VAS tool often become difficult to analyse because respondents may lack the capacity to understand such scales (Cardello et al., 2005).

Holt (1995) developed a horizontal, 100 mm VAS that was verbally anchored at the end-points and at five equally spaced intervals between the end-points, creating a seven-point scale labelled ‘extremely hungry’, ‘hungry’, ‘semi-hungry’, ‘no particular feeling’, ‘semi-satisfied’, ‘satisfied’, and ‘extremely full’ and used it in their studies (Holt et al., 1995; Holt et al., 2001). However, this scale has been critiqued in the past. Cardello and colleagues suggested that this scale violated the psychophysical advantage of a VAS because it used labels that did not necessarily represent a single measurement dimension, and the terms used in the scale were not all appropriate to show the intensity of hunger/fullness. Considering these disadvantages, the Satiety Labeled Intensity Magnitude (SLIM) scale was developed to determine the intensity of sensations along a linear graphic scale at specific locations that reflect the numerical ratios among their perceived intensities (Cardello et al., 2005). The SLIM scale is commonly utilized in many research fields to assess perceived satiety, including the effect of DF (Haub et al., 2012; Savastano et al., 2014).

This SLIM scale fixed the end-point sensation, and participants can express their intensity of sensation without receiving additional instructions (Cardello et al., 2005). This scale is a valid and unbiased measurement tool that has reliability and sensitivity equal to or better than the current category and VAS scales and provides ratio-level data, which shows the intensity of satiety sensations, e.g. ‘twice as hungry’, ‘one-third as full’, etc (Cardello et al., 2005) for regular use in standard practice (Blundell et al., 2013).

1.5 Study objective and specific aims

This thesis is a pilot study for larger clinical trials on the physiological effects of three novel RS4s, in which the RS4s will be incorporated into foods before they were served (provided

to the participants). Before novel RS4s are utilized in the future clinical trials or in the food supply, a systematic investigation into the tolerability of RS4s is essential. Additionally, previous studies have compared the health benefits and effects on GI microbiome between two different types of RS (e.g. RS2 and RS3, RS2 and RS4); however, the different method used to chemically modify the starch, plus the source of native starch, could potentially result in similar yet structurally distinct RS4s, it is unknown to what degree differences in the chemical structure of RS4s impacts the GI tolerability and physiological health effects, thus research investigations are needed. The amount of 50 g/day of RS4s was used in this thesis, which allows the possibility of the daily DF intake to exceed 60 g/day with the background diet.

Confounding effects were considered by measuring physical activity before and after the intervention to assure the stability of energy expenditure. Perceived stress, and total fibre from background diet was also determined to prevent confounding effects on GI tolerability.

The objective of the study was to characterize the dose-dependent effect of chemically-distinct RS4s on GI symptoms, and perceived satiety. A further objective, to compare the effects of different RS4s on GI microbiota composition and function was done in parallel with a study conducted by another student, results from this second study are not the focus of this thesis.

Specific aims and research questions are as follows:

(1) To perform a randomized, placebo-controlled, double-blind, parallel-four-arm human intervention study to assess the GI tolerance (GI symptoms and bowel habits) of three novel RS4s added to people's regular food at a dose increasing to 50 g/day over four weeks, as compared to a control condition that contained digestible corn starch.

- Maize RS4 (*VERSAFIBETM 2470 resistant starch*)
- Potato RS4 (*VERSAFIBE™ 1490 resistant starch*)
- Tapioca RS4 (*VERSAFIBE™ 3490 resistant starch*)

(2) To assess the effects of the increasingly higher doses of different RS4s have on perceived satiety when consumed with food or beverages.

The hypotheses related to each objective were:

(1) Providing 50 g/day of novel RS4 with the dose escalating over time would be well tolerated in healthy adults.

(2) The GI tolerability score would not increase as the consumption of RS4 increased over time.

(3) Higher doses of RS4 (35 to 50 g/day) would not increase perceived satiety.

Chapter 2. Methods

2.1 Study design

A randomized, double-blinded, placebo-controlled, parallel four-arm study design was employed. Sample size (n=10/arm) was determined by referencing previous studies that successfully assessed the effect of DF on GI tolerability and GI microbiome composition (Martínez et al., 2010; So et al., 2018; Calame et al., 2008). Briefly, interested participants were recruited, stratified based on sex, and then equal numbers of healthy adult men and women (5 of each) were randomly assigned to one of three treatment arms (three types of novel RS4) or placebo arm (digestible corn starch). Participant were further asked to consume the assigned supplement for four weeks with a dose escalation from 10 grams per day in the first week to 50 grams per day in the fourth week (**Figure 2-1**). The protocol of this randomized controlled trial was approved by the University of Alberta Health Research Ethics Board – Biomedical Panel (Pro00069884). The study was registered with ClinicalTrials.gov: NCT03255603.

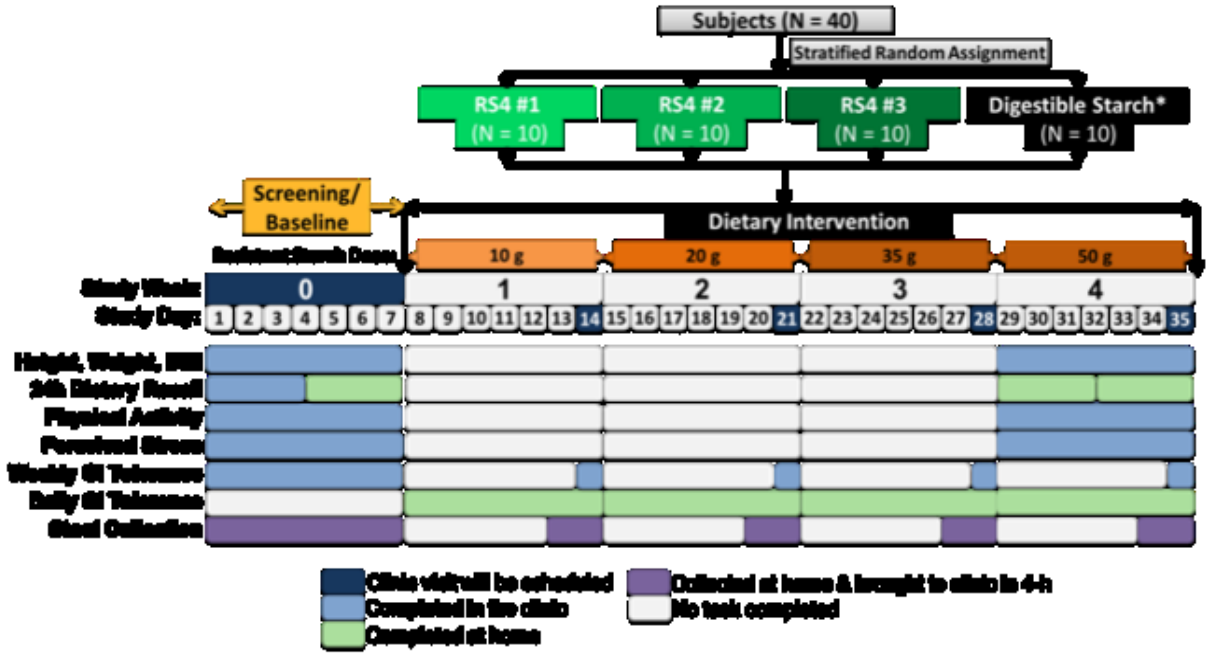

Figure 2-1. Study design of the human intervention trial

2.2 Participant recruitment

Healthy male and pre-menopausal, non-pregnant or lactating female volunteers aged 18 to 50 years were recruited using campus-wide flyers, mailings to specific Listservs, local events, and word of mouth. An example of the recruitment poster is shown in Appendix A. Participants were screened and enrolled from September 2017 to January 2018 on an ongoing basis. The exclusion criteria were as follows: 1) acute or chronic GI illnesses, conditions, or issues; 2) history of GI surgical intervention; 3) chronic or current use of anti-hypertensive, lipid-lowering, anti-diabetic, analgesic, or laxative medications; 4) antibiotic treatment in the three months prior to the study period; 5) use of prebiotic or probiotic supplements; 6) known allergies or intolerances to fibre sources; 7) vegetarianism; 8) a smoking habit; 9) alcohol intake of more than 8 drinks/week; and 10) 5 hr/wk or less of moderate-vigorous exercise.

2.3 Study randomization and blinding

2.2.1 Randomization

A study investigator not involved in the assignment of participants to their respective intervention arm (Starch 1 to 4) generated the randomization sequence before the onset of the clinical trial using a randomization website that uses a method of randomly permuted blocks (www.randomization.com).

Once participants were enrolled in the study, they were sequentially allocated to the subsequent intervention arm (Starch 1 to 4) in the randomization scheme by a separate study investigator blinded to the randomization sequence.

2.2.2 Blinding

The supplements were nearly identical white powders, which allowed a double-blinded study design. The packaging process of the starch supplements and the subsequent blinding (assignment of supplements to Starch 1 to 4) were carried out by a third party that was not involved in the study. Trained personnel within the Human Nutrition Research Unit kitchen at the University of Alberta first packaged the supplements into daily ready-to-use sachets, which were then packaged into labelled opaque bags (Starch 1 to 4 and Week 1 to 4) prior to being provided to the investigators and participants. All study investigators remained blinded to the treatment allocation until the intervention was complete.

2.4 Starches

Ingredion Inc. (Bridgewater, NJ, USA) provided all starch products used in the study, including the Maize RS4, Potato RS4, and Tapioca RS4, as well as the digestible, high amylopectin corn starch, which was utilized as the control. The product specifications are provided in **Table 2-1**. The total DF content (as RS) was measured using AOAC 2009.01, which is the method of DF measurement used for food labels in Canada (Health Canada, 2012).

Table 2-1. Supplement Specifications

Treatment Name	Product Name	Technical Description	Sample Processing	Total Fibre (dwb[1] - %)	Total Fibre (as-is[2] - %)
Maize RS4	VERSAFIBE ™ 2470	Modified Maize Starch	Acid hydrolysis and heat treatment of raw high-amylose maize starch.	65.0	58.2
Potato RS4	VERSAFIBE ™ 1490	Modified Potato Starch	Phosphorylation of raw potato starch with phosphorus oxychloride.	90.0	78.7
Tapioca RS4	VERSAFIBE ™ 3490	Modified Tapioca Starch	Phosphorylation of raw tapioca starch with phosphorus oxychloride.	96.0	85.3
Corn Starch	AMIOCA TF	Digestible Corn Starch	None	0.0	0.0

[1]dwb, dry weight basis; [2]as-is, adjusted for moisture content

Potato RS4 was modified from raw potato starch. Phosphorus oxychloride was added to the slurry, and the starch was cross-linked while maintaining the reaction pH, which was neutralized with acid after phosphorylation. Then the starch was washed, dewatered, and dried to

a moisture content that did not exceed 18% (Stewart & Zimmer, 2017a). The raw tapioca starch was processed by a similar method to increase RS content. High-amylose maize RS4 was derived from raw high-amylose maize starch that underwent further acid hydrolysis and then heat treatment to increase the RS content. As the control, AMIOCA TF is a highly purified amylopectin that should be rapidly digested and absorbed proximally in the small intestine, which prevents its availability for microbial fermentation in the colon, making it an ideal placebo when characterizing the microbial response to RS later.

The "as-is" DF contents (controlling for moisture) of the three RS4 were taken into consideration when calculating the daily dose of each supplement for each participant to standardize DF amounts. For the control arm, the amount of corn starch provided was equal to the mean amount of total starch supplied across the three treatment arms. Supplement specification and the actual weight of the starch provided are shown in Appendix B.

Participants received a handout (Appendix C) with instructions on how to incorporate the supplements into the foods that they normally choose before the intervention started to reduce participant burden. In addition, a package with the seven individual bags of daily doses of the supplement for the following week was provided to participants at the beginning of each intervention week, with instructions to consume one bag each day in two to three servings. The daily dosage was 10 grams in the first week, 20 grams in the second week, 35 grams in the third week, and 50 grams in the fourth week. Participants were instructed not to heat up the supplements, but they could mix the starch with water, coffee, soup, or any other food they preferred and consume it at any time during the day. Participants were asked to bring the empty supplement packages back to the next study visit and were requested to keep the unconsumed

starch in the bag so that the study investigators could weigh the remaining amount of the supplement to calculate their compliance with the study protocol each week.

Participants were also asked to avoid consuming excessive amounts of food items that could cause GI distress while maintaining their habitual diet and physical activity level during the intervention period. A list of foods was provided for their reference and included: cabbage, artichoke, onions, beans, lentils, wheat bran, prunes, and plum juice.

2.5 Study procedure

2.5.1 Telephone pre-screening

Individuals interested in participating contacted the study investigator via study email, who provided them with a list of general pre-screening questions and a summary of study information. Potential participants who met the inclusion criteria completed a follow-up pre-screening questionnaire over the phone to determine further study enrolment eligibility. The pre-screening questionnaire is shown in Appendix D. Upon confirmation of eligibility by telephone pre-screening, participants were booked for their in-person screening/baseline visits and study visits on the last day of each week during the intervention period.

2.5.2 In-person screening/baseline visit

After obtaining a signed consent form and personal identification form from each participant, the study staff collected relevant demographic information such as age, sex, ethnicity, education, employment status, and income with a questionnaire. The participants' medical history, current use of medications and supplements, anthropometric measurements, physical

activity, smoking, alcohol use, and dietary information were confirmed and evaluated to ensure eligibility. Height and weight were measured by a single study investigator to calculate body mass index (BMI). Height was measured using a digital stadiometer and recorded to the nearest 0.01 cm (QuickMedical Digital Stadiometer, Issaquah, WA, USA). Height obtained at the screening visit was used for the duration of the study period. Body weight was measured using a digital scale (752KL, Health-o-metre Professional, Bridgeview, IL, USA) with participants' shoes removed and was recorded to the nearest 0.1 kg. All anthropometric measurements were completed twice, and the mean values were then calculated and entered into the database.

Perceived stress, physical activity, short-term diet, and GI symptoms were assessed using questionnaires during the visit. At the end of the visit, participants received a hunger-fullness SLIM questionnaire and a bowel habit questionnaire. They were instructed to complete the surveys at home and bring them back before the intervention started. A copy of the information letter and consent form and a list of study questionnaires relevant to the thesis are found in Appendices E – K.

2.5.3 Weekly study visits

After random allocation to each of the 4 study groups (n=10 in each group), participants received a study journal, which included a bowel habits questionnaire and a hunger-fullness SLIM questionnaire each week. The participants were asked to complete these two questionnaires during the 5th and 6th day of the study week and return them to the study team on their day seven visits. An example of the study journal is shown in Appendix L. The participants were then asked to attend four study visits, one at the end of each study week during the

intervention period, to return the questionnaires and receive their starch supplements for the next study week.

During weekly visits, GI symptoms were assessed using a weekly GI tolerability questionnaire that assessed the severity of GI symptoms over the past 7 days, and the bowel habits questionnaire and the hunger-fullness SLIM questionnaire were reviewed by the study investigators to ensure that they were completed as instructed (Cohen & Williamson, 1988; Maki et al., 2013). The investigators also kept a record of how participants were incorporating the supplements in their daily diet during the respective week. The source document for study visits is shown in Appendices M and N.

In addition, perceived stress and physical activity were assessed at the week four visit with the Perceived Stress Scale and the International Physical Activity Questionnaire (Mäder et al., 2006). Weight was measured again at the week four visit using the same method as the baseline visit and was used to calculate BMI after the intervention.

2.6 Protocol details

2.6.1 Perceived stress

At the screening/baseline visit and week four visit, participants were requested to complete a Perceived Stress Scale (Cohen & Williamson, 1988). In the survey, participants were asked about their feelings and thoughts during the last month. The answer "0 = never" to "4 = very often" represented how often they felt or thought a certain way. Total Perceived Stress Score (TPSS) was the sum of the numerical values for all responses to four questions.

2.6.2 Physical activity

At the screening/baseline visit and week four visit, participants were requested to complete an International Physical Activity Questionnaire (Mäder et al., 2006). Participants were asked about their level of physical activity over the past seven days, which varied from vigorous activity to moderate activity to walking. Then, total metabolic equivalent of task (MET) was calculated for seven days by a trained study investigator using the following equation:

Vigorous MET-minutes/week: 8.0 * vigorous-intensity activity minutes/day * vigorous-intensity days/week

Moderate MET-minutes/week: 4.0 * moderate-intensity activity minutes/day * moderate days/week

Walking MET-minutes/week: 3.3 * walking minutes/day * walking days/week

Total physical activity MET-minutes/week: Walk (METs*min*days) + Moderate (METs*min*days) + Vigorous (METs*min*days)

2.6.3 Dietary assessment

Diet was assessed at baseline and week four. Participants were requested to complete two ASA24 hour recalls within a week before the intervention started, and two during week four. The first one was completed during the screening/baseline visit, while the other three were completed at home.

The ASA24-Canada is a free web-based dietary assessment tool developed by Health Canada in collaboration with NCI that allows self-administered 24-hr recalls to be performed, automatically coded, and then analyzed. (https://epi.grants.cancer.gov/asa24/respondent/canada.html, http://asa24.ca/about.html).

2.6.4 Gastrointestinal symptoms

The severity of GI symptoms was assessed at the screening/baseline visit and at all four study visits during the intervention period. Participants were requested to complete the Gastrointestinal Tolerability Questionnaire (Maki et al., 2013; Stewart & Zimmer, 2017b) during their weekly visit. The questionnaire asked the participants to rate the severity of six GI symptoms, including nausea, bloating, GI rumbling, gas/flatulence, abdominal pain, and diarrhoea (watery stool). The severity/frequency of the symptoms was reported on a three-point scale, with "0" indicating no symptoms or no more than usual, "1" indicating somewhat more than usual, and "2" indicating much more than usual. The composite GI tolerability score was then calculated by summarizing the score of six individual symptoms. It ranged from "0" to "12", with a lower score indicating less severe symptoms and a higher score indicating more severe symptoms.

2.6.5 Bowel movement habits

Bowel movement habits were evaluated at baseline and on the 5^{th} and 6^{th} day of each study week during the intervention using the bowel habit questionnaire (Maki et al., 2013; Stewart & Zimmer, 2017b). Participants were asked to record the date and time of their bowel movements, noting the faecal hardness, straining, discomfort, and sensation of incomplete evacuation during

each bowel movement within those two consecutive days. Faecal hardness was rated as 1=soft, 2=normal, 3=hard, and 4=very hard while the descriptions for the other sensations were rated as 1=none, 2=mild, 3=moderate, and 4=severe. Bowel movement consistency was assessed in the questionnaire as well by using the Bristol Stool Scale. The average of the two daily scores was calculated prior to statistical analyses.

If participants did not experience any bowel movements during the two days when their bowel habit questionnaires needed to be completed, nothing was reported for their faecal hardness, stool consistency, or sensations during the bowel movements at those time points. In these cases, the data for the previous study week would be brought forward to fill in the absent data in the present study week for statistical analysis, assuming these properties did not change from the previous week. For the unknown baseline data, the answers for week 1 were used for the same purpose, considering that baseline was the very first time point and week 1 was the closest to baseline compared to other time points.

2.6.6 Perceived satiety

Satiety was evaluated at baseline and on the 5th or 6th day of each study week during the intervention. Participants were requested to complete a hunger-fullness SLIM questionnaire at each time point. The questionnaire asked the participants to rate their feelings of hunger or fullness by marking a slash (/) on the SLIM scale at three different times within a day: 0-30 minutes after waking up; 2 hours ± 30 minutes after the reported meal with the supplement added; and 4 hours ± 30 minutes after this meal. The SLIM scale is a 100 mm line with descriptions of sensation anchored at varying lengths along the VAS. "Neither hungry nor full" is in the middle of the SLIM scale (0 mm), while the top part of the scale reflects increasing satiety with “greatest

imaginable fullness" (50 mm) as the maximal perception of satiety and the bottom part of the scale reflects increasing sensations of hunger with "greatest imaginable hunger" (-50 mm) as the maximal sensation. The SLIM score was used to evaluate the hunger and fullness rate, which was calculated by the following equation:

SLIM score = 2 * the length between 0 and the point marked by participants (mm).

If a participant failed to complete questions at the correct times, the data were accepted if the time when the answer was reported was less than 1 hour different from the time that was requested (expected time ± 1 hour). If an omission was greater than 1 hour, data from the previous week were used to replace it and considered for analysis instead.

In addition to the SLIM score, participants were also asked: "At what time after the reference meal did you feel hungry?" and "When did you eat again after the meal?".

The means ± SD of the amount of time after a meal including the supplements to feel hungry again and the time after this reference meal to eat again reported by the participants at baseline were both between three to four hours, although both were closer to four and could therefore be rounded up to four. Thus, we categorized these two characteristics into two groups: "≥ four hours" and "< four hours". If data were missing, data from the previous week were brought forward. If participants went to bed and answered the question on the following day, the answers were considered to have occurred more than four hours after the meal.

2.7 Statistical analysis

To determine the main effect of treatment and dose on GI tolerability, Generalized Estimating Equation (GEE) models were applied using the 'geepack' package in R (Højsgaard et

al., 2006). Cumulative link models were alternatively applied to test the main effect of treatment and dose for ordinal data in repeated measurement using the 'original' package in R (Christensen & Brockhoff, 2013). If a significant effect of supplement dose was detected with GEE or Cumulative link models, pair-wise comparisons were performed within each of the four treatment arms using the estimated marginal means in the 'emmeans' package in R (Lenth, 2018).

Two-way ANOVA with Holm-Sidak's multiple comparisons test (Sokal & Rohlf, 1969) was applied to determine the difference in perceived satiety between each treatment arm and its baseline, which were continuous data with repeated measurement. Furthermore, for each RS4 treatment arm, the average change during the intervention was determined (considering Δ Week 1-Baseline to Δ Week 4-Baseline) and then compared to the average change reported in the control arm by using one-way ANOVA with Holm-Sidak's multiple comparisons test (Bartko, 1976).

Across the treatment arms, participants' baseline demographic characteristics, anthropometric measurements, compliance rate, changes in weight, BMI, stress, physical activity, energy and total fibre intake during the study were compared using one-way ANOVA (Bartko, 1976). To assess differences in count data, for instance, the number of people who used the starch supplement, between the four independent treatment arms, Fisher's Exact test was applied due to small sample size, using the 'exact2x2' package in R (Fay, 2010).

ANOVAs are considered to be a robust statistical approach for the analysis of data that may not be normally distributed (Schmider et al., 2010). Nevertheless, normality of the residuals was checked by using Shapiro-Wilks tests and through inspection of QQ Plots. If indicated, $\log_2$ transformation of data was performed prior to analysis.

GraphPad Prism version 7.00 statistical software (GraphPad Software, Inc., San Diego, CA, USA) was used for both data visualization and ANOVAs. R version 3.4.4 (R core team, 2018, Vienna, Austria) was used for the GEE model, cumulative link models, and Fisher's exact tests. The results are presented as the mean ± SD unless otherwise noted, and a *p-value of* <0.05 was considered to indicate statistical significance.

Chapter 3. Results

3.1 Participant characteristics

From September 1, 2017, to January 10, 2018, 58 participants were screened for eligibility, of whom 43 were eligible and randomized to one of the four treatment arms to reach the target sample size (n=10/arm complete the intervention according to protocol) (**Figure 3-1**). During the intervention period, one participant from the Tapioca RS4 treatment arm and two participants from the Potato RS4 treatment arm withdrew from the study due to personal reasons (*n*=1) or low compliance caused by reasons that were unrelated to GI tolerance (*n*=2), respectively. Three additional participants were screened, enrolled and then randomly assigned to one of the two groups. In total, 40 participants completed the intervention study with 10 participants in each arm: 5 males and 5 females.

The baseline demographic characteristics and physical characteristics of the study participants are shown in **Table 3-1**. The majority of participants self-identified as Caucasian (50%), followed by Asian (42.5%) and other ethnicities (7.5%) and were between 20 and 39 years old (77.5%). More than half of the participants were students who possessed a bachelor's degree (37.5%) and high school diploma (27.5%), which was expected considering that the recruitment was mainly conducted at the University of Alberta. No significant differences in the assessed demographic characteristics were found among the four treatment groups. In addition, no significant differences were found in age, height, weight, BMI, perceived stress, or physical activity status at baseline. Furthermore, the range of BMI values of participants in all arms was between 19.6 and 29.8, with the majority falling within the normal healthy BMI category.

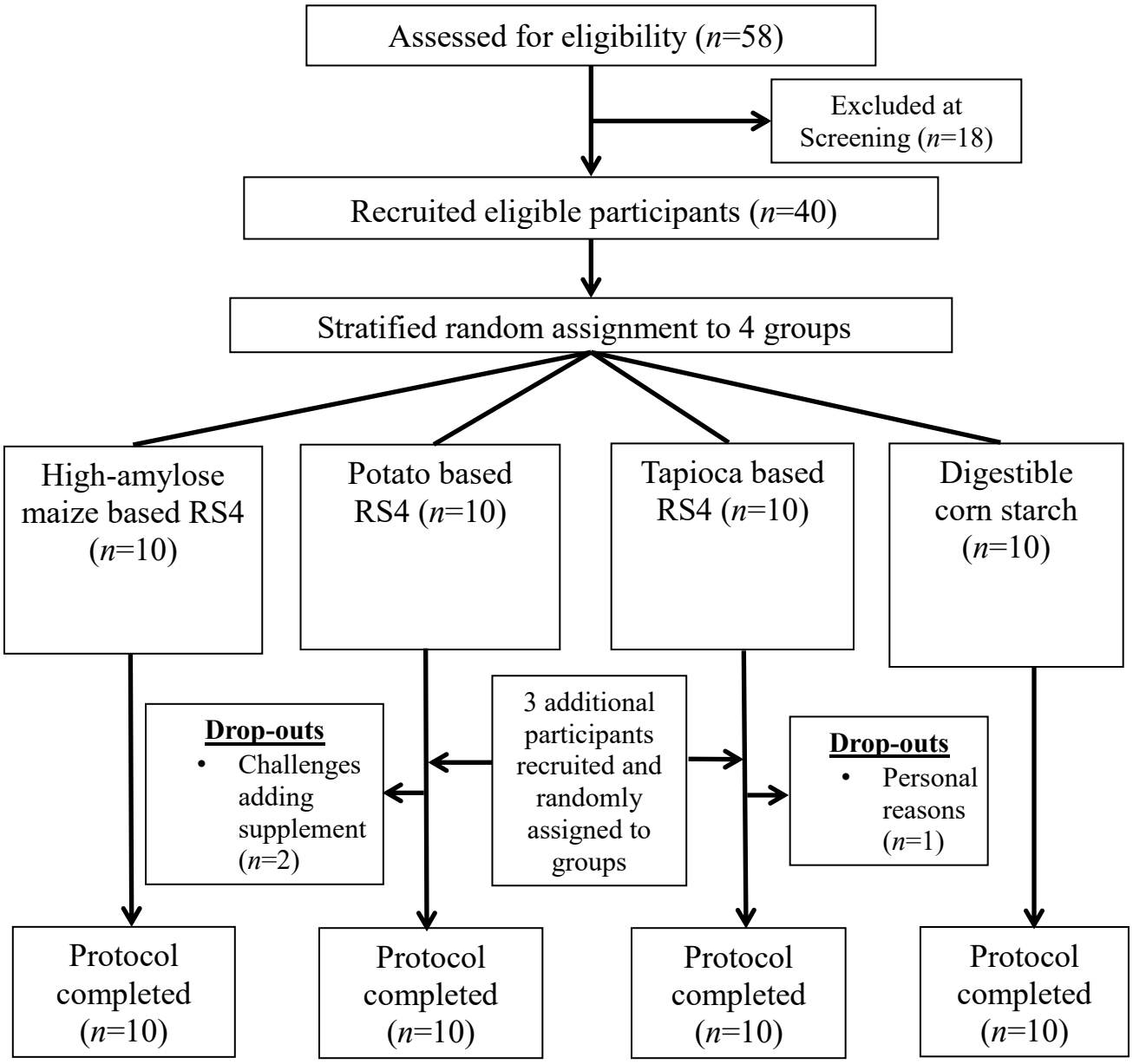

Figure 3-1. Flowchart of all the participants in the human intervention trial

Table 3-1. Baseline characteristics of participants[1]

Characteristic	Treatments				Between Group
	Corn Starch	Maize RS4	Potato RS4	Tapioca RS4	*P* value
Population (*n*)	10	10	10	10	
Gender (M/F)	5/5	5/5	5/5	5/5	
Age (y)[2]	27 ± 7.8	25 ± 8.3	31 ± 8.4	29 ± 7.7	0.41
Height (cm)	173.5 ± 10.1	170.5 ± 9.3	169.5 ± 9.0	173.2 ± 10.0	0.74
Weight (kg)[2]	72.3 ± 10.4	66.7 ± 12.7	71.6 ± 14.0	73.4 ± 18.2	0.68
BMI (kg/m^2)[2]	24.1 ± 3.7	22.8 ± 2.1	24.7 ± 2.8	24.3 ± 3.9	0.56
Perceived stress score	4.2 ± 3.1	4.1 ± 1.8	4.7 ± 3.2	5.4 ± 2.2	0.68
MET score[2]	3741 ± 2395	2794 ± 1568	2326 ± 2402	2507 ± 1837	0.16
Marital status					0.50
Single/never married	7 (70%)	9 (90%)	7 (70%)	6 (60%)	
Married/common-law	3 (30%)	1 (10%)	3 (30%)	4 (40%)	
Ethnicity					0.13
Caucasian	8 (80%)	3 (30%)	4 (40%)	5 (50%)	
Asian	1 (10%)	7 (70%)	5 (50%)	4 (40%)	
Other	1 (10%)	0 (0%)	1 (10%)	1 (10%)	
Education level					0.96
High school diploma	2 (20%)	4 (40%)	2 (20%)	3 (30%)	
Bachelor's degree	5 (50%)	3 (30%)	4 (40%)	3 (30%)	
Graduate degree	2 (20%)	1 (10%)	3 (30%)	3 (30%)	
Other	1 (10%)	2 (20%)	1 (10%)	1 (10%)	
Employment status					0.31
Student	7 (70%)	6 (60%)	5 (50%)	3 (30%)	
Employed	3 (30%)	3 (30%)	5 (50%)	7 (70%)	
Unemployed	0 (0%)	1 (10%)	0 (0%)	0 (0%)	
Household income					0.23
Less than $40,000	5 (50%)	3 (30%)	2 (20%)	3 (30%)	
$40,000 -- $69,000	0 (0%)	5 (50%)	3 (30%)	2 (20%)	
$70,000 -- $99,000	2 (2%)	2 (20%)	1 (10%)	2 (20%)	
$100,000 or more	3 (30%)	0 (0%)	4 (40%)	3 (30%)	

[1] Values are presented as the mean ± SD or count (%)
P values indicate the difference among treatment arms. One-way ANOVA or Fisher's exact test were applied.
[2] Log base 2 transformation of data prior to statistical analysis with one-way ANOVA. No significant differences at baseline were observed.
Abbreviation: BMI, body mass index; MET, metabolic equivalent.

3.2 Compliance

Mean overall compliance during the intervention, assessed by the amount (weight) of returned supplement, was high at 98.9 ± 2.9%, with no significant difference between supplementation groups, except for one participant assigned to the Potato RS4 arm consumed 82% of the total supplement provided (**Figure 3-2**). This participant had 100% compliance during the first three weeks, but the compliance was much lower (37.3%) in week four. This participant may not have tolerated the dose of 50 g/day, although exacerbation of an underlying illness cannot be excluded. Considering this factor, a more conservative approach was applied to analyse the tolerability of the supplement, and the participant was not removed from the analysis. When comparing all of the treatment arms to the control, no difference was found in the overall compliance rate.

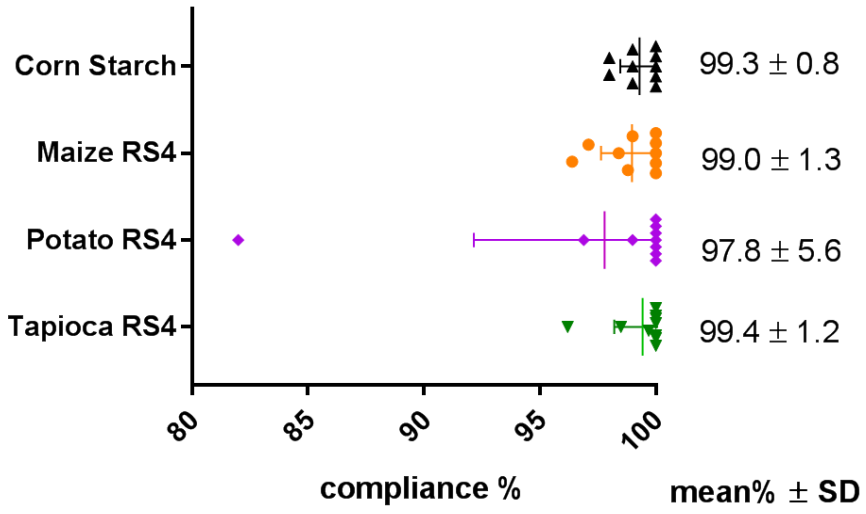

Figure 3-2. The overall compliance rate for total supplements in each treatment arm throughout the intervention. The values presented in the figure are the compliance rates of total supplements for each subject. The overall compliance in each arm was the percentage of the amount of supplements provided consumed by participants throughout the study, and the overall compliance of the study was the average of the overall compliance in the four treatment arms.

3.3 Incorporation of starch supplement

During the intervention period, most participants separated the supplement into two to three portions a day as directed (**Table 3-2**). Only a few participants reported consuming the supplement in one portion for convenience even after being encouraged not to do so by research staff. Additionally, no significant difference in daily portions of supplements was found among participants in the RS4 treatment groups and the control group.

Participants tended to add the supplements to either their meals or their drinks during the day. The methods of supplementation were significantly different between the 4 intervention arms (Fisher's exact test, P=0.02). Participants assigned to the Potato RS4 arm consumed the supplement in a significantly different manner from those in the control group, as a majority of participants who consumed Potato RS4 added the supplement in their meals or both their meals and drinks, while most of the participants assigned to the control group added the supplements to drinks. The other two groups used two supplementing methods evenly.

Table 3-2. Supplement incorporation[1]

	Treatments				Between Group
Parameter	Corn Starch	Maize RS4	Potato RS4	Tapioca RS4	*P* value
Portions/d					0.26
1	0 (0%)	3 (30%)	3 (30%)	0 (0%)	
2-3	7 (70%)	4 (40%)	5 (50%)	8 (80%)	
≥ 4	3 (30%)	3 (30%)	2 (20%)	2 (20%)	
Method of Supplementation			‡		0.02
Drinks	8 (80%)	5 (50%)	1 (10%)	4 (40%)	
Solid food	2 (20%)	3 (30%)	4 (40%)	1 (10%)	
Both	0 (0%)	2 (20%)	5 (50%)	5 (50%)	

[1] Values are counts (%)
P values show the difference among groups. Fisher's exact test was used.
‡: Significant difference in the treatment arm compared to the control arm, P<0.05

3.4 Changes in weight, perceived stress, physical activity, and diet

The changes in weight and BMI of the participants in the three RS4 treatment arms after the four-week intervention did not differ from those in the control group **(Table 3-3)**. Additionally, no significant effect of treatment type, dose, treatment-dose interaction was observed on perceived stress scores, physical activity, calorie intake, macronutrient intake, or total fibre intake (g) between baseline and week four, except for the additional DF provided as RS4 in the three treatments groups.

Table 3-3. **Change in Characteristics During Dietary Intervention.**

Δ in Characteristic (W4 - BL)	Treatments				Treatment *p*-value	Time *p*-value	Interaction *p*-value
	Corn Starch	Maize RS4	Potato RS4	Tapioca RS4			
Weight (kg)	0.0 ± 1.1	0.9 ± 1.1	-0.3 ± 1.5	0.4 ± 1.1	0.753	0.195	0.174
BMI (kg/m^2)	0.0 ± 0.4	0.3 ± 0.4	-0.1 ± 0.5	0.1 ± 0.4	0.620	0.163	0.224
Perceived stress score	1.4 ± 2.5	-0.2 ± 2.1	-0.3 ± 2.2	0.4 ± 1.6	0.624	0.337	0.269
MET score	105 ± 1162	-230 ± 1326	-852 ± 1268	-423 ± 594	0.168	0.057	0.303
Energy (kcal)	-107 ± 569	348 ± 897	-103 ± 518	234 ± 391	0.652	0.350	0.258
Carbohydrate (g)	4.1 ± 57.5	18.7 ± 102.4	-14.0 ± 49.1	16.7 ± 68.2	0.914	0.582	0.730
Total fibre[a] (g)	1.2 ± 6.8	-0.2 ± 6.9	-0.2 ± 9.0	-2.2 ± 9.1	0.521	0.856	0.939
Protein (g)	-13.5 ± 41.0	27.6 ± 49.9	-17.1 ± 26.2	20.1 ± 33.3	0.212	0.488	0.025
Total fat (g)	-2.0 ± 45.4	17.4 ± 46.9	3.0 ± 36.0	4.5 ± 28.0	0.382	0.369	0.733

[a]Total dietary fibre provided by the diet without the added fibre supplement.
Data presented as mean ± SD, no significant changes within or between each group after Holm-Sidak's multiple comparison test (two-way repeated measures ANOVA).
BMI: body mass index; MET: metabolic equivalent.

3.5 Overall gastrointestinal tolerability of resistant starch type 4

3.5.1 Composite gastrointestinal tolerability

The effect of treatment type and dose on composite GI tolerability score was determined (**Figure 3-3**). The results suggested that the overall effect of supplementation dose on composite GI tolerability score was significantly (GEE model, main dose effect $P<0.0001$), yet 10 to 20 g/day of supplements were well tolerated by all participants except for those who consumed Maize RS4. As the dose increased from 35 to 50 g/day, GI symptoms notably increased in participants who consumed corn starch, Maize RS4 and Tapioca RS4 (GEE models, $P<0.05$). In addition, the effect of dose was significant for participants who consumed the Maize RS4 at 10 g/day compared to the baseline (GEE models, $P<0.05$). However, only one participant rated four on a twelve-point scale, while the other nine participants rated their GI symptoms zero, one, or two. Surprisingly, a significant effect of dose and treatment on the composite GI tolerability score was also observed in participants assigned to the control group (GEE models, $P<0.05$). Further, no detectable overall effects of dose and treatment were observed in a comparison of the GI tolerability score between participants assigned to the RS4 groups and the controls (GEE models, $P<0.05$).

Interestingly, extensive inter-individual variability was observed in supplement tolerance with some participants reporting no change in symptoms and others reporting symptoms at each dose. When considering all the participants, 77.5% and 72.5% of them tolerated supplements well up to 35 g/day or even 50 g/day, respectively, with their tolerability scores below three on a twelve-point scale. Moreover, some of the participants did not report a change in GI symptoms

throughout the intervention period, while some participants experienced GI symptoms coinciding with the supplements.

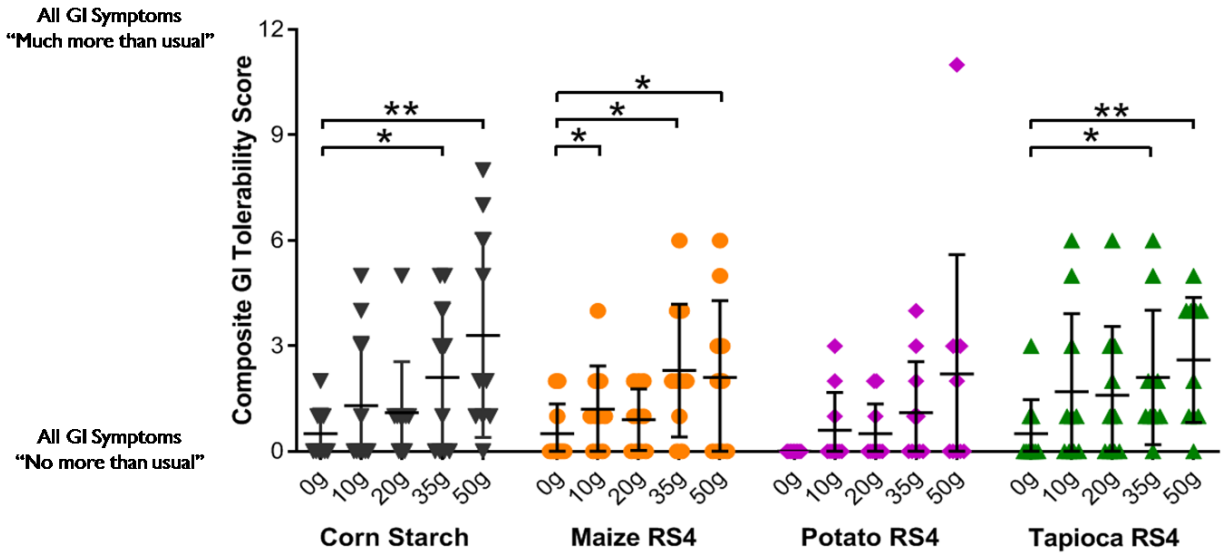

Figure 3-3. **The composite GI tolerability score of individuals.**
Data are presented as the mean ± SD; Generalized estimating equation assessed the effect of treatment type and dose with post hoc test. Pair-wise comparison within treatment arm noting significant change from baseline, * P<0.05 ** P<0.01

3.5.2 Individual gastrointestinal symptoms

When assessing all six GI symptoms individually, bloating and flatulence were the primary symptoms reported to significantly increase by participants throughout the supplement intervention. In addition to the overall effectiveness of dose on all four arms, an increase in supplement dose significantly increased the frequencies of bloating (b) and flatulence (d) symptoms (cumulative link models, P<0.05), with no difference between participants assigned to the RS4s or placebo (cumulative link models, P >0.05) (**Figure 3-4**). An increase in supplement dose also significantly increased GI rumbling (c) symptoms in participants from all treatment arms during week 1, week 3 and week 4 compared to the baseline (cumulative link models, P

<0.05), with no difference between participants assigned to the RS4 s or placebo. Interestingly, the mean score of bloating and GI rumbling symptoms in all participants decreased in week 2 and then significantly increased again compared to baseline. Abdominal pain (e) symptoms were significantly affected by a supplement dose higher than 35 g/day when considering participants from all four treatment arms (cumulative link models, $P<0.05$), with no difference between participants assigned to the RS4 and control arms. In contrast, supplement dose did not have a significant effect on nausea (a) or diarrhoea (f) symptoms over the course of the intervention, while Maize RS4 and Tapioca RS4 caused less diarrhoea than the placebo. Only one subject experienced nausea "somewhat more than usual" in week 2, and no more than three out of all 40 participants reported "somewhat more than usual" or "much more than usual" during weeks 3 and 4.

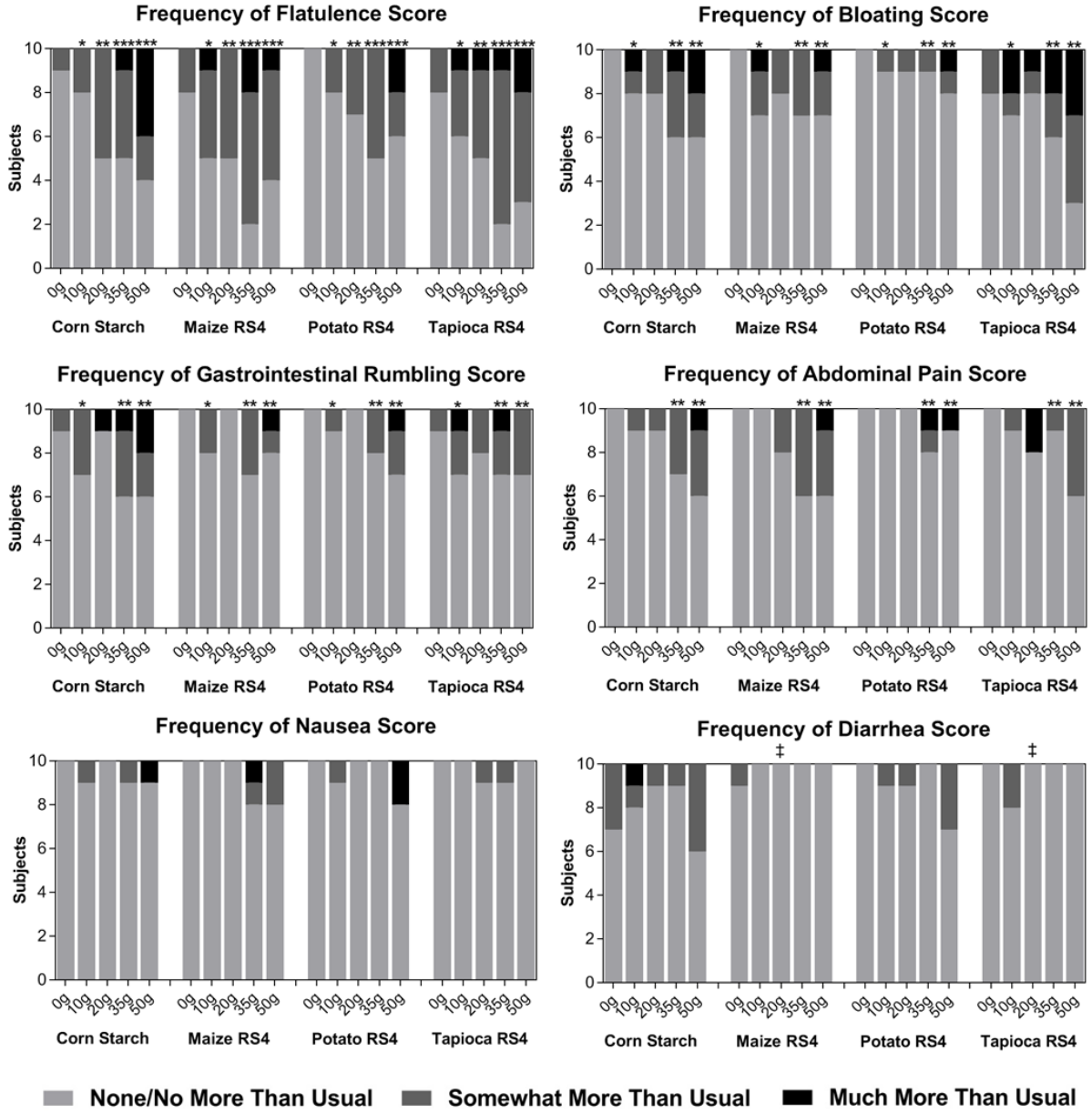

Figure 3-4. Changes in the frequency of GI symptom rates over the intervention period. Data were analysed by cumulative link models relative to baseline (considering all groups) and to placebo (corn starch; considering all time points). All symptoms assessed, with the exception of nausea and diarrhea, were reported more frequently as supplementation dose increased. Compared to baseline: **P*<0.05, ***P*<0.01, ****P*<0.001; compared to placebo: ‡ *P*<0.05.

3.5.3 Bowel habits

The effect of supplement dose was significant at 50 g/day on stool frequency (GEE, $P<0.01$), and at 20 g/day on faecal hardness (GEE, $P=0.04$) when considering all participants from the four treatment arms. However, the effect of dose was not found in other bowel movement habits include straining, discomfort during bowel movement, or incomplete evacuation (GEE, $P>0.05$). Participants consuming Potato RS4 reported a significant increase in bowel movement frequency and decrease in fecal hardness at the 50 g/d dose as compared to the baseline (GEE, $P<0.05$) (**Table 3-4**), while this effect was not observed in participants in the other three treatment arms (GEE, $P>0.05$). Additionally, 35 g and 50 g of Potato RS4 per day significantly reduced faecal hardness (**Table 3-4**). Moreover, no significant differences in bowel habits were reported between participants in the RS4 treatment arms and the control arm, except significantly less discomfort during a bowel movement was reported by participants in the Tapioca RS4 group compared to the participants consuming the control (digestible starch) (GEE, $P<0.05$). Last but not least, stool consistency was assessed using the Bristol Stool Scale, which remained unchanged over the course of intervention compared to the baseline for all participants in the four treatment arms (GEE, $P>0.05$).

Table 3-4. Effect of RS4 supplementation on bowel habits[1]

Treatments	Study Weeks					Treatment Effect *P* value
	Baseline (0g)	Week 1 (10g)	Week 2 (20g)	Week 3 (35g)	Week 4 (50g)	
Frequency (stools/day)						
Corn Starch	1.5 ± 0.7	1.7 ± 0.7	1.9 ± 1.4	1.7 ± 0.6	1.6 ± 0.7	–
Maize RS4	1.1 ± 0.6	1.2 ± 0.6	1.4 ± 0.8	1.1 ± 0.7	1.3 ± 0.7	0.077
Potato RS4	1.6 ± 0.6	1.8 ± 0.6	1.3 ± 0.3	1.8 ± 1.1	**1.9 ± 0.7***	1.000
Tapioca RS4	1.2 ± 1.0	1.3 ± 0.9	1.6 ± 1.0	1.3 ± 0.9	1.6 ± 0.9	0.416
Bristol stool scale						
Corn Starch	4.1 ± 1.0	4.1 ± 0.7	4.2 ± 1.1	3.4 ± 1.1	4.1 ± 1.0	–
Maize RS4	4.0 ± 0.8	4.0 ± 1.0	3.8 ± 0.8	3.7 ± 0.9	3.5 ± 0.8	0.520
Potato RS4	3.8 ± 1.1	3.8 ± 1.0	3.7 ± 0.9	3.9 ± 0.8	4.1 ± 1.1	0.690
Tapioca RS4	3.8 ± 1.1	4.1 ± 1.0	3.9 ± 0.6	4.2 ± 0.7	3.8 ± 0.9	0.930
Fecal hardness						
Corn Starch	1.8 ± 0.6	1.6 ± 0.5	1.6 ± 0.6	2.0 ± 0.8	1.7 ± 0.6	–
Maize RS4	1.6 ± 0.5	1.8 ± 0.6	1.6 ± 0.4	1.9 ± 0.6	1.6 ± 0.6	0.679
Potato RS4	2.1 ± 0.5	1.8 ± 0.4	1.9 ± 0.6	**1.8 ± 0.4***	**1.6 ± 0.5***	0.574
Tapioca RS4	2.0 ± 0.7	1.7 ± 0.5	1.7 ± 0.4	1.6 ± 0.5	1.7 ± 0.5	0.965
Straining						
Corn Starch	1.3 ± 0.4	1.6 ± 0.5	1.4 ± 0.4	1.8 ± 0.8	1.5 ± 0.3	–
Maize RS4	1.6 ± 0.5	1.5 ± 0.6	1.4 ± 0.5	1.6 ± 0.4	1.8 ± 0.7	0.682
Potato RS4	1.7 ± 0.6	1.6 ± 0.4	1.6 ± 0.7	1.6 ± 0.5	1.4 ± 0.5	0.713
Tapioca RS4	1.8 ± 0.8	1.4 ± 0.5	1.5 ± 0.6	1.4 ± 0.5	1.5 ± 0.5	0.920
Discomfort						
Corn Starch	1.4 ± 0.4	1.7 ± 0.8	1.2 ± 0.4	1.7 ± 0.7	1.7 ± 0.6	–
Maize RS4	1.3 ± 0.5	1.4 ± 0.7	1.3 ± 0.4	1.3 ± 0.4	1.6 ± 0.6	0.419
Potato RS4	1.3 ± 0.5	1.3 ± 0.4	1.4 ± 0.5	1.2 ± 0.4	1.2 ± 0.5	0.183
Tapioca RS4	1.2 ± 0.5	1.2 ± 0.3	1.2 ± 0.5	1.1 ± 0.2	1.2 ± 0.5	**0.023**
Incomplete evacuation						
Corn Starch	1.5 ± 0.5	1.7 ± 0.8	1.5 ± 0.6	1.6 ± 0.6	1.3 ± 0.4	–
Maize RS4	1.4 ± 0.7	1.3 ± 0.6	1.1 ± 0.1	1.2 ± 0.2	1.6 ± 0.9	0.330
Potato RS4	1.5 ± 0.6	1.3 ± 0.4	1.6 ± 0.6	1.5 ± 0.5	1.6 ± 0.5	0.880
Tapioca RS4	1.6 ± 0.7	1.2 ± 0.4	1.6 ± 0.8	1.3 ± 0.4	1.3 ± 0.5	0.400

Data presented as mean ± SD. Generalized estimating equation assessing the effect of treatment type and dose, with post-hoc test. Pair-wise comparison within treatment arms noting significant change from baseline, * $P<0.05$.

3.6 Perceived satiety

At baseline, no significant between-group differences were detected among SLIM scores reported upon awaking or 2-hours after a meal (one-way ANOVA, $P<0.05$). However, SLIM scores reported 4-hours after a meal were significantly different between-groups (one-way ANOVA, $P=0.018$) with participants assigned to Potato RS4 reporting more hunger than the other groups. In addition, extensive inter-individual variation was noted at baseline, with a standard deviation similar to or higher than the mean values (**Table 3-5**). Therefore, caution should be taken when interpreting the findings, especially those assessing the perceived satiety four hours after supplementation.

Table 3-5. **Differences in SLIM scores at different time points between the four intervention arms at baseline** [1]

Time points	Treatments				*P* value
	Corn starch	Maize RS4	Potato RS4	Tapioca RS4	
Upon Awaking	-20.7 ± 16.0	-9.5 ± 11.4	-2.2 ± 39.8	-10.9 ± 36.2	0.555
2-hrs After Meal	-9.4 ± 32.9	13.2 ± 30.5	3.8 ± 33.6	1.1 ± 31.2	0.480
4-hrs After Meal	-21.5 ± 22.1	-10.7 ± 35.6	-31.2 ± 29.1	12.0 ± 31.8	**0.018**

[1] Values are presented as the mean ± SD
One-way ANOVA was used. *P* values provided are the differences compared between all treatment arms. Significant difference: $P<0.05$.

Perceived satiety upon waking and two hours after the reference meal did not change significantly as the supplement doses increased for participants within all four treatment arms (two-way ANOVA, $P>0.05$) (**Figure 3-5, a; Figure 3-6, a**), and no significant differences were observed between the RS4 treatment arms and the control (one-way ANOVA, $P>0.05$) (**Figure 3-5, b; Figure 3-6, b**). Participants consuming Potato RS4 did however report a significantly increase in perceived satiety 4-hours after a meal when consuming the 35 g/d dose ($P<0.05$, two-way ANOVA); no other significant effects were reported (**Figure 3-7, a; Figure 3-7, b**)

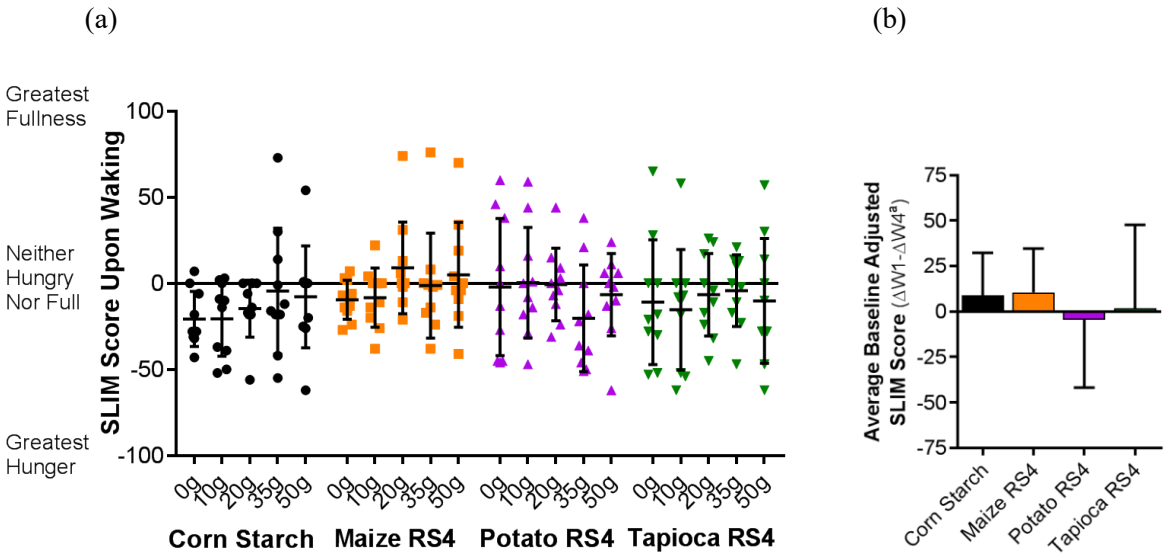

Figure 3-5. SLIM scores upon waking for participants in each treatment arm over the course of intervention (a) absolute SLIM score of all participants, (b) change in average SLIM score adjusted for baseline. Data are presented as the mean ± SD, two-way ANOVA with Holm-Sidak's multiple comparison tests to assess change from baseline within the intervention arm. [a] One-way ANOVA with Holm-Sidak's multiple comparison tests comparing the average baseline adjusted composite GI tolerability score for each treatment to the control while considering the delta values together (ΔW1-ΔW4).

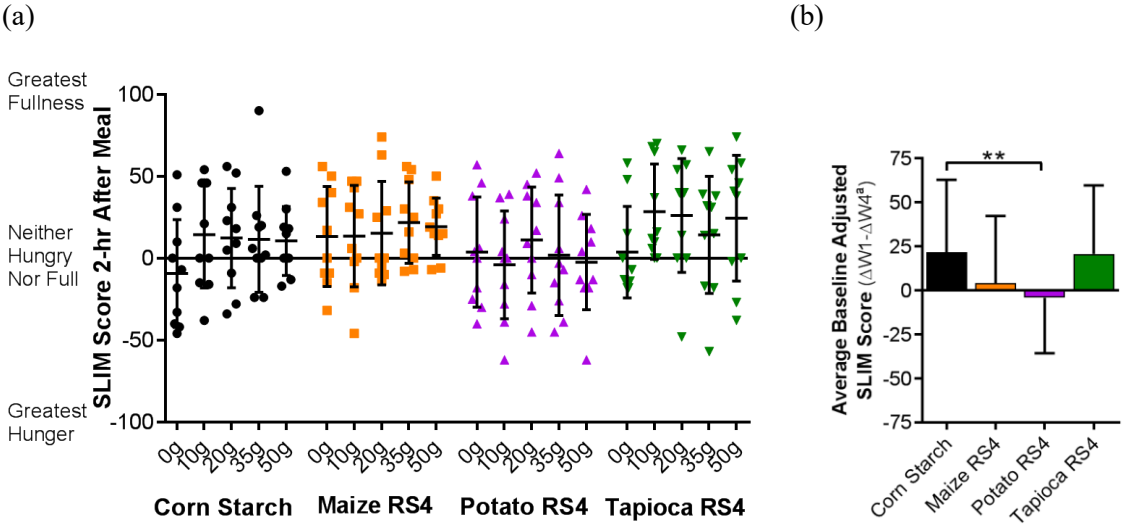

Figure 3-6. SLIM scores two hours after the reference meals of participants in each treatment arm over the course of intervention (a) absolute SLIM score of all participants, (b) change in average SLIM score adjusted for baseline. Data are presented as the mean ± SD, two-way ANOVA with Holm-Sidak's multiple comparison tests to assess change from baseline within the intervention arm. [a]One-way ANOVA with Holm-Sidak's multiple comparison tests comparing the average baseline adjusted composite GI tolerability score for each treatment to the control while considering the delta values together (ΔW1-ΔW4). * P<0.05, ** P <0.01.

(a) (b)

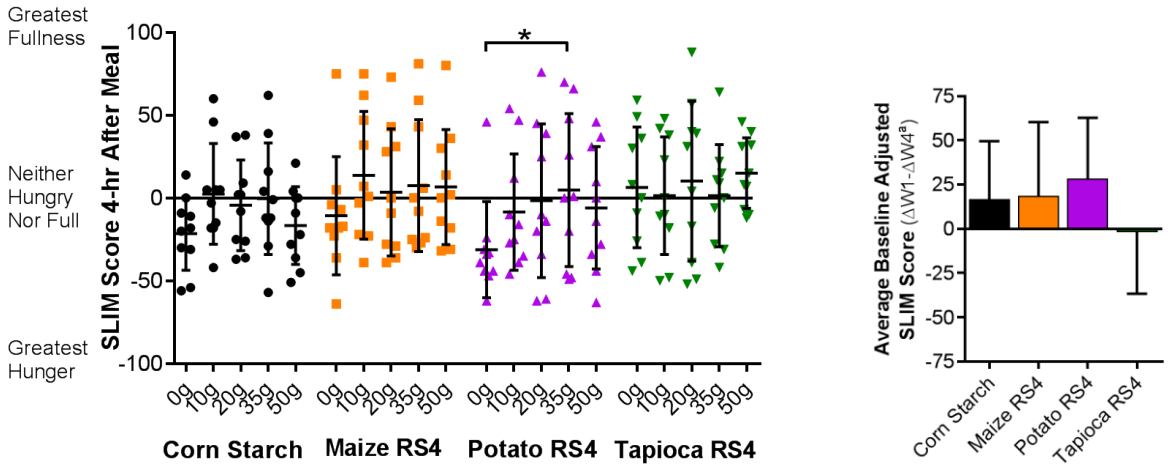

Figure 3-7. SLIM score four hours after the reference meals of participants in each treatment arm over the course of intervention (a) absolute SLIM score of all participants, (b) change in average SLIM score adjusted for baseline. Data are presented as the mean ± SD, two-way ANOVA with Holm-Sidak's multiple comparison tests to assess change from baseline within the intervention arm. * $P<0.05$. [a]One-way ANOVA with Holm-Sidak's multiple comparison tests comparing the average baseline adjusted composite GI tolerability score for each treatment to the control while considering the delta values together (ΔW1-ΔW4).

We found no significant effect of supplementation dose or effect of treatment (cumulative link models, $P>0.05$) on the time to getting hungry after a meal with the supplements for participants in the four treatment arms **(Figure 3-8)**. Also, no significant effect of supplementation dose was observed in all four treatment arms in the time participants waited to eat again after a meal with the supplements, (cumulative link models, $P>0.05$) (**Figure 3-9**). Significantly fewer participants in the Potato RS4 group ate again less than four hours after the reference meal compared to participants in the control group (cumulative link models, $P<0.05$). However, no difference was found between participants who consumed Maize RS4, Tapioca RS4, and corn starch (cumulative link models, $P>0.05$).

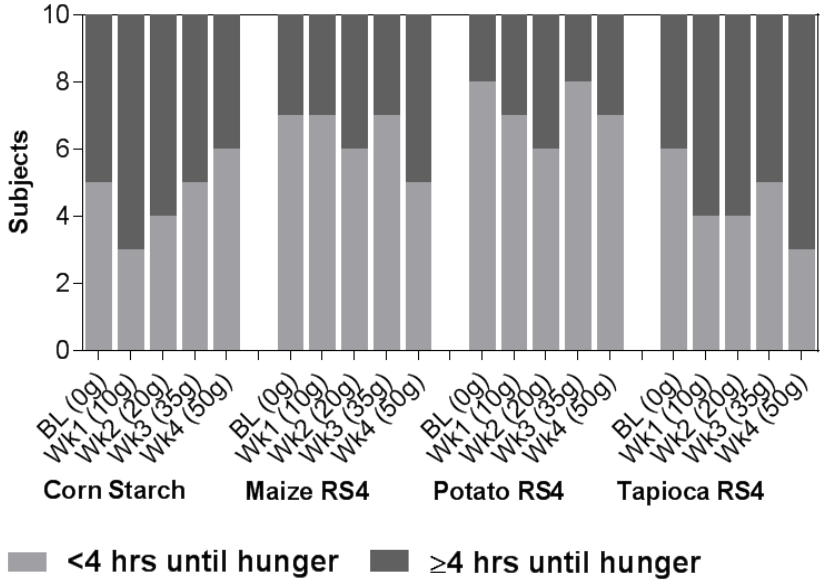

Figure 3-8. Time after the reference meal for the participants to feel hunger again. Cumulative link models assessing the effect of treatment dose and type.

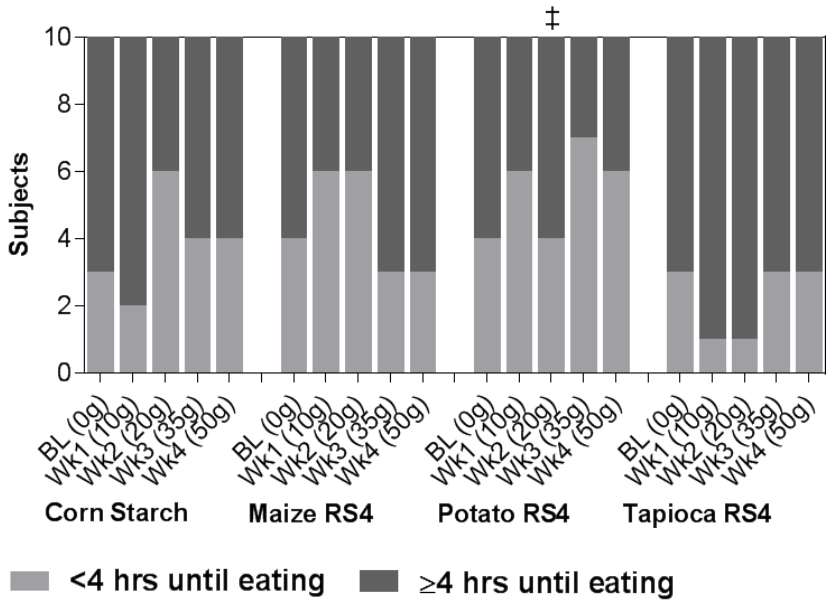

Figure 3-9. Time after the reference meal for the participants to eat again. Cumulative link models assessing the effect of treatment dose and supplement type; more participants in the Potato RS4 group ate again in less than four hours compared to those in the control group, ‡: $P<0.05$.

Chapter 4. Discussion and Conclusions

4.1 Discussion

4.1.1 Gastrointestinal symptoms

Overall, no detectable difference was found in the effect of dose or treatment on the tolerability of the RS4s compared to the digestible corn starch, which suggested that all three RS4s were tolerated no worse than the control. Maize RS4 and Tapioca RS4 were tolerated well with only moderate increases in symptoms, considering that supplementation with 50 g/day increased GI symptoms only "somewhat more than usual" in a majority of participants and had no significant effect on bowel habits, while Potato RS4 caused no GI symptoms but led to looser yet normal stool (BSS at 4) at higher doses (35-50g/day), which indicated a good tolerability. The GI tolerability of other DF, such as fructo-oligosaccharides and inulin, has been studied extensively (Bonnema et al., 2010; Grabitske & Slavin, 2009; Ripoll et al., 2010; Housez et al., 2012). Only 10-15 g/d of these DF could produce gases rapid enough to cause bloating, cramping, and flatulence (Grabitske & Slavin, 2009). The effect of RS4s on GI symptoms suggests a good tolerability when compared with these DFs.

Noticeable symptoms reported when consuming 50 g/day of RS4 were flatulence, bloating, GI rumbling, and abdominal pain, and they were directly related to an increase in gas in the large intestine as a direct result of the fermentation of DF by the GI microbiome (Livesey, 2001), which was consistent with the findings reported in the literature (Crincoli et al., 2016; Stewart et al., 2010; Storey et al., 2007). With an increasing amount of RS4 consumed and reaching the colon, fermentation is likely enhanced, and gas production is intensified. The good tolerability

shown in the present study could be attributed to the slower fermenting nature of RS in the colon resulting in fewer GI symptoms compared to more rapidly fermented DFs, such as oligosaccharides (Wang et al., 2017; Nordgaard et al. 1995; Fässler et al., 2006). Considering that the Potato RS4s was tolerated better than the Maize and Tapioca RS4, its fermentation rate may be slower. However, a previous *in vitro* faecal fermentation study by Erickson and colleagues showed that the Potato and Tapioca RS4s were fermented at similar rate, which was significantly much faster than Maize RS4 (Erickson et al., 2018), and it was not consistent with the present study. Therefore, it is possible that Potato RS4 can not be fermented in vivo. Additionally, mixing RS4 with solid food could postpone RS4 fermentation and gas production, considering slower gastric emptying would compare to liquids, such as when combined with drinks such as water and coffee (Kelly, 1980). To improve the GI tolerability of RS4s in future studies, the supplements could be provided with an escalating dosage, as in the present study, and incorporated into a food matrix, such as baked foods, which makes supplementation method of RS4 consistent for all participants and conforms to daily life.

A great deal of inter-individual variation was observed in the reported composite GI tolerability scores, which may be due to differences in microbiome composition among the participants. For example, a keystone species for degrading RS2 and RS3 found in the human colon is *Ruminococcus bromii*, and without it, the utilization of these RS is significantly reduced (Abell et al., 2008; Salonen et al., 2014; Ze et al., 2012). In the present study, those who experienced few GI symptoms throughout the study may lack specific key species necessary to utilize these RS4s. Further, the ability of background microbiome to utilize hydrogen could also explain the difference in tolerability as it is one of the main gas produced through fermentation (Livesey, 2001). For example, methanogens can utilize hydrogen and reduce carbon dioxide, and

leads to less gas production, which indicates less symptoms (Chaudhary et al., 2017). However, this suggestion cannot be confirmed until the microbiome analysis, which is currently being conducted by a post-doc and PhD student in the Walter laboratory (Maria Elisa Perez-Muñoz and Edward Deehan), is completed for the present study. The background diet may also be associated with GI symptoms severity. Consumption of DF or RS rich foods (i.e. whole grains, starchy vegetable, legumes, nuts, and seeds) is correlated with microbiome composition, which affects the ability of the host to ferment the supplements (Conlon & Bird, 2015; Sawicki et al., 2017), thus affect GI symptoms severity.

There was no effect of dose or treatment observed on nausea or diarrhoea during the four-week intervention. Only a few participants reported minus nausea (less than 2 in each group), while and less diarrhoea was recorded by participants who consumed Maize RS4 and Tapioca RS4. This result is similar to that of other studies, in which only a few participants reported nausea at the highest dose (50 - 60 g/day) (Crincoli et al., 2016; Storey et al., 2007). The result also showed that diarrhoea was not affected by the supplement dose, which could fit with the notion that RS has less potential to affect laxation, as they have a smaller osmotic effect and hence less water reaches the large intestine compared to DF with lower molecular weights (Grabitske & Slavin, 2009). Study showed that even 1.5-2 g/day of inulin can have laxative effect (Hond et al., 2000), yet 20 g/day of RS4 resulted in no significant symptoms in the present study.

Surprisingly, we found that the digestible corn starch used as a control was not as well tolerated as expected, as participants showed similar or worse GI tolerability scores compared to those who consumed the RS4 starches. The primary symptoms reported were bloating and

flatulence, which were also the main symptoms shown in the three RS4 treatments. A plausible explanation for this finding was the placebo effect (Hammami et al., 2016). All the participants were fully aware of the symptoms they might experience if they were assigned to consume one of the RS4s. Therefore, as they consume the supplement powder every day, symptoms due to their perception may be noticed and recorded. On the other hand, related symptoms could draw more attention than usual from participants and thus be overstated. In the 1950s, Beecher developed an "additive model", indicating that "The total 'drug' effect is equal to its 'active' effect plus its placebo effect" (Beecher, 1953). Although statistically too simplistic for clinical studies, the placebo effects on treatment arms are also not negligible. The symptoms observed in participants who consumed RS4 may have been partially due to psychological effects. Also, the participants were asked not to cook with the supplements, therefore, the control, which is a type of high-amylopectin corn starch, was consumed as intact starch granules, which behave differently in the GI tract than when it is gelatinized starch or starch in a food matrix such as in baked foods (Holm et al., 1988; Smith et al., 2015). Intact starches are less digestible than gelatinized starch solution, as flexible α-glucans would be exposed in the periphery of the starch granules after gelatinization of the starch (Bello-perez et al., 2018; Dreher et al., 2009). They are the primary substrate of α-amylase and can be rapidly cleaved in the initial stage of hydrolysis. Further, the digestibility of starch granules could be different in vitro and in vivo (Dreher et al., 2009). Thus, the corn starch may have been a more effective placebo if it had been consumed gelatinized.

4.1.2 Bowel movement habits

The present study showed that 35 to 50 g/day of Potato RS4 significantly increased bowel movement frequency and stool consistency, while the other three RS4s did not affect bowel movement habits, yet bowel movement frequency remained regular (one to three bowel movements daily) and there was no presence of diarrhoea or constipation since the average BSS was in the mid-range of the Bristol Stool Chart ratings throughout the intervention. It is not surprising that bowel movement frequency did not change in the study, since it has a plateau effect (Burkitt et al.,1972; Wrick et al., 1983), and participants had their bowel movements in regularity at baseline. Once the bowel movement is regular, the effect of DF is mainly to increase stool weight, and faecal volume, rather than bowel movement frequency (Haack et al., 1998; Shen et al., 2017).

Previously, at least 20 to 25 g/d of RS has been suggested to be required for achieving significant changes in laxation (Maki et al., 2009). In another study on three different types of Potato RS4, only one of them significantly increased stool frequency and led to looser stool in healthy adults at 30 g/day, while the others did not (Dahl et al., 2016). The present study shows that this proposed dose might be higher or dependent on the type of RS and that the chemical structure of RS could have an impact on its laxative effect. Potato RS4 has been shown to be well fermented in vitro (Erickson et al., 2018), yet in vivo study has not been conducted. Previous studies have demonstrated that when DF is not fully fermented in the large intestine, the unfermented DF particles will remain bound to water and will be discharged together within faeces, which increases faecal bulk (Stephen, 1991) and may contribute to looser and more watery stool (Livesey, 2001). According to the results, Potato RS4s may undergo a gradual and

non-complete fermentation at higher doses from 35 to 50 g/day, resulting in osmotic pull from unfermented material. However, further evidence from microbiome analysis will be needed.

4.1.3 Perceived satiety

The data showed that Potato RS4 significantly increased the perceived satiety four hours after the meal, while Maize RS4 and Tapioca RS4 did not have an impact on perceived satiety during the intervention. However, due to baseline variation, the results of the present study showed no effect of RS4 on perceived satiety.

Foods with lower glycaemic index, such as RS4 supplements, have been suggested to produce greater satiety two to six hours following consumption of the reference meal compared with foods that have a higher glycaemic index, such as digestible corn starch (Bornet et al., 2007), indicating that the three RS4 were thought to have a more similar and greater impact on perceived satiety than the corn starch, yet the findings of the present study proposed different effects. This is similar to previous studies that had participants consumed 30 g/day of RS2, RS3, or two novel potato RS4s, no supplement effect on satiety responses was found when compared with the control (de Roos et al., 1995; Haub et al., 2012). The different effects of Maize RS4, Potato RS4, and Tapioca RS4 on perceived satiety indicated that the chemical structure of RS4 might be responsible for this observation. Moreover, considering the study design, participants in the study were free-living individuals; therefore, the time when the supplements were consumed and the time when perceived satiety was recorded varied among participants, which could influence the result as well. Further, in the present study, considering the high variation in the SLIM score four hours after the reference meal and the low sample size (n=10 in each arm), interpretations of findings on satiety should be made with caution.

4.2 Strengths and limitations of this study

There are several strengths of this study, the first being the randomized double-blind placebo-controlled study design, which reduces the likelihood that unknown or unmeasured confounding variables, such as biological differences among participants, affect the results. The supplements provided to the participants looked identical and were all in powder form without any smell. Therefore, participants would not be able to identify which supplement they consumed. Moreover, the structures of three RS4s are different. They originated from different sources and were processed in two different ways, allowing us to conduct a novel investigation of differences in structure within a sub-category of a DF and to explore whether consuming these different subgroups of DF leads to differences in GI tolerability and satiety.

The use of supplements instead of food products containing RS is an important limitation to acknowledge. The participants needed to add the powdered supplements to their diet daily, and although the participants were asked to try not to change their normal diet. This limitation could be addressed in future studies by enriching foods with the supplements and then having participants replace food items in their normal diet. Additionally, to avoid GI symptoms caused by their background diet, participants were asked to avoid consuming food that could cause GI distress, which could be a change in diet and a limitation for the generalizability of the results. Another limitation of the study is the relatively small sample size, with only ten participants in each treatment arm. However, Grabitske and Slavin (2009) reviewed five studies that used subject numbers from eight to twelve to assess the tolerability of RS and fifteen studies with populations from six to twelve to assess the tolerability of sugar alcohols and non-starch polysaccharides. They found the effects on GI symptoms to be large enough to be successfully

detected in these studies by the statistical tests, therefore ten participants per arm is likely sufficient to detect meaningful differences between study groups. In addition, the placebo control in the present study was not a physiologically inert control, as it unexpectedly yet significantly worsened the participants' GI symptoms. Therefore, future studies should avoid using raw starch as the control when assessing GI tolerance. Lastly, the extensive variation in perceived satiety at baseline, in combination with the small subject numbers, might have prevented the detection of significant effects. Therefore a stricter study design specifically designed to assess satiety as the primary outcome, with a larger sample size, would be necessary to appropriately assess the effect of these RS4's on perceived satiety.

4.3 Conclusions and implications on future clinical trials

The present study provides important evidence for the purpose of designing further clinical trials that high doses of these three novel RS4s can be well tolerated at least over the short term. Moreover, as discussed previously, more than 50 g/day of total DF intake has been suggested to be required to promote notable physiological benefits, supplementing with these RS4s at a 50 g/day dose can and perhaps should be recommended for future clinical trials aimed at assessing the health benefits of these RS4s.

In summary, these three novel RS4s are very promising for closing the "fibre gap". Enriching food products with up to 10 g/serving of these RS4s would likely be a practical option for incorporating higher doses of DF in the diet, assuming that three to five servings could be consumed daily. This process will make it possible for the industrialized population to achieve 50 g/day of total DF or higher, considering their current background diet. However, to provide rationale for their use in the food supply and to be considered a DF in Canada and the US, future

human studies need to be conducted with supplementation of higher daily doses (i.e., 50 g/day) while concomitantly assessing their physiological effect on relevant health markers and how these effects are related to compositional and functional responses in the GI microbiome.

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Appendices

Appendix A. Sample recruitment poster

Version 3 - July 31, 2017

How would your gut tolerate increasing fibre?

Particp ate in a study on how your gut tolerates different amounts of a fibre supplement.

Study PI: Dr. Jens Walter

You'll receive compensation upon completion of the i 5 week study.

Contact: (780) 293-3449 or guthealth@ualberta.ca

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Appendix B. Products specifications

technical specification

Ingredion

VERSAFIBE™ 2470 Dietary Fiber 22457B00

VERSAFIBE™ 2470 dietary fiber is a modified food starch based on high amylose corn. VERSAFIBE™ 2470 dietary fiber is an easy to use, process tolerant resistant starch for use in bakery applications. It tests as dietary fiber via AOAC method 2009.01.

Chemical and Physical Properties

	Min.	Max.
Moisture, %	-	13.0
pH (20% w/w slurry)	5.0	7.0
Total Dietary Fiber, % as is (AOAC 2009.01)	65	-

Physical Appearance

Color	White to Off-White
Form	Fine Powder

Screen Test

	Typical
% thru U.S.S. #100	>90
% thru U.S.S. #200	>75

Microbiological Limits

	Max.
Total Plate Count, cfu/g	10,000
Yeast, cfu/g	200
Mold, cfu/g	200
E. coli	Negative
Salmonella	Negative

Nutritional Data/100 g

	Typical
Calories (US)[1]	109
Total fat, g	1.0
Total Carbohydrate, g	89.9
Dietary Fiber, g (US)	65
Other Carbohydrate, g (US)	24.9
Total Sugars	0
Protein, g	0
Sodium, mg	62
Vitamin C, mg	<0.5
Calcium, mg	124
Iron, mg	<1
Ash, g	0.17

[1] based on 4 kcal/g carbohydrates, insoluble fiber non-caloric
Not a significant source of cholesterol, vitamins, or other minerals .

Certification

Kosher pareve
Halal

Packaging and Storage

VERSAFIBE™ 2470 dietary fiber is packaged in multi-ply kraft paper bags with a net weight of 50 lbs. VERSAFIBE™ 2470 dietary fiber should be stored in a clean, dry area at ambient temperature and away from heavily aromatic material.

Shelf Life

The best before date for VERSAFIBE™ 2470 dietary fiber is 24 months from the date of manufacture.

Regulatory Data

Source High Amylose Corn

United States

Meets FCC (Food Chemical Codex) requirements
Labeling Food Starch-Modified

Features and Benefits

VERSAFIBE™ 2470 dietary fiber is a cost effective, high fiber resistant starch type 4 (RS4). The product can be used to add fiber to a variety of bakery applications including extruded cereal and snacks, breads, pasta, and cookies. It is an easy to use fiber with little impact to formulation or process. Due to its high process tolerance and exceptional fiber retention, VERSAFIBE™ 2470 is ideal for a variety of applications including those with high temperature and shear processes.

VERSAFIBE™ 2470 dietary fiber is a corn based, making it ideal for gluten-free products.

Effective Date: July 26, 2016 Next Review Date: July 26, 2019

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www.ingredion.us

Take the lead in fibre fortification

Adding fibre to many commonly consumed foods continues to be a challenge. You must balance the positive health benefits and label claims with the potentially negative impact of fibre on taste, texture and appearance. Not anymore. New NOVELOSE™ dietary fibres are a family of novel insoluble fibres that have little or no impact on taste, colour or texture. They can improve the texture of breads, noodles, pasta, biscuits, cereals and snacks while reducing calories and offering processing ease and reduced cost-in-use.

Meet the growing fibre trend

Complementing the trend toward disease prevention, consumer awareness about dietary fibre and the health benefits associated with its increased consumption are on the rise. According to a 2015 Nielsen Global Health & Wellness report, 36% of respondents rated foods high in fibre as very important, with Asia Pacific closely mirroring global averages for the desire for foods that are high in fibre, and are low in carbohydrates and reduced calories[1]. In fact, the region is projected to lead the growth of dietary fibre demand, making up more than 17% of the global dietary fibre market share by 2020[2].

Boost content and strengthen claims

You can raise fibre content and win in the label-to-label comparisons taking place in grocery aisles. Your products may be able to feature claims like "good source of fibre" or "excellent source of fibre" as well as "gluten-free", stimulating trial and repeat purchases. With some NOVELOSE™ dietary fibres, you can also promote "grain-free" on your labels. And health-conscious consumers will appreciate potential calorie and carbohydrate reduction.

Cost-effective NOVELOSE™ dietary fibres perform in a wide range of applications

	NOVELOSE™ 3490
Excellent performance in key applications	Breads Cakes, muffins and baked goods Extruded cereals and snacks Pastas and noodles
Raw material source	Tapioca
Label declaration	Food starch modified Modified food starch Modified tapioca starch
Typical total dietary fibre (dry basis)	⩾90%
Non-GMO available	Always
Alternative bases available	Potato and wheat

NOVELOSE™

Removing obstacles. Uncovering solutions.

You can answer the HEALTH & NUTRITION trend with confidence when you leverage the research and expertise of Ingredion. We're making fibre more cost-effective and easier to work with so you can boost fibre content or introduce fibre in new places. Collaborate with us at Ingredion Idea Labs™ innovation centres to create fibre advantage in consumer-winning baked goods, snacks, pastas, noodles, breakfast bars, breakfast cereals, instant soups – even mashed potatoes and much more.

Innovate with HEALTH & NUTRITION

To learn more about the benefits of NOVELOSE™ dietary fibres, contact your Ingredion representative or visit us online.
Asia Pacific: +65 6872 6006 | apac.ingredion.com

Ingredion Singapore Pte Ltd
21 Biopolis Road #05-21/27
Nucleos Singapore 138567

Ingredion™

1. Nielsen Global Health & Wellness Survey, 2015
2. Mordor Intelligence, Global Dietary Fiber Market - Growth, Trends and Forecasts (2016 - 2021), August 2016.

technical specification

VERSAFIBE™ 1490 Dietary Fiber 06400400

VERSAFIBE™ 1490 dietary fiber is a modified food starch based on potato. VERSAFIBE 1490 dietary fiber can be used to increase total dietary fiber and reduce caloric content. It is bland in flavor and cannot be detected organoleptically in most applications. It tests as dietary fiber via AOAC method 991.43.

Chemical and Physical Properties

	Min.	Max.
Moisture, %	-	16.0
pH (20% w/w slurry)	5.5	7.5
Total Dietary Fiber, % (d.s.) (AOAC 991.43)	85	-

Physical Appearance

Color	White to Off-White
Form	Fine Powder

Screen Test

	Typical
% thru U.S.S. #200	>95

Microbiological Limits

	Max.
Total Plate Count/g	10,000
Yeast/g	200
Mold/g	200
Coliform/g	100
E. coli	Negative
Salmonella	Negative

Nutritional Data/100 g

	Typical
Calories (US)[1]	47
Total Fat, g	0
Sodium, mg	382
Total Carbohydrate, g	86
Dietary Fiber, g (US)	74.2
Total Sugars, g	0
Other Carbohydrate, g (US)	11.8
Protein, g	0
Vitamin A, IU	0
Vitamin D, mcg	0
Calcium, mg	43
Iron, mg	0
Potassium, mg	2
Ash, g	1.8

[1] based on 4 kcal/g carbohydrates, insoluble fiber non-caloric
Not a significant source of fat, cholesterol, vitamins, or other minerals

Certification

Kosher pareve
Halal
Non-GMO

Packaging and Storage

VERSAFIBE 1490 dietary fiber is packaged in multi-ply kraft paper bags with a net weight of 50 lbs. VERSAFIBE 1490 dietary fiber should be stored in a clean, dry area at ambient temperature and away from heavily aromatic material.

Shelf Life

The best before date for VERSAFIBE 1490 dietary fiber is 24 months from the date of manufacture.

Regulatory Data

Source Potato

United States

Meets 21CFR 170.30 (GRAS) requirements
Labeling Food Starch-Modified

Features and Benefits

VERSAFIBE 1490 dietary fiber is a cost effective, high fiber resistant starch type 4 (RS4). The product can be used to add fiber to a variety of bakery applications including snacks, breads, pasta, and cookies. It is an easy to use fiber with little impact to formulation or process. It contributes minimally to the viscosity of food systems, has low water holding capacity, and improves the texture of crackers, cereals, pasta, and snacks.

VERSAFIBE 1490 dietary fiber is potato based, making it non- allergen and ideal for gluten-free products.

Effective Date: August 24, 2016 Next Review Date: August 24, 2019

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Westchester, Illinois 60154
U.S.A.
708.551.2600

1600 – 90 Burnhamthorpe Rd., West
Mississauga, Ontario L5B 0H9
Canada
905.281.7950

www.ingredion.us

technical specification

AMIOCA TF 04400108

AMIOCA TF starch is food grade and consists primarily of amylopectin -- a naturally occurring branched glucose polymer. It is typically used as a natural thickener and texturizing agent. This product is available under Ingredion Incorporated's TRUETRACE® Identity Preserved Program for non-GM products.

Chemical and Physical Properties

	Min.	Max.
Moisture, %	-	14.0
pH (20% w/w slurry)	4.5	6.7
Viscosity (CML-M105)		
Peak, MVU	850	1200
End, MVU	400	650

Physical Appearance

	Typical
Color	White to Off-White
Form	Fine Powder

Screen Test

	Typical
% thru U.S.S. #100	>95
% thru U.S.S. #200	>85

Microbiological Limits

Initial testing is done on a single composite sample against a limit of m. If result is above m, the three class sampling and acceptance below is used.

	n	c	m	M
Total Plate Count/g	5	3	10,000	100,000
Yeast/g	5	3	200	1,000
Mold/g	5	3	200	1,000
Enterobacteriaceae	5	3	100	1,000

Where n = # of samples tested; c = maximum allowable number of results between m and M; m = upper target limit; M = maximum acceptable value.

E. coli	Negative
Salmonella	Negative

Nutritional Data/100 g

	Typical
Calories	358
Calories from fat	0
Total Fat, g	<0.1*
Cholesterol, mg	0
Sodium, mg	12
Total Carbohydrate, g	89.3
Dietary Fiber, g	0
Total Sugars, g	<0.1*
Added Sugars, g	0
Other Carbohydrate, g	89.3
Protein, g	0.1
Vitamin D, mcg	0
Calcium mg	15
Iron, mg	<0.2*
Potassium, mg	<10*
Ash, g	<0.1*

* Not present at level of quantification.

Certification

Kosher pareve
Halal

Packaging and Storage

AMIOCA TF starch is packaged in multi ply kraft paper bags with a net weight of 50 lbs. AMIOCA TF starch should be stored in a clean, dry area at ambient temperature and away from heavily aromatic material.

Shelf Life

The best before date for AMIOCA starch is 24 months from the date of manufacture.

Regulatory Data

Source	Waxy Maize
CAS No.	9037-22-3

United States

Meets FCC (Food Chemical Codex) requirements.

Labeling	Corn Starch

Canada

CFDA Regulation	B.13.011
Labeling	Corn Starch

Features and Benefits

Most commercially available starches such as corn, potato, wheat, rice and tapioca are composed of two types of glucose polymers: amylose, a linear polymer, and amylopectin, a branched chain. The linear fraction contributes gelling properties to the starch. Since AMIOCA TF starch is essentially composed of amylopectin, it develops viscosity without the gelling characteristics generally associated with natural starches. AMIOCA TF starch develops a clear, cohesive long texture when cooked. On cooling, it remains clear and cohesive while developing a higher viscosity. High acid, shear, or extended cooking times will dramatically reduce the starch's viscosity.
This product is available under Ingredion Incorporated's TRUETRACE® Identity Preserved Program for non-GM products.

Effective Date: April 27, 2017 Next Review Date: April 27, 2020

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Westchester, Illinois 60154
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708.551.2600

1600 – 90 Burnhamthorpe Rd., West
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Canada
905.281.7950

www.ingredion.us

Packaging Amount for the Starches

Starch #: Versafibe 1490

- Amount to WEEK 1 for 10g: **12.8 g**
- Amount to WEEK 2 for 20g: **25.5 g**
- Amount to WEEK 3 for 35g: **44.6 g**
- Amount to WEEK 4 for 50g: **63.6 g**

Starch #: Versafibe 2470

- Amount to WEEK 1 for 10g: **17.2 g**
- Amount to WEEK 2 for 20g: **34.4 g**
- Amount to WEEK 3 for 35g: **60.2 g**
- Amount to WEEK 4 for 50g: **85.9 g**

Starch #: Versafibe 3490

- Amount to WEEK 1 for 10g: **11.8 g**
- Amount to WEEK 2 for 20g: **23.5 g**
- Amount to WEEK 3 for 35g: **41.1 g**
- Amount to WEEK 4 for 50g: **58.7 g**

Starch #: Amioca TF

- Amount to WEEK 1 for 10g: **13.9 g**
- Amount to WEEK 2 for 20g: **27.8 g**
- Amount to WEEK 3 for 35g: **48.6 g**
- Amount to WEEK 4 for 50g: **69.4 g**

Appendix C Instructions on how to incorporate supplements

How to Add the Fiber to your Diet

Do not eat the fiber on its own!

Make sure you eat the fiber with other foods and preferably with your meals.

Spread out the fiber evenly throughout the day.

Add part of the fiber to all your meals in similar amounts. The more spread out it is the better!

Drink plenty of fluids!!!

Make sure to drink fluids not only with the fiber, but also throughout the day.

It is recommended that you drink more than _____ liters per day of fluids.

Key Things to Keep in Mind

Overview of the Fiber:

A fine white powder that **may taste a little starchy** depending on how much is added to foods. It mixes quite well into most foods without really clumping; however, it may add a white or creamy tint to the food; this is normal.

Bottom line … add 1 package of fiber over the course of each day

- Spread the fiber **into at least two to three meals** during the day.
- Other than that how you decide to add it is up to you, and we will check in with you during the study to see how you end up adding the fiber daily.

Works well in semisolids

- **Mix the fiber into foods like:** yogurt, pudding, smoothies, thicker soup, stew, chili, curry.
- It may absorb some of the liquid in the food when you add it. If it does just add a little bit more liquid.
- It does not dissolve or mix well into thin liquids, and will just fall to the bottom on the glass. If you add it this way, just **stir it well right before drinking**.

It is fine to heat or to cook with

- Mix the fiber in well towards the end of the preparation or right before serving.
- When adding the fiber to leftovers, it may taste better to add it before reheating the dish.
- Try and **keep the fiber in its original powdered form**, as in avoid baking with the fiber.

Be Creative!

- We will provide you with ideas that we found work well, but feel free to experiment to find out ways that work best for you. The amount of fiber that you add to any dish is up to you.
- Please let us know what works/doesn't work for you, and don't hesitate to ask for more ideas!

DON'T HESITATE TO ASK US FOR ANYTHING!

Appendix D. Pre-screening questionnaire

Instructions: This pre-screening questionnaire will be completed over the phone to determine if interested individual meet study inclusionary/exclusionary criteria. Subjects that pass this pre-screening questionnaire will be scheduled for an in-person screening clinic visit.

Study Summary

The purpose of this study is to learn how well different types and amounts of resistant starch (a type of dietary fibre) are tolerated and how they affect our gut bacteria. This study will help determine the ideal dose and type of these fibres to use in future studies.

During the study you will be randomly assigned to 1 of 4 different groups. Three of the groups will receive 1 of 3 different types of resistant starch. The 4th group will receive a starch that our bodies can break down as the placebo. You will be asked to add the assigned powdered starch to your normal diet daily for 4 weeks. Each week the amount of resistant starch added to your diet will increase. Over the 4 weeks you will add doses of 10g, 20g, 35g, and 50g of the fibre daily, increasing dosage each week respectively.

You will be asked to attend at least 5 clinic visits over a 5 week period. During the study we will ask for you to provide 5 stool samples (once a week) and complete different diet and lifestyle questionnaires. The study staff will be in regular contact with you during the study.

Participant Information

First Name: _____ Last Name: _____ M.I.: _____

Phone Number: _____ Email: _____

Where did you hear about the study? : _____

Screening Questions

1. What is your current age in years ***(18 – 50 years)***: _____

2. What is your gender: MALE FEMALE

3. What kind of physical activity do you usually do per week?

Describe Physical Activity:

○ < 5 hour per week of vigorous ○ **> 5 hours per week of vigorous (ineligible)**

4. Do you currently smoke cigarettes?
○ **Yes (ineligible)** ○ No

5. Would you consider yourself a vegetarian or vegan?
○ **Yes (ineligible)** ○ No

6. Do you have any food allergies or intolerances *(specifically wheat)*?
○ **Yes (ineligible)** ○ No

7. Are you taking any digestive aids? (*i.e. probiotics, fibre supplement/bars, digestive enzymes, Beano*)
○ **Yes** ○ No
○ If yes, please specify: _____
○ If yes, would be willing to discontinue the supplement during the trial with a 4 week washout period?

8. Are you taking any dietary supplements? (*i.e. multivitamin, vitamin D, fish oil, herbals*)
○ **Yes** ○ No

If yes, please specify supplements:

9. Are you pregnant, planning to become pregnant in the next 6 months, or breast feeding?
○ **Yes (ineligible)** ○ No

10. Are you post-menopause?
○ **Yes (ineligible)** ○ No

11. Have you take any antibiotics within the last 3 months?
○ **Yes (ineligible)** ○ No

12. Do you have any history of GI disorders or surgeries? (*i.e. IBS, IBD, celiac, chronic constipation*)
○ **Yes (ineligible)** ○ No

List your current medical diagnoses :

13. Are you currently taking any medications for your blood pressure, cholesterol, or blood sugars?

○ **Yes (ineligible)** ○ No

14. Are you chronically taking any analgesic (pain killers like aspirin, ibuprofen, Motrin [weekly use >1 month]) or laxative/stool softener medications?

○ **Yes (ineligible)** ○ No

List current medications taking:

<u>Follow Up-Questions/Information:</u>

1. Does the individual meet the inclusion/exclusion criteria?

○ Yes ○ **No**

2. Does the individual wish to continue with the trial?

○ Yes ○ **No**

3. Screening Visit Scheduled for: _____ at: _____hrs.

4. Participant was assigned a **Screening ID #:** _____

Appendix E. Information sheet

Information Sheet

Project Title: Gastrointestinal Assessment of Three Novel Resistant Starch Type 4
Department: Agricultural, Food and Nutritional Science [AFNS]

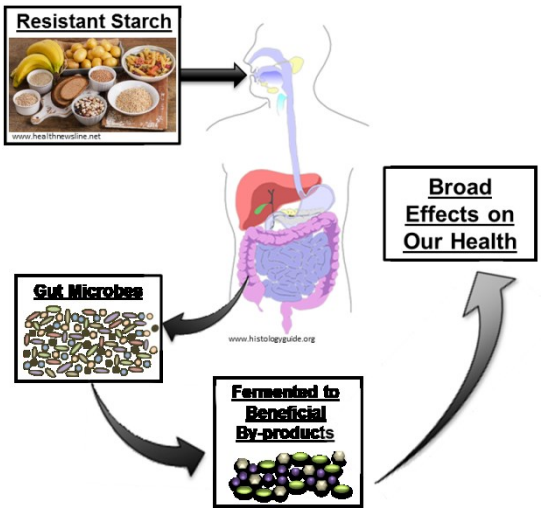
Investigator	Position	Phone Number	E-mail Address
Dr. Jens Walter	Associate Professor	780-492-1182	jwalter1@ualberta.ca
Adele Gagnon	Research Coordinator	780-492-9506	aegagnon@ualberta.ca
Edward Deehan	PhD Candidate	780-221-4356	deehan@ualberta.ca

Background

It is known that a high fibre diet can benefit our health and the health of our gut bacteria. We also know that these gut microbes can help cause and prevent diseases. When fibre is eaten, it gets broken down by our gut microbes. During this fermentation process different by-products are made. These by-products have been shown to promote health. Therefore, it is thought that more fibre will help our gut microbes to produce more by-products, which may improve our overall health **(Fig. 1)**.

Our current refined diet greatly lacks fibre. There is a 'fibre gap' between the amount of fibre we actually eat and the amount we should eat. In order to aid our microbes and improve our heath we need to find ways to reduce this 'fibre gap'. One possibility is to add fibre to our refined diet. One promising type of fibre for this is resistant starch. Resistant starch is a type of starch that our bodies cannot break down. Therefore it reaches our gut where our gut bacteria do break it down. Before we enrich our refined diet with these fibres we must first learn how different fibres perform in our gut. This includes how our gut tolerates increasing amounts of fibre, and how our microbes respond.

Figure 1: The Effect of Fibre Including Resistant Starch



What is the purpose of the study?

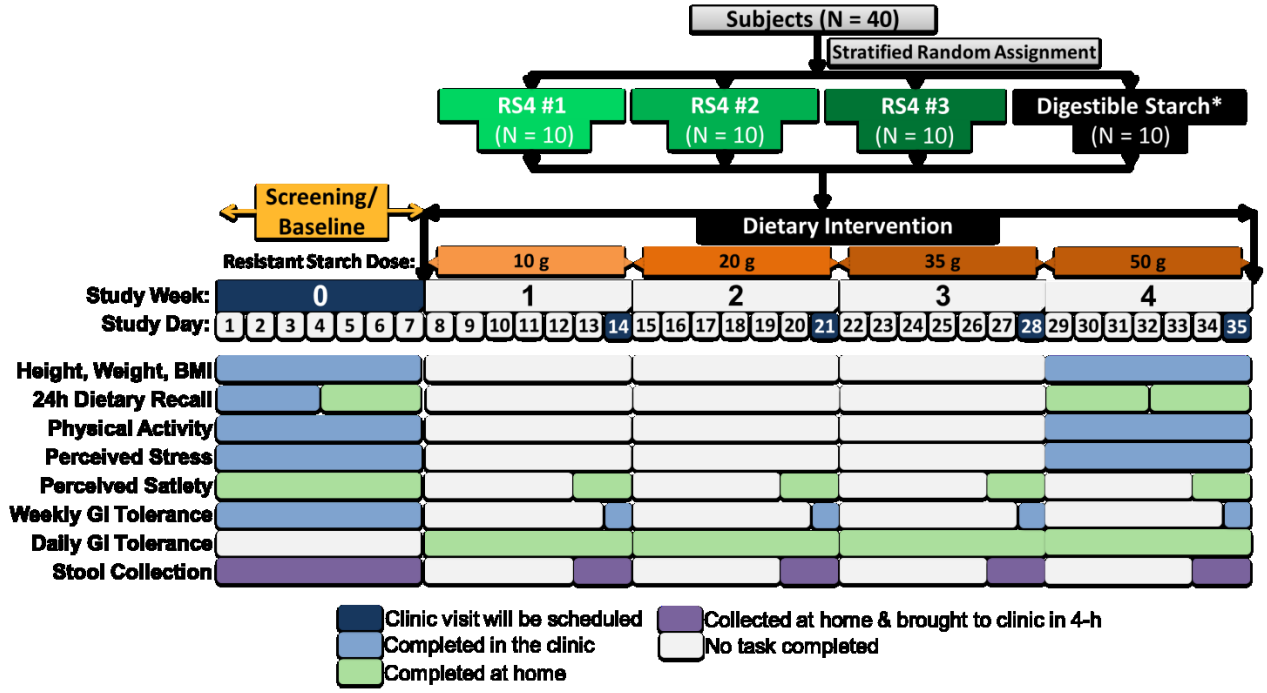
The purpose of this study is to learn how well different types and amounts of resistant starch are tolerated and how they affect our gut bacteria. This study will help determine the ideal dose and type of these fibres to use in future studies.

What do participants do?

Participants will be randomly assigned based on gender to 1 of 4 different groups. Three of the groups will receive 1 of 3 different types of resistant starch. The 4th group will receive a starch that our bodies can break down as the placebo. Participants and researchers will both be blinded and will not know what starch treatment they are assigned to. Participants will be asked to add the assigned fibre to their normal diet daily for 4 weeks. The fibre will be provided pre-packaged in powdered form. Each week the amount of fibre added will increase. Over the 4 weeks participants will add doses of 10g, 20g, 35g, and 50g of the fibre daily, increasing dosage each week respectively. Consume the full amount of the provided starch daily. Do not exceed this daily provided dose. No further lifestyle changes are needed. Maintaining your normal medication regime and physical activity level is required. The study coordinators will teach you how to add the fibre to your diet. They will also provide you with a handout to further help introduce the fibre to your diet.

Participants will be asked to attend at least 5 clinic visits over a 5 week period **(Fig. 2)**. The day and time of your visits will be decided by you and the study staff. Stool samples will be collected at the end of each study week during the trial. This is the only way to study your gut microbes. Different questionnaires will also be completed during study visits and at home. The study staff will be in regular contact with you during the study.

Figure 2: Resistant Starch GI Tolerance Study Design



<u>Study visit 1</u> *(Screening/Baseline)***:** Estimated time: < 2 hours.

Potential participants will be asked to come into the clinical research unit [CRU]. The study will be explained and questions will be answered. You will be asked to sign this consent form if you want to participate. Following this, demographic and lifestyle information, and

height/weight will be collected by study staff. We will ask you questions about your normal diet. You will then complete a 24-h recall using the web-based ASA24 program. The program will ask you to record everything you ate and drank the day before the visit. In addition, you will be given some questionnaires to complete regarding physical activity, stress and gut symptoms.

You will then be randomized to one of the 4 fibre groups. The study staff will educate you on how to add the assigned fibre to your diet. This information will be tailored to your normal diet. We will also provide you with a study journal. This journal will be used to keep track of fibre intake. It will also allow for you to record any symptoms that may occur during the trial.

Before you start adding the fibre to your diet, we will ask you to collect your first stool sample at home. You will have a full 2 day window to collect the sample. We will provide you with a stool collection kit to collect the sample at home. The study staff will provide direction for this simple process. It is important that the stool samples are received as soon as possible to prevent microbes from dying. The sample should be returned to us within 4 hours of collection. Samples can be collected at any time of day. If the sample is dropped off outside of regular hours, arrangements can be made to have a team member meet you.

Within these 2 days we will have you complete one additional 24-hr recall using the ASA24 program. We will also have you complete both a bowel-habits and a hunger-fullness questionnaire over these 2 days. Upon completing and dropping off these items you will receive your first week of fibre packages. The day to start the fibre will be decided upon by you and the study staff.

Study visits 2, 3, & 4 *(study weeks 1, 2, & 3)***:** Estimated time: ~30 minutes.

One week after starting the fibre, you will attend a visit at the CRU, and each week following. The study staff will review your study journal and answer any questions you may have. If there are any issues adding the fibre to your diet, we will help troubleshoot any problems with you. They will also have you complete a gut symptoms questionnaire and comment on how the fibre has been incorporated.

Please bring all (empty and full) packaging to the visit with you. Any fibre that was not consumed will be weighed to measure compliance. If you have not eaten enough of the fibre (<80%) you will be removed from the study. You will be given the fibre packages and stool collection kit for the following week. A stool sample will be collected at this visit, or the day before. We will need to collect the stool sample before continuing on with the next study week's fibre dose.

Study visit 5 *(study week 4)***:** Estimated visit time: ~45 min.

This visit is four weeks after you have started the fibre, you will attend the final visit CRU. The study staff will review your study journal and answer any questions you have. Please bring all packaging to the visit with you. Any fibre that was not consumed will be weighed to measure compliance. Your weight will be measured again. We will also have you complete some questionnaires, similar to the first visit. During this week you will be asked to complete 2 more 24-hr recalls using the ASA24 program. These need to be completed before or during the last visit. You will be sent a reminder to complete these at the start of the 4th study week. Your last stool sample will also be collected at this visit, or the day before. Upon completion of the study you will be provided with the honorarium.

Stool Samples for Future Research

As part of the study, the stool samples collected during the study will be kept for future studies. We would like to use them in future studies that continue from this tolerance study. This would include projects that closely look at how the microbes are able to use the resistant starch. The samples will be stored frozen in UofA Buildings until needed for future studies. These samples may be stored up to 10 years after the project is done, and they will be stored without using your name to protect your privacy. If you prefer that we not keep your stool samples for future studies, please let the study team know. You can also change your mind at any time and ask for these samples to be destroyed. However, if any testing of the samples has been done, the results will still be kept and used.

How do I benefit? You may or may not benefit directly from participating in this study.

Are there any Risks if I participate?

Some side effects may occur when increasing the amount of fibre in your diet. These symptoms include, but are not limited to, stomach pain, bloating, gas, softer stools, and constipation. To reduce this risk we will provide guidance on how to add the fibres. However, we are unsure how individuals will respond to the fibres. Therefore, please contact the study coordinator if, at any point, you experience unusual or unexpected symptoms. If symptoms persist once the intervention is stopped, please visit your doctor. The study coordinator will be in regular contact with you. This purpose of this contact is to monitor changes and help with tolerance.

We would like to inform your family doctor about your study participation and the possible symptoms. We will provide you with a brief information sheet that you can provide your doctor. If desired we can also contact them directly if you provide us with your doctor's name.

Do I have to participate?

No, taking part in this study is your choice. You may stop participating in the study at any time. You can withdraw from the study by contacting a study staff.

Phone (office): 780-492-9506 **Phone (cell):** 780-221-4356

Will I be paid if I Participate?

You will not be paid for participating in the study and there are no costs to you. You will be reimbursed for the cost of parking or transportation, up to a maximum of $10 per visit. To reimburse and thank you for your time, you will receive $100 upon completion of the study.

Will my records be kept Private?

During the study we will be collecting health data about you. We will do everything we can to make sure that this data is kept private. No data relating to this study that includes your name will be released or published by the study team. Sometimes, by law, we may have to release your information with your name, so we cannot guarantee absolute privacy. However, we will make every legal effort to make sure that your health information is kept private.

All 24-h recall data collected using the ASA24 program will be saved on National Cancer Institute`s (NCI) servers in the United States. This information will be completely de-identified, meaning that your name will not be included. The stool samples will be studied to assess the type of microbes in the gut (called gut microbiome research). It is encouraged to share gut bacteria

data among researchers to promote our knowledge of the gut microbiome. Therefore, only the information about what gut bacteria are present in stool samples will be uploaded completely de-identified to the National Center for Biotechnology Information Sequence Read Archive (NCBI SRA). Both the NCI and NCBI SRA are part of the United States' National Institutes of Health which is a government research agency. During research studies it is important that the data we collect is accurate. For this reason your health data, including your name, may be looked at by people from the University of Alberta auditors or Health Research Ethics Board. By signing this consent form you are giving permission for the study staff to collect your health information and use it for research purposes.

After the study is done, we will still need to securely store your health data that was collected as part of the study. We will keep data stored for at least 10 years after the end of the study at the University of Alberta. If you leave the study, we will not collect any new information from you, but we will need to keep the data that we have already collected, unless you specifically request it to be destroyed.

What if I suffer a research-related injury?

If you become ill or injured as a result of being in this study, you will still be able to receive necessary medical treatment. This will occur at no additional cost to you. By signing this consent form you are not releasing the investigators, institution, or sponsors from their legal and professional duties.

Who can I contact for more information?

If you have further question related to this research, please contact Dr. Jens Walter: **780-492-1182**. If you have any concerns about any part of this study, please contact the Health Research Ethics Board office at the University of Alberta **(780-492-9724)**. This office has no connection with the study investigators.

Appendix F. Consent form

Consent Form

Project Title: Gastrointestinal Assessment of Three Novel Resistant Starch Type 4
Department: Agricultural, Food and Nutritional Science (AFNS)

Investigators:

Investigator	Position	Phone Number	E-mail Address
Dr. Jens Walter	Associate Professor	780-492-1182	jwalter1@ualberta.ca
Adele Gagnon	Research Coordinator	780-492-9506	aegagnon@ualberta.ca
Edward Deehan	PhD Student	780-221-4356	deehan@ualberta.ca

Please circle your answers:

Do you understand that you have been asked to be in a research study? YES NO

Have you read and received a copy of the attached information sheet? YES NO

Do you understand the benefits and risks involved in taking part in this research study? YES NO

Have you had an opportunity to ask questions and discuss this study? YES NO

Do you understand that you can stop taking part in this study at any time by notify the researchers of your wishes? YES NO

Has confidentiality been explained to you? YES NO

Do you understand who will be able to see your study information? YES NO

Do you want the investigator(s) to inform your family doctor that you are participating in this research study? YES NO If so, give his/her name:_____

I agree to take part in this study.

Name (please print):_____

Signature:_____ **Date:**_____
(mm/dd/yy)

I believe that the person signing this form understands what is involved in the study and voluntarily agrees to participate.

Signature of Investigator:_____ **Date:**_____
(mm/dd/yy)

Appendix G. GI tolerability questionnaire

RS4 GI Assessment Study
Gastrointestinal Tolerability Questionnaire

Participant ID: _____
Screening ID: RS-_____
Study Week: _____

Complete by marking **one** response for each question that describes your digestive habits over the last week. If you answer yes, please mark the associated follow-up question.

1.) Did you experience any nausea in the past 7 days?
○ Yes ○ No
If yes, how would you rate the amount of nausea?
○ No more than usual
○ Somewhat more than usual
○ Much more than usual

2.) Did you experience any bloating in past 7 days?
○ Yes ○ No
If yes, how would you rate the amount of bloating?
○ No more than usual
○ Somewhat more than usual
○ Much more than usual

3.) Did you experience any gastrointestinal rumblings in the past 7 days?
○ Yes ○ No
If yes, how would you rate the amount of gastrointestinal rumblings?
○ No more than usual
○ Somewhat more than usual
○ Much more than usual

4.) Did you experience any gas/flatulence in the past 7 days?
○ Yes ○ No
If yes, how would you rate the amount of gas/flatulence?
○ No more than usual
○ Somewhat more than usual
○ Much more than usual

5.) Did you experience any abdominal pain in the past 7 days?
○ Yes ○ No
If yes, how would you rate the amount of abdominal pain?
○ No more than usual
○ Somewhat more than usual
○ Much more than usual

6.) Did you experience any diarrhea (watery stools) in the past 7 days?
○ Yes ○ No
If yes, how would you rate the amount of diarrhea (watery stools)?
○ No more than usual
○ Somewhat more than usual
○ Much more than usual

Coding: *no more than usual = 0, somewhat more than usual = 1, much more than usual = 2*
GI Tolerability Score:

Source: *Maki et al. Int J Food Sci Nutr. 2013;64:274-81. (MB Clinical Research and Consulting, LLC)*
Version 1 - May 1, 2017

Appendix H. Bowel habit questionnaire

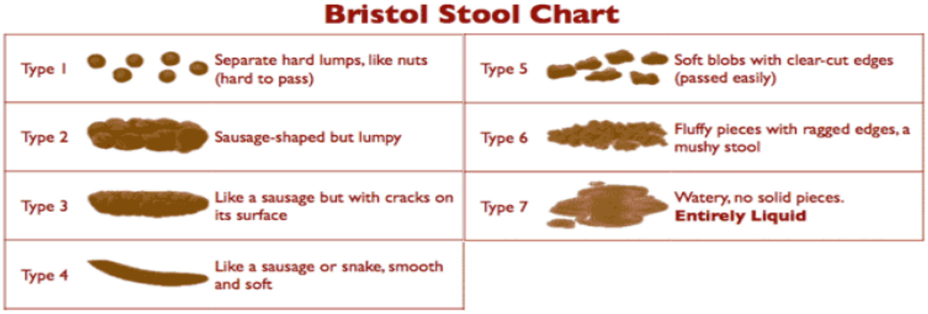
Dates completed: __/__/____ and __/__/____ **Participant ID:** ___________

Please complete this **bowel habit questionnaire** over the next 2 full days by recording each bowel movement you have. If you do not have a bowel movement on a given day, then just note the date and circle 'no'. Please return at your next visit.

Bowel Movement			**Fecal Hardness**	**Straining During Bowel Movement**	**Discomfort During Bowel Movement**	**Sensation of Incomplete Evacuation**	**Bristol Stool Scale**
DATE M/D/Y	**Bowel Movement**	**TIME AM/PM** *(hr:min)*	**1=Soft 2=Normal 3=Hard 4=Very Hard**	**1=None 2=Mild 3=Moderate 4=Severe**	**1=None 2=Mild 3=Moderate 4=Severe**	**1=None 2=Mild 3=Moderate 4=Severe**	**Rate fecal consistency as 1 through 7 per the Bristol**
	YES NO	: AM PM	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4 5 6 7
	YES NO	: AM PM	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4 5 6 7
	YES NO	: AM PM	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4 5 6 7
	YES NO	: AM PM	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4 5 6 7
	YES NO	: AM PM	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4 5 6 7
	YES	: AM	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4 5 6 7
	YES NO	: AM PM	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4 5 6 7

○ Please check if these two days do not represent your normal bowel habits.

Bristol Stool Chart



RS4 GI Study - Screening Visit Handout *Version 2 – June 19, 2017*

Appendix I. Hunger-fullness SLIM questionnaire

We want to know your normal level of hunger/fullness, so we can see how the added the dietary fibre affects your average level of hunger/fullness.

- Please rate how hungry/full you are feeling at the 3 given times by **marking ON THE LINE below with a slash (/)** and then note what time you became hungry and ate after the chosen meal.
- Complete the questionnaire on the **same day** within the **next 2 days**, and at the **3 times noted below**
- Remember to write down the date/times you completed the form.

Please bring back to your next visit completed.

Completed ***Once Awake***

Please complete this questionnaire
0 min - 30 min after you wake up; before you eat.

GREATEST IMAGINABLE FULLNESS

EXTREMELY FULL
VERY FULL

MODERATELY FULL

SLIGHTLY FULL

NEITHER HUNGRY NOR FULL

SLIGHTLY HUNGRY

MODERATELY HUNGRY

VERY HUNGRY
EXTREMELY HUNGRY

GREATEST IMAGINABLE HUNGER

Date Completed: __/__/_____
(mm/dd/yy)

Time Completed: __:__ AM / PM

For Scoring Use

SLIM #: _____

Completed ***2 hours After*** a Meal

Time of Meal: __:__ AM / PM

Please complete **2 hours** ***(± 15 min)*** **after a <u>meal</u>**.

GREATEST IMAGINABLE FULLNESS

EXTREMELY FULL
VERY FULL

MODERATELY FULL

SLIGHTLY FULL

NEITHER HUNGRY NOR FULL

SLIGHTLY HUNGRY

MODERATELY HUNGRY

VERY HUNGRY
EXTREMELY HUNGRY

GREATEST IMAGINABLE HUNGER

Time Completed:
__:__ AM / PM

For Scoring Use

SLIM #: _____

Completed ***4 hours After*** a Meal

Please complete **4 hours** ***(± 15 min)*** **after the <u>same meal</u>.**

GREATEST IMAGINABLE FULLNESS

EXTREMELY FULL
VERY FULL

MODERATELY FULL

SLIGHTLY FULL

NEITHER HUNGRY NOR FULL

SLIGHTLY HUNGRY

MODERATELY HUNGRY

VERY HUNGRY
EXTREMELY HUNGRY

GREATEST IMAGINABLE HUNGER

Time Completed:
__:__ AM / PM

For Scoring Use

SLIM #: _____

- **At what time after this meal did you become hungry?** __:__ AM / PM
- **At what time after this meal did you eat again?** __:__ AM / PM
- **Was this a snack or a meal?** SNACK / MEAL

Appendix J. Perceived stress scale

As part of this study, we would like to know the level of stress participants are feeling. The following questions ask you about your feelings and thoughts during THE LAST MONTH. In each case, please indicate your response by placing an "X" over the circle representing HOW OFTEN you felt or thought a certain way.

1.) In the last month, how often have you felt that you were *unable* to control the important things in your life?

○ 0 - Never
○ 1 - Almost Never
○ 2 - Sometimes
○ 3 - Fairly Often
○ 4 - Very Often

2.) In the last month, how often have you felt confident about your ability to handle your personal problems?

○ 0 - Never
○ 1 - Almost Never
○ 2 - Sometimes
○ 3 - Fairly Often
○ 4 - Very Often

3.) In the last month, how often have you felt that things were going your way?

○ 0 - Never
○ 1 - Almost Never
○ 2 - Sometimes
○ 3 - Fairly Often
○ 4 - Very Often

4.) In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?

○ 0 - Never
○ 1 - Almost Never
○ 2 - Sometimes
○ 3 - Fairly Often
○ 4 - Very Often

For Scoring Use

TPSS: _____

Appendix K. International physical activity questionnaire

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.

1. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling?

   _____ **days per week**

   ☐ No vigorous physical activities → ***Skip to question 3***

2. How much time did you usually spend doing **vigorous** physical activities on one of those days?

   _____ **hours per day**

   _____ **minutes per day**

   ☐ Don't know/Not sure

Think about all the **moderate** activities that you did in the **last 7 days**. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.

3. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

   _____ **days per week**

   ☐ No moderate physical activities → ***Skip to question 5***

4. How much time did you usually spend doing **moderate** physical activities on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

Think about the time you spent **walking** in the **last 7 days**. This includes at work and at home, walking to travel from place to place, and any other walking that you have done solely for recreation, sport, exercise, or leisure.

5. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?

_____ **days per week**

☐ No walking → ***Skip to question 7***

6. How much time did you usually spend **walking** on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

The last question is about the time you spent **sitting** on weekdays during the **last 7 days**. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the **last 7 days**, how much time did you spend **sitting** on a **week day**?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

This is the end of the questionnaire, thank you for participating.

For Scoring Use

Walking MET-minutes/week = _____	Moderate MET-minutes/week = _____
Vigorous MET-minutes/week = _____	Total MET-Minutes/week = _____
Median minutes of sitting/day = _____	

Appendix L. Source document -- Baseline

Date of Visit: _____ **Arrival:** _____ hrs. **SCREENING ID #: RS - _____**
(mm/dd/yy) **PARTICIPANT ID #: GI_____**

❑ **Consent form:** Reviewed with the Candidate and signed by the Candidate (*copy provided*)
❑ **Participant completed personal identification form** (*started other questionnaires*)

DEMOGRAPHIC INFORMATION

DOB (mm/dd/yyyy):	**Age:**	**Gender:** ❑ Male ❑ Female
Marital Status:	❑ Married/Common-Law ❑ Single/Never Married	❑ Separated/Divorced ❑ Widowed

● Age between 18 and 50 years? ❑ Yes *(continue)* ❑ No *(ineligible)*

What is your ethnicity?

❑ Aboriginal (e.g. First Nations, Métis, or Inuit) ❑ Arab (e.g. Egyptian, Kuwaiti, Libyan) ❑ Black (e.g. African, Nigerian, Somali) ❑ Caucasian / White	❑ Chinese ❑ Filipino ❑ Japanese ❑ Korean ❑ Latin American / Hispanic (e.g. Chilean, Costa Rican, Mexican)	❑ South Asian (e.g. Bangladeshi, Punjabi, & Sri Lankan, Pakistani) ❑ Southeastern Asian (e.g. Vietnamese, Cambodian, Malaysian, Laotian) ❑ West Asian (e.g. Afghan, Iranian, Syrian) ❑ Other (Specify: _____)

What is the highest level of formal education you have completed?

❑ Less than High School Diploma	❑ High School Diploma	❑ Some College
❑ Undergraduate or Bachelor's degree	❑ Trade, Technical, or Vocational School	❑ Graduate Degree
❑ Professional Degree	❑ None	❑ Other (Specify: _____)

What is the candidate's employment status?

❑ Employed	❑ Unemployed	❑ Student	❑ Retired
❑ Work from Home or Homemaker	❑ On Disability or Unable to Work	❑ Other (Specify:_____)	

Please describe your occupation:

What was the total income, before taxes and deductions, of <u>all household members</u> from all sources in the past 12 months?

❑ Less than $20,000	❑ $21,000 - $39,000	❑ $40,000 - $69,000
❑ $70,000 - $99,000	❑ $100,000 - $129,000	❑ $130,000 or more

MEDICAL HISTORY

Has the candidate been diagnosed with or treated for any disease/disorders affecting these organ/body systems? *Check all tha*

➲ Diabetes Mellitus	❑ Yes or ❑ No	➲ **<u>Digestive / GI</u>**	❑ Yes or ❑ No
➲ Cardiovascular	❑ Yes or ❑ No	➲ Kidney / Renal / Liver	❑ Yes or ❑ No
➲ Immune / Lymphatic	❑ Yes or ❑ No	➲ Thyroid / Endocrine	❑ Yes or ❑ No
➲ Lungs / Respiratory	❑ Yes or ❑ No	➲ Nervous System	❑ Yes or ❑ No
➲ Musculoskeletal	❑ Yes or ❑ No	➲ Food Allergies	❑ Yes or ❑ No
➲ Psychiatric / Neurologic	❑ Yes or ❑ No	➲ Other:_____	❑ Yes or ❑ No

↯ *Please provide details, type, date of occurrence, interventions if any.*

Comments:

❑ **Surgeries or Surgical Interventions?** ❑ Yes ⇨ *(Please comment)* ❑ No ⇨ *(Proceed to medications)*

↯ *Please comment on date, type etc.*

Comments:

- Has medical history of <u>IBD</u> or <u>other GI disorder</u>. ❑ Yes *(ineligible)* ❑ No *(continue)*
- Has history of <u>GI surgical interventions</u>. ❑ Yes *(ineligible)* ❑ No *(continue)*

MEDICATIONS AND DIETARY SUPPLEMENTS

- Taken antibiotics in the last 3 months. ❑ Yes *(ineligible)* ❑ No *(continue)*
- Takes **digestive aids** like Beno, fibre, prebiotics, or probiotics. ❑ Yes ↯ ❑ No *(continue)*
  - If candidate is taking digestive aids, <u>are they willing to discontinue</u> use during the trial, with a one month washout period? ❑ Yes *(continue)* ❑ No *(ineligible)*
- On lipid-lowering, anti-hypertensive, anti-diabetic, analgesics (chronic NSAID - aspirin, ibuprofen [weekly use >1 month]), or laxative/stool softener medications? ❑ Yes *(ineligible)* ❑ No *(continue)*

Currently taking any <u>medication</u>, herbal or dietary supplements, vitamins or minerals? (*Prescribed or over the counter*)

❑ Yes ↯ (*Comment below*) ❑ No ⇨ *(Proceed to next section)*

Please indicate medication name, dose, route, and indication:

ANTHROPOMETRIC MEASURMENTS

Height (cm) 1st: 2nd: **Average Height (cm):**	**Weight (kg) 1st: 2nd:** **Average Weight (kg):**	**Average BMI (kg/m²):**

- Pregnant or lactating female, pregnancy planned? ❑ Yes *(ineligible)* ❑ No *(continue)* ❑ N/A *(male)*

PHYSICAL ACTIVITY, SMOKING, & ALCOHOL USE

Please Comment on Level of Activity:

- Candidate does >5 hours/week of vigorous physical activity. ❑ Yes *(ineligible)* ❑ No *(continue)*

(*Vigorous physical activities refer to activities that take hard physical effort and make you breathe much harder than normal, like heavy lifting, dancing, aerobics, or fast bicycling.*)

- Candidate is willing to maintain a stable physical activity level during the study. ❑ No *(ineligible)* ❑ Yes *(continue)*

Smoking Type: ❑ Never Smoked ➪ *(Alcohol)* ❑ Current Smoker ⇨ *(Ineligible)* ❑ Ex-Smoker ⇨*(Complete →→→)*	Duration: ___|___ Years ❑ N/A Amount: ____|____ Cigs/Day Quit: ___|___|___|___ Year
Alcohol Usage: ❑ Yes ⇨ *(Complete →→→)* ❑ No ➪ *(Dietary)*	**Frequency of Alcohol Use:** ❑ N/A ❑ Less than once a month ❑ 1 to 3 times a month ❑ 1 to 3 times a week ❑ 4 to 7 times a week ❑ 8+ times a week (*Ineligible)*

DIETARY INFORMATION

- Candidate is a vegetarian or a vegan. ❑ Yes *(ineligible)* ❑ No *(continue)*
- Candidate has known food allergies or intolerances to **corn, potato, tapioca** ❑ Yes *(ineligible)* ❑ No *(continue)*
- Currently participating in or has participated in a food intervention study within the last 1 month. ❑ Yes *(ineligible)* ❑ No *(continue)*

CANDIDATE ELIGIBILITY

❑ **ELIGIBLE** - Randomize ➪ ❑ **NOT ELIGIBLE:** Reason: _____

Comments:

If eligible, explain questionnaires and have the participant complete them prior to leaving screening visit.

- ❑ Physical activity questionnaire
- ❑ Perceived stress questionnaire
- ❑ Gastrointestinal tolerability questionnaire

24 hour recall process: *(if eligible)*

- ❑ Candidate was explained the process of the 24-h recalls, and how the ASA-24 will be used.
- ❑ Candidate was explained when *(within the next 2 days)* the 24-hour recalls will occur.

- ❑ **1st screening visit ASA24 hour recall completed** *(During visit*)
- ❑ **2st screening visit ASA24 hour recall completed** *(Within next 2 days)*

  Participant ASA24 **User Name**: _____

  Participant ASA24 **Password**: _____

- ❑ Participant will receive a reminder email to complete 2nd recall.

ADDITIONAL INSTRUCTIONS

❑ Participant was provided with the ***Hunger-Fullness Questionnaire***, and instructed how to complete on 1 of the next 2 days and informed to return at the next visit.

❑ Participant was provided with the ***Bowel Habits Questionnaire***, and instructed how to complete over the next 2 days and informed to return at the next visit.

❑ Participant was provided with stool collection kit (*pre-labeled*).

❑ Participant was instructed on how to collect stool samples *(within the next 3 days)* at home and to return the sample within 4 hours of collection.

❑ If eligible, assigned Participant ID # :_____

and assigned treatment fibre:_____

(assigned and provided once WEEK 0 sample is collected)

❑ Participant reimbursed for transportation and signed signature sheet.

Additional Comments:

Appendix M. Study journal – Only week 1 is shown as an example

Gastrointestinal Tolerance Study

Study Journal

Please use this journal to keep track of items to complete during each study week.
Bring this to each of the study visits.

Participant ID: _____ **Fiber Treatment #: ____**

Date Range of Study: ___/___/___ (mm/dd/yy) **To** ___/___/___ (mm/dd/yy)

If at any time you have questions or issues please feel free to contact the study coordinators:

Adele Gagnon
Telephone Number: 780-492-9506

Edward Deehan
Cellphone Number: 780-221-4356

Email Address: guthealth@ualberta.ca

When dropping off a stool sample, especially after clinic hours, please call/text: 780-221-4356

In case of a medical emergency please call 911!

INSTRUCTIONS

Instructions for Collecting Stool Samples

The stool collection kit contains:

1 commode specimen collection container with a pre-labeled lid

1 double-lock zipper plastic bag

Stool Collection Process:

1) Raise the toilet ring and place the frame and plastic specimen container on the toilet seat towards the rear of the bowl, then lower the toilet ring to hold it in place.
2) Have your bowel movement into the specimen container, with the stool sample size being around the size of a half of a banana or more. **Be careful not to get water or urine in the container.**
3) Once collected, remove the specimen container from the frame and firmly seal it with the pre-labeled lid. Discard the frame, as you get a new one each time.
4) Please fill out the label on the lid or bag with the **time of collection**.
5) Place sealed container into the plastic bag, remove the excess air, and then seal the double-lock zipper.
6) Once the sample is collected, please return the sample as soon as possible and **within 4 hours** to the clinic.
   - The clinical research unit hours: **8:00am to 4:00pm**.
   - Please give your sample to the clinic receptionist or the study coordinator. If the door is locked, please ring the clinic doorbell to provide the sample.
   - **Please call or text the study cell when you are ready to drop off the sample**, especially if it outside of this time frame, so the study coordinator can be ready to meet you at the clinic. **Cell:** 780-221-4356

INSTRUCTIONS

Bristol Stool Chart - Needed for Bowel Habit Questionnaires

Bristol Stool Chart

Type 1	Separate hard lumps, like nuts (hard to pass)	Type 5	Soft blobs with clear-cut edges (passed easily)
Type 2	Sausage-shaped but lumpy	Type 6	Fluffy pieces with ragged edges, a mushy stool
Type 3	Like a sausage but with cracks on its surface	Type 7	Watery, no solid pieces. **Entirely Liquid**
Type 4	Like a sausage or snake, smooth and soft		

The Bristol stool chart is a scale relating to stool consistency, please use this chart to rate your stool consistency 1-7 (solid – liquid) in your daily diary. For example, a rating of -4- used in the diary example would relate to "like a sausage or snake, smooth and soft."

Foods to limit during the study since they are linked to GI distress.

- Cabbage
- Artichoke
- Onions
- Beans
- Lentils
- Wheat Bran
- Prunes
- Plum Juice

Schedule Overview

The "Study Week" column lists items that are to be done over the week. While the other columns list things that are to be done on specific days. Use this calendar to help keep track of everything that needs to be done over the next 4 weeks.

Study Week	Day 1 ____	Day 2 ____	Day 3 ____	Day 4 ____	Day 5 ____	Day 6 ____	Day 7 ____
WEEK 1 • **Clinic Meeting** • **Provide Stool Sample** • **Bowel Habits/Hunger Questionnaires** • Fiber dose: 10 g/day	**Start the Fiber** **Date:** ____/___/____				-Bowel Habits Questionnaire -Hunger/Fullness Questionnaire	-Bowel Habits Questionnaire -Hunger/Fullness Questionnaire -Provide WEEK 1 Stool Sample*	**-Clinic Meeting** -Provide WEEK 1 Stool Sample*
WEEK 2 • **Clinic Meeting** • **Provide Stool Sample** • **Bowel Habits/Hunger Questionnaires** • Fiber dose: 20 g/day					-Bowel Habits Questionnaire -Hunger/Fullness Questionnaire	-Bowel Habits Questionnaire -Hunger/Fullness Questionnaire -Provide WEEK 1 Stool Sample*	**-Clinic Meeting** -Provide WEEK 2 Stool Sample*
WEEK 3 • **Clinic Meeting** • **Provide Stool Sample** • **Bowel Habits/Hunger Questionnaires** • Fiber dose: 35 g/day					-Bowel Habits Questionnaire -Hunger/Fullness Questionnaire	-Bowel Habits Questionnaire -Hunger/Fullness Questionnaire -Provide WEEK 1 Stool Sample*	**-Clinic Meeting** -Provide WEEK 3 Stool Sample*
WEEK 4 • **Last Clinic Meeting** • **Provide Stool Sample** • **Bowel Habits/Hunger Questionnaires** • Fiber dose: 50 g/day					-Bowel Habits Questionnaire -Hunger/Fullness Questionnaire	-Bowel Habits Questionnaire -Hunger/Fullness Questionnaire -Provide WEEK 1 Stool Sample*	**-Last Clinic Meeting** -Provide WEEK 4 Stool Sample*
	Complete two 24-hr recalls using the online ASA-24 program						

* Provide each WEEK stool samples on the 6th day (+ 1 day in needed) of each study week.

WEEK 1 – WHAT TO DO?

Participant ID:_____

In the table below please check the amount you consumed each day, and any symptoms or comments. Please record ***anything*** that changes from adding the fiber, such as side effects or any other observations. If you are unable to consume all or part of a package, please bring the remaining fiber package, and any empty packages from other days. to your next appointment.

Date	Dosage Taken	Symptoms and Comments
Day 1 ____	○ None of it ○ 25% ○ Half ○ 75% ○ All of it	
Day 2 ____	○ None of it ○ 25% ○ Half ○ 75% ○ All of it	
Day 3 ____	○ None of it ○ 25% ○ Half ○ 75% ○ All of it	
Day 4 ____	○ None of it ○ 25% ○ Half ○ 75% ○ All of it	
Day 5 ____	○ None of it ○ 25% ○ Half ○ 75% ○ All of it	
Day 6 ____	○ None of it ○ 25% ○ Half ○ 75% ○ All of it	
Day 7 ____	○ None of it ○ 25% ○ Half ○ 75% ○ All of it	

Week Overview & Appointments

Day 1 ____	Day 2 ____	Day 3 ____	Day 4 ____	Day 5 ____	Day 6 ____	Day 7 ____
Start Day				-Bowel Habits -Hunger/ Fullness	-Bowel Habits -Hunger/Fullness -Provide WEEK 1 Stool Sample*	**-Clinic Meeting Time:** ___:___ AM / PM -Provide WEEK 1 Stool Sample*

WEEK 1 Checklist

○ Eat 1 package of fiber/day. At the end of each day, check off the amount that you ate and note any symptoms/changes noticed.

○ Keep all packaging and uneaten fibers, and bring them back at your next clinic visit *(on the 7th day).*

○ Describe each bowel movement you have during days 5 & 6 by using the bowel habits questionnaire *(on the next page)*.

○ Complete hunger/fullness questionnaire on either day 5 or day 6.

○ Drop off stool sample, as soon as possible, **within 4 hours** of collection. Collect the 6th day of the study week, +1 day if needed.

○ Meet with study coordinator at the clinic on the 7th day.

WEEK 1 – BOWEL HABITS QUESTIONNAIRE

Participant ID:___________

We want to know how participants are tolerating the fiber. This would include any changes in your digestive system or related symptoms. Please record and **describe each bowel movement you have during days 5 & 6** of WEEK 1 by using this bowel habits questionnaire. If you do not have a bowel movement on any of these given days, then just note the date and circle 'no'. If more space is need, please record on another piece of paper.

Bowel Movement			**Fecal Hardness**	**Straining During Bowel Movement**	**Discomfort During Bowel Movement**	**Sensation of Incomplete Evacuation**	**Bristol Stool Scale**
DATE M/D/Y	**Bowel Movement**	**TIME AM/PM** *(hr:min)*	**1=Soft 2=Normal 3=Hard 4=Very Hard**	**1=None 2=Mild 3=Moderate 4=Severe**	**1=None 2=Mild 3=Moderate 4=Severe**	**1=None 2=Mild 3=Moderate 4=Severe**	**Rate fecal consistency as 1 through 7 per the Bristol Stool Chart**
	YES NO	: AM / PM	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4 5 6 7
	YES NO	: AM / PM	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4 5 6 7
	YES NO	: AM / PM	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4 5 6 7
	YES NO	: AM / PM	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4 5 6 7
	YES NO	: AM / PM	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4 5 6 7
	YES NO	: AM / PM	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4 5 6 7
	YES NO	: AM / PM	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4 5 6 7
	YES NO	: AM / PM	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4 5 6 7
	YES NO	: AM / PM	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4 5 6 7

WEEK 1 – HUNGER/FULLNESS QUESTIONNAIRE

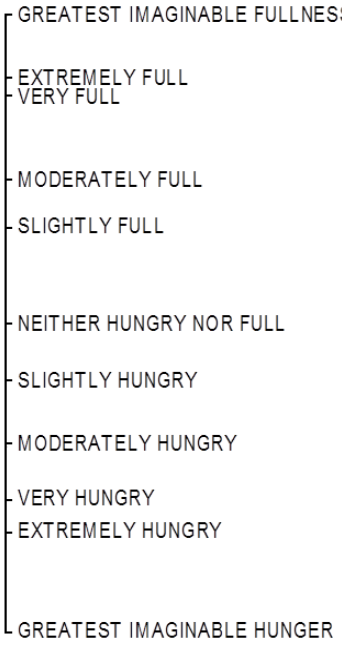
Participant ID: _____

As part of this study, we want to know how the fiber is affecting participants' level of hunger/fullness.

- Please rate how hungry/full you are feeling at the 3 given time by **marking ON THE LINE below with a slash (/)**, and then note what time you became hungry and ate after the chosen meal.
- Complete the questionnaire on the **same study day *(either day 5 or day 6)*** and at the **3 times noted below**
- Remember to write down the date/times you completed the form.

Completed ***Once Awake***

Please complete this questionaire
0 min - 30 min after you wake up, but before you eat.



Date Completed: __/__/____
(mm/dd/yy)

Time Completed: __:__ AM / PM

For Scoring Use
SLIM #: _____

WEEK 1 – HUNGER/FULLNESS QUESTIONNAIRE

Participant ID: __e_____

Completed *2 hrs After* a *Meal with Fiber*

Time of Meal: __:__ AM / PM
Amount of Fiber Eaten at Meal: ___ of bag

Please complete **2 hours** ***(± 15 min)*** **afte a meal with the studyfib r**.

- GREATEST IMAGINABLE FULLNESS
- EXTREMELY FULL
- VERY FULL
- MODERATELY FULL
- SLIGHTLY FULL
- NEITHER HUNGRY NOR FULL
- SLIGHTLY HUNGRY
- MODERATELY HUNGRY
- VERY HUNGRY
- EXTREMELY HUNGRY
- GREATEST IMAGINABLE HUNGER

Time Completed:
__:__ AM / PM

Completed *4 hrs After* a *Meal with Fiber*

Please complete **4 hours** ***(± 15 min)*** **afte the same meal with the study fiber**.

- GREATEST IMAGINABLE FULLNESS
- EXTREMELY FULL
- VERY FULL
- MODERATELY FULL
- SLIGHTLY FULL
- NEITHER HUNGRY NOR FULL
- SLIGHTLY HUNGRY
- MODERATELY HUNGRY
- VERY HUNGRY
- EXTREMELY HUNGRY
- GREATEST IMAGINABLE HUNGER

Time Completed:
__:__ AM / PM

At what tim after this meal with the fiber did you become hungry? __:__eAM / PM

At what tim after this meal with the fiber did you eat again? __:__ AM / PM

Was this a snack or a meal? SNACK / MEAL

Did you add more fiber at this meal? YES / NO

For Scoring Use
SLIM #: _____

For Scoring Use
SLIM #: _____

Appendix N. Source documents – Only week 1 is shown as an example

Date of Visit: _____ **Arrival:** _____ hrs. **PARTICIPANT ID #: GI_____**
(mm/dd/yy)

TO COMPLETE

- ❑ **Review and collect WEEK 1 of the Study Journal**
- ❑ Participant to complete **WEEK 1** gastrointestinal tolerability questionnaire
- ❑ Collect all empty/unused packages of fibre from **WEEK 1**, assess compliance/tolerance
  - ❑ Address any concerns or difficulties noted around adding the fibre to the diet

Comment on Compliance/Tolerance:

Weight of Uneaten Fibre:
WEEK 1 (Compliant if weight < ____ g) Weight:

○ < 50% compliant
○ < 75% compliant
○ ≥ 75% compliant
○ 100% compliant

Comment on how fibre is added *(food items & frequency)**:***

Number of portions/day: TWO / THREE / OTHER :

- ❑ **Confirm collection of WEEK 1 stool sample**

If not collected then remind that it is to be collected tomorrow

- Date: _____ Time Collected: _____ Time Froze: _____ AM / PM
(mm/dd/yy)
  - Note the individual's onset date of their **last menstrual cycle**: *Date:* _____ ❑ N/A

TO PROVIDE

- ❑ Participant was reinstructed on completing the **Study Journal**.
- ❑ Participant was provided with **WEEK 2** of the assigned treatment fibre.
  Assigned Treatment Fibre: _____
- ❑ Participant has been provided with another stool collection kit for **WEEK 2**.
- ❑ Participant has been reinstructed on how to collect stool samples and to return within 4 hours.
- ❑ Participant reimbursed for transportation and signed signature sheet.

WEEK 2 Visit Scheduled for: _____at: _____hrs.

Additional Comments: