PHYSICOCHEMICAL PROPERTIES OF ARABINOXYLANS AND THEIR EFFECT ON APPETITE

By

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ABSTRACT

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Dietary fibers with high viscosities may promote greater satiety and weight loss than fibers with low viscosities suggesting that the physicochemical properties of a fiber contribute to its satiety promoting effects. We measured physicochemical properties of arabinoxylans both separate and incorporated in a flaked cereal, and related these properties to appetite in two clinical trials.

The impact of a steam pressure-cooking process on physicochemical properties of ready-to-eat (RTE) cereal with 15% added fiber as an intact arabinoxylan from flax (FLAX) or an enzyme hydrolyzed arabinoxylan from wheat (AXOS) versus a low fiber control RTE cereal was evaluated. Peak molecular weights of intact and hydrolyzed fibers were ~2.9×10^6 and ~800 g/mol, respectively, with a ~400-fold higher viscosity for intact fiber. As a result of the steam pressure-cooking process used, the molecular weight of intact (FLAX) fiber was reduced to approximately the molecular weight of hydrolyzed (AXOS) fiber. Consistent with molecular weight reduction, there was only a 2-fold difference in viscosity between the 2 high-fiber RTE cereals. The low fiber (4%) control RTE cereal, due to greater starch content, had higher viscosity than either of the 2 high fiber RTE cereals.

Effects of these two high-fiber RTE breakfast cereals as compared with a lower fiber RTE cereal on perceived appetite, hormone responses, and lunch meal energy intake in overweight women (BMI 25.0-29.9 kg/m^2) were evaluated. Two randomized, double-

blind, crossover design trials (n=30, n=35 subjects, respectively) were completed. All 3 breakfast meals (100 g RTE cereal) were standardized for a total mass of 500 g with milk and water. Perceived appetite was assessed before and after breakfast, at specific times throughout the testing day. No differences in perceived appetite were observed among breakfast meals in either trial. At 240 min, the subjects were provided with an ad libitum macaroni and cheese lunch. Subsequent lunch energy intake was not different after any of the breakfast meals in either trial. In addition to the previous procedures, blood samples were collected up to 4 h after breakfasts in trial 2 for assessments of plasma glucose, insulin, active ghrelin, active GLP-1, and total PYY concentrations. Both high-fiber breakfast meals increased post-breakfast GLP-1 and PYY concentrations compared with the low-fiber RTE cereals possibly due to fermentation and production of short chain fatty acids.

Cereal processing reduced the molecular weight of intact fiber and viscosity of the RTE cereal from a predicted >160,000 to ~5,000 mPa·s. This viscosity was lower than in the control RTE cereal (~9,000 mPa·s), and significantly less than viscosity demonstrated in beverages to affect satiety. Fifteen grams of soluble low molecular weight fiber added to RTE cereal did not increase viscosity enough to affect perceived appetite or energy intake at lunch despite differences in satiety hormone signaling in overweight females. Subjects were not sensitive enough to the different satiety hormone signaling cues to affect their perceived appetite or lunch energy intake.

This dissertation is dedicated to my family for their support and understanding, and especially to my wife Linda for her love, patience, and encouragement throughout this journey.

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KEY TO ABBREVIATIONS

AACC, American Association of Cereal Chemists

AOAC, Association of Analytical Chemists

AXOS, Wheat bran arabinoxylan fiber

DP, degree of polymerization

FLAX, intact flax fiber extract from flax seed

GLP-1, glucagon-like peptide 1

HPLC, high pressure liquid chromatography

LDL, low density lipoprotein

niAUC, net incremental area under the curve

PYY, peptide YY

RTE, ready to eat

RVA, Rapid Visco Analyzer

SCFA, short chain fatty acids

SEC-MALS, size exclusion chromatography with multiple angle light scattering

VAS, visual analogue scale

INTRODUCTION

Obesity is increasing in the US and globally, and has become a major health concern in most developed countries. About a third of the adult population in the US is considered obese at a BMI of over 30, and about one third are considered overweight (BMI 25-29.9 kg/m^2) (Office of the Surgeon General, 2010). Dietary fiber can be used as an effective means of reducing the caloric density of foods, and thereby assist in control of energy intake. The benefits of dietary fiber however, are greater than simple calorie dilution. Dietary fibers with increased viscosities may promote greater satiety and weight loss than fibers with lower viscosities. This suggests that the physicochemical properties of fiber, in addition to caloric density lowering properties, contribute to the satiety promoting effects of fiber. New novel fibers are being created by hydrolysis or by other chemical modifications of existing fibers to facilitate their addition to food, as well as minimize their effects on the texture and flavor characteristics of the finished product. It is not certain whether these new fibers will still have a beneficial effect on satiety beyond calorie dilution.

In response to the on-going obesity epidemic, the food industry is increasing the level of fiber in its ready to eat (RTE) breakfast cereals and snack food products in order to help address the obesity problem and the fiber deficit in the diet. Wheat, corn, and rice are the major grains used in the US, and wheat represents a significant fiber source (Slavin, Martini, Jacobs Jr., & Marquart, 1999). Whole kernel wheat is only about 12% fiber thus limiting its ability to significantly increase fiber levels (Hoseney, 1994). Wheat bran fiber is composed of the hemicellulose arabinoxylan as well as cellulose and lignins and is

much higher in fiber than the whole-wheat kernel. However, bran is mostly insoluble fiber, limiting its ability to be added to food without adversely changing product attributes (Oriz & Lafond, 2012). Processes to increase the solubility of bran such as hydrolysis would enable production of a higher fiber food, however there are concerns regarding functional properties and nutritional benefits of such a food.

Over the past 20 years, there have been many studies examining the role of increased dietary fiber intake on satiety and weight management. A link between fiber and satiety was proposed as early as 1987 (Blundell & Burley, 1987). Howarth et al. (2001) reviewed 20 published studies on the acute effects of dietary fiber on satiety, and in a majority of the studies, a positive correlation between increased fiber consumption and increased satiety was reported. Pereira & Ludwig (2001) reviewed epidemiological studies from 1984-2000 and found 16 out of 27 studies supported a beneficial effect of fiber consumption on satiety. Dosages of fiber used have varied considerably, with more consistent satiety effects above 10 grams at a single eating occasion (Wanders, et al., 2011). However, mean daily fiber intake across the day for adult women in the U.S. is only slightly above this threshold (about 14 g/d) (What we eat in America NHANES 2007-2008., 2010) and only about 50% of the recommended 25 g/day (Institute of Medicine of the National Academies, 2005). More fiber is needed for satiation and to meet the recommended daily intake.

It is difficult to incorporate different fiber sources into existing breakfast cereal food products without changing desirable food texture and flavor characteristics. Fiber may be added either as part of an ingredient, or as an isolated fiber (Oriz & Lafond, 2012). When fiber is added as part of an ingredient such as wheat bran, other components in

the ingredient may contribute color, flavor, and texture, as well as additional calories to the finished food. In the case of wheat bran, these components are largely starch and protein from the endosperm included in the bran mill fraction. An isolated fiber contributes significantly fewer calories to the cereal, and less change to food properties.

The goal of this research was to obtain hydrolyzed arabinoxylan fiber from wheat with a low molecular weight distribution and compare it to an intact arabinoxylan having a longer chain length and thus different physical properties. The specific properties of these two fibers were characterized before incorporating them in flaked RTE cereal, and the physical characteristics of the resulting cereal products were determined. The effect of these RTE cereals on perceived appetite in overweight females was also measured. This research was intended to improve our understanding of how changes in physical properties of a specific fiber may affect perceived appetite.

CHAPTER 1 - Literature Review

Cereal Fibers

Fiber is not a group of structurally similar compounds (Figure 1). The only common factor is that they are all non digestible by humans. We can digest α 1→4 and 1→6 linked hexose and pentose rings, however α1→2, 1→3, and 1→5 as well as β linkage forms are non-digestible by human enzymes (Fennema, 1996). Fibers are produced by plants to serve different functions such as fructans like inulin that are an alternative storage form of energy for some plants, and cellulose which forms long microfibrils embedded within plant cell walls to reinforce its structure (Lee, Marcus, & Knox, 2011).

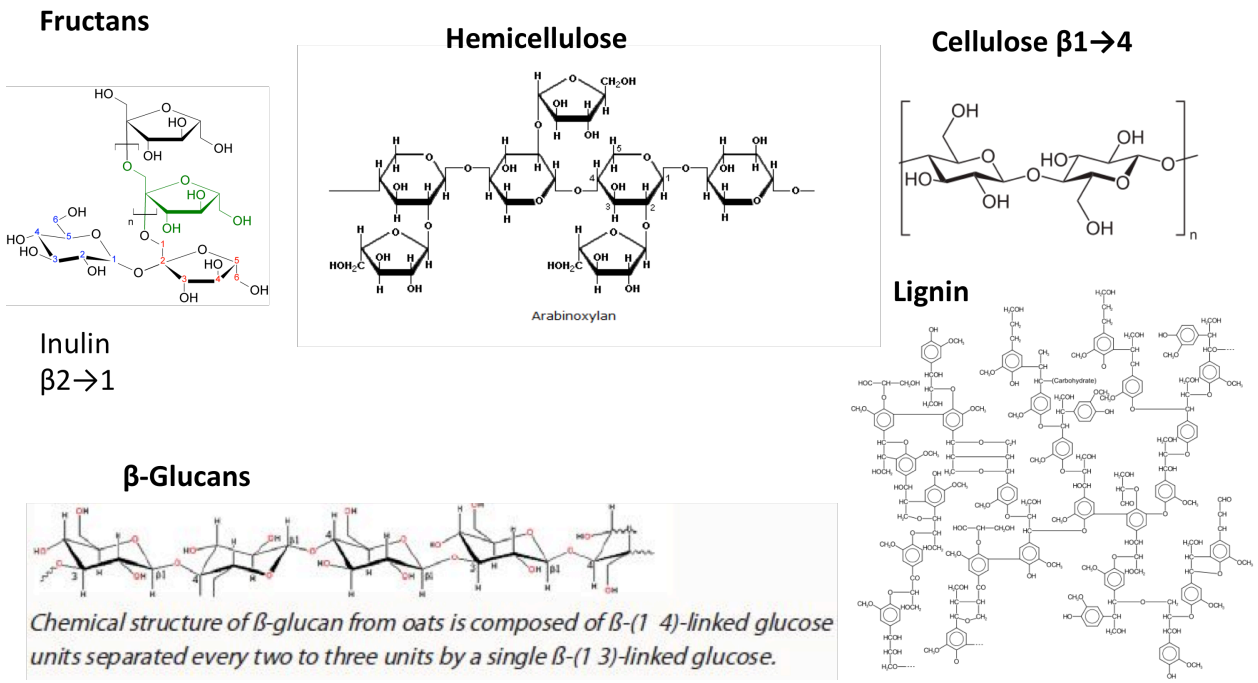


Figure 1. **Fiber is not a group of structurally similar compounds.** *Each of these types of carbohydrates is fiber based on the current definition in the US. Their structures and hence their physical properties are very different from each other* (Oriz & Lafond, 2012).

The major grains consumed in the world are wheat (~33%) and rice (~25%) (Slavin, Martini, Jacobs Jr., & Marquart, 1999). In the US, consumption is mainly from wheat, corn, and oats. These grains have very different amounts of fiber depending on the specific grain. Table 1 illustrates the fiber content in each of these grains as well as the relative proportion of components. Fiber content varies based on growing region, environmental conditions, and specific grain variety but typically wheat and oats have the highest levels of fiber from whole grain although the fiber chemical structure is different. Wheat is high in arabinoxylans whereas oats are high in β-glucans.

Table 1. Compositional differences of the major grains

Component (%)	Wheat	Rice	Corn	Oats[1]
Endosperm	83.0	90.0	82.0	67.0
Germ	3.0	2.5	11.0	-
Bran	14.0	7.5	7.0	33.0
Total Fiber	12.2	2.8	7.3	10.6

[1]There is no commercial mill fraction for oat germ. The germ is not present as a discrete layer that can be removed thru milling. (Hoseney, 1994; USDA, 2010)

Cereal Fibers are Located in the Cell Walls of the Caryopsis

Plants have two types of cell walls that differ in both chemical composition and botanical function (Keegstra, 2010). Primary cell walls surround growing and dividing plant cells. In addition to providing strength to cells, they must also be flexible and allow the cell to grow and divide. Secondary walls are much thicker and more rigid. They are deposited once the cell has stopped growing and account for most of the fiber in plants.

Cell wall polysaccharides account for about 10% of the dry weight of mature wheat (*Triticum aestivum*) grain, and about 2% to 3% dry weight of the white flour fraction (Oriz & Lafond, 2012). Primary and secondary cell walls of wheat are composed of hemicellulose (mainly arabinoxylan), β-glucan, cellulose, and lignin, in different proportions (Khan & Shewry, 2009). Most of the analytical research in this area is focused on mill fractions of wheat as opposed to cell wall type so the amount of data represented in Table 2 is limited.

Table 2. Distribution (% dry weight) of fiber types within various wheat cell wall components

Component[1]	Arabinoxylan	β-Glucan	Cellulose	Lignin
Endosperm	70	20	2	ND[2]
Aleurone	65	29	2	ND
Bran	64	6	29	8.3

[1]Numbers in this table were compiled from multiple studies and totals do not add up to 100%. [2]None detected, (Khan & Shewry, 2009)

Table 2 shows the principle fibers of wheat cell walls. Cell walls also contain smaller amounts of glycoproteins, and phenolic esters (ferulic and coumaric acids). Cellulose fibrils are embedded within a network of hemicellulose and lignin (Figure 2). Cellulose is composed of only 1, 4-linked β-D-glucose units in long linear polymers (Fennema, 1996). Lignin is composed of highly cross-linked phenolic molecules, and is also a component of secondary walls. Lignin serves to form links between cellulose fibrils and hemicellulose polymers to form cell wall structure.

Hemicelluloses, a Major Fiber in Wheat

Hemicelluloses are branched polysaccharides that have some structural similarity to cellulose because their backbone is composed of 1, 4-linked β-D sugar monomers (Oriz & Lafond, 2012). Hemicelluloses however, contain a variety of sugar monomers, pentoses, and hexoses, and their corresponding uronic acids as opposed to only glucose in the backbone of cellulose.

Hemicelluloses are typically highly branched as opposed to the linear cellulose structure. Hemicelluloses are found in both primary and secondary cell walls and include arabinoxylan, glucuronoxylan, glucomannan, and galactomannan (Keegstra, 2010).

They are present in grain hulls, plant stems, and fruit and represent a major source of plant based dietary fiber. The complete cell wall structure model is still evolving although the composition is fairly well known.

Arabinoxylans, the Major Hemicellulose in Wheat

During wheat milling, outer layers that make up bran are separated from starchy endosperm, which becomes flour (Oriz & Lafond, 2012). Wheat bran is a combination of many distinct layers of specialized cells (Figure 3). The outermost layers or pericarp are composed of epidermis, hypodermis, cross and tube cells. The innermost layers include nucellar and aleurone layers. The aleurone layer is composed of a single layer of cells whose cell walls are high in arabinoxylans linked to ferulic acid. Thus

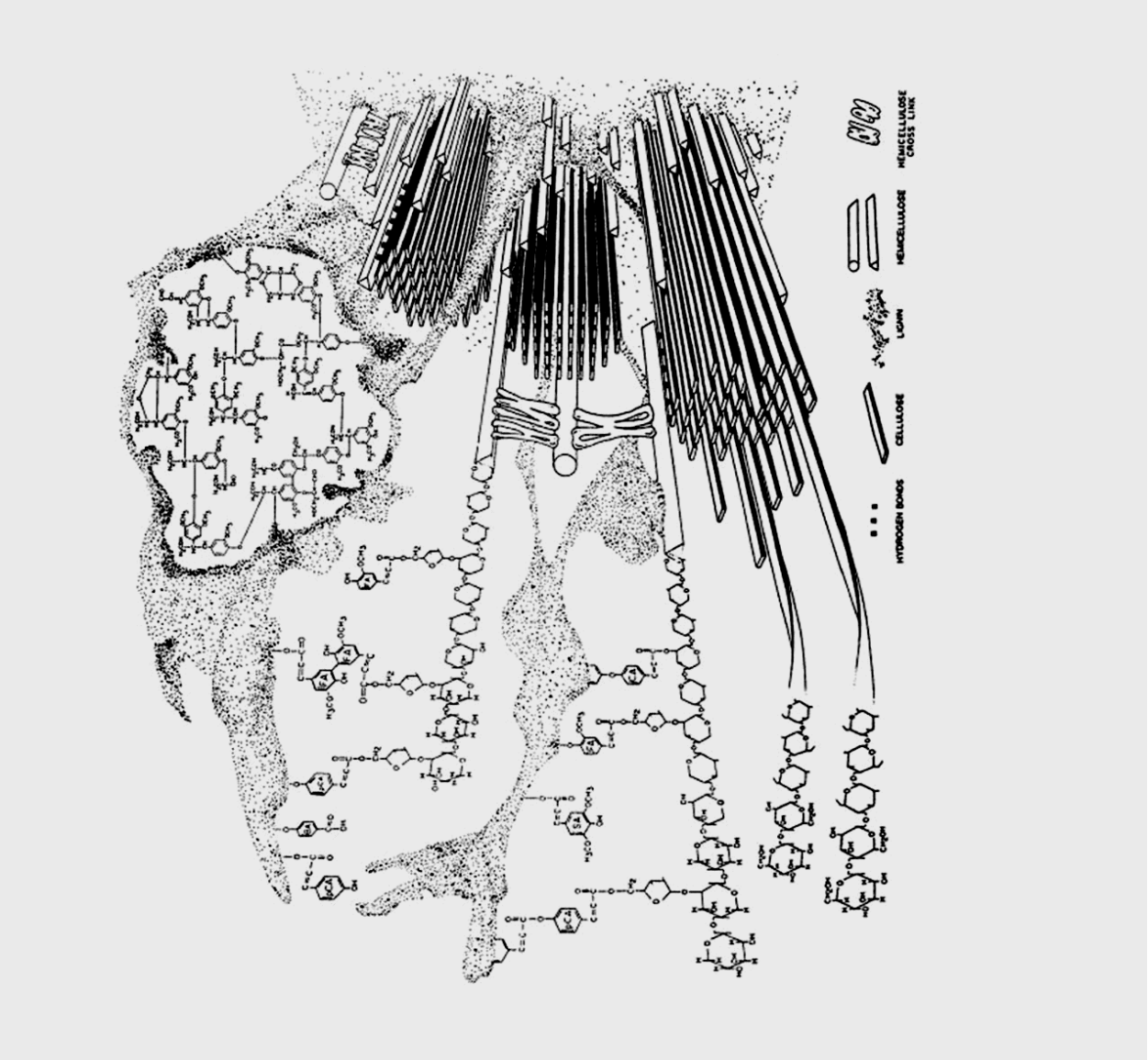


Figure 2. Secondary cell wall structure (CW). *Components are arranged so that the cellulose microfibrils and hemicellulosic chains are embedded in lignin. Specific linkages and components of non-core lignin are shown for a generalized grass secondary CW. Non-core lignin components include p-coumaric (pCA), ferulic (FA), p-hydroxybenzoic (BA), sinapic (SA), and cinnamic (CA) acids.* (Oklahoma State University Digital Library)

wheat bran is composed of cell walls from peripcarp, seed coats, and aleurone layers with some attached remnants of endosperm.

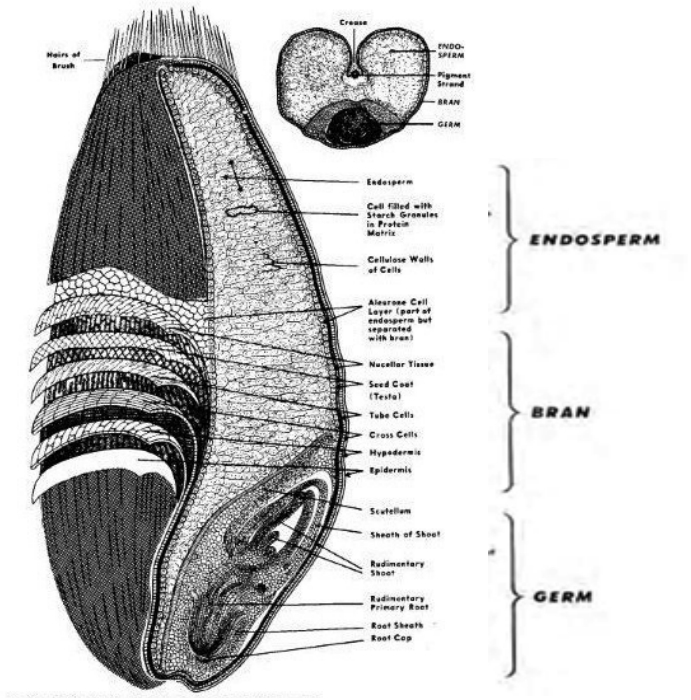


Figure 3 Longitudinal and cross sections of a wheat kernel (Hoseney, 1994)

The proportion and structure of arabinoxylans vary in wheat bran by tissue layer (Beaugrand, Reis, Guillon, & Chabbert, 2004). The majority of arabinoxylans is found in the pericarp (38%), nucellar epidermis (25%), and aleurone layer (25%) (Swennen, Courtin, Lindemans, & Delcour, 2006).

Arabinoxylans are pentosans (made from pentose sugar monomers) found in the bran of grains such as wheat, rye, and barley. They consist of a xylan backbone (see Figure 4) with L-arabinofuranose (L-arabinose) attached randomly by α1→2 and/or 1→3 linkages to xylose units throughout the chain (Ring & Selvendran, 1980).

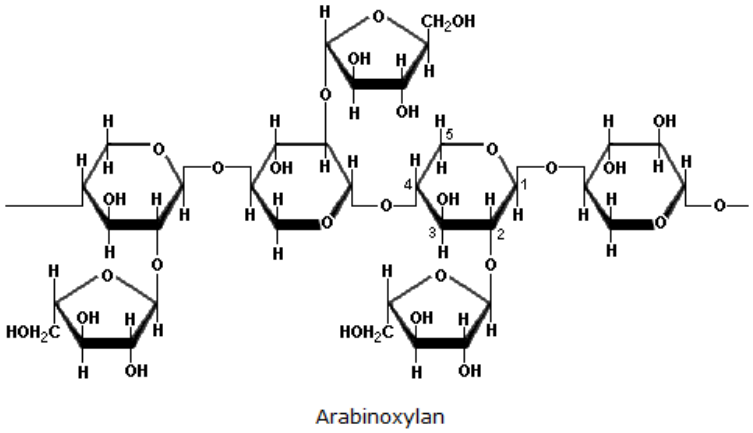


Figure 4. Arabinoxylan structural unit

Arabinoxylans are present in cross-linked form to cellulose, to other arabinoxylan chains, ferulic acid and *p*-coumaric acid at some arabinofuranosyl units, and to lignins (Oriz & Lafond, 2012). Arabinoxylans are important in the baking industry because of their water binding properties that affect viscosity of dough, retention of gas bubbles from fermentation, and final texture of baked products.

Fiber Definition Controversy

The definition of what is considered fiber has moved from one based on physiological responses to one based on chemical properties and then back to physiological over the last 60 years (Figure 5). Hipsley (1953) is generally credited with the first reference to dietary fiber. He defined it as simply the non-digestible plant cell wall materials. Others expanded the definition of fiber to include gums and mucilage, and developed

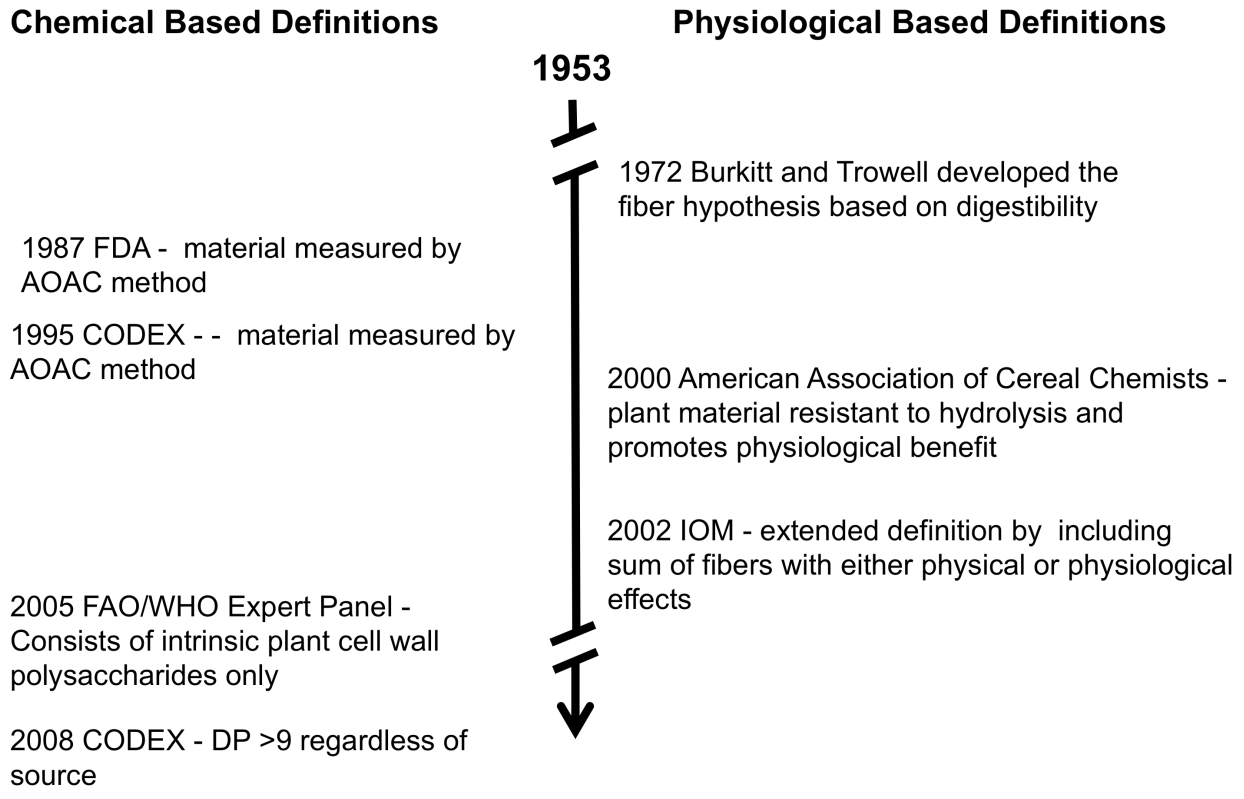


Figure 5. Fiber definition history - *Dietary Fiber moved from physiological to chemical back to physiological. IOM is the Institute of Medicine and AOAC is the American Organization of Analytical Chemists, WHO is the World Health Organization*

the fiber hypothesis linking dietary fiber to disease, based on digestibility (Burkitt, Walker, & Painter, 1972; Trowell H. , 1972; Trowell H. , 1974; Trowell, Southgate, & Wolever, 1976). This set the course for a debate between chemical and physiological based definitions of dietary fiber that has been continued by researchers for many years and is still going on today.

The result of this controversy could have an impact on which fibers may be used by food companies to increase fiber in existing products. At the center of the controversy is whether to allow fibers that have been modified, particularly hydrolyzed fibers, to be

included in the definition. These "novel" or new fibers are smaller in molecular weight and more soluble, making them easier to be added to food without detrimental organoleptic or processing effects. However, less is known about their physiological effects than many intact fibers, making it more difficult for novel fibers to qualify as a fiber on food product labels if the definition includes a proven effect. Since my research evaluated a physiological effect of a hydrolyzed fiber for the potential purpose of using it in a food product as a fiber source, the outcome of this controversy could affect the value of my work.

In 1987 the US FDA defined dietary fiber as the material isolated by Association of Official Analytical Chemists International (AOAC) method 985.29 which was an enzymatic gravimetric measurement using a phosphate buffer system. Total dietary fiber was obtained by digesting duplicate samples with heat stable alpha-amylase at 100°C to remove starch followed by treatments of protease and amyloglucosidase to digest protein and residual starch and maltodextrins resulting from the alpha-amylase. Fiber is precipitated with four volumes of ethanol, then dried and weighed. This method was refined to separate the insoluble dietary fiber from the soluble fiber based on precipitation in ethanol, approved as AOAC 991.43, and adopted by Codex.

A problem with these two methods (985.29 and 991.43) is that they don't include some of the "newer" fiber sources like inulin, fructooligosaccharides, polydextrose, extensively hydrolyzed arabinoxylans and resistant maltodextrins. AOAC has approved a number of methods for these specific fiber types, but until 2009, no single method was approved that could account for all of these fibers (Figure 6). The McCleary method AOAC 2009.01 is the first method designed to include all of these fiber types within a single

method. This gives the food industry a single method for measuring all types of fibers available today, and a common method for food labeling.

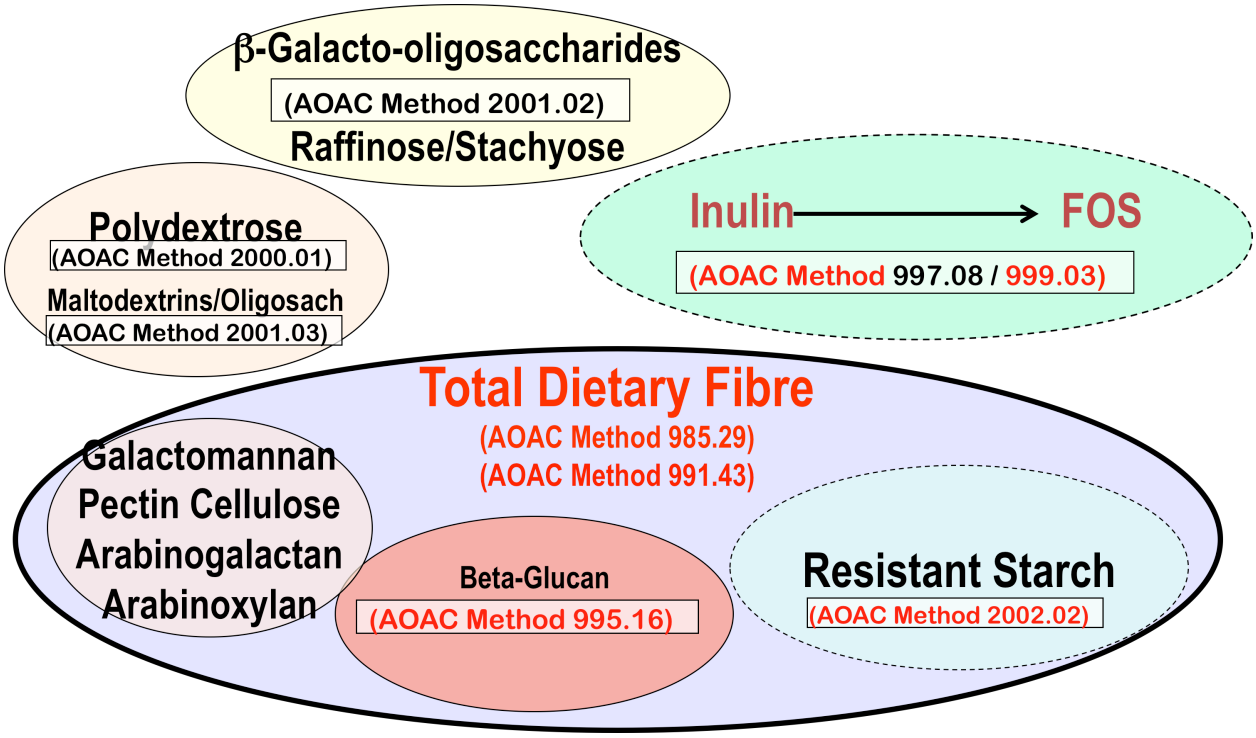


Figure 6. Total dietary fiber methods - *There is no one method approved today that measures all fibers that potentially are in food products.* (McCleary B. , 2008)

According to Code of Federal Register 21 CFR 101.9 Nutrition labeling of food for the US, labeling laws require manufacturers to validate fiber content levels in a product by an official AOAC method, but the US FDA does not yet specify a single method. The FDA's current definition of fiber includes non-digestible carbohydrates with a degree of polymerization of 3 and above, and naturally occurring as well as man-made and isolated fiber. Therefore manufacturers are free to choose which method suits their needs as long as they meet the FDA definition. A manufacturer augmenting a product's

fiber content with inulin for example would choose to use AOAC method 997.08 as opposed to AOAC method 991.43 in order to fully account for the increased fiber coming from inulin. This means that when comparing food labels from different companies with respect to fiber content, the analytical methods used, the ingredient accounted for as fiber, and it's physical properties may all be different. Because of this flexibility in regulations regarding fiber, several groups have been lobbying for labeling reform through more concise definitions of dietary fiber.

In the US in 2000, the American Association of Cereal Chemists introduced the concept that fiber should be defined as plant material that is both non-digestible and physiologically beneficial (functional) (The definition of dietary fiber, 2001). In 2002, the Institute of Medicine defined fiber as the total of dietary fiber plus functional fiber (Institute of Medicine of the National Academies, 2005). The FAO in 2005 proposed that dietary fiber consists of intrinsic plant cell wall polysaccharides only (Cummings & Stephen, 2007). This definition did not include non-digestible oligosaccharides, resistant starch, or carbohydrate polymers that have been obtained from plant products by physical, chemical or enzymatic means. It restricts the definition of dietary fiber to intact polymers isolated from plants, and eliminates all new or novel fibers that have been produced by hydrolyzing polymers to make them more soluble and easier to incorporate into food products. The FDA is considering making a distinction for fibers, between dietary fiber (described as intrinsic and intact fibers from plant cell walls of cereals, fruits and vegetables) and functional fiber (described as novel fibers that are isolated, modified or manufactured) partly due to these groups lobbying for change in the definition (FDA, 2014).

There is a lack of scientific consensus on the definition and analytical methodologies of fiber, causing debate and confusion within the food industry. There is ambiguity about what ingredients contribute to fiber content and what levels of fiber appear on the product label, inevitably leading to confusion on what is fiber, dietary fiber and functional fiber.

The European Commission included a DP >3 as part of the definition of fiber until recently. In 2008, The Codex Alimentarius Commission, a global organization created by The World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO) to provide standards and guidelines undertaken by international governmental and non-governmental organizations, defined fiber as carbohydrate polymers with ten or more monomeric units, which are not hydrolyzed by the endogenous enzymes in the small intestine of humans and are either naturally occurring in food, have been shown to have a physiological benefit, or are synthetically produced and shown to have a physiological benefit (Codex Committee on Nutrition and Foods for Special Dietary Uses, 2008).

In a footnote to the definition, Codex allows for each government to decide to include non-digestible carbohydrates with a degree of polymerization between 3 and 9. If the footnote is ignored, this might mean that fiber ingredients composed of a blend of hydrolyzed carbohydrates would need to be analyzed, and their fiber content adjusted to reflect only the fraction that contains a degree of polymerization >9. This definition would seriously restrict the use of the more soluble hydrolyzed fibers even though they may provide a physiological benefit.

The controversy is critical to food manufacturers engaged in international commerce because different fiber definitions could lead to different fiber content labels that are country specific on the same food product. It might lead to changing the fiber content of some ingredients used today because of changes to the definition, for example fibers that are sold as a blend of intact and hydrolyzed fiber.

Today, short chain oligosaccharides like hydrolyzed inulin and long chain branched complex carbohydrates like pectin or arabinoxylans are all included on food labels as fiber on US food products, even though their physical and chemical properties are very different, and potentially so are the physiological responses generated by the different fiber types. In this research I have determined that a physiological benefit, as measured by PYY and GLP-1 satiety hormone concentrations, is present with an arabinoxylan isolated from wheat that has been hydrolyzed to produce a low molecular weight non-digestible carbohydrate with a degree of polymerization primarily between 3 and 9. This could allow the food industry the flexibility of utilizing hydrolyzed arabinoxylans as fiber because they fit the current definition of non digestible carbohydrates with a degree of polymerization greater than 3, and might fit the possible future definition by having a demonstrated physiological benefit.

Dietary Fiber Intake

The Dietary Reference Intake (DRI) for fiber in the US is set at 38 grams per day for adult men and 25 grams per day for adult women (Institute of Medicine of the National Academies, 2005). This level was recommended by the IOM based on epidemiologic data showing a protective effect in cardiovascular disease at these consumption levels. It does not specify which fiber type, only fiber amount based on 14 grams/1000 calories

consumed. Since fiber provides other physiological benefits beyond cardiovascular disease (see next section on benefits), it is possible that a specific type of fiber at different levels than currently consumed may be needed to achieve these additional benefits.

Mean intake of fiber in the US from 2007-2008 was found to be 13.3 grams/day for women aged 20-29 (What we eat in America NHANES 2007-2008., 2010). Mean fiber intake for all individuals across all ages is estimated at only 15.1 grams per day. These estimates were based on respondent surveys, as there are no biomarkers currently available to measure fiber consumption objectively. Thus the data and its accuracy are dependent on subject recall. Most fiber containing foods range between 1-4 grams per serving according to the USDA Nutrient Database (Table 3).

White flour and white potatoes provide the most fiber in the US diet, due to high consumption rates not because of high fiber content (Slavin J. , 2008). For a person to meet current fiber requirements consuming white flour and white potatoes, they would need to consume a high number of calories given the number of calories per gram of fiber from these sources. For a woman to close the current fiber gap of 11.2 grams/day she would acquire an additional 147 – 242 calories. Grain based, high fiber breakfast cereals like Kellogg's All-Bran® are a much more effective way to close the fiber gap with only 90 additional calories acquired (Oriz & Lafond, 2012). Adding additional fiber to these cereals provides an even more calorie efficient means of raising dietary fiber consumption.

Table 3. Fiber and calories in some common foods

Food	Weight (grams)	Calories	Fiber (grams)	Calories/g Fiber
Medium raw apple with skin	182	95	4.4	21.6
Medium sized raw carrot	61	25	1.7	14.7
Broccoli (1 stalk)	151	51	3.9	13.1
Oatmeal (1 cup cooked)	234	166	4.0	41.5
Pinto Beans, (1 cup cooked)	171	245	15.4	15.9
Medium baked potato with skin	138	130	3.0	43.3
French fried potato (large)	169	531	5.9	90.0
Potato Chip, Plain Salted (8 oz.)	227	1230	10.0	123.0
White wheat flour enriched	125	455	3.4	133.8
Kellogg's All-Bran®	31	80	10	8.0

(USDA, 2010)

Benefits of Fiber

Fiber has been linked to several health benefits possibly related to its physical properties. Many studies over the last 20 years have linked fiber to weight loss and satiety. Blundell et al. proposed a link between fiber and satiety as early as 1987 (Blundell & Burley, 1987). Since that time there has been a significant amount of research relating fiber to satiety. Howarth et al. reviewed 20 published studies on the acute effects of dietary fiber on satiety and in a majority of the studies found a positive correlation of increased fiber consumption and increased satiety (Howarth, Saltz, & Roberts, 2001). Pereira & Ludwig (2001) reviewed fiber literature from epidemiological studies from 1984-2000 and found 16 out of 27 studies supported a beneficial role of fiber consumption on satiety. Several investigators have suggested that satiety benefits from fiber are related to higher viscosity (Burton-Freeman, 2000; Slavin & Green, 2007; Zijlstra, Mars, Wijk, Westerterp-Plantenga, & Graaf, 2008; Juvonen, et al., 2009).

A number of additional benefits of dietary fiber include improved gastrointestinal health, and reduced risk for cardiovascular disease, diabetes, and colon cancer (Slavin J. , 2008). A link between dietary fiber consumption and cholesterol levels in ischemic heart disease was proposed by Trowell almost 40 years ago (Trowell H. , 1972). The FDA approved a health claim in 1997 based on numerous studies linking consumption of beta-glucan from oats and barley as well as psyllium (psyllium seed husk) to serum LDL and total cholesterol reduction (21 CFR 101.81). The claim allows manufacturers to state that in the context of a low fat diet and with specific formula restrictions, fiber may help reduce the risk of heart disease as indicated below:

Fiber

Development of heart disease depends on many factors. Eating a diet low in saturated fat and cholesterol and high in fruits, vegetables, and grain products that contain fiber may lower blood cholesterol levels and reduce your risk of heart disease (FDA 21 CFR 101.77).

Soluble Fiber

Beta-glucans and psyllium fibers are soluble and although their chemical structures are different, they are very viscous because of their high molecular weight. It is believed that the beneficial effects for serum cholesterol reduction are related to viscosity (Dikeman & Fahey, 2006).

Because of the inability of normal human digestive enzymes to hydrolyze fiber, it passes from the stomach into the intestinal tract largely intact, and is responsible for laxation effects and modification of gut transit time. Some fibers, termed prebiotics, are

fermentable and thus serve as substrate for beneficial microorganisms to preferentially populate the intestinal tract (Cummings, Macfarlane, & Englyst, 2001).

Issues Adding Whole Grain Wheat as a Source of Fiber to Food

If used as an ingredient intended to significantly raise fiber levels in a finished product, whole grains are limited in the amount of fiber that they add, and they bring other calorie contributing components with them (Oriz & Lafond, 2012). For example, if you were to make a breakfast cereal using only whole grain wheat, the highest level of fiber you could commercially attain is ~12 grams/100 grams (this number might be increased slightly through extensive drying to ~13.5 grams/100 grams). In a typical 30 gram serving, this would equate to 3.6 grams of fiber, much less than the level required for an excellent fiber source claim (5 grams/ 30 gram serving), but sufficient for a good source claim (3 grams/30 gram serving). Most of the wheat kernel is starch coming from the endosperm, which provides considerable energy to the growing plant and calories to food it is added to. In order to increase the amount of fiber that can be added without raising calories, a more concentrated fiber source is required. Grains are milled to remove the seed coat from the kernel, and to separate out the endosperm from the bran and germ layers, to provide commercially available fractions of the grain. In the case of wheat kernels, the bran fraction provides a higher level of fiber than the whole grain and with fewer calories (Table 4).

Properties of Fiber in Foods

Fiber imparts textural, flavor, and color properties to foods; these properties become more noticeable and typically less desirable as the fiber concentration increases (Oriz & Lafond, 2012). Adding fiber to foods affects the food texture by making it harder, more

Table 4. Typical analysis of mill fractions

Analysis	Whole Wheat	Patent Flour	Germ	Bran
Protein (%)	12.0	11.0	30.0	14.5
Ash (%)	1.8	0.4	4.0	6.0
Fiber (%)	2.5	ND[1]	2.0	10.0
Fat (%)	2.9	0.88	10.0	3.3
Digestible Carbohydrate (by difference) (%)	80	87	54	66
Calories/100 grams	397	402	426	352
Calories/ gram Fiber	158	-	213	35

[1]Not detected, adapted from (Ziegler & Greer, 1971)

brittle, or rougher depending on the application. Fiber may change the appearance of the food, making it darker and browner or speckled, particularly in a bland flavored food or a lightly colored product like a cracker or a rice cake. In dough products where water is added to the mix, fiber may increase the water needed to hydrate the dough, making stickier dough with more water to remove in baking or drying in later unit operations.

Fibers provide solids, viscosity, and water binding properties for use in food product development. They are added to a food product either as part of an ingredient or as an isolated fiber. When fiber is added as an ingredient such as bran, it is part of an existing matrix that impacts the fiber's accessibility to water. The physical structure of the matrix can limit water reaching the fiber, and other components in the ingredient compete for water. Other components in the ingredient may contribute additional calories to the finished product. In the case of wheat bran, this would include starch and protein from the endosperm included in the bran mill fraction.

Isolated fibers are generally classified as soluble or insoluble in water although when considered soluble, they are present as a colloidal suspension as opposed to a true solution. Solubility is influenced by the chemical structure of the fiber (Tungland & Meyer, 2002). The more branching a fiber has, the greater its solubility in water, as this structural feature limits the amount of interchain interactions and allows water to interact with fiber. A high molecular weight linear fiber such as cellulose with its repeating structure of β 1-4 linkages is insoluble because of strong interchain interactions allowing it to form an ordered crystalline structure of polysaccharide chains held together by hydrogen bonding, whereas high molecular weight gums such as guar are highly branched and are very soluble. Arabinoxylans also have branched structures and are soluble as well. Ionizing groups and varied sugar positional bonding (for example, β-glucans in oats with mixed β 1-3 and β 1-4 linkages) impact solubility because they too affect interchain interactions (Tungland & Meyer, 2002).

Solubility has a large impact on food processing and finished product attributes. Insoluble fibers may hydrate and physically entrap water, but are still present in the food matrix as discrete particles. The more coarse ground fibers are abrasive in processing equipment such as extruders. Insoluble fibers often require more water in the dough system to hydrate the particles. Once in a food system, the insoluble fiber particles may interrupt the food macro structure causing potential weak points in the three dimensional food matrix. Particles may also impart a gritty mouthfeel if the size has not been reduced low enough.

Soluble fibers interact more readily with water and other food matrix components than insoluble fibers. Gums such as guar and acacia are very soluble, and are often used to

stabilize water in a food system favorably impacting appearance, mouthfeel and shelf life stability (Tungland & Meyer, 2002).

The viscosity of a fiber ingredient refers to its ability to thicken or form gels in fluid due to polymer entanglement within the fluid. It is generally related to molecular weight or chain length, and viscosity increases as these increase. There is a positive non-linear relationship between molecular weight and solution viscosity (Dikeman & Fahey, 2006). Long chain polysaccharides bind or entrap significant amounts of water and thus exhibit high viscosities in solution. These fibers are typically used as thickening agents at low concentrations in food systems. For example, fibers like pectin, guar gum, or locust bean gum, may be used to bind water and provide thickening to food systems. Highly soluble fibers that are either short polymer chains or highly branched polymers have low solution viscosities. These fibers can be added to foods at much higher concentrations and used to affect moisture migration or modify food texture with less effect on viscosity.

The interaction between water and fiber in a food system has been described as water binding or water holding. These terms differ by the impact of physical stress and amount of water affected. Water binding of a fiber refers to retention of water after physical stress, whereas water holding refers to water retained within a fiber's structure without stress (Tungland & Meyer, 2002). Many of the unit operations used to make food products exert physical stresses on the food system (for example processes such as extrusion, mixing, pumping) (Oriz & Lafond, 2012). These processes affect the amount of water retained by the fiber, possibly due to changes in the fiber's molecular structure. Processing may impact the stickiness of insoluble fibers in the dough of cereals due to the fiber’s water binding properties. Adding wheat bran to a cereal

formula will increase the amount of water held by the bran versus the remainder of the dough ingredients due to insoluble fiber present in the bran. This would require more water to be added in the cooking stage. More water at this stage makes the dough difficult to handle and physically move from the cooker to the next processing stage. The stickier dough affects remaining unit operations. Hydrolyzed arabinoxylans are much more soluble than wheat bran and require less water at the dough stage, resulting in drier dough and more efficient processing downstream.

Processing Effects on Hydrodynamic Properties

Food processing affects the physical and chemical structure of fibers and thus changes its hydrodynamic properties. Several recent studies have shown the effects food processing has on fiber. Regand et al. demonstrated that oat β-glucan in different food products had different molecular weights due to processing procedures used to make food products, and that this affected the fiber's viscosity and its serum glucose attenuation ability (Regand, Tosh, Wolever, & Wood, 2009). They prepared several food products, porridge, granola, crisp bread, and pasta (each containing 4 grams of oat β-glucan) in order to assess various cooking processes on molecular weight, viscosity, and physiological properties related to blood glucose response. They were able to demonstrate that viscosity increased with increasing molecular weight, and that little degradation of molecular weight occurred during processing of porridge and granola. However, there were significant reductions in molecular weight of the oat β-glucan in crisp bread and pasta possibly due to the presence of enzymes in wheat flour used to make these products. The solubility of fiber was highest in the crisp bread and the molecular weight was very low. Oat β-glucan molecular weights were compared from

several food products and extruded flakes, macaroni, and muffins all had high average oat β-glucan molecular weights, whereas fresh pasta and teacake contain reduced molecular weight distributions of oat β-glucan (Aman, Rimsten, & Andersson, 2004). These research studies demonstrate that other food components in the food matrix and the processes used to make the finished product can alter physicochemical properties of fiber used, and thus should be measured and confirmed prior to making statements relating ingredient properties to physiological effects. I have measured the molecular weight and viscosity of the fiber alone as well in combination with other water binding ingredients in a cereal product model system to assess that interaction. I observed a molecular weight and viscosity reduction due to RTE cereal processing.

Tosh, et al. (2010) showed that increased shear and decreased water in producing extruded cereal resulted in lower molecular weight oat β-glucan and lowered its viscosity. In this study, extrusion parameters (temperature and mechanical energy) were adjusted to cause a depolymerizaton of the oat β-glucan structure. They were able to reduce the molecular weight of oat β-glucan from 1,930,000 to 251,000 g/mol, and this reduced the apparent viscosity of the cereal extract from 2,900 to 131 mPa·s (at $30s^{-1}$). The cereal extract was obtained by grinding the cereal, dispersing it in phosphate buffer, and subjecting it to enzymes designed to simulate the digestion process, in order to estimate the affect of the cereal on gastric viscosity. They demonstrated that the same formula and unit operation subjected to different operating conditions reduced the molecular weight of oat β-glucan fiber and had a significant impact on its viscosity. If viscosity is an important hydrodynamic property with respect to its physiological benefit, then measuring the viscosity after processing is critical in

terms of understanding its effects on the benefit. I have measured the impact of the different fibers on the viscosity of the finished flaked cereal product in water and in acid and with enzymes, to assess the effects cereal processing has on viscosity.

Wolever, et al. (2010) demonstrated that the blood LDL reducing capacity of oat β-glucan in human subjects was dependent on its molecular weight and concentration in the food, which affected the fiber's viscosity. They compared oat β-glucan from extruded breakfast cereals with processing treatments designed to produce variables with different molecular weights. They fed cereals to 345 people with blood LDL cholesterol levels between 3 and 5 mmol/L over 4 weeks and compared effects on LDL cholesterol relative to a control cereal without oat β-glucan. They found that the cholesterol lowering effect was dependent on the molecular weight of the oat β-glucan and was a significant determinant of LDL cholesterol. They also demonstrated that the log (MW x C) was positively related to the log (viscosity) of the solutions obtained in vitro digestion of the cereals. Three grams of oat β-glucan/day with molecular weights of 2,210,000 g/mol and 530,000 g/mol both lowered LDL cholesterol by 0.2 mmol/L, but efficacy was reduced by 50% with the 210,000 g/mol molecular weight oat β-glucan variable. These studies show that the molecular weight of the fiber and its dependent physical properties should be considered in addition to the total amount of oat β-glucan in the food product. I have measured the molecular weight of test fibers before and after processing of the flaked cereal in order to correlate molecular weight with viscosity and other hydrodynamic properties, and relate them to satiety effects in a clinical trial.

Satiety Rationale

The prevalence of obesity in the US has increased from 13.4% in 1980 to 34.3% in 2008 among adults and 5 to 17% among children. Today two thirds of adults and nearly one in three children are overweight or obese (Office of the Surgeon General, 2010). In 2006 an estimated 97 million adults were overweight or obese (Schwartz & Byrd-Bredbenner, 2006). The Center for Disease Control has been tracking the levels of obesity in the US by state since 1985. Figure 7 is a graphical representation of the rise in obesity levels as a percent of state population.

In 1990, of the states participating in the Behavioral Risk Factor Surveillance System, ten states had a prevalence of obesity less than 10% and no states had prevalence equal to or greater than 15%. By 2009, only one state (Colorado) and the District of Columbia had a prevalence of obesity less than 20%. Thirty-three states had prevalence equal to or greater than 25% and nine of these states had a prevalence of obesity equal to or greater than 30% (Obesity and Overweight for Professionals: Data and Statistics: U.S. Obesity Trends).

Obesity is a major cause of morbidity and mortality in the US (Mokdad, Bowman, Ford, Vinicor, Marks, & Koplan, 2001). It has been linked to other diseases such as coronary heart disease, diabetes and metabolic syndrome. Obesity related deaths in the US are estimated at over ¼ million adults per year (Bowman & Spence, 2002). Obese adults have increased risk for high blood pressure, high cholesterol, diabetes, coronary heart disease, and stroke. Overweight children have an increased risk for insulin resistance, high blood pressure, and high levels of fats and other lipids. Obese children often become obese adults.

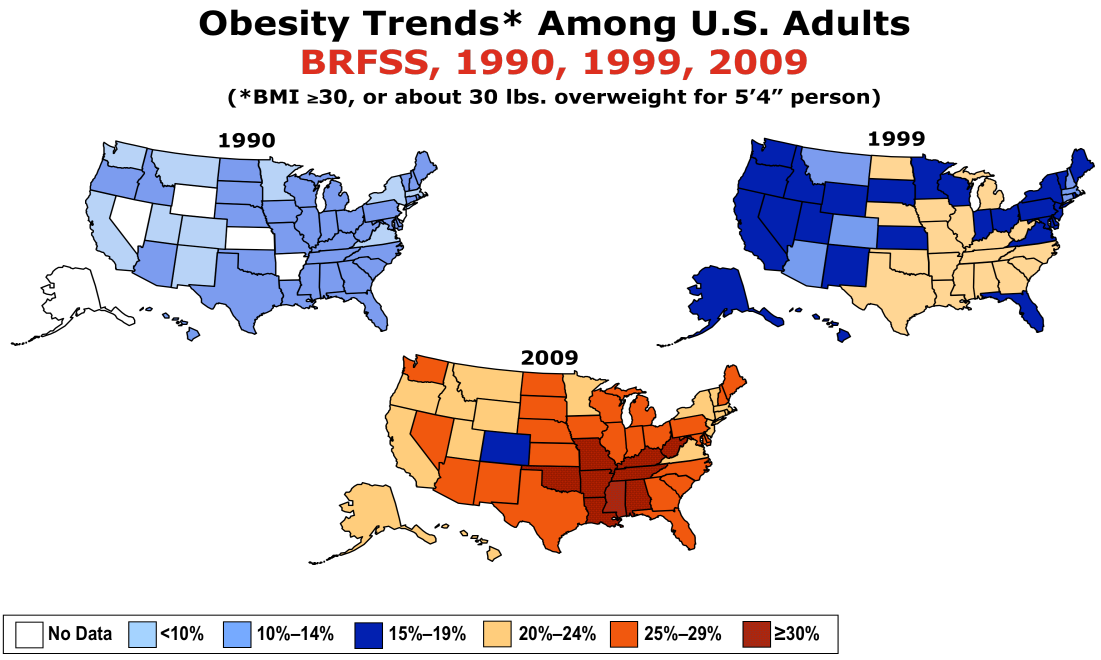


Figure 7. Obesity trends in the US by percent of state population (Obesity and Overweight for Professionals: Data and Statistics: U.S. Obesity Trends). *For the interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this dissertation.*

Lifestyle modifications, primarily exercise and diet, are the major interventions for weight management of which a high fiber diet may be an effective part. As indicated previously, consumers are not getting enough fiber in their diet. Some RTE cereals are relatively low in caloric density and provide consumers with a source of fiber.

Fiber Mechanism for Short Term Satiety

Heaton proposed almost four decades ago that fiber reduces energy intake by 3 mechanisms (Heaton, 1973). The first was that fiber induced increased chewing which in turn increases salvia and gastric juice secretions distending the stomach. This

stretching of the stomach was proposed to signal satiety. The second was based on a reduction of caloric density. Fiber as part of a food product provides food volume

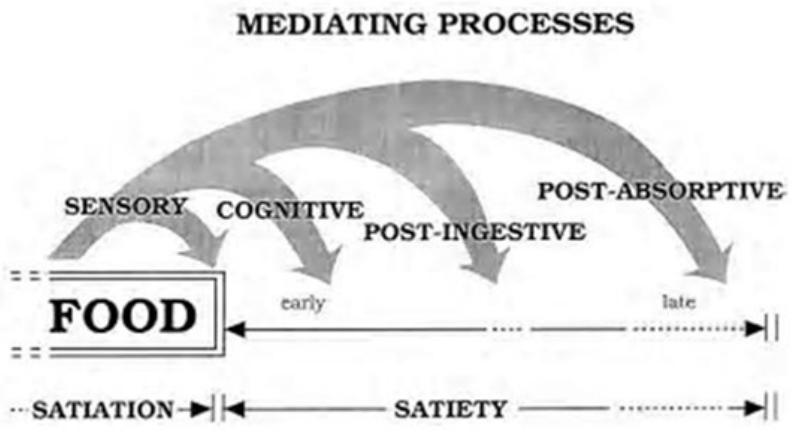


Figure 8. The satiety cascade illustrating the events that constitute satiety signals after consumption of food *(Blundell 1999).*

without adding calories, so for a given amount of food consumed to fill the stomach, less energy is available. The third mechanism was based on increased viscosity to reduce efficiency of nutrient absorption in the small intestine. Blundell & Burley (1987) examined these possible mechanisms and introduced the concept of behavioral and hedonic aspects of fiber and satiety. They proposed that there was a cascade of signaling that occurred pre and post consumption and that these represented events that together signaled satiety (Figure 8).

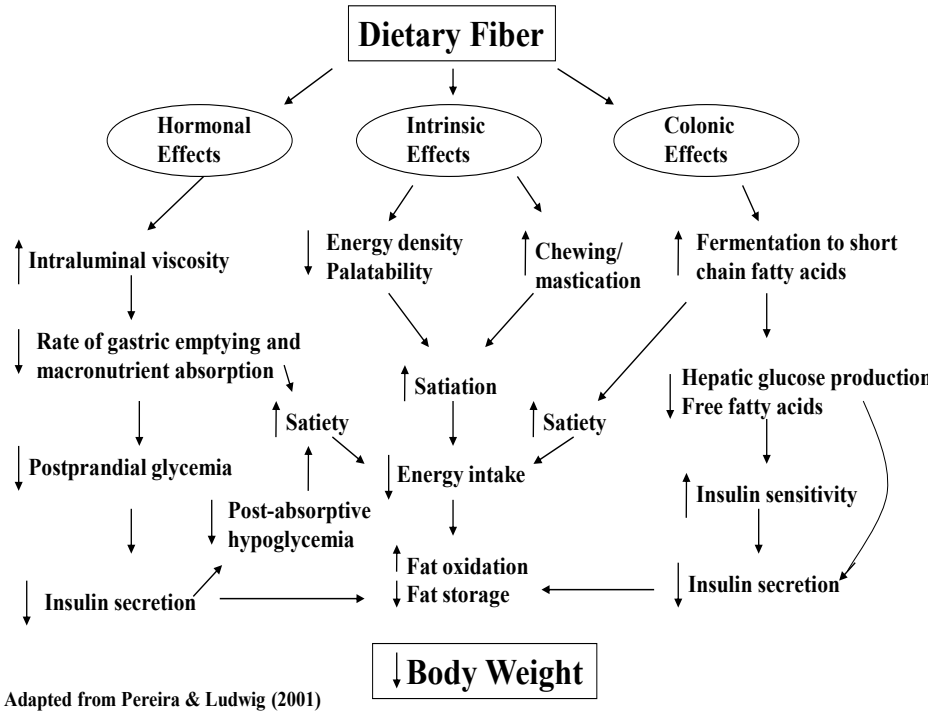


Figure 9. Proposed mechanisms for fiber and satiety.

Pereira & Ludwig (2001) reviewed epidemiological studies involving fiber and satiety, and proposed a more complex model (Figure 9) based on their observations of high fiber diets. Their model was based on hormonal, intrinsic (energy), and colonic effects of dietary fiber on satiety. The hormonal and intrinsic effects of dietary fiber are likely to be relatively short term or acute, as opposed to colonic effects which involve food transit to the colon and fermentation time (although some fermentation may occur short term). My research has focused on the acute affects of arabinoxylans on hormonal responses and perceived appetite.

Intrinsic Effects

Haber, Heaton, & Murphy (1977) studied physical structure effects of a food system related to fiber. In a small study with 10 subjects, 60 grams of available carbohydrate was provided as apples, apple puree, or apple juice. They found that juice without fiber could be consumed 4X faster than fiber disrupted puree, and 11X faster than intact apples. With rate of ingestion equalized, juice was less satisfying than puree and puree less than apples, and the effects lasted 2 hours. Plasma glucose rose to similar concentrations on all 3 but showed a very fast rebound fall after juice, less with puree and none with apples. They concluded that "serum insulin rose to higher concentrations after juice and for puree than after apples" and suggested that satiety was partly dependent on chewing. This finding was confirmed in a similar study where whole apples increased satiety more than apple sauce or juice when measured over a 5 week period (Flood-Obbagy & Rolls, 2009). This might also explain effects of whole grain on satiety, as more physical structure of the whole grain would increase the need for chewing. Possibly physical structure has an effect on the energy the stomach needs to digest food.

In a separate study, test lunches were prepared with carrots (200 grams) as whole carrots, blended carrots, or carrot nutrients in order to compare physical structure effects on satiety (Moorhead, et al., 2006). The carrot nutrients had the same energy and mass as the other two variables, but were made of only the nutrients of the carrots without the physical structure component. They found that whole carrots and blended carrots resulted in significantly higher satiety compared to carrot nutrients as assessed by Visual Analogue Scales (VAS). Ad libitum meal plus food intake for the remainder of

the day was found to be highest with the carrot nutrients, followed by blended carrots, and the lowest with whole carrots. This suggests that both fiber content and food structure are important to these satiety effects.

In a series of studies on volume and energy density, Rolls et al. assessed the impact of each on satiety. They compared 3 milk drinks (300, 450, 600 ml) with equal energy content but different volumes by the addition of water (Rolls, et al., 1998). They observed that volume affected energy intake at lunch, with energy intake lowest with the larger volume 600ml drink. They concluded volume consumed affects satiety independent of its energy content.

In a different study water given as a preload with a food, was compared to in a food using chicken rice casserole, casserole with water, or diluted in soup (Rolls, Bell, & Thorwart, 1999). They found that the soup was the most satiating, reduced hunger, and lowered energy intake at lunch. Drinking water with the casserole did not increase satiety as much.

In a third study, increased volume of food again in 3 milk drinks (300, 450, 600 ml) was compared with equal energy content but different volumes this time by the addition of air (Rolls, Bell, & Wauh, 2000). They found that volume affected energy intake at lunch, energy intake was lowest with the larger volume 600 ml drink. Satiety scores were significantly higher in the 600 ml drink than 300 ml one. They concluded that volume independent of energy density influenced satiety.

These studies indicate that volume is important to satiety effects regardless if driven by water or air. In my research the volume of the preload, and the amount of solid to liquid

ratio has been controlled in order to eliminate volume and chewing variation in the study. The texture of the food variables was designed to be similar through product and process adjustments.

Gastric Emptying

A common belief is that viscous fibers slow gastric emptying, and that this is partially responsible for its satiety effect. Clinical evidence for a role of viscosity in gastric emptying and satiety is not firmly established (Kristensen & Jensen, 2010). Many studies have been conducted relating the short-term effects of fiber on satiety covering numerous types, dosages, sources, and food systems. However, in most cases the simple description of the fiber used is insufficient to characterize the fiber because source, extraction procedure, food system, and processing among other things determine physicochemical characteristics, information needed to understand the role fiber has in satiety. Although the levels and types of fiber used could produce high viscosity, most investigators did not measure the viscosity of the fiber or confirm viscosity in the final food product. This makes it very difficult to make conclusions on the effect viscosity has on gastric emptying.

Table 5 shows a summary of recent intervention studies evaluating the effects of fiber viscosity on gastric emptying rate (GER) and satiety. Only those studies where a viscosity number was reported are shown, which greatly limits the scope of research in this area. The methods used to measure viscosity in the various studies are not the same, so a direct viscosity comparison is not possible.

Bennett et al. studied the effects of locust bean gum in 5 healthy adults and 5 post Swedish adjustable gastric banding subjects (Bennett, et al., 2009). A 3% solution of locust bean gum with a viscosity of 171 mPa·s was compared to water (1 mPa·s). They found that satiety was higher, and the gastric emptying rate as measured by MRI was slower, after the high viscosity beverage in both treatment groups, suggesting that higher viscosity slows gastric emptying and increases satiety.

Juvonen, et al. (2009) measured viscosity, gastric emptying, and satiety with both hydrolyzed and unhydrolyzed oat β-glucan (10.2 g). Viscosity was reduced by beta glucanase from >3,000 to <250 mPa·s. Contrary to expectations, hydrolyzed fiber with low viscosity had greater satiety even with faster gastric emptying rate and the higher viscosity induced smaller glucose and insulin responses.

The low viscosity treatment induced greater satiety, faster gastric emptying and higher serum glucose, insulin, CCK, GLP-1, PYY and a decrease in ghrelin than the higher viscosity treatment (Juvonen, et al., 2009). This suggests that lower viscosity increases gastric emptying rate and increases satiety, the opposite of what Bennett et al. found. Also both studies were done with beverages, so chewing of food was not a factor.

Guar gum (viscosity not affected by pH) was compared to sodium alginate, a fiber that gels at low pH in the presence of calcium (Hoad, et al., 2004). They fed 12 subjects a 325 mL beverage at various viscosities and measured appetite ratings with a visual analogue scale. They used MRI to look at stomach images. They found that guar was the only meal to stay homogenous in the stomach. The sense of fullness was significantly greater for all viscous meals versus control and hunger was delayed with

Table 5. Summary of recent intervention studies evaluating the effects of fiber on gastric emptying rate (GER) and satiety

Reference	Study Design	Product	Viscosity η	Results	η	GER	Satiety
(Bennett, et al., 2009)	Crossover RCT n=5 healthy & 5 post SAGB, 90 min.	1000 ml of water vs. 3% locust bean gum beverages	1 vs.171 mPa·s at 37˚C shear rate from 0.01-500/s	Hunger and GER were lower after high viscosity beverage for both groups	↑	↓	↑
(Juvonen, et al., 2009)	Crossover RCT n=20 healthy adults, 180 min.	2 beverages with 10.2 g Oat β-Glucan vs. Hydrolyzed Oat β-Glucan	<250 or >3,000 mPa·s at 20˚C shear rate 50/s	Satiety, CCK, PYY, GLP-1, GER and next meal energy intake increased and ghrelin decreased with low viscosity beverage	↓	↑	↑
(Hoad, et al., 2004)	Crossover RCT n=12 healthy adults, 240 min.	4 beverages with no DF, 1% weak gel alginate, strong gel alginate or guar (3.25 g DF)	1.6, 17,000, 39,100, or 50,000 mPa·s at 37˚C zero shear rate calculated	Fullness increased and hunger decreased with strong gelling alginate and guar gum but not with weak gel alginate; no change in GER	↑	-	↑
(Rigaud, Paycha, Meulemans, Merrouche, & Mignon, 1998)	Crossover RCT n=14 normal adults	High or low viscosity beverage with psyllium 7.4 g	23,000 vs. 695,000 mPa·s	No delay in gastric emptying at high viscosity but significant hunger and energy intake reduction	↑	-	↑
(Marciani, et al., 2001)	Crossover RCT n=12 healthy adults, 90 min.	High or low viscosity beverage with locust bean gum with or without nutrients	60 vs. 295,000 mPa·s at 37˚C	Fullness increased more with high viscosity, GER was delayed more with nutrients than viscosity	↑	↓	↑

Adapted from (Kristensen & Jensen, 2010)

higher viscosity meals. However, there was no difference in gastric empting rate. This study provides evidence that viscous fiber may not affect gastric emptying even though it affected satiety.

Psyllium (7.4 g) was assessed versus a gelatin and sweetener placebo mixed into 100 mL water 15 min prior to meal consumption (Rigaud, Paycha, Meulemans, Merrouche, & Mignon, 1998). They measured the viscosity of the solutions at 37 ˚C at 695,000 mPa·s vs. 23,000 mPa·s in the placebo. While they found no delay in gastric emptying at relatively high viscosity, there were significant hunger (-13%) and energy intake (-17%) reductions observed. In this case viscosity showed no affect on gastric emptying, but it did induce satiety.

High and low viscosity locust bean gum based beverages with and without added nutrients were compared in a study with 12 healthy adults (Marciani, et al., 2001). Although they did not disclose the level of fiber, they did provide viscosity measurements. The high viscosity beverage was 29,500 mPa·s compared to the low at 60 mPa·s. They found that increasing the nutrient content of the high viscosity beverage delayed gastric emptying more than viscosity alone, and that both were slower than the low viscosity variables. Meal viscosity significantly delayed gastric emptying however, the presence of nutrients almost doubled the time needed to empty one half of the initial gastric volume. Fullness increased more with viscosity than with the presence of nutrients, and that satiety was higher with all high viscous treatments

than low viscous treatments. This suggests that both caloric density and viscosity are impacting gastric emptying and satiety.

All of the above studies were conducted using a beverage system as opposed to a solid food system. It is possible that viscosity in a beverage system has less impact on satiety than viscosity in a solid food system. Mattes et al. compared viscosity in a study of shakes with equivalent nutrient content and different viscosities. They found significantly greater and more prolonged increased satiety with the higher viscosity shake (Mattes & Rothacker, 2001). However, Rothacker et al. demonstrated that although a solid bar product contained an additional 30 calories over the previous shake study it provided an additional 2 hours of satiety suggesting that solid food is more effective at providing satiety than liquid (Rothacker & Watemberg, 2004). Satiety scores were consistently greater for more solid preloads. This agrees with results presented in earlier sections of this paper relating physical structure to satiety. Viscosity provides some structure to beverage products however solid foods provide more structure to break down in the digestive system. A small number of studies evaluated the effects of fiber on gastric emptying; however these studies did not measure the viscosity of the fiber or the finished product used, limiting definite conclusions that may be made on the effects of viscosity.

Wheat bran flakes were compared with whole meal oat flakes (Hlebowicz, Darwiche, Bjorgell, & Almer, 2008). They measured satiety and gastric emptying rate of 12 healthy subjects and found that gastric emptying rate was slower with the wheat bran flakes versus the whole oat or the control flakes. They did not measure viscosity in the study however, Dikeman et al. have reported that wheat bran is not viscous in water or

gastric juice, and oat β-glucan is (Dikeman & Fahey, 2006). This suggests that the oat flakes may have contributed more to gastric viscosity than the wheat bran, yet the wheat bran delayed gastric emptying more, agreeing with the Juvonen et al. study. Factors other than viscosity may be involved. One possible factor is that wheat bran fiber is largely insoluble versus soluble fiber in oats. This might impact the amount of energy the stomach uses to grind and digest the food and possibly delay gastric emptying. Marciani et al. suggest that the physical form of the food interacts with the calorie content to alter fullness and satiety perceptions (Marciani, et al., 2001). Haber et al. was able to show that fullness in whole apples lasted longer than the same apples pureed or in an equicaloric apple juice beverage (Haber, Heaton, & Murphy, 1977). My study controlled for product texture and ingredient particle size of the fibers used when comparing cereal flakes with different fiber physicochemical properties.

Darwiche et al. found that 6 g locust bean gum in rice pudding increased viscosity, and gastric emptying was slower. However, when viscosity was decreased with 100 ml. added water to the meal, gastric emptying rate was not affected (Darwiche, Björgell, & Almer, 2003). If gastric emptying was based solely on viscosity there should have been a change in gastric emptying rate. This suggests that other factors are involved and that simply adding viscous fiber to a meal may not induce slower gastric emptying or satiety. If viscosity were the sole factor for gastric emptying the results would be more consistent between similar studies. I have characterized the physicochemical properties of the fibers, including viscosity, in my research and have confirmed those properties in the finished cereal flake.

Gut Hormone Signaling

Nutrient composition is an important determinant driving satiety signaling via hormones released from enteroendocrine cells in the intestine walls (Murphy & Bloom, 2006). Endocrine cell types and hormones produced vary along the mucosal microvilli of the intestine distributed among the absorption enterocytes. These endocrine cells are believed to sense the nutrient composition of food released from the stomach. As food passes, these cells release peptides in response to food composition. I-cells release cholecystokinin (CCK), K-cells release gastric inhibitory polypeptide (GIP), and L-cells secrete glucagon like peptide (GLP-1 and GLP-2) and polypeptide Y (PYY). I-cells are located in the duodenal and jeujenal mucosa whereas L-cells are located in the distal ileum and colon. These peptides have all been studied for their effects on energy regulation.

Ghrelin

Ghrelin is the only known orexigenic gut hormone shown to stimulate appetite. Ghrelin is released primarily by the stomach, but also from the duodenum, ileum, and colon. It is a 28 amino acid peptide with 2 major forms, acylated (n-octanoic acid on serine 3) and non-acylated ghrelin. Acylated ghrelin has been shown to be positively correlated with appetite scores, whereas the non-acylated form has been shown to have the opposite effect (Delzenne, et al., 2010). Acylation might be a trigger for appetite stimulation. This suggests that suppression of ghrelin may induce satiety (Cummings, E., & Overduin, 2007). I measured the acylated form of ghrelin and have correlated it to perceived appetite scores.

GLP-1

L-cells secreting GLP-1 are largely distributed in the ileum, colon, and rectum (Murphy & Bloom, 2006). The active form of GLP-1 is a 7-36 amino acid peptide. It is quickly inactivated by dipeptidyl peptidase IV, an enzyme produced in the intestinal epithelial cells (Wren & Bloom, 2007). The half-life of the active form of GLP-1 is about 30 seconds, making it a very fast acting and difficult hormone to measure.

When glucose is given orally, gastric emptying is activated, and the rate is controlled in part by GLP-1. These effects demonstrate the role GLP-1 has in regulating the onset and rate of gastric emptying. Plasma concentrations increase 10-20 minutes after a meal and reach a peak in 60, which corresponds to about the time it takes for food to reach the L-cells. Therefore GLP-1 may only contribute to intermeal satiety and not necessarily meal termination, since most meals are terminated before 60 minutes. GLP-1 might act to prolong the satiety effects of the previous meal and inhibit eating at the next (Blundell & Naslund, 1999).

CCK

CCK is produced by I-cells in duodenal and jejunal mucosa, as well as by the brain and enteric nervous system. Satiation effects have been shown in numerous studies over more than 30 years. It is released in response to nutrients in the gut with levels rising as much as 5 fold. In addition to inducing satiety, CCK also delays gastric emptying. It may serve to regulate digestion by slowing the rate at which nutrients flow from the stomach into the intestine allowing for matching the nutrient absorption rate with the nutrient delivery rate. It is only active in the sulphated form that binds with high affinity

to the CCK-A receptor (Wren & Bloom, 2007). It has a half-life of only 1-2 minutes and may interact with other satiety regulators or be a satiety initiator.

PYY

PYY is secreted as a 36 amino acid peptide by the L-cells in the distal ileum and colon (Wren & Bloom, 2007). Once present in the blood, it is rapidly degraded by dipeptidyl peptidase IV to the 3-36 peptide form. This form is an agonist for the Y2 receptor, which has been shown to reduce food intake. Therefore its action on satiety is to block the receptor found in the hypothalamus arcuate nucleus. Secretion of PYY by the intestine is proportional to the caloric density of nutrients ingested.

I looked for differences in active GLP-1 and total PYY concentrations over time and related them back to physicochemical properties of the fiber in a flaked cereal.

Fiber Effects on Gut Hormones

Burton-Freeman, Davis, & Schneeman (2002) compared low fiber low fat, high fiber low fat, and low fiber high fat isoenergetic breakfast meals effects on CCK and satiety in 7 men and 8 women. The meals consisted of a variety of foods from different food groups commonly consumed for breakfast. Plasma was drawn over a 6 hour period after consumption and analyzed. In women, they found the meals with higher fiber or fat resulted in greater satiety and significantly higher CCK levels than did the low fat low fiber meals. In men, results were less consistent however, since my research was designed to measure satiety effects in women this study was included in the review. The investigators concluded that in women, increasing either fiber or fat content of a low fat low fiber meal enhanced satiety and was correlated to the release of CCK. This

suggests that fiber may be as effective as fat in stimulating CCK release and satiety in women.

Effects of resistant starch on satiety and hormonal responses were evaluated in 10 healthy normal weight males (Raben, Tagliabue, Christensen, Holst, & Astrup, 1994). Test meals contained 50 g of pregelatinized starch, or raw potato starch (54% resistant starch) together with 500 g artificially sweetened syrup. They found that glucose, insulin and GLP-1 were significantly lower after the resistant starch meal versus the control. Scores for satiety were significantly lower after the resistant starch meal as well. They concluded that resistant starch resulted in significant reduction in postprandial serum glucose, insulin, and satiety.

Five isoenergetic meals were compared, 1 white wheat bread (low fiber 3.4 g) and 4 test meals of various combinations of soy protein and psyllium fiber in a bread product with 16 healthy non obese subjects (13 female and 3 male) in a single blind randomized crossover study (Karhunen, et al., 2010). They found that fiber enriched meals with 23 grams psyllium decreased glucose, insulin, ghrelin, and PYY responses and prolonged PYY secretion versus other meals. GLP-1 concentration was significantly reduced after both the fiber and protein rich meal versus the other meals. Postprandial appetite ratings were similar however across all the meals. Although they found significant hormonal responses with a very large dose of psyllium, they were not able to find a difference in perceived appetite. The number of subjects in the study was small, and they could have missed differences in satiety. I included at least 30 subjects in my study in order have 80% power to detect a difference of 10 mm in the average appetite

composite score between treatments assuming a standard deviation of 15.9 mm. This assumes a nominal p-value of 0.017 to account for up to three primary comparisons.

A mixture of fibers in a muffin was compared at levels of 0, 4, 8, and 12 grams with 20 healthy adults (10 female and 10 male) (Willis, Thomas, Eldridge, Harkness, Green, & Slavin, 2010). The fiber blend was pectin, barley β-glucan, guar gum, pea fiber, and citrus fiber in equal proportions. They measured satiety using visual analogue scales and serum ghrelin, GLP-1, and PYY. Although they found that satiety did not differ between treatments, they found that ghrelin was higher after the 12 gram fiber dose versus the 4 and 8 gram dose, GLP-1 was higher after the 0 gram fiber dose, and PYY did not differ among the fiber doses. This was a relatively small study with low doses of fiber, low enough to be within the noise of the satiety assays and could be responsible for these fluctuations in hormone levels.

All of these studies have severe limitations as they used a small number of subjects and none of the studies showed a direct link between fiber consumption, satiety and hormonal response. There are numerous studies showing fiber has a satiety effect, that viscous fibers also induce satiety, and that CCK, GLP-1, and PYY have an effect on satiety, but there are very few studies where fiber was related to these hormones and satiety in the same study. Those studies that did were limited by small subject size or low levels of fiber, low enough to be within the noise level of the satiety measurements. I have measured perceived appetite and these plasma hormone concentrations in a controlled study with a large enough subject size, and a high enough fiber dosage to be able measure effects of a viscous and a non-viscous fiber added to the same food system.

Conclusions

As food is consumed, complex signaling from the stomach and intestine as well as the brain are initiated to regulate digestion and perceived appetite. Fiber may have an effect on this regulation thru various mechanisms. One impact fiber has on satiety may be related to its physicochemical properties. Gastric satiation may be more related to volumetric or physical structure, properties that increase the amount of energy needed to chew the food and for the stomach to grind food. Fiber might also have an effect on the amount of gastric juice the stomach produces in response to more viscous food. Intestinal satiation may be more related to nutrient composition and rate of nutrient absorption in the small intestine affecting hormonal release.

Some studies have characterized soluble fibers with different viscosities based on their chemical structure, and suggested a link between soluble viscous fiber and satiety. However, certain high shear processes used to make finished foods can disrupt the chemical structure of fiber and thus have an impact on its physical properties that may influence satiety. Limited work has been done in the same study to characterize physicochemical properties of specific fibers, and to also measure satiety effects of those fibers in a food product at the same usage level in order to relate satiety back to specific physical properties. I have addressed this limitation and related physicochemical properties of fiber both separate and incorporated in a flaked cereal, and related these properties to satiety and its possible mechanism of action.

HYPOTHESIS

A mechanism by which fiber affects appetite is related to its physical structure and hydrodynamic properties. An intact arabinoxylan was compared to a hydrolyzed

arabinoxylan to assess the impact of different physicochemical properties on perceived appetite. Hydrolysis of arabinoxylans by endoxylanase alter its chemical structure changing its solubility, apparent viscosity, and ability to hold water and thus may alter the mechanism by which the fiber affects appetite.

SPECIFIC AIMS

1) Ingredient

Obtain an enzyme hydrolyzed arabinoxylan from wheat and an intact arabinoxylan from flax for use in the study and characterize these fibers for molecular weight and viscosity.

2) Flaked Cereal Food Product

Measure fiber molecular weight, viscosity, and water retention capacity of the flaked cereal. Also measure texture and flavor properties in the food product and adjust food products based on sensory properties to be similar across variables for the clinical trials.

3) Clinical Trials

Phase I

Measure differences in perceived appetite, and lunch meal energy intake at 4 hours after the breakfast meal among the control and test cereals.

Phase II

In addition to the previous procedures, measure plasma glucose, insulin, active ghrelin, active GLP-1 and total PYY concentrations in the subjects fed the test cereals made with different fibers.

CHAPTER 2 - Processing Affects the Physicochemical Properties of Arabinoxylans in Ready-to-Eat (RTE) Flaked Cereal.

Abstract

Wheat bran fiber is mostly insoluble, making addition in high amounts to a food difficult without adversely affecting product attributes. One approach to increasing the level of wheat fiber in food is to hydrolyze intact fiber to more soluble forms through processing. This study was designed to evaluate the impact of a steam pressure-cooking process on physicochemical properties of RTE cereal with 15% added intact or hydrolyzed arabinoxylan. Peak molecular weights of intact and hydrolyzed fibers were ~2.9×10^6 and ~800 g/mol, respectively with a ~400-fold higher viscosity for intact fiber. Molecular weight of intact fiber was reduced to approximately the molecular weight of hydrolyzed fiber as a result of the low shear steam pressure-cooking process used, and consistent with molecular weight results, there was only a 2-fold difference in viscosity of the cereal remaining. The low fiber control RTE cereal had the highest viscosity due to starch content.

Introduction

Obesity has been classified as a disease in the US by the American Medical Association, and it has become a major health problem in developed countries. About two thirds of the adult US population is overweight (Office of the Surgeon General, 2010). In addition to a positive net energy balance, consumption of dietary fiber in the US is lower than the recommended Dietary Reference Intake. The cereal food industry has been increasing the level of fiber in food products like breakfast cereals and snacks to help reduce the dietary fiber gap. Wheat, corn, and rice are the major grains used in the US, and wheat represents a significant fiber source. However, whole kernel wheat contains only about 12% fiber, limiting its ability to appreciably increase fiber levels. Wheat bran fiber is mainly composed of the hemicellulose arabinoxylan, as well as cellulose and lignins, and bran is much higher in fiber content than whole kernel wheat. However, wheat bran fiber is mostly insoluble, limiting its ability to be added to a food without adversely changing product attributes. Utilization of processes such as hydrolysis to increase the solubility of bran would enable production of a higher fiber food with wheat fiber.

Arabinoxylans from wheat have been hydrolyzed with xylanase into very low molecular weight fibers, and this process has increased the solubility of the fiber (Swennen, Courtin, Lindemans, & Delcour, 2006). Increased solubility facilitates incorporation of arabinoxylan fiber into food, making a higher fiber level possible, while maintaining product texture, flavor and appearance attributes. Hydrolyzed arabinoxylans have been shown to improve gastrointestinal health by increasing bifidobacteria levels and short chain fatty acids after a daily intake of 10 g in a beverage (Francois, et al., 2012). Thus,

hydrolysis of arabinoxylan fiber not only facilitated addition to a beverage, it also resulted in a product with improved nutritional (i.e., prebiotic) value.

Another method to hydrolyze fiber is through processing with heat, shear and moisture. Manufacture of grain-based breakfast cereals typically involves hydration of the grains by cooking them at high temperatures, either using a high shear extrusion process or using a low shear process such as steam pressure cooking. Extensive research has been done on the effects of extrusion processing on oat β-glucan. Tosh et al. showed that increased shear and decreased water in producing extruded cereal caused a depolymerizaton of the oat β-glucan structure, and a lower viscosity cereal (Tosh, et al., 2010). The cereal with reduced β-glucan molecular weight was less effective than cereal with intact β-glucan at reducing LDL cholesterol in humans (Wolever, et al., 2010). In this case, fiber hydrolysis resulted in a product with reduced health benefit because some of that benefit was based on viscosity, a physicochemical property partially dependent on molecular weight. Steam pressure-cooking is a much lower shear process and may not be expected to assert the same depolymerizing effect as extrusion.

The first objective of this study was to characterize the effects of steam pressure-cooking on fiber molecular weight and cereal viscosity of two RTE cereals with 15% added arabinoxylan (hydrolyzed versus unhydrolyzed), and on a third low fiber control RTE cereal. The RTE cereals were exposed to hydration and steam cooking under significantly less shear conditions than typical extrusion processes. A second objective was to characterize how exposure of these three RTE cereals to water would affect their viscous properties.

Materials And Methods

Materials.

The purified hydrolyzed wheat bran arabinoxylan (AXOS) was obtained from Fugeia NV (Belgium). The fiber was enzyme hydrolyzed and purified by a proprietary process utilizing several treatment steps designed to remove the digestible carbohydrates, cellulose, and proteins from the bran yielding a concentrated wheat arabinoxylan (Swennen, Courtin, Lindemans, & Delcour, 2006). This resulting extract was a soluble form of wheat bran, and was available in sufficient quantities to produce RTE cereal on the pilot plant line. Sugar composition of AXOS was 13.9% arabinose, 0.49% galactose, 16.5% glucose, 0.23% mannose, 68.4% xylose (per supplier).

However, an isolated unhydrolyzed wheat bran arabinoxylan was not available in quantities needed for this work. Therefore, a flax seed extract composed of arabinoxylans and rhamnogalacturonan was used instead. The intact fiber (FLAX) was a flax fiber extract obtained from Biogin Biochemicals Co. Ltd. (China). The FLAX was extracted from flax seed mucilage using water and ethanol. Sugar composition of FLAX was rhamnose 21.7%, fucose 5.2%, arabinose 13.0%, xylose 32.3%, galactose 22.8% and glucose 4.5% (per supplier).

Fiber Analysis.

The percent fiber of the AXOS and FLAX was determined by AOAC 2009.01. This method is based on digestion of samples designed to simulate human digestion, and the fibers are then fractionated by ethanol solubility into high and low molecular weight components. This method was selected because it captures low molecular weight

fibers that the traditional dietary fiber method (e.g. AOAC 985.29) does not. Another method (AOAC 2001.03) also captures low molecular weight fibers; however, its digestion method based on AOAC 985.29 applies a thermostable α-amylase at ~95°C for 30 minutes rather than 37°C for 16 hours in AOAC 2009.01.

RTE Flaked Cereal.

RTE cereal was produced by a proprietary process. The main unit operations were steam cooking under pressure, then cooling and drying the cereal, forming pellets, drying and tempering and finally flaking and toasting, all under relatively low shear conditions as compared with extrusion conditions. The cooking unit operation was done in a batch pressure cooker to heat the food to a temperature of 123°C at 117 kPa pressure for 55 minutes to hydrate and cook the grains (Hoseney, 1994). The arabinoxylans were added to the cereal before cooking by replacing the same amount of rice in the formulae (Table 6).

Table 6. Flaked RTE cereal formulae

ingredients (%)	low fiber control	AXOS	FLAX
wheat, soft white, cracked	25.5	25.5	25.5
sugar	12.3	12.3	12.3
liquid malt extract	2.9	2.9	2.9
second head milled rice	57.8	34.7	35.3
salt	1.5	1.5	1.5
AXOS		23.1	
FLAX			22.5
total	100.0	100.0	100.0

Molecular Weight Distribution.

Size exclusion chromatography was used to assess the apparent molecular weight distribution. The molecular weight distribution of the fibers was obtained using Agilent Technologies 1200 series high-performance size-exclusion chromatography coupled to a Wyatt miniDAWN TREOS multiple-angle light scattering detector and a Wyatt OptiLab rEX differential refractometer (SEC-MALS). Two size exclusion columns, SB-802.5 HQ and SB-804 HG, from Shodex were used and kept at 70ºC. The mobile phase was 50 mM sodium nitrate flowing at 0.2 mL/minute with 180 minute run times per injection. Dextran reference materials from Fluka were used for molecular weight calibration on the high pressure liquid chromatography (HPLC) system. Dextran materials had molecular weights of 1,270, 5,000, 48,600, 147,600, and 667,800 g/mol.

Fiber Ingredient Molecular Weight.

A 1% solution of AXOS fiber and a 0.1% FLAX fiber solution were prepared in distilled water, and 100 µL of fiber solution, filtered through a 0.2 µm membrane filter, was injected into the HPLC.

RTE Cereal Fiber Molecular Weight.

Samples were prepared for SEC-MALS analysis after starch and protein digestions (conditions adopted from AOAC 2011.25), followed by a de-salt and de-protein step by mixed ion exchange resins treatment. Ground RTE cereal sample (500 ± 5 mg) was treated with pancreatic α-amylase and amyloglucosidase dissolved in sodium maleate buffer (50 mM, pH 6.0, containing 2 mM $CaCl_2$ and 0.02% sodium azide), and incubated for 16 hours at 37°C. After incubation, the samples were heated to 100°C to inactivate the enzymes, and cooled to approximately 60°C. Samples were treated with protease

solution (*50 mg/mL; approximately 350 tyrosine units/mL, Megazyme)*, followed by an addition of 2M acetic acid, and incubated for 30 minutes. A portion of the sample digest (10 mL) was purified with ion exchange resins and filtered through a 0.2µm syringe filter before injection into the column.

It is very difficult to selectively extract all of the arabinoxylans from the RTE cereals, and as a result there is some loss in fiber recovery, as well as inclusion of other components in the same molecular weight range as the fibers. Thus, a model system was also used to better determine the effects of heat processing on molecular weight of fibers. Solutions of the AXOS and of the FLAX were prepared at 1 g/100 mL. An autoclave was used to simulate the effects of the batch pressure cooker stage of the flaked cereal process at 125°C for 60 minutes at 152 kPa. The molecular weight distribution of the fibers in solution before and after autoclaving was determined by SEC-MALS to measure effects of the heat process on molecular weight.

Viscosity Analysis. A rheometer and a Rapid Visco Analyser (RVA) were used to assess viscosity. The rheometer allows for precise measurements across a wide shear rate, while the RVA enables measurements to be made on samples containing particulates, and with a programmable time temperature profile for the viscosity measurements.

Fiber Ingredient Viscosity.

Viscosity data on AXOS and FLAX fibers were generated using a cup and bob concentric cylinder, at 24˚C, with an ARES G2 controlled strain rheometer. AXOS and FLAX were dispersed in DI water by a magnetic stirrer for at least 30 minutes until no

visible lumps were observed. AXOS was evaluated at 0.5% and 30% up to a shear of 1000 S^{-1} whereas the FLAX was evaluated at 0.5% up to a shear of 100 S^{-1}. The starch from the milled rice ingredient was extracted and evaluated at the same concentrations it was presented in the control (2.31g/ 24 mL) and high fiber (1.31 g/ 24 mL) RTE cereals prepared for RVA measurements. A Rapid Visco Analyzer model RVA-5 with a paddle and cup assembly was also used to assess viscosity.

RTE Cereal Viscosity.

The flakes were ground for 1 minute in a food processor to simulate mastication. Next, the powdered flakes were passed thru a screen-cut between 80 mesh (180 microns) and 170 mesh (90 microns) to ensure viscosity data consistency. Ground flakes (3.5 g) were added to 24.5 g of distilled water, and the viscosity measured with the Rapid Visco Analyzer. The run protocol was 960 rpm for 10 seconds at 30˚C, then at 160 rpm for 3 minutes to increase to 37˚C. Further measurements were at 160 rpm with data collected for 14 minutes at 37˚C. Viscosity data were taken between 8 and 13 minutes where the viscosities were stabilized.

RTE cereals were also exposed to acid and enzyme solutions based on the McCleary method (McCleary B. V., 2007) of fiber sample preparation, prior to measuring viscosity to assess which of the food components in the flakes contributed most to viscosity. This method was designed to remove starch with α-amylase and amyloglucosidase, and to remove protein with acid and pepsin. RTE cereal flakes were ground and screened as outlined earlier, and 8 g was used for each analysis. Viscosity data were taken between 20 and 150 minutes where the viscosities were stabilized.

The amylase/amyloglucosidase solution was prepared as reported by McCleary (McCleary B. V., 2007) and an acid pepsin solution was prepared as reported by Tagliazucchi et al. (Tagliazucchi, Verzelloni, & Conte, 2005). Amylase (50 units/mL) and amyloglucosidase (3.4 units/mL) solution was equilibrated at 37˚C, and 24 mL was added to the cup. The suspension was mixed with a spatula for 2 minutes in a 37˚C water bath, and the viscosity assessed on a model AR 2000 stress control rheometer at a constant temperature of 37°C by a starch pasting cell. The prepared RTE cereal samples were assessed for viscosity at a shear rate at 5 S^{-1} for 150 minutes.

Acid pepsin solution (3.2 g pepsin/7 mL HCl) was diluted with water to 1000 mL, and 16 mL at 37˚C was added to ground RTE cereals and mixed for 1 minute. The prepared RTE cereal samples were assessed for viscosity at a shear rate at 5 S^{-1} for 150 minutes.

Water Retention Capacity.

Water retention capacity, as assessed by a modified AACC Method (56-11), measured the water holding capacity of the RTE cereal. Five grams of ground cereal were weighed into centrifuge tubes to which 25 g of water was added to suspend the cereal. The RTE cereals were allowed to swell 20 minutes, shaking at 5, 10, 15, and 20 minutes. The soaked cereals were centrifuged at 1,000 x g for 15 minutes at 20°C. The supernatant was removed. The cereal pellets were weighed, and the amount of absorbed water was calculated as a percent of sample weight.

Results

Fiber Ingredients.

AXOS and FLAX ingredients contained about 80% total fiber as determined by AOAC 2009.01 (Table 7). As expected, AXOS contained largely low molecular weight components (i.e., fiber fraction soluble in 78% ethanol aqueous solution). FLAX was almost entirely high molecular weight fiber, which was insoluble in the ethanol solution, also as expected given it had not been hydrolyzed.

Fiber Ingredient Molecular Weight.

The molecular weight distributions, as determined by SEC-MALS, of AXOS and FLAX fibers are shown in Figure 10. The distribution data indicated a wide difference in molecular weights between the two fibers. The apparent

Table 7. Fiber analysis of AXOS and FLAX

analysis[a]	AXOS	FLAX
total dietary fiber (%)	79.6	81.3
78% ethanol insoluble fraction (%) (high molecular weight dietary fiber)	13.1	100
78% ethanol soluble fraction (%) (low molecular weight dietary fiber)	86.8	<0.3

[a] Fiber was measured by AOAC method 2009.01. The proportion of high versus low molecular weight fiber is shown based on ethanol solubility and is reported as a percent of the total dietary fiber.

peak molecular weight (g/mol) of AXOS was about 849 versus about 2,888,567 for FLAX. AXOS was predominantly (67%) short-chain polymers with a degree of polymerization (DP) of 3-9, with about 15% mono- and disaccharides (DP<3), and very little (1.4%) above DP 50 (Table 8). FLAX was predominantly (53%) long-chain polymers (DP 4,000-50,000), with a very small fraction below DP 9 (15%).

Fiber Ingredient Viscosity.

A rheometer was used to measure viscosity of these two fibers in solution (Table 9). At a shear rate of 10 S^{-1} and a concentration of 0.5% fiber,

Table 8. Degree of polymerization of AXOS and FLAX

AXOS		FLAX	
range	**%[a]**	**range**	**%**
DP <3	15.5		
DP3-9	67.5	DP <9	14.9
DP10-20	11.8	DP10-300	4.1
DP20-50	3.8	DP300-4000	28.4
DP>50	1.4	DP4000-50000	52.6
total	100	total	100

[a] Calculated from molecular weight distribution data as assessed by SEC-MALS.

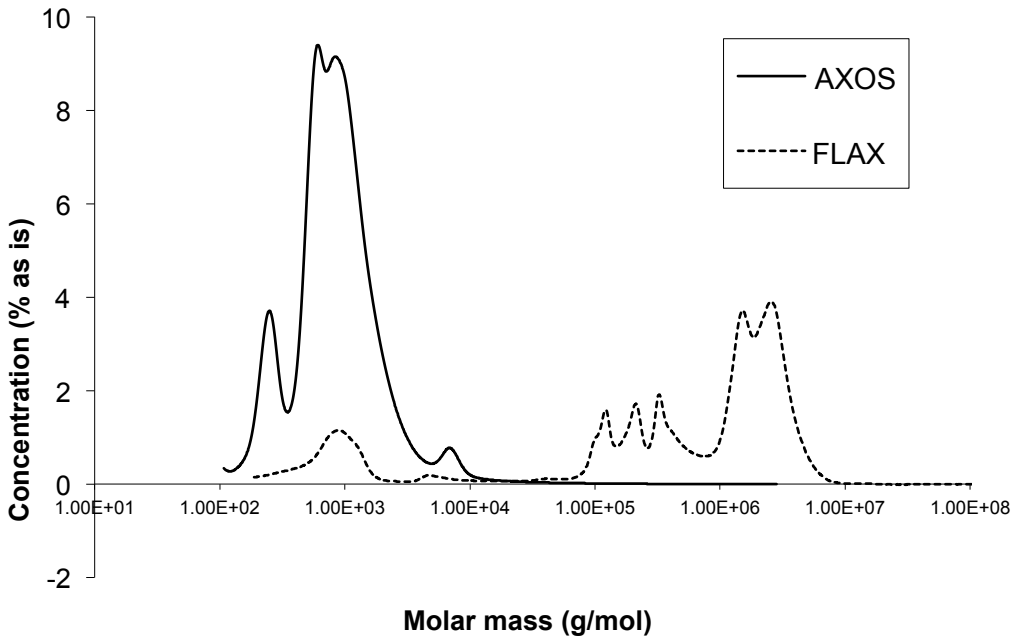


Figure 10. Molecular weight distributions by high-performance size-exclusion chromatography with post column multi-angle light scattering (SEC-MALS) comparing AXOS and FLAX in solution. *The AXOS was measured in a 1% water solution and the FLAX was measured in a 0.1% water solution each in duplicate.*

the viscosity of the FLAX was approximately 400 times higher than AXOS (374 versus 1.03 mPa•s). AXOS exhibited a Newtonian behavior (independent of shear rate) across the shear rates from 10 S^{-1} – 1000 S^{-1}, while FLAX was shear thinning, indicating a non-Newtonian behavior. Viscosity of AXOS was measured at several higher concentrations (10%, 20%, and 30%; only the 0.5% and 30% concentrations are presented).

Table 9. Viscosity of AXOS and FLAX

	0.5% AXOS	**30% AXOS**		**0.5% FLAX**
	***n*=3**	***n*=1**		***n*=2**
shear rate	viscosity[a]	viscosity	shear rate	viscosity
(1/s)	(mPa•s)	(mPa•s)	(1/s)	(mPa•s)
10	1.03 (0.09)	23.36	1.0	1513 (50.0)
16	1.05 (0.05)	23.20	1.6	1106 (12.7)
25	1.02 (0.03)	23.13	2.5	844 (2.6)
40	1.04 (0.02)	23.08	4.0	648 (1.2)
63	1.06 (0.01)	23.04	6.3	493 (0.4)
100	1.09 (0.01)	23.05	10.0	374 (0.1)
159	1.13 (0.00)	23.13	15.9	283 (0.4)
251	1.16 (0.00)	23.29	25.1	213 (0.3)
398	1.21 (0.00)	23.48	39.8	159 (0.6)
631	1.28 (0.00)	23.74	63.1	119 (0.4)
1000	1.37 (0.00)	24.04	100.0	89 (0.3)

[a] Standard error of the mean is shown in parenthesis. The number or replicates (*n*) is indicated. Measurements were taken in solution at 24˚C with an ARES G2 controlled strain rheometer[a].

The viscosity increased at higher concentrations across the shear rate range, exhibiting the same non-Newtonian behavior. Higher concentrations were not feasible with FLAX given its extremely viscous nature. Viscosity of AXOS and FLAX was also measured in a Rapid Visco Analyzer (RVA) (Figure 11). A comparison of viscosities in water and in acid solution are shown. The acid was used to estimate viscosity in the gastric environment. The FLAX had a much higher viscosity than the AXOS in both water and in acid. FLAX was about 16 times more viscous than AXOS in water, and about 22 times more viscous than AXOS in acid.

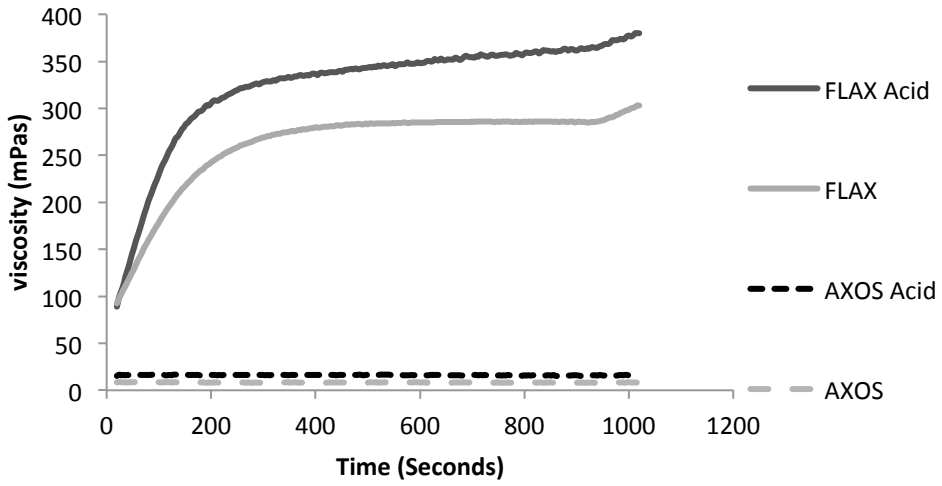


Figure 11. Viscosity measurement on a Rapid Visco Analyzer (RVA) of AXOS and FLAX at 1% solution in water and in 0.08M HCl*, in duplicate.*

RTE Cereal.

Two separate methods for fiber analysis were completed on the 3 RTE cereals formulated to have 4% fiber included in the control RTE cereal, and an additional 15% fiber from AXOS or FLAX in the high fiber RTE cereals. Both methods yielded similar results, control RTE cereal contained ~6% fiber and the two test cereals contained ~21% fiber (Table 10).

RTE Cereal Fiber Molecular Weight.

Most of the fiber extracted from processed RTE cereal made with AXOS was low molecular weight fiber (Table 10). The two methods yielded similar results for the low molecular weight fraction of the total dietary fiber (76% by AOAC 2009.01 and 75% by AOAC 2001.03). This is consistent with data in Table 7, which shows the AXOS fiber to

be mostly low molecular weight fiber (~87%). These results suggest that the RTE cereal process did not affect the molecular weight of the AXOS.

In contrast to what was observed with AXOS during processing, both fiber analysis methods showed a consistent and unexpected difference in fiber molecular weight from FLAX RTE cereal. FLAX was a high molecular weight fiber ingredient (Table 7, Table 8, and Figure 10), but after processing FLAX into RTE cereal, the FLAX was recovered mostly as a low molecular weight fiber (Table 10). After processing, the FLAX RTE cereal was now similar to the AXOS RTE cereal in terms of the ratio of high to low molecular weight fibers (Table 10). Size exclusion chromatography of the fiber extracted from the cereal also indicated that the molecular weight distributions of fibers from AXOS and FLAX RTE cereals were very similar (Figure 12). These results suggest that the RTE cereal process reduced the FLAX fiber molecular weight.

In a simulation of the batch pressure cooking stage of RTE flaked cereal process, FLAX fiber was dissolved in water and heated in an autoclave. Molecular weight distributions of the FLAX by SEC-MALS before and after heating, showed that some of the high molecular weight material shifted to lower molecular weight after this heat treatment (Figure 13).

RTE Cereal Viscosity.

The 3 RTE cereals were ground and assessed for viscosity by RVA in water (Figure 14). Unexpectedly the highest viscosity was observed with the control RTE cereal (662 mPa•s); and the addition of either FLAX (145 mPa•s) or AXOS

Table 10. Fiber analysis of RTE cereals

analysis	Control flake (*n*=1) %	AXOS flake (*n*=1) %	FLAX flake (*n*=2) %
McCleary [a]			
total dietary fiber	5.9	20.2	19.8 (0.4)
78% ethanol insoluble fraction (%) (high molecular weight dietary fiber)	3.6	4.9	6.1 (0.1)
78% ethanol soluble fraction (%) (low molecular weight dietary fiber)	2.4	15.3	13.7 (0.3)
Fibersol [b]			
total dietary fiber	5.4	21.9	21.0 (1.0)
78% ethanol insoluble fraction (%) (high molecular weight dietary fiber)[c]	3.3	5.4	7.6 (0.7)
78% ethanol soluble fraction (%) (low molecular weight dietary fiber)	2.1	16.5	13.5 (0.4)

[a]Results from the McCleary fiber analysis method (AOAC method 2009.01) versus Fibersol method (AOAC 2001.03) are compared. Standard error of the mean is shown in parenthesis. The number of replicates (*n*) is indicated.

[b] As determined by the sum of AOAC 985.29 and low molecular weight dietary fiber.

[c] As determined by AOAC 985.29.

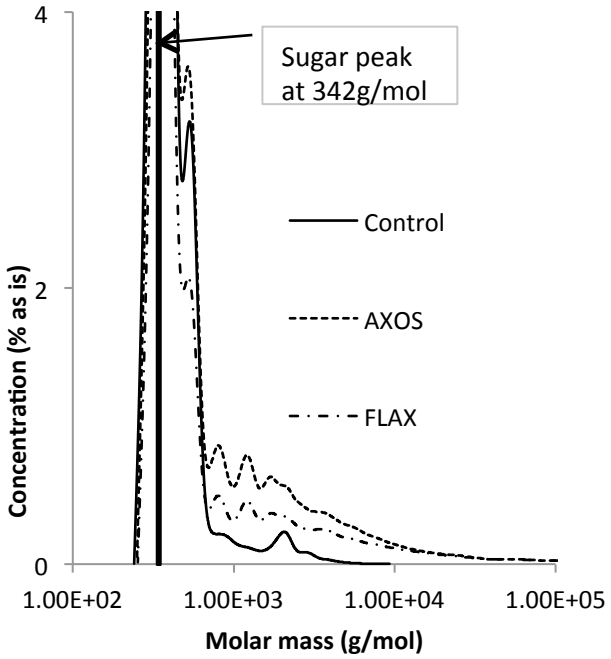


Figure 12. Molecular weight distributions of fibers extracted from control RTE cereal and test cereals made with AXOS and FLAX assessed by high-performance size-exclusion chromatography with post column multi-angle light scattering (SEC-MALS).

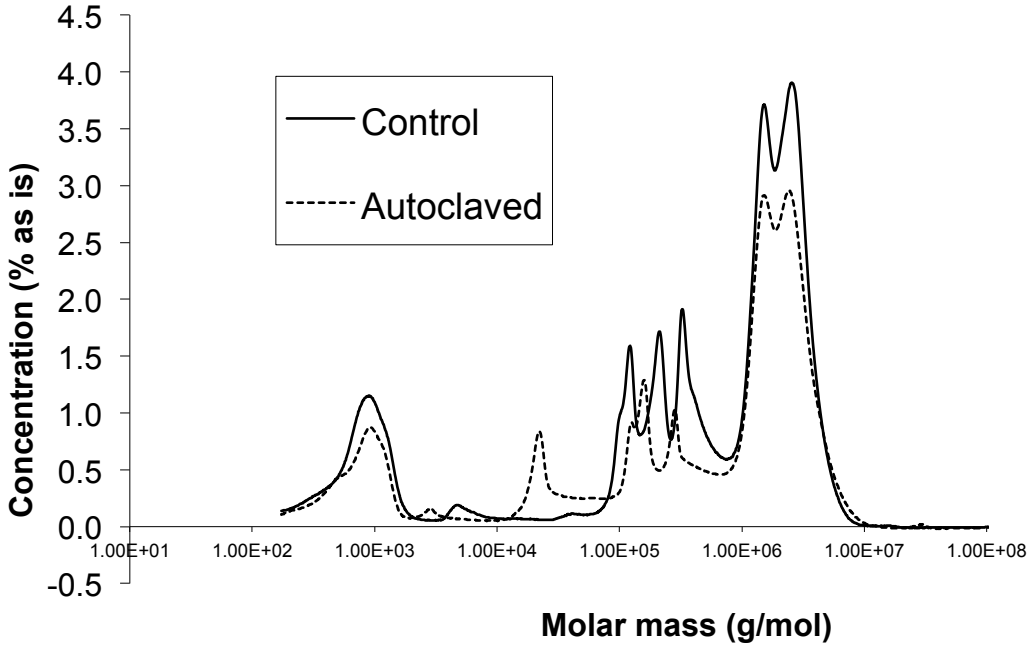


Figure 13. Molecular weight distributions by high-performance size-exclusion chromatography with post column multi-angle light scattering (SEC-MALS) for FLAX before and after autoclaving at 125°C for 60 minutes.

(69 mPa•s) at the expense of an equal amount of rice reduced the slurry viscosity. In acid solution (data not shown), a similar viscosity order was observed among the RTE cereals (549, 137, and 65 mPa•s for control, FLAX, and AXOS RTE cereals, respectively). The viscosities of all three RTE cereals were lower in acid than in water, presumably due to the effect of pH on behavior of the charged molecules such as protein in the matrix.

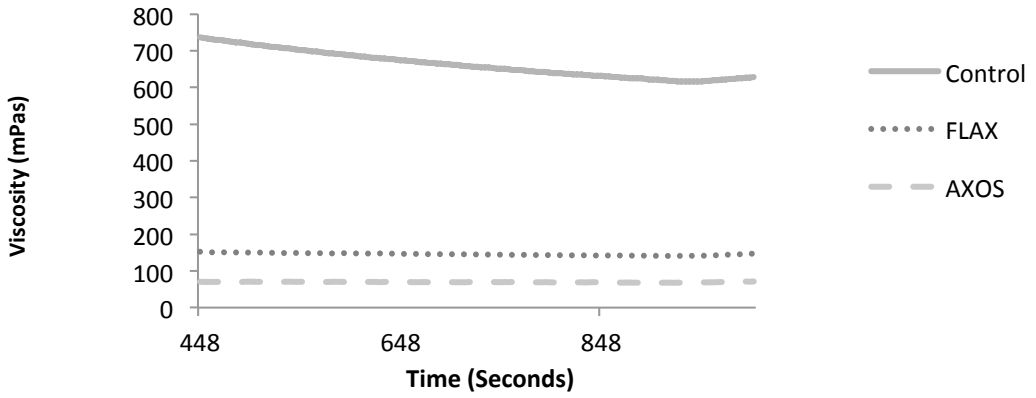


Figure 14. Viscosity measurement with Rapid Visco Analyzer (RVA), on control, AXOS and FLAX RTE cereal. *Cereals were ground and suspended in water (14 g/100 g water), data in triplicate.*

Table 11 details the effects of sample preparation using enzymes and acid to remove starch and protein components from the flakes, similar to the sample analysis in AOAC 2009.01. Enzymes, in water and in acid, were evaluated for their effects on viscosity as assessed by a stress-controlled rheometer. The control RTE cereal had the highest viscosity in water, followed by FLAX and AXOS RTE cereals. The α-amylase/ amyloglucosidase in combination hydrolyzed the available starch to glucose, and resulted in a large drop in viscosity in all three cereals indicating that starch was a large contributor to cereal viscosity. Viscosity of the rice starch (6,982 ± 85 mPa•s) was

measured after gelatinization, and at the same concentration that it was present in the RTE control cereal. The viscosity was almost as high as the RTE control cereal (8,976 mPa•s) shown in Table 11, again indicating starch was a major contributor to cereal viscosity. The viscosity of the rice starch at a concentration equal to that of the fiber in RTE cereals was 517 ± 9 mPa•s by comparison. The viscosity of all 3 RTE cereals did not change in acid and pepsin solution relative to water, suggesting that proteins in the cereals are not contributing to viscosity at low pH.

Table 11. Flake viscosity (mPa•s) in water, amylase/amyloglucosidase solution, and acid pepsin solution

RTE cereal	water alone (mPa•s) *n*=2	amylase solution (mPa•s) *n*=2	acid pepsin solution (mPa•s) *n*=2
control[a]	8,976 (209)	42 (4)	8,155 (530)
AXOS	2,927 (234)	30 (3)	2,590 (61)
FLAX	5,461 (416)	40 (1)	5,033 (52)

[a]Standard error of the mean is shown in parenthesis. The number of replicates (n) is indicated.

RTE Cereal Water Retention Capacity.

Consistent with the viscosity results where the control had a higher viscosity than the high fiber containing RTE cereals, the control RTE cereal also held significantly more water (almost 4X its weight) than the two high fiber containing RTE cereals (Table 12). The control RTE cereal had twice as much starch as the high fiber RTE cereals (14% versus 7%), which might account for the higher water retention capacity, and the higher

viscosity. The FLAX RTE cereal water retention capacity was about 20% higher than for the AXOS RTE cereal. Although FLAX had a reduced molecular weight after cereal processing, the molecular weight was still higher than AXOS and this may have contributed to more water holding capacity.

Table 12. Water retention capacity of RTE cereals

RTE cereal	% water retention capacity mean [a] (± SEM)
control	379 (0.7)
AXOS	204 (0.3)
FLAX	248 (4.5)

[a] Expressed as the percent of the sample weight retained as water. Standard error of the mean is shown in parenthesis. Data from triplicate measurements.

Discussion

Wheat bran fiber is mostly insoluble, which makes it difficult to add to a food in high amounts without adversely affecting product attributes. One approach to increasing the level of wheat fiber in food is to hydrolyze insoluble fiber to more soluble forms through processing. This study was designed to determine the impact of a low shear steam pressure-cooking process on soluble fiber molecular weight and viscosity of a RTE cereal to better understand the implications of processing on fiber physicochemical properties important to nutritional benefits. The present study demonstrated a reduction in soluble fiber molecular weight resulting from the relatively low shear steam pressure-

cooking process we used. Unexpectedly, FLAX containing RTE cereal had an even lower viscosity than the low fiber control RTE cereal.

The molecular weight of the AXOS and FLAX ingredients were characterized before processing (Figure 10). AXOS DP was similar to results presented by Francois et al. (Francois, et al., 2012). They found an estimated average molecular weight of 660 g/mol based on DP of 5, compared to our measurements with 68% of AXOS between DP 3-9 (396-1188 g/mol), primarily DP 4 and 6 (528 and 792 g/mol, respectively) (Table 8). Cui *et al.* used size exclusion chromatography to determine molecular weight for flax (Cui, 1994). Although their column resolution was less than ours, they demonstrated that most of the flax had a molar mass greater than 466,000 g/mol, which is consistent with what we found (72% > 528,000 g/mol) (Figure 10). Warrand et al. identified a fraction of flax fiber at about 5,000,000 g/mol (Warrand, et al., 2005) also consistent with what we found (2,900,000 g/mol).

High shear processes such as extrusion, ultrasonication, and jet cooking in combination with heat, have been shown to decrease molecular weight of several soluble fibers, i.e. oat β-glucan, and dextran (Aman, Rimsten, & Andersson, 2004; Tosh, et al., 2010), (Wolever, et al., 2010; Cote, 1999). However, very few studies have investigated effects of low shear steam pressure-cooking on soluble fiber, particularly on arabinoxylans from flax and wheat. We extracted AXOS and FLAX from the RTE cereals after low shear processing to assess changes in molecular weight. Since it is possible that extraction of the fiber from RTE cereal samples might have affected the fiber molecular weight results, we used several methods, as well as a model system, to compare changes.

Ethanol solubility of carbohydrates is related to their molecular weight, with increasing solubility as an indication of lower molecular weight. Our findings showed a major shift in solubility after processing (Table 7, Table 10). Both AXOS and FLAX fiber showed an increase in solubility, with the FLAX changing the most significantly. SEC-MALS data also indicated that there was little high molecular weight fiber recovered after processing (Figure 12). SEC-MALS data from the model system study showed a similar shift. Each method supported a similar conclusion, that the steam pressure-cooking unit operation decreased the molecular weight of flax arabinoxylans studied in RTE cereals.

The viscosity of the AXOS and FLAX ingredients were also characterized before processing. Since AXOS was extensively hydrolyzed, the low viscosity of a 0.5% solution (1.03 mPa•s) (Table 9) was consistent with expectations. There are several reported results for flax viscosity at concentrations used in this study. Under comparable conditions, Cui et al. reported 300 mPa•s (Cui, 1994), for flax slightly lower than what we found (374 mPa•s). Mazza et al. and Qian, et al. used a different instrument than ours to measure viscosity of various fractions of flax extracts and observed lower viscosities than our study (Mazza & Biliaderis, 1989; Qian, Cui, Wu, & and Goff, 2012), although all studies reported the same shear thinning behavior. Extraction procedures have also been shown to have an impact on the resulting viscosities as demonstrated by Wang et al. (Wang, Wang, Li, Xue, & and Mao, 2009) and might account for these differences as well.

Viscosity is a physicochemical property partly dependent on molecular weight of the fiber. Since we observed a molecular weight change in the FLAX, a lower viscosity

after processing is then not surprising. The viscosity of the FLAX ingredient was substantially higher than AXOS as measured by rheometry (374 versus 1.03 mPa•s) (Table 9), and by RVA (285 versus, 8 mPa•s) (Figure 11) before processing. However, the magnitude of viscosity differences between the RTE cereals containing FLAX and AXOS by rheometry (5,461 and 2,927 mPa•s, respectively) (Table 11), and by RVA (145 versus 69 m.Pas, respectively) (**Figure 14**) was much less. These results were anticipated based on our molecular weight measurements, further supporting physical changes in the fiber.

Surprisingly, the most viscous RTE cereal was the control low fiber cereal (8,976 mPa•s) as measured by rheometry (Table 11), and by RVA (687 mPa•s) (**Figure 14**). Results measured by rheometry from the digestion study indicated that when amylase treatment reduced the starch components in the cereal, its viscosity in water was markedly reduced as well. The viscosity of all three cereals dropped to ~40 mPa•s after exposure to the amylase treatment. Treatment with acid and pepsin (to hydrolyze protein) resulted in similar viscosities to water alone. Starch from milled rice was heated to gelatinize the starch, and its viscosity was compared with AXOS both at 2.31 g /24 mL water. The viscosity, as measured by RVA, of rice starch was 6,892 versus only 6.3 mPa•s for AXOS. The greater starch content of the low fiber control RTE cereal is the major contributor to the higher viscosity in the control RTE cereal versus the higher fiber cereals. Water retention capacity (Table 12) of the control high starch containing RTE cereal was also greater than for the 2 fiber containing cereals. The gelatinized starch component of the flaked RTE cereal appears to hold more water and affect the viscosity more than the fiber component. These high fiber RTE cereals may

not contribute significantly to gastric viscosity relative to a low fiber control as previously thought.

Several studies by Tosh et al. demonstrated that a reduction in molecular weight of oat β-glucan can occur in extruded cereal, and that this is associated with a reduction in viscosity of the cereal. This physicochemical property impaired oat β-glucan to lower LDL cholesterol, and thus its health benefit. Reduction of FLAX fiber molecular weight seen in this study was not expected under lower shear steam pressure-cooking conditions used, and might have an impact on its nutritional value.

Fiber viscosity is believed to influence satiety by affecting gastric emptying, or by delaying nutrient absorption in the gastrointestinal tract affecting hormone production that reduces the perception of hunger (Kristensen & Jensen, 2010). Results from this study suggest that soluble viscous fiber in flaked RTE cereals made using steam pressure cooking, may not have a high viscosity as previously thought, and might be partially responsible for the wide variation of results in satiety studies using flaked cereals as seen in the literature (Howarth, Saltz, & Roberts, 2001). Further work should be done to assess the effects of processing on fiber physicochemical properties to better interpret clinical trial results using viscous soluble fibers.

CHAPTER 3 - Effects of Two Dietary Fibers as Part of Ready to Eat Breakfast Cereals on Perceived Appetite and Gut Hormones in Overweight Women

Abstract

This study was designed to evaluate the effects of an enzyme hydrolyzed arabinoxylan from wheat (AXOS) versus an intact arabinoxylan from flax (FLAX) added to a ready to eat (RTE) cereal on perceived appetite, on intake of a subsequent meal, and on satiety hormone responses in overweight women (BMI 25.0-29.9 kg/m^2). Two randomized, double-blind, placebo controlled, crossover design studies were completed. RTE cereal breakfasts with 4 g fiber versus an additional 15 g of either AXOS or FLAX in 100 g RTE cereal were prepared with 2% milk, and diluted with water to standardize the total weight consumed (500 g) for each breakfast meal. An additional low-fiber control (70 g RTE cereal) matched to the same energy as the high-fiber breakfasts provided an iso-caloric comparison in trial 2. Perceived appetite assessment was completed before and after breakfast, at specific times, throughout the 270 min testing day. An ad libitum lunch meal was offered 4 hours after the breakfasts. In addition to the previous procedures, blood samples were collected throughout the testing day in trial 2 for assessments of plasma ghrelin, GLP-1, PYY, glucose, and insulin concentrations. Results in both trials indicate no differences in perceived appetite for the low-fiber breakfasts versus the high-fiber breakfasts. No differences were observed in lunch meal energy intake after all breakfasts for either trial, thus the lower energy density of the high-added fiber RTE cereals was not compensated for at lunch. Both high-fiber

breakfasts increased GLP-1 and PYY plasma concentrations (hormones known to increase satiety) relative to the low-fiber iso-caloric control RTE cereal, possibly due to fermentation and production of short chain fatty acids. Fifteen grams of low molecular weight fiber added to RTE cereal did not affect perceived appetite or energy intake at lunch despite differences in satiety hormone signaling in overweight females. The subjects in this study may not have been sensitive enough to the satiety hormone signaling cues to affect perceived appetite or lunch energy intake.

Introduction

Obesity is increasing in the US and globally, and has become a major health concern in most developed countries (Office of the Surgeon General, 2010). In response to the ongoing obesity epidemic, food companies are reducing the caloric content of their product offerings by reducing caloric density and serving size. Addition of fiber containing ingredients to a processed food is an important approach the food industry is using to help reduce caloric density, and thus overall calories consumed. The addition of fiber may have other benefits relating to digestive health, coronary heart disease, cholesterol lowering, attenuation of glucose concentrations, and appetite reduction (Jenkins, 2000; Eastwood & Morris, 1992; Regand, Tosh, Wolever, & Wood, 2009; Kristensen, 2013).

Over the last 20 years there have been many studies examining the role of increased dietary fiber intake on satiety and weight loss. A link between fiber and satiety was proposed as early as 1987 (Blundell & Burley, 1987). Since that time there has been a significant amount of research relating fiber to satiety (Howarth, Saltz, & Roberts, 2001; Pereira & Ludwig, 2001). Dosages of fiber used have varied considerably, with more consistent satiety effects above 10 grams at a single eating occasion. However, mean daily fiber intake for adult women in the U.S. is only slightly above this threshold (about 14 g/d) (US Department of Agriculture Agricultural Research Service, 2010) and only approximately 50% of the recommended 25 g/day (Institute of Medicine of the National Academies, 2005). Increased fiber intake is needed for satiety benefits and to meet the recommended daily intake.

It is difficult to incorporate fiber sources into existing breakfast cereal food products without changing desirable food texture and flavor characteristics. Fiber may be added either as part of an ingredient, or as an isolated fiber (Oriz & Lafond, 2012). When fiber is added to food as part of an ingredient such as wheat bran, other components in that ingredient can contribute color, flavor, and texture, as well as additional calories to the finished food. In the case of wheat bran, these components would include starch and protein from the endosperm included in the bran mill fraction. An isolated fiber would contribute significantly fewer calories to the cereal, and less change to food properties.

Isolated fibers are generally classified as insoluble or soluble in water, and this property is influenced by the chemical structure of the fiber (Dikeman & Fahey, 2006). High molecular weight linear fibers such as cellulose are insoluble; however, high molecular weight fibers that are highly branched, or low molecular weight fibers, are generally soluble (Tungland & Meyer, 2002). Addition of insoluble fibers to a breakfast cereal will increase the amount of water needed to process it, water that ultimately must be removed, adding process complexity and costs. Viscous soluble fibers added to cereals may increase the viscosity of the dough stage, making it harder to process through the unit operations needed to make the cereal (extrusion, mixing, pumping, drying) (Oriz & Lafond, 2012). One approach to reducing viscosity of soluble fibers is to hydrolyze them into shorter polymers, which then avoids these process issues.

Novel concentrated fibers are being created by reducing their molecular weights through enzymatic hydrolysis, or by chemical modifications of existing fibers, to facilitate their addition to food, as well as to minimize effects on finished product (Liu, 2010; Swennen, Courtin, Lindemans, & Delcour, 2006). Overweight women that are conscious

of their body shape, and desire to lose weight, are seeking food solutions. To help these consumers, the food industry is customizing foods that are lower in calories and higher in fiber content, many containing novel fibers (Slavin & Green, 2007; Hiza, 2007). Viscous high molecular weight soluble fibers added to beverages have been shown to increase satiety (Wanders, et al., 2011). Although having optimized food quality characteristics, it is not certain whether lower molecular weight modified fibers will still have beneficial effects on satiety.

Two clinical trials were undertaken to evaluate the effects of a hydrolyzed low molecular weight wheat arabinoxylan fiber as part of a ready to eat (RTE) cereal on subjective appetite and on energy intake at a subsequent meal in overweight women. The first trial compared this RTE cereal to a high molecular weight fiber containing RTE cereal, and to a low-fiber control RTE cereal. Weight of food consumed is a factor in appetite measurements due to gastric distention (Howarth, Saltz, & Roberts, 2001; Kristensen & Jensen, 2010). To keep the weight of the diets the same, the control cereal by necessity had more energy, because adding fiber reduced the energy density of the RTE cereals (fiber replaced milled rice in the high-fiber RTE cereals). Weight of breakfast (100 g RTE cereals with 400 g of 2% milk/water), physical density, solid/liquid ratio, texture, protein, total carbohydrates and fat were held constant among diets so they would not impact appetite assessment.

The second trial was designed to evaluate subjective appetite in conjunction with blood hormone and glucose responses. An additional control provided a comparison between the low-fiber RTE cereal used in trial 1 (control A; 461 kcal), and a low-fiber RTE cereal

matched to the energy in the high-fiber breakfasts. This was accomplished by reducing the amount of cereal consumed from 100 g to 70 g (control B; 341 kcal).

Materials and Methods

Subjects

For both trials, subjects were overweight women, 18-29 years of age, each with body mass index (BMI) of 25.0-29.9 kg/m^2, inclusive. Exclusion criteria included recent weight loss of > 4.1 kg within 4 weeks of screening visit; history or presence of cancer, renal, hepatic, endocrine (including diabetes mellitus), pulmonary, biliary, gastrointestinal, pancreatic, or neurologic disorders; recent use of any weight loss drugs; weight-reducing surgery or a diagnosed eating disorder; and pregnancy or planning to become pregnant during study period. Subjects were regular consumers of breakfast cereal and did not dislike macaroni and cheese.

Subjects were recruited through approved advertisements, flyers, and emails. Before starting the trial, all applicants completed an assessment for inclusion in the study. This included body weight, vital signs, evaluations of inclusion/exclusion criteria, concomitant medication use, a first day of last menses query, as well as medical history, Eating Habits Questionnaire (to exclude unusual eating patterns), in-clinic urine pregnancy test, and Vein Access Scale assessments. Signed written informed consent for participation in the study was obtained from all subjects before protocol-specific procedures were carried out. Subjects were informed of their right to withdraw from the study at any time. These trials were conducted according to Good Clinical Practice Guidelines, the Declaration of Helsinki (2000), and US 21 CFR. The trials were

approved by Quorum Review IRB (25787/1), an appropriately constituted Institutional Review Board in accordance with the requirements of 21 CFR 56. For trial 1 and 2, 30 and 36 subjects, respectively were recruited and completed the studies. Five subjects participated in both trials. Women were only tested during the luteal phase of their menstrual cycles in order to improve sensitivity of satiety testing (Both-Orthman, 1988; Davidsen, Vistisen, & Astrup, 2007). Therefore, before starting each treatment visit, subjects again underwent assessments of body weight, vital signs, evaluations of inclusion/exclusion criteria, concomitant medication use, and a first day of last menses query. For trial 1, treatment visits were a minimum of 7 days apart and for trial 2, visits were a minimum of 4 days apart to enable more treatment visits within the luteal phase of the menstrual cycle and because 4 days was considered to be a sufficient period for wash out of any carry over from the previous treatment.

Diets

Wheat bran is a major component of some high-fiber breakfast cereals with arabinoxylans being the predominant fiber in wheat bran (Khan & Shewry, 2009). The hydrolyzed fiber included in this study was a wheat bran arabinoxylan extract (AXOS), enzyme hydrolyzed and purified by a proprietary process and obtained from Fugeia N.V. (Belgium). Several treatment steps designed to remove the digestible carbohydrates, cellulose, and proteins from the bran yielded a concentrated wheat arabinoxylan (Swennen, Courtin, Lindemans, & Delcour, 2006) with a degree of polymerization between 3 and 9. The percent fiber in the hydrolyzed AXOS was determined by AOAC method 2009.01 and was 79.6% (Lafond, Jin, Cho, & Romsos, 2014).

Since an isolated unhydrolyzed wheat bran arabinoxylan was not available in quantities needed for this work, a flax seed extract composed of arabinoxylans and rhamnogalacturonan was used. The intact fiber (FLAX) was a flax fiber extract obtained from Biogin Biochemicals Co. Ltd. (China). The FLAX was extracted from flax seed mucilage using water and ethanol. The percent fiber in the FLAX was determined by AOAC method 2009.01 and was 81.3% (Lafond, Jin, Cho, & Romsos, 2014).

RTE Flaked Cereal

RTE cereal was produced by a proprietary process in the Kellogg Company pilot plant. The main unit operations were cooking under pressure, then cooling and drying the dough, forming pellets, drying and tempering, and finally flaking and toasting. Cooking was done in a batch pressure cooker to heat food to a temperature of 123°C and 117 kPa pressure to hydrate the grains. The nutrient composition of the cereals is shown in Table 13 and the formulae are referenced (Lafond, Jin, Cho, & Romsos, 2014).

In trial 1, subjects consumed 100 g of one of three RTE cereals with 2% milk on three separate days in random order. The milk was diluted with water to standardize the weight consumed (500 g/ breakfast meal). The target level of test fiber desired was 15 g per breakfast meal based on variation in perceived appetite responses observed in the literature at dosages less than 15 g (Howarth, Saltz, & Roberts, 2001; Pereira & Ludwig, 2001). The maximum quantity of test fiber which could feasibly be incorporated into the cereal and ensure that all products had similar properties was 15%, thus the amount of RTE cereal required to deliver the target fiber was 100 g (approximately 3 times a typical RTE cereal labeled serving size). The amount of liquid (milk and water) added to the test cereals was also is approximately 3 times the amount recommended for a

typical RTE cereal labeled serving size. One hundred grams of the high fiber RTE cereals provided an additional 15 g of dietary fiber from AXOS (345 kcal) or FLAX (347 kcal) versus control A RTE cereal (4.0 g fiber, 461 kcal).

In trial 2, subjects consumed of one of four RTE cereals with 2% milk. Again the milk was diluted with water to standardize the weight consumed (500 g each breakfast meal). Control A, AXOS, and FLAX RTE cereals were the same as in trial 1 and all were consumed on four separate days in random order. The fourth breakfast meal was a low-fiber RTE cereal, energy matched to the high fiber RTE cereals by reducing the amount of low fiber cereal from 100 g to 70 g (control B; 341 kcal).

Cereal Flake Texture and Sensory Measurements

RTE cereal (59 mL) was placed in a Kramer shear press and peak force (g) was assessed using TX.XT Plus Texture Analyzer with 50 kg load cell at a test speed of 2 mm/s. For milk soaked samples, RTE cereal (59 mL) was placed into 52 mL of 2% milk for 7 minutes. The soaked sample was assessed at a speed of 5 mm/s with a square probe. A trained panel of 8 people also evaluated the three RTE cereal samples for texture attributes in a sequential monadic fashion both at first bite and after chewing (<15 times). The data were collected by panel consensus (Meilgaard, Civille, & Carr, 1999).

Table 13. Nutrient composition of RTE cereals

RTE Cereals	Low Fiber Control A	Low Fiber Control B	AXOS	FLAX
Flaked Cereal (g)	100	70	100	100
Water (g)	220	260	200	200
2 % Milk (g)	180	170	200	200
Mass (g)	500	500	500	500
Total Carbohydrate (g)	95	69	82	82
Fiber (g)	4	3	19	19
Protein (g)	13	11	12	12
Lipid (g)	5	4	5	5
Calories (kcal)	461	345	345	347

Clinical Trial Design

Clinical trials 1 and 2 were both randomized, double-blind, placebo-controlled, crossover trials. In both trials, subjects fasted 9-13 hours before the start of RTE cereal product consumption (t = 0 min). Subjects recorded all foods and beverages consumed after 1400 h the day prior to the first treatment visit. These records were collected and reviewed, and subjects were provided a copy of the record and instructed to replicate the same diet from 1400 h the day prior to each subsequent treatment visit. Following the overnight fast, the subjects randomly consumed, on separate days, one of the breakfasts. Perceived appetite was assessed before and after breakfast at specific times throughout the 4 ½ h testing day. After the 120 min rating, subjects were allowed 200 mL of water. The amount of water consumed was recorded, and subjects were asked to consume a similar amount of water at subsequent treatment visits.

In addition to the previous procedures, trial 2 also included blood sample collection. An intravenous catheter was inserted in the forearm for collection of venous blood. To

maintain patency of the intravenous catheter, the catheter was flushed with 10 mL normal saline solution hourly. A baseline blood sample to assess active ghrelin (acylated form; n-octanoic acid on serine 3), active glucagon-like peptide-1 ($GLP\text{-}1_{7\text{-}36}$), total PYY ($PYY_{3\text{-}36} + PYY_{1\text{-}36}$), glucose, and insulin was drawn at t = -10 ± 5 min. Subsequent blood samples were collected at t = 15, 30, 45, 60, 90, 120, 180 and 240 min ± 5 min.

Ad libitum Lunch

A macaroni and cheese lunch (40 oz.) was provided in coded ceramic pots to subjects at 240 minutes after the breakfast meal (Stouffer's Family Size 40 oz., Nestles U.S.A., Solon OH). Subjects ate directly from the pots and were provided with a standard amount of water during lunch. The quantity of water consumed was recorded. Subjects were allowed 25 minutes for lunch and were instructed to eat until comfortably full. Food was weighed prior to and following consumption, and energy intake was assessed based on nutrition facts panel data (Total Fat 17.0 g, Carbohydrates 30.0 g, Dietary Fiber 2.0 g, Sugars 4.0 g, Protein 15.0 g, 330 kcal/225 g).

Appetite Measurements

Perceived appetite was assessed using a visual analogue scale (VAS). The scale was 100 mm in length with descriptions expressing the most positive and most negative ratings for desire to eat, hunger, fullness, and prospective consumption. Subjects placed a mark crossing the line connecting descriptors and the distance in mm from the left side was used as the rating. Perceived appetite was assessed prior to breakfast meal consumption (t = -15 and -5 min). At t = 0 minutes, subjects consumed one of the RTE cereals between 0700 and 1000 h. Subjects were allowed 15 minutes to consume

breakfast meal. Subsequent VAS ratings were completed by the subjects at t = 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, 180, 195, 210, 225, 240, and 270 min. A Consolidated Appetite Score was calculated as follows [desire to eat + hunger + (100 – fullness) + prospective consumption]/4]. This was done to minimize possible variability in scoring due to individual interpretation of specific rating scales. Net incremental area under the curve (niAUC) was calculated using the trapezoid method for 2 hours and 4 hours.

Hormonal Analyses

Blood was collected in test tubes containing EDTA and protease inhibitors (AEBSF and DPP-IV) to reduce protein degradation. Within 10 min of collection, samples were centrifuged at 4°C for 10 min. The plasma was separated and stored at -80°C for future analysis. Plasma active ghrelin (acylated form; n-octanoic acid on serine 3), active GLP-1 ($GLP\text{-}1_{7\text{-}36}$), total PYY ($PYY_{3\text{-}36} + PYY_{1\text{-}36}$), and insulin, were prepared using the Milliplex MAP magnetic bead-based multi-analyte, metabolic panel, 4-plex immunoassay (HMHMAG-34K; Millipore, St. Charles, MO) and measured using Luminex Magpix with xPONENT software (Luminex Corporation, Austin, TX). Intra- and inter-assay CV were 2 and 8%, 7 and 10%, 2 and 11%, and 3 and 6% for active ghrelin (acylated form), active GLP-1 ($GLP\text{-}1_{7\text{-}36}$), total PYY ($PYY_{3\text{-}36} + PYY_{1\text{-}36}$), and insulin, respectively. The detection limit of the assay was 2, 7, 8, and 58 pg/ml for active ghrelin (acylated form), active GLP-1 ($GLP\text{-}1_{7\text{-}36}$), total PYY ($PYY_{3\text{-}36} + PYY_{1\text{-}36}$), and insulin, respectively. Plasma glucose was measured with a glucose oxidase assay (Thermo Fisher Scientific, USA). Intra- and inter-assay CV for glucose were 2 and 5%. The detection limit of the assay was 1.0 mg/dL.

Gastrointestinal Tolerability

Assessment was by self-reported scoring on a 6-point scale with 0 being not experienced, and 5 being most severe experience for each attribute. Five different gastrointestinal symptoms were measured (cramping, flatulence, gas/bloating, loose stools, and nausea).

Statistics

VAS Appetite Scores: All values are reported as means with their respective standard errors. Statistical analyses were conducted using SAS for Windows (version 9.1.3 or higher, Cary, NC). Descriptive statistics are presented for the outcome parameters for each RTE cereal. Response differences among RTE cereals were assessed using repeated measures analysis of variance (ANOVA) including subject as a random variable, and test diet as a fixed effect. The model was reduced until only test diet and any significant ($p < 0.05$) terms remained. Pairwise comparisons between all treatment conditions were conducted using Tukey's adjustment for multiple comparisons.

All tests of significance, unless otherwise stated, were performed at alpha = 0.05, two-sided. Assumption of normality of residuals from the final model of each outcome parameter was investigated by the Shapiro-Wilk test (Shapiro & Wilk, 1965).

Hormones and Glucose: All values are reported as means with their respective standard errors. Statistical analyses were conducted using PROC MIXED in SAS for Windows (version 9.2 Cary, NC). Repeated measures over time were modeled with a heterogeneous banded (Toeplitz) covariance structure using the repeated statement of SAS. For all main effects, significance was declared at $P<0.05$, and tendencies were

declared at P<0.1. For interactions, significance was declared at P<0.1 and tendencies were declared at P<0.15. Descriptive statistics are presented for the outcome parameters for each test diet using least squares means. Response differences among breakfast meals were assessed for each time point using mixed model analysis including subject as a random variable, and treatment as a fixed effect. The model was reduced until only test diet and any significant ($p < 0.05$) terms remain. Pairwise comparisons between all treatment conditions were conducted using Tukey's adjustment for multiple comparisons. Net incremental area under the curve (niAUC) was calculated using the trapezoid method and results reported at 2 and 4 hours. Per protocol, hormone analysis values were screened to reject subjects with at least 1 plasma hormone niAUC value greater than 3 times the standard deviation from the mean in at least 2 breakfast meals. Subjects were also rejected with fasting plasma glucose concentrations greater than 100 mg/dL. Some hormone concentration assays were not completed, so the number of data points per hormone and per breakfast meal varied.

Sample Size: In trial 1, with an evaluable sample of 30 subjects, the study has 80% power to detect a difference of 10 mm in the consolidated appetite composite score (pre-meal to 240 min) between treatments assuming a standard deviation of 15.9 mm. This assumes a nominal p-value of 0.017 to account for up to three primary comparisons using Šidák adjustment (Sidak, 1971). Rolling recruitment was employed until the desired 30 subjects had been randomized. In trial 2, with an evaluable sample of 33 subjects, the study has 80% power to detect a difference of 9.8 mm in the average appetite composite score (pre-meal to 240 min) between treatments assuming a

standard deviation of 15.9 mm. This assumes a nominal p-value of 0.0127 to account for up to four primary comparisons using Šidák adjustment. A sample of 36 subjects was randomized to allow for attrition and non-compliance. After consuming control A RTE cereal, 27 subjects were included for GLP-1, and 30 subjects for the remaining hormone assays and glucose. After consuming the remaining breakfast RTE cereals, 28 subjects were included for GLP-1, and 31 subjects for the remaining hormone assays and glucose.

Results

RTE Cereal

The composition of RTE cereals was similar in protein, fat (Table 13), and flake density (133, 136, and 166 kg/m^3 for the control, AXOS and FLAX RTE cereals, respectively) to minimize impact of these factors in the comparisons. RTE cereal texture was evaluated by both instrumental and sensory methods. The 3 RTE cereals had similar texture based on peak force measurements both dry and in milk (dry: 9,744 ± 1,158, 7,882 ± 722, and 7,369 ± 1288 g of force, and in milk: 35,588 ± 7,305, 35,506 ± 8,196, and 30,667 ± 3,598 g of force for control, AXOS and FLAX RTE cereals, respectively). There were slight texture differences in sensory evaluation of the 3 RTE cereals (Table 14). The control flake exhibited a more cohesive mass after chewing and in milk. The AXOS RTE cereal absorbed the least moisture/saliva after chewing. Finally, the FLAX

Table 14. Sensory characteristics of RTE flaked cereal dry and with milk Results were collected as panel consensus [a].

Attribute Title	Control Low Fiber Flake	AXOS Fiber Flake	FLAX Fiber Flake
First Chew (Dry Cereal)			
Hardness, Molars	5.0	5.5	5.0
Fracturability	4.5	5.0	4.0
Chew Down (Dry Cereal)			
Moisture Absorption	11.0	7.5	10.0
Cohesiveness of Mass	8.5	7.5	8.0
Gritty/Particles	4.0	3.0	2.0
3 Minutes in Milk			
Fracturability	0.0	0.0	0.0
Cohesiveness of Mass	5.5	4.5	4.0

[a] 15-point scale divided into ½ point increments, with 0 meaning “none” and 15 meaning “extremely strong”.

RTE cereal was the least gritty. Flavor was also judged to be very similar (data not presented).

Subject characteristics for each trial are outlined in Table 15. Characteristics of the participants were controlled with inclusion of females within a narrow range of BMI and age. Since the high fiber diets contained 19 grams of fiber per serving (15 g test fiber and 4 g base cereal fiber), gastrointestinal tolerability was assessed in both trials (data not presented for trial 1) at each treatment (Buemann, Toubro, & Astrup, 1999).

Table 15. Subject characteristics at baseline

Parameter	Trial 1	Trial 2
Female	30	36
Race/ethnicity		
Non-Hispanic White	20	23
Black/African American	8	8
Asian or Pacific Islander	1	2
Multiracial	1	3
Smoking Status		
Non-Smoker	24	27
Current Smoker	2	5
Past Smoker	4	4
Age (years)	22.5 (0.6)[a]	24.3 (0.5)
Weight (kg)	72.9 (1.2)	74.3 (1.2)
Body Mass Index (kg/m^2)	27.0 (0.3)	27.4 (0.3)
Systolic Blood Pressure (mm Hg)	109 (2)	113 (2)
Diastolic Blood Pressure (mm Hg)	66 (1)	71 (2)
Heart Rate (bpm)	73 (2)	77 (2)

[a] standard error of mean in parentheses

Gastrointestinal symptoms were not observed in a majority (66-75%) of the participants. However, for those who did experience symptoms during the study, flatulence was

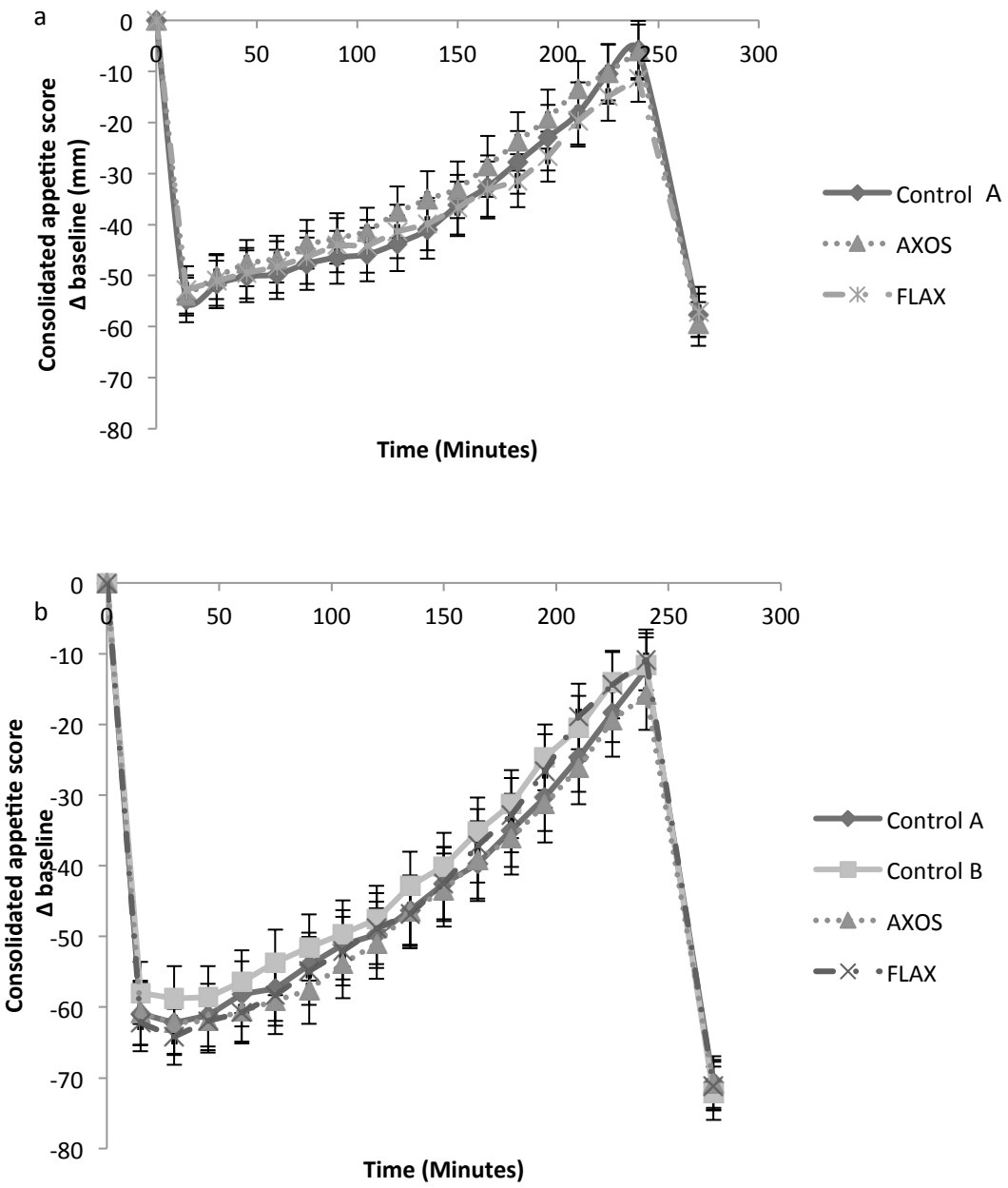


Figure 15. Consolidated Appetite Score change from baseline following consumption of each breakfast. *a) Clinical Trial 1. Data are presented as mean ± SEM, based on 30 subjects. b) Clinical Trial 2. Data are presented as mean ± SEM, based on 36 subjects. There were no significant differences between treatments at any time point (p > 0.05) in either trial.*

higher with the high-fiber containing RTE cereals (1.3±0.3 and 1.1±0.3 for AXOS and FLAX RTE cereals, respectively) compared with both control diets (0.3±0.2 and 0.2±0.1 for control A and B RTE cereals, respectively). Gas/bloating experienced was also

higher with the high-fiber containing RTE cereals (1.5±0.3 and 1.4±0.3 for AXOS and FLAX RTE cereals, respectively) compared with both controls (0.3±0.2 and 0.1±0.1 for control A and B RTE cereals, respectively).

Clinical Trial 1

Increasing fiber content from 4 g/serving in the control cereal to 19 g/serving in the two high-fiber cereals did not affect perceived appetite ratings. Results for VAS scores (mm) of Hunger, Fullness, Desire to Eat, and Prospective Consumption showed no significant differences ($p>0.05$) among treatment conditions in appetite ratings at any time point (data not presented). The Consolidated Appetite Score (CAS) also showed no significant differences at any time point (Figure 15). Total $niAUC_{0\text{-}240}$ values for CAS over the pre ad libitum lunch phase were -8,737 ± 1,214, -7,961 ± 1,134, and -8,810 ± 1,191 mm x minutes ±SEM for the control, AXOS, and FLAX RTE cereals, respectively. No significant differences were observed among conditions.

The energy intake subjects consumed during the ad libitum lunch did not differ significantly among treatments ($p = 0.96$), and thus subjects did not compensate for lower calorie intakes with the high-fiber breakfasts (breakfast calories 461, 345, 347 kcal for control, AXOS and FLAX RTE cereals, respectively). Mean energy intakes during the ad libitum lunch were 552 ± 37 545 ± 37, and 559 ± 36 kcal ± SEM for the control, AXOS, and FLAX RTE cereals, respectively. Mean total energy intakes from the combined RTE cereal breakfast and lunch meals were 1,013 ± 36, 907 ± 37, and 906 ± 36 kcal ± SEM for the control, AXOS, and FLAX RTE cereals, respectively ($p=0.065$ across breakfasts). Consequently participants consumed ~10% fewer kcal

for breakfast and lunch combined when fed the AXOS ($p = 0.109$) and FLAX ($p = 0.101$) RTE cereals versus the low fiber RTE cereal because the control RTE cereal breakfast contained more calories. When both fiber treatments were averaged together, the difference in energy intake reached significance versus control ($p = 0.036$).

Clinical Trial 2

In this trial, 3 of the diets were the same as in trial 1. An additional control was added to compare a low-fiber control RTE cereal with high calories (control A; 4 g fiber; 461 kcal), to a low-fiber control RTE cereal (control B; 3 g fiber; 341 kcal) energy matched to the high-fiber RTE cereals. As with trial 1, increasing fiber content from 3-4 g/serving in the two control cereals to 19 g/serving in the two high-fiber cereals did not affect appetite ratings. Results for VAS scores of Hunger, Fullness, Desire to Eat, and Prospective Consumption showed no significant differences ($p > 0.05$) among treatment conditions in appetite ratings at any time point (data not presented). The Consolidated Appetite Score (CAS) also showed no significant differences at any time point (Figure 15). Total $niAUC_{0\text{-}240}$ values for the CAS over the pre ad libitum lunch phase were -10,468 ± 987, -9,730 ± 998, -10,754 ±1,066, -10,321 ± 996 mm x minutes ± SEM for the control A, control B, AXOS, and FLAX RTE cereals, respectively. No significant differences were observed among RTE cereals.

As in trial 1, subjects consumed similar amounts of macaroni and cheese during the ad libitum lunch in the four treatment conditions. Again, just as in the first clinical trial, they did not compensate for the lower calories in the isocaloric breakfasts (461, 341, 345, 347 kcal for the control A, control B, AXOS and FLAX RTE cereals, respectively). Energy intakes from lunch were 533 ± 37, 558 ± 38, 544 ± 37, and 547 ± 36 kcal (mean

± SEM) for the control A, low energy control B, AXOS, and FLAX RTE cereals, respectively and were not significantly different between treatments ($p = 0.77$). Total energy intakes from the combined RTE cereal and lunch for the low energy control B 899 ± 38, AXOS 907 ± 37, and FLAX 894 ± 36 kcal (mean ± SEM) conditions were significantly lower than for control A, 994 ± 37 kcal ($p \leq 0.006$ for each), again suggesting that the participants did not compensate for the lower energy intake at the breakfast meal.

Since the 2 high-fiber cereals and the high-energy control cereal were the same in both clinical trials, the niAUC CAS data were pooled for these test conditions and were -10,165 ± 851, -10,352 ± 816, and -10,338 ± 822 mm x min. for control A, AXOS and FLAX RTE cereals, respectively. Although this served to increase the number of subjects included in the analysis (n=65), there was still no significant difference in CAS among these test cereals ($p > 0.05$) at any time point.

Hormones and Glucose

Blood samples were collected from each of the subjects after the overnight fast prior to each day they ate the 4 different breakfasts. Five subjects were removed from the data pool per protocol. One subject was removed due to high fasting plasma glucose concentrations (> 200 mg/dL) on all four days. The other 4 subjects were removed because one or more of their niAUC hormone concentrations were > 3 standard deviations from the mean for at least 2 breakfast meals. Baseline values for each of the hormones and glucose, after these subjects were removed, were similar prior to consumption of the 4 breakfasts (Table 16). All change from baseline values were calculated from these results. Fasted average glucose concentrations were in the

Table 16. Baseline plasma hormones and glucose taken after overnight fast

Blood Parameter[ab]	Control A	Control B	AXOS	FLAX	p-value
Ghrelin (pg/mL)	81 (7)	72 (7)	72 (7)	75 (7)	0.38
GLP-1 (pg/mL)	38 (7)	31 (7)	30 (7)	23 (7)	0.21
Total PYY (pg/mL)	87 (8)	92 (8)	91 (8)	90 (8)	0.81
Glucose (mg/dL)	84 (1)	86 (1)	86 (1)	86 (1)	0.41
Insulin (pg/mL)	877 (181)	1,072 (179)	730 (181)	744 (179)	0.17

[a]Blood samples were taken from each subject after an overnight fast prior to consuming each of the 4 cereals on different days and averaged for the subject pool (±SEM).
[b]Number of subjects varied: Control A – GLP-1, 27; Ghrelin, Total PYY, Glucose, Insulin, 30; remaining breakfasts - GLP-1, 28; Ghrelin, Total PYY, Glucose, Insulin, 31.

normal range, consistent with screening criteria for the study to exclude subjects that had diabetes or pre-diabetes.

Ghrelin: Similar post-meal reductions in plasma ghrelin were observed for all 4 breakfast meals (Figure 16a). Ghrelin concentrations all decreased during the first 45 minutes. Ghrelin then began to return to baseline values at approximately 60 min and continued to increase until lunch was consumed. The greatest reduction of ghrelin after consumption of breakfast, as well as the longest delay to return to baseline concentrations, tended to occur when subjects consumed the high-energy control A RTE cereal. A treatment x time interaction was observed with repeated measures, P = 0.01. At t = 45 and t = 90 minutes, ghrelin concentrations in subjects consuming control A RTE cereal were significantly lower than when they consumed FLAX RTE cereal. At t = 120 minutes, change from baseline measurements for subjects consuming control A RTE cereal were significantly lower than when they consumed control B and AXOS RTE cereals. At t = 180 minutes, ghrelin concentrations in subjects consuming control A RTE cereal were significantly lower than when they consumed control B RTE cereal.

Composition of the RTE cereals did not affect mean net area under the curve measurements of plasma ghrelin concentrations in subjects after breakfast consumption (Table 17).

GLP-1: GLP-1 concentrations immediately increased after consumption of each of the 4 breakfast meals, peaked after 30 minutes, and slowly decreased towards baseline, remaining above baseline values throughout the remainder of the testing day (Figure 16b). Overall, GLP-1 concentrations tended to be lower when control B RTE cereal was consumed. When subjects consumed control B RTE cereal, plasma concentrations of GLP-1 were significantly lower than when subjects consumed FLAX RTE cereal at t = 90, and 120 minutes. Concentrations of GLP-1 were lower in subjects consuming control B RTE cereal than when they consumed control A RTE cereal at t = 15, 90, 120, and 240 minutes. Concentrations of GLP-1 were also lower in subjects when they consumed control B RTE cereal than when they consumed AXOS RTE cereal, with significant differences in the later portion of the time curve at t = 15, 45, 60, 90, and 120 minutes. Concentrations of GLP-1 were the same in subjects consuming control A RTE cereal as when subjects consumed either AXOS or FLAX RTE cereal at all time

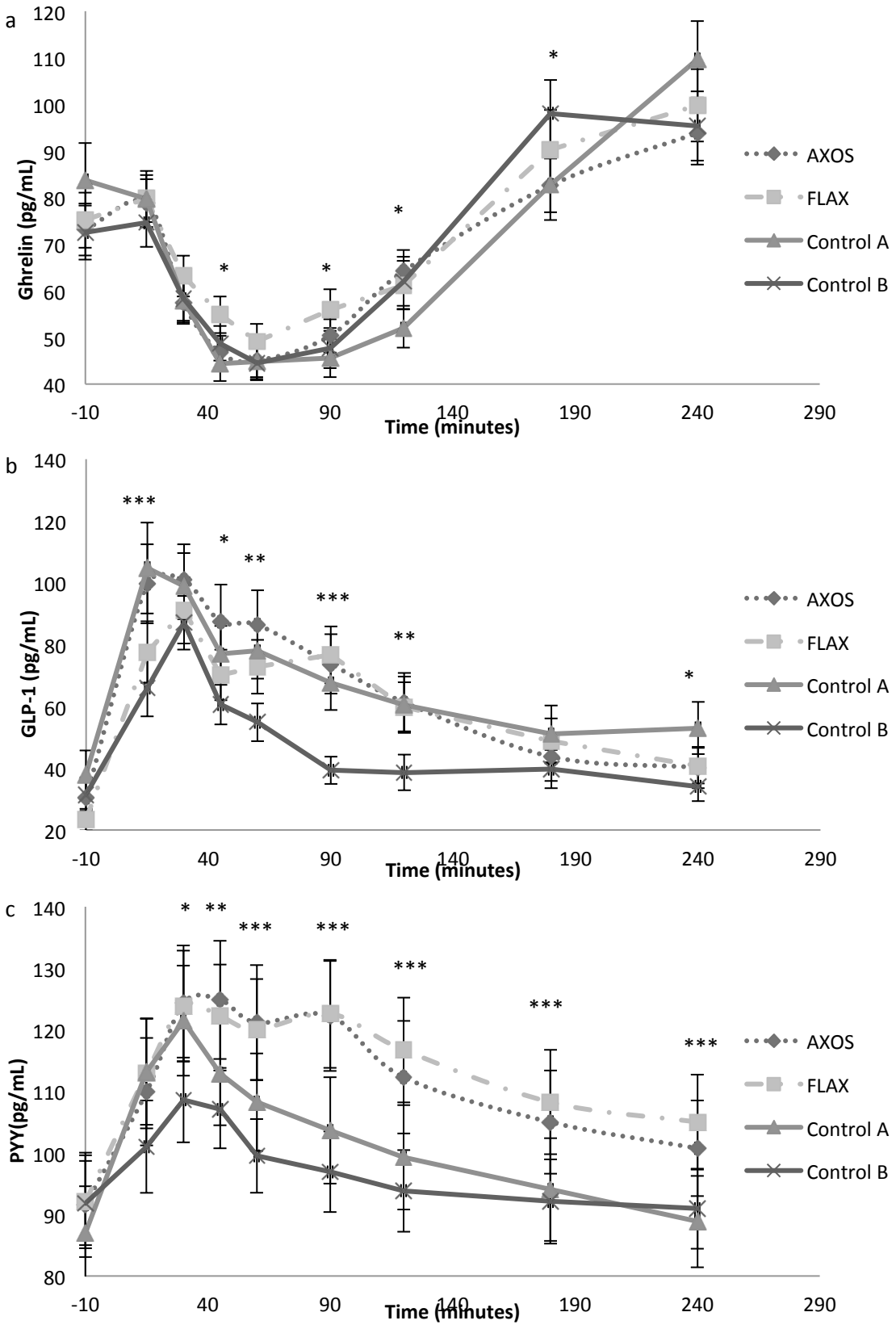


Figure 16. Plasma hormone concentrations at selected time points. a) active ghrelin, b) active GLP-1, c) total PYY. *Significant differences among groups are signified as follows: ***P<0.001; **P<0.01; *P<0.05.*

points. Mean net area under the curve measurements of GLP-1 values demonstrated that subjects consuming control B RTE cereal had a lower plasma concentration of GLP-1 than when consuming the other 3 treatment cereals at 2 and 4 hours (Table 17).

Table 17. Net incremental area under the curve for each hormone at 2 and at 4 hours, a) 2 hour niAUC, b) 4 hour niAUC

a)

Blood Parameter [a] (mean x 10^3)	Control A	Control B	AXOS	FLAX	p-value
Ghrelin (pg/mL x min.) [b]	6.45 (.51) ^{A}	6.77 (.51) ^{A}	6.67 (.51) ^{A}	7.19 (.51) ^{A}	0.33
GLP-1 (pg/mL x min.)	8.81 (1.11) ^{A}	6.27 (1.11) ^{B}	9.25 (1.11) ^{A}	8.35 (1.11) ^{A}	<0.01
Total PYY(pg/mL x min.)	12.76 (1.02) ^{AB}	11.87 (1.01) ^{B}	13.93 (1.02) ^{A}	13.93 (1.01) ^{A}	<0.01
Glucose (mg/dL x min.)	11.59 (.32) ^{A}	11.80 (.32) ^{A}	11.29 (.32) ^{A}	11.18 (.32) ^{A}	0.052
Insulin (pg/mL x min.)	327.90 (32.62) ^{A}	299.80 (32.52) ^{AB}	282.80 (32.62) ^{B}	269.00(32.52) ^{B}	<0.01

b)

Blood Parameter (mean x 10^3)	Control A	Control B	AXOS	FLAX	p-value
Ghrelin (pg/mL x min.)	11.32 (.88) ^{A}	12.06 (.88) ^{A}	11.40 (.89) ^{A}	12.25 (.88) ^{A}	0.33
GLP-1 (pg/mL x min.)	12.06 (1.56) ^{A}	8.27 (1.56) ^{B}	11.96 (1.56) ^{A}	11.27 (1.55) ^{A}	<0.01
Total PYY(pg/mL x min.)	18.41 (1.49) ^{BC}	17.40 (1.49) ^{C}	20.19 (1.49) ^{AB}	20.44 (1.49) ^{A}	<0.01
Glucose (mg/dL x min.)	16.16 (.37) ^{A}	16.41 (.36) ^{A}	16.01 (.37) ^{A}	15.75 (.36) ^{A}	0.12
Insulin (pg/mL x min.)	436.00 (42.19) ^{A}	382.30 (42.08) ^{B}	360.30 (42.19) ^{B}	348.70 (42.08) ^{B}	<0.01

[a] number of subjects varied: Control A – GLP-1, 27; Ghrelin, Total PYY, Glucose, Insulin, 30; remaining breakfasts - GLP-1, 28; Ghrelin, Total PYY, Glucose, Insulin, 31.
[b] standard error of mean in parentheses (±SEM)), values within rows with different letters represent significant differences using Tukey multiple comparisons.

PYY: PYY concentrations immediately increased after consumption of each of the 4 breakfast meals, peaked after 30 minutes, and then slowly decreased throughout the remainder of the testing day (Figure 16c). A treatment x time interaction was observed with repeated measures, P = 0.01. Subjects consuming the two high-fiber RTE cereals had significantly higher PYY concentrations than when they consumed the two control cereals suggesting a fiber effect on PYY concentrations. Subjects consuming AXOS RTE cereal also had plasma PYY concentrations higher than when they consumed control B RTE cereal at almost every time point, with significant differences at t = 30, 45, 60, 90, 120, and 180. Overall, PYY concentrations tended to be lowest when subjects consumed control B RTE cereal. When subjects consumed FLAX RTE cereal, PYY concentrations were higher than when they consumed control B, with significant differences at t = 30, 45, 60, 90, 120, 180, and 240. When subjects consumed control B RTE cereal, they tended to have lower plasma PYY concentrations than when they consumed the high-energy control A RTE cereal. Subjects consuming AXOS RTE cereal had a significantly higher PYY concentration than when they consumed control A RTE cereal at t = 90. Mean net area under the curve measurements of PYY concentrations were higher in subjects consuming FLAX RTE cereal than when they consumed control B RTE cereal at 2 hours, and when they consumed control B or control A RTE cereals at 4 hours (Table 17). Mean net area under the curve measurements of PYY concentrations were also higher in subjects consuming AXOS RTE cereal than when they consumed control B RTE cereal at both 2 and 4 hours.

Glucose and Insulin: Glucose concentrations increased immediately after consumption of each of the 4 breakfast meals, reached a peak at 30 minutes, and then decreased

during the remainder of the test (Figure 17a). Subject's glucose concentrations all returned to baseline between 120 and 180 minutes. Plasma glucose concentrations were higher when subjects consumed control B than when they consumed FLAX RTE cereal, with significant differences at t = 45, and 60 minutes. Glucose concentrations in subjects consuming all 4 breakfast cereals were not significantly different at the other time points. Mean net area under the curve measurements of glucose concentrations of subjects consuming all 4 RTE cereals were not statistically different at 2 or 4 hours (Table 17).

Insulin concentrations also increased immediately after consumption of each of the 4 breakfast meals, reaching a peak between 30 - 45 minutes, and then decreased during the remainder of the test (Figure 17b). Unlike glucose, subject's insulin concentrations did not return to baseline until approximately 240 minutes. Insulin concentrations were higher in subjects consuming the high energy control A RTE cereal than when they consumed the other breakfasts, with significant differences versus control B RTE cereal at t = 120, and 180 minutes; versus AXOS RTE cereal at t = 90, 120, 180, and 240 minutes; and versus FLAX RTE cereal at time points t = 60, 90, 120, and 180 minutes. Mean net area under the curve measurements of insulin concentrations were higher in control A RTE cereal than AXOS and FLAX RTE cereals at 2 and higher than control B, AXOS, and FLAX RTE cereals at 4 hours (Table 17).

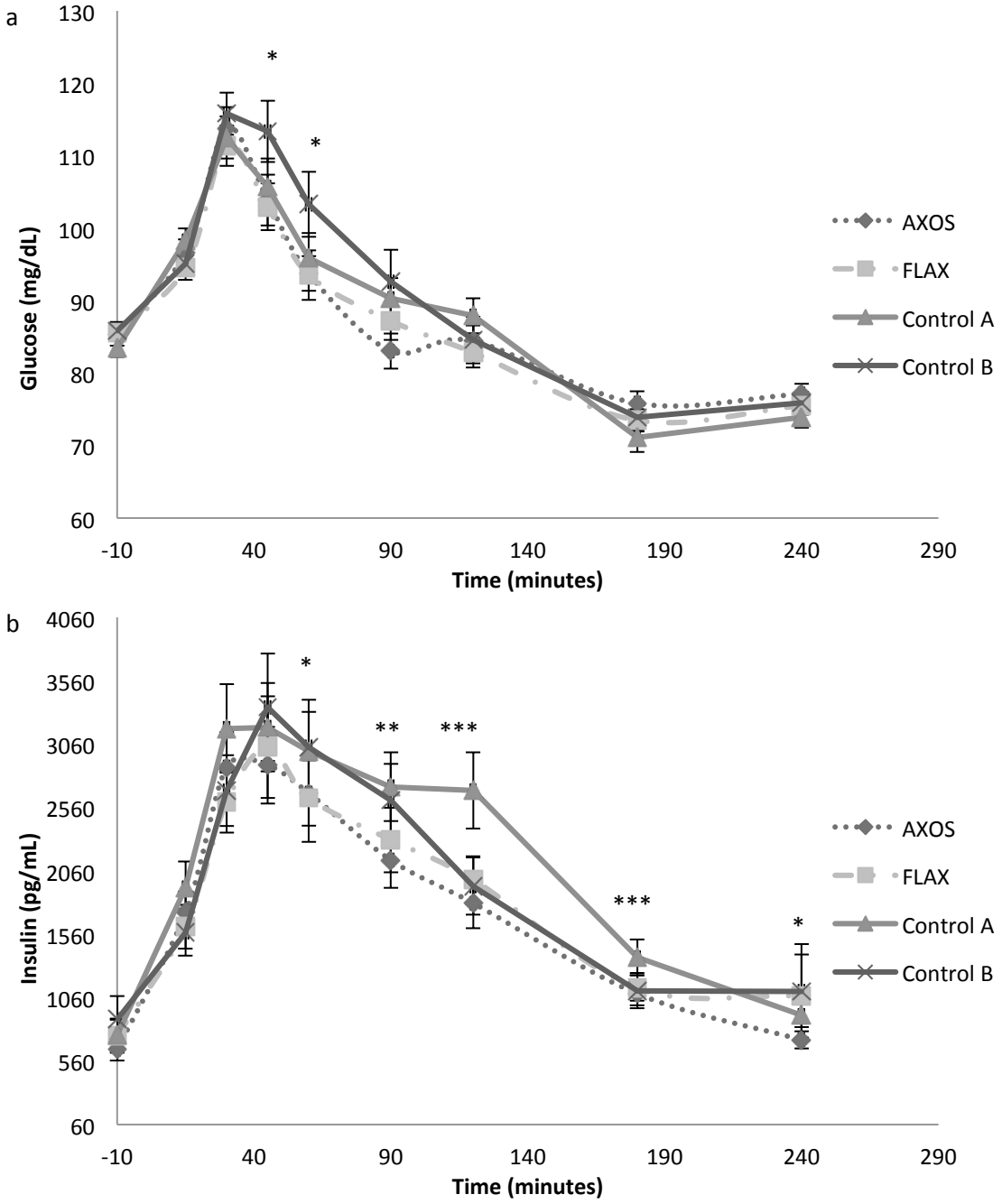


Figure 17. Plasma glucose and insulin concentrations at selected time points. a) glucose, b) insulin.
*Significant differences among groups are signified as follows: ***P<0.001; **P<0.01; *P<0.05.*

Discussion

The effects of increased dietary fiber on satiety are inconsistent, potentially due to type of fiber, dosage, and delivery form incorporated in these studies as well as the

physicochemical properties of the fibers (Blundell & Burley, 1987; Howarth, Saltz, & Roberts, 2001; Pereira & Ludwig, 2001). Also, fibers can be modified by hydrolysis to facilitate incorporation into food, making their effects on satiety even more uncertain. It is important to further understand how physicochemical properties of fiber and fiber modifications influence satiety and energy intake parameters. This study was designed to evaluate the effects of an enzyme-hydrolyzed arabinoxylan from wheat (AXOS) versus an intact arabinoxylan from flax (FLAX) added to a RTE cereal on perceived appetite, on subsequent meal energy intake, and on hormonal responses in overweight women. A comparison was provided between RTE cereal breakfasts with 4 grams fiber versus 15 grams of additional fiber (AXOS and FLAX) in 100 g RTE cereals with 2% milk, diluted with water to standardize the weight consumed (500 g) for each breakfast meal. Since the addition of fiber reduced the caloric content of the cereal, an additional control product was added in trial 2 to provide a comparison between the low-fiber RTE cereal used in trial 1 (control A; 461 kcal), and a low-fiber breakfast (control B; 341 kcal) matched for energy content to the high-fiber breakfasts (~345 kcal). No differences in perceived appetite in a consolidated appetite score, or in subsequent meal energy intake for the low-fiber breakfast meals versus the high-fiber breakfast meals were observed in two clinical trials. However, the high fiber breakfast meals led to elevated plasma GLP-1 and PYY concentrations.

Perceived Appetite

There have been numerous studies comparing perceived appetite of RTE cereals with added fiber. No effect was found in perceived hunger or fullness (AUC) when comparing RTE cereal with 41 g fiber, 250 kcal, and a 550 g meal size with a low-fiber

control cereal (1.5 g fiber) 60 or 120 minutes after the breakfast meal (Freeland, Anderson, & Wolever, 2009). Samra & Anderson (2007) provided a RTE cereal with 33 g fiber and a meal size of 500 g, and found that average appetite scores 75 minutes later were not statistically different versus a low-fiber (1.5 g) RTE cereal. They did observe an increase in fullness with the higher fiber RTE cereal. Other studies compared RTE cereals with lower fiber concentrations on perceived appetite (Hamedani, Akhavan, Samra, & Anderson, 2009; Delargy, Burley, O'Sullivan, Fletcher, & Blundell, 1995; Hlebowicz, Wickenberg, Fahlstro, Almer, & Darwiche, 2007; Delargy, O'Sullivan, Fletcher, & Blundell, 1997). The majority of these studies indicate that there was no effect of fiber on perceived appetite in this food form. The results from the current study support this conclusion. This is in conflict with many studies where a modest amount of fiber added to beverages increased perceived appetite (Wanders, et al., 2011). Food form may have an impact on the ability of fiber to increase satiety.

In a beverage system, as little as 2.5 g fiber has been shown to affect perceived appetite (Solah, et al., 2010; Hoad, et al., 2004). Significant hunger reduction (-13%) with a psyllium (7.4 g) containing beverage relative to no fiber control was found (Rigaud, Paycha, Meulemans, Merrouche, & Mignon, 1998). These studies demonstrated a perceived appetite effect of beverages at much lower added fiber levels than we used in RTE cereals. One possible explanation is that fibers need to be hydrated before their viscous properties can be fully realized, and this is more likely to occur in a beverage food form than a solid one, or than in a meal with liquid added to a solid form, like milk added to RTE cereal. In the current study ~80% of the meal mass

was liquid (Table 13); however, the physical form of the RTE flake may have attenuated the fiber's ability to affect perceived appetite.

Viscosity of the fiber is believed to be responsible for some of the satiety effects of beverages (Hoad, et al., 2004; Wanders, et al., 2011; Mattes & Rothacker, 2001). Kristensen & Jensen (2010) reviewed 9 published studies on the effect of fiber and viscosity on satiety of beverages. They found that in most cases increased viscosity led to increased satiety with fiber levels tested ranging from 3-10 grams. Many of these beverage studies used very viscous fibers like alginates and gums that have a very high molecular weight and impact viscosity at a low concentration. The FLAX ingredient we used to produce the high-fiber RTE cereal had a high molecular weight and a very high viscosity (Lafond, Jin, Cho, & Romsos, 2014). We anticipated that it would produce a higher viscosity RTE cereal; however, we found that processing reduced FLAX fiber molecular weight and viscosity of the cereal. The most viscous cereal at the time of consumption was the control A RTE cereal (from the higher available gelatinized starch present) however, that viscosity might quickly be reduced by digestive amylases in the intestine. Most RTE cereal studies used a low-fiber, corn flake control RTE cereal that had a relatively high level of gelatinized starch. Although viscosity of cereal is seldom reported, high-fiber RTE cereals may not be more viscous than low-fiber, high-starch control RTE cereals. This may contribute to the reported inconsistency of high-fiber cereals to enhance satiety.

Lunch Meal Energy Intake

The overweight female subjects in the current study were offered the *ad libitum* lunch meal 4 hours after the breakfast meals because this represented a typical time interval between breakfast and lunch meals for most adults. In the current study, subjects consumed a similar amount of calories at lunch regardless of breakfast calories consumed. By 4 hours, effects of the breakfast cereals at the lunch meal may have been diminished, since perceived appetite scores were almost at baseline. In addition, both clinical trials studied overweight female subjects, who may be less responsive to appetite cues (Adam & Westerterp-Plantenga, 2005). Other investigators also found similar energy intake at lunch offered between 3 - 3.5 hours after subjects consumed RTE cereal with high fiber content (Delargy, Burley, O'Sullivan, Fletcher, & Blundell, 1995; Hamedani, Akhavan, Samra, & Anderson, 2009). However unlike these studies, some investigators using RTE cereals did observe a reduction in second meal energy intake when they employed a shorter time interval between breakfast and lunch (Samra & Anderson, 2007; Delargy, O'Sullivan, Fletcher, & Blundell, 1997; Levine, Tallman, Grace, Parker, Billington, & Levitt, 1989). This may explain why they observed a lower energy intake in the second meal.

GLP-1 and PYY

Plasma GLP-1 and PYY concentrations were significantly higher with the high-fiber breakfasts than with an equal calorie low-fiber control RTE cereal. Secretion of GLP-1 and PYY has been shown to be co-located within the enteroendocrine L cells located predominately in the distal small intestine and colon (Habib, Richards, Rogers, Reimann, & Gribble, 2013) and carbohydrates and protein are strong stimulants of

these hormones within this segment of the intestinal tract (Gribble, Williams, Simpson, & Reiman, 2003; Little, et al., 2006; van der Klaauw, et al., 2013; Lomenick, Melguizo, Mitchell, Summar, & Anderson, 2009; Batterham, et al., 2006). The high fiber content of the AXOS and FLAX RTE cereals may have caused carbohydrate and protein residues to reach the distal small intestine within the first hour after consumption (van der Klaauw, et al., 2013; Ou, Kwok, Li, & Fu, 2001). Digestion and absorption of carbohydrate and protein in the distal small intestine would then contribute to greater observed elevation of GLP-1 and PYY when the high fiber RTE cereals were consumed than when the low fiber RTE cereals were consumed.

In the later portion of the interval between breakfast and lunch consumption, it is likely short chain fatty acids (SCFA) were produced from fermentation of the AXOS and FLAX high-fiber containing RTE cereals, and that the SCFA stimulated L cells in the distal small intestine and colon to cause GLP-1 and PYY secretion (Tolhurst, et al., 2012; Cani, et al., 2009). Our subjects reported an increase in flatulence and gas/bloating after consuming the high-fiber RTE cereals indicating fermentation was occurring, and SCFA were likely being formed (Francois, et al., 2012; Neyrinck, Van Hee, De Backer, Toussaint, Cani, & Delzenne, 2012). The high solubility and low molecular weight of the 2 fibers added to our high-fiber RTE cereals likely contributed to rapid fermentation and SCFA production (Lafond, Jin, Cho, & Romsos, 2014; Slavin J. , 2013).

Plasma Ghrelin, Glucose and Insulin

Plasma ghrelin concentrations were similar after consumption of all 4 breakfast cereals and followed an expected postprandial pattern consistent with our reported VAS appetite scores. Ghrelin is strongly associated with perceived hunger (Delzenne, et al.,

2010), and we observed a time dependent change in appetite (CAS) and ghrelin concentration. We did observe a lower plasma active ghrelin concentration at the 2 and 3 hour time points with the high-energy control RTE cereal versus the low-energy control RTE cereal, suggesting the greater available calories in the high-energy RTE cereal might have delayed the return to baseline concentrations in ghrelin. The ghrelin difference observed was relatively small, and may not have been sufficient to elicit a perceived appetite change nor a change in lunch meal energy intake. These lower ghrelin concentrations with the high-energy control RTE cereal at 2 and 3 hours also corresponded to a higher concentration of insulin at the same time points. Blom et al. (2005) demonstrated a significant negative correlation between plasma ghrelin and insulin concentrations between 30-180 minutes and concluded that insulin may contribute to ghrelin suppression.

Viscous fiber consumption has been shown to attenuate plasma glucose and insulin concentrations (Regand, Tosh, Wolever, & Wood, 2009; Dikeman & Fahey, 2006). The steam pressure-cooking used to make the RTE cereal reduced FLAX fiber molecular weight and viscosity of the cereal such that cereals containing FLAX and AXOS each had a relatively low viscosity (Lafond, Jin, Cho, & Romsos, 2014). This reduced the FLAX fiber's ability to attenuate plasma glucose and insulin concentrations.

Conclusions

No differences in perceived appetite as measured by VAS, or in lunch meal energy intake were observed with 15 grams of added AXOS or FLAX fiber in a RTE cereal breakfast when consumed by overweight women. Since steam pressure-cooking of the

cereal reduced the viscosity of the FLAX RTE cereal, our comparisons were between two low-viscosity RTE cereals with added fiber versus a low-fiber control RTE cereal, and the possible physiological benefit from adding a viscous fiber was reduced. It is also possible that the large meal mass (500 g) and the protein content may have negated the ability to detect a difference in perceived appetite from the fibers in the RTE cereals. Additionally, timing of the second meal (4 hours after breakfast) may have been delayed too long to see an effect of the fixed breakfast meal differences on energy intake in overweight females. Both AXOS and FLAX fibers added to RTE cereals increased GLP-1 plasma concentrations relative to a low-fiber iso-caloric control RTE cereal, and increased PYY plasma concentrations relative to the low-fiber RTE cereals. It is possible that despite observed hormonal changes, overweight females in this study may not have been sensitive enough to the satiety hormone signaling cues to affect perceived appetite after breakfast or energy intake at lunch. Collectively these data suggest that 15 g of low molecular weight fiber added to RTE cereal did not affect perceived appetite as measured by VAS or energy intake at lunch 4 hours after breakfast despite differences in satiety hormone signaling in overweight females.

CHAPTER 4 – SUMMARY AND FUTURE DIRECTION

The current research demonstrated that the process used to make RTE flaked cereal reduced fiber molecular weight, and viscosity from cereal made with 15 g of added FLAX. After RTE cereal processing, the viscosity from cereal with added FLAX was much closer to AXOS containing RTE cereal, and the control low-fiber RTE cereal was more viscous than either of the high-fiber RTE cereals (Chapter 2). This had the impact of altering our original intended comparison between high and low molecular weight fibers in RTE cereal on perceived appetite.

It is possible that other researchers working with flaked RTE cereals in clinical trials experienced the same molecular weight change with other added soluble fibers. If they were investigating physiological benefits of fiber dependent on physicochemical properties like molecular weight, I expect they would have had the same depolymerization effects that we observed, resulting in altered physiological benefits. This further supports the need for measuring the physicochemical properties of the fibers under investigation prior to investing time and money in clinical trials.

The minimum viscosity needed from fiber to affect perceived appetite in RTE cereal is uncertain. This study was not able to demonstrate a large viscosity difference, however in a beverage food form, perceived appetite was associated with very high viscosity (>39,000 mPa·s) (Hoad, et al., 2004). Cereal processing reduced the molecular weight

of intact fiber and viscosity of the RTE cereal from a predicted >160,000 to ~5,000 mPa·s. This viscosity was lower than in the control RTE cereal (~9,000 mPa·s), and significantly less than viscosity demonstrated in beverages to affect satiety. It is unlikely that enough viscous fiber could be added to flaked RTE cereal to achieve such high viscosities based on the results from this study.

However, we were still able to demonstrate that a RTE cereal with added fibers elevated plasma GLP-1 and PYY concentrations relative to low-fiber control RTE cereal (Chapter 3). The hormone elevation was probably due to fermentation of soluble fibers producing SCFA that stimulated L cells to produce the hormones.

While we did not observe a difference in perceived appetite or in energy intake at lunch with the different fibers investigated, we did see a fiber effect on plasma hormone concentrations. The subjects in this study were overweight women who may have been less sensitive to these hormonal changes over the 4 h time period. It is still possible that these same plasma hormone concentrations would be sufficient to affect perceived appetite in other subjects groups with different characteristics, and potentially different sensitivity to GLP-1 and PYY signaling. This would support conclusions from other investigators that fiber consumption increases satiety and reduces body weight.

Physicochemical Properties

A major technical challenge in this study was to prepare RTE flaked cereals with 15 g of added fiber delivered in a single meal, and be similar to a low fiber control RTE cereal with respect to color, flavor and texture. This criterion was necessary in order to minimize affects of liking, or chewing among the breakfast meals and thereby reduce

confounding factors in perceived appetite scores. Another challenge was to identify an intact soluble arabinoxylan source. Several ingredients were evaluated however, they were either not concentrated enough, they generated a very hard RTE cereal texture, or they did not have a high molecular weight. Once an intact arabinoxylan source (FLAX) was identified, the next challenge was to produce RTE flaked cereal. At a 15% fiber addition, the properties of the two high fiber cereals behaved very differently from the low fiber control cereal in hydration, cooking, and toasting stages requiring large process modifications in order to produce comparable products for use in this study.

Since there has not been a reported molecular weight reduction in soluble fiber from flaked RTE cereal processing, this study illustrates the importance of measuring the effects of food form and processing on the physicochemical properties of fiber used. The study also demonstrates techniques that could be used to measure the physicochemical properties of fiber after processing in RTE cereal. This research prompts the question: are other soluble fibers susceptible to the same molecular weight reduction with a RTE flaked cereal process? The autoclave based model system described in the current study could be used to test soluble fibers and determine if this reduction occurs with other fiber structures. Soluble fibers such as psyllium and inulin, which are used in commercial RTE cereals, could be evaluated. Analytical ultracentrifugation was evaluated as an additional technique to measure relative molecular weight of the fibers however, the technique required modifications in order to accommodate the range in molecular weight from hydrolyzed to intact fiber and was abandoned for use in this study.

If depolymerization does occur with other soluble fibers, then a viscosity reduction from cereal in the final food is expected as well. Viscosity reduction could be measured using the techniques outlined in this research. The effects of flaked RTE cereal processing on insoluble fibers should be evaluated to determine if their structures are also susceptible to reduction in molecular weight. Many commercial RTE cereals use insoluble fibers like wheat bran, and these results would be important to manufacturers attempting to demonstrate physiological benefits of fiber in the finished product.

Another question that is prompted by this research is: how much of the change in molecular weight observed was due to low shear steam cooking versus the other unit operations needed to produce the flaked RTE cereal, such as pelletizing, flaking, toasting and drying? A study that measures the fiber molecular weight before and after each individual unit operation is needed to determine if low shear steam pressure-cooking is the only unit operation to have this effect on fiber.

If a high viscosity is needed in the RTE cereal to affect perceived appetite, it might be possible to add the fiber to the cereal after the cooking process rather that beforehand. Studies would be needed to assess fiber hydration and its impact on the food matrix with respect to texture, flavor, and appearance. Further experiments could evaluate if changes in pressure, time and temperature might reduce the depolymerization effect observed, and keep more of the fiber intact and able to maintain viscosity.

Since we observed this fiber depolymerization effect in RTE flaked cereal, then an additional question to answer is: would we see the same change in fiber molecular weight in other food systems that employ heat and water? Since only one

time/temperature condition was used to cook the grains, it is possible that this depolymerization effect occurs in other food systems that have similar conditions. This could impact many food products and processes that utilize a grain hydration step or an ingredient isolation process that uses high temperature and pressure.

Most research into physiological benefits on fiber has been done with food, but without fiber characterization after processing. This future research would continue to improve the understanding of the effect of fiber structure on physiological benefit. It is likely that some variation in satiety results reported in studies using steam pressure-cooked viscous fiber is due to processing effects. As the FDA and other regulatory agencies move to label new fibers based on demonstrated physiological benefits, food processing could impact how new fibers are labeled, and possibly cause researchers to revisit existing fibers as well, given that any fiber benefit dependent on structure may be impacted.

Perceived Appetite

Although the methodology for measuring perceived satiety has been used for over 20 years, the sensitivity of the method for detecting a perceived appetite difference in foods like RTE cereal is less certain. The method is subjective and highly dependent on subjects' understanding the 4 questions, evaluating how they feel, and putting a magnitude to that sensation. Some subjects may not be in tune with their own appetitive signals during or shortly after eating a meal, especially the overweight female subjects that participated in our study. Could a more sensitive measure be to look for the first occurrence of hunger? An additional question that might reduce the interpretation bias might be to ask subjects for their first feeling of hunger. The time of

first hunger could be recorded and used to identify and compare the onset of hunger feelings, and relate the time to meal composition. It may be less difficult to determine the first time a subject feels hunger, rather than interpreting a magnitude of hunger sensation, or fullness, prospective consumption or desire to eat.

In the current study, only one measurement method was used for perceived appetite. If we allowed ad libitum breakfast consumption to continue until subjects were comfortably full (giving them the option to eat as much as they wanted), we might have collected a better measure of satiety and could compare RTE breakfast cereals with fiber on the basis of total breakfast consumption rather than by a fixed breakfast amount.

If we measured time of first hunger and allowed subjects to begin eating lunch when they were hungry and ready to eat versus at a fixed time of 4 h, we might have obtained a more realistic measure of satiety, and seen differences among the breakfasts in this study.

Energy Intake

Would we see a difference between fibers if we offered the second meal at an earlier time point? Although 4 hours represents a typical time interval between breakfast and lunch, it does not take into account mid morning snacking behavior. It may have been too long an interval to identify differences in energy intake. It may be more relevant to test when a snack is desired, how much of the snack is eaten, and what is the effect on the lunch meal (kcal, mass). Although it may be difficult to control these conditions, designing a study that measures a variable amount of breakfast, allows for snacking mid-morning, and measures lunch intake when the subject is hungry would approximate

eating behavior more closely. Methodology to mask timing cues and still collect data would need to be developed. Future research should also evaluate offering the subsequent meal at different time points.

The current study did not evaluate insoluble fibers however, other investigators have determined that high content of insoluble fiber can decrease energy intake (Samra & Anderson, 2007). It is possible that the different physicochemical properties of soluble and insoluble fibers affect satiety by different mechanisms. Insoluble fiber may promote decreased energy intake whereas soluble viscous fibers may decrease perceived appetite. A study evaluating a high molecular weight soluble fiber and a similar high molecular weight insoluble fiber with at least 15 g fiber in the meal is needed to determine the effects of fiber solubility on satiety. Delargy (1997) compared soluble and insoluble fiber in various ratios in RTE cereal and found no effect on perceived appetite but found a decreased energy intake with the soluble fiber breakfast at 1.5 hours. However, the RTE cereal process used was not disclosed, therefore the properties of the fiber in the RTE cereal are unknown. A commercially beneficial result from this future research could be to identify an optimal blend of low and high molecular weight fibers to affect satiety. Once an optimum molecular weight blend is characterized, it is possible that the process to make the food could be used to improve rather than destroy the fiber nutritional benefits by reducing soluble fiber molecular weight to a target value through manipulation of time, temperature and pressure conditions, and achieve desired fiber physicochemical properties.

Gut Hormones

This research has inferred that the mechanism responsible for the elevated GLP-1 and PYY plasma concentrations observed was due to the production of SCFA from fiber fermentation in the colon. To validate the mechanism, future research could measure breath hydrogen to determine the start of fermentation, and study fibers with different molecular weights to identify an optimum size for maximum hormone elevation. SCFA concentrations in stool samples could also be used to validate this assumption. Identifying an optimum fiber molecular weight range to induce elevated hormone concentrations could serve as part of a fiber blend. The blend could be made up of an optimum amount of low molecular weight fibers for hormone elevation, large molecular weight fibers for high viscosity, and insoluble fiber for satiety. The combination could help promote satiety in food products like RTE cereals and help consumers maintain or reduce body weight.

APPENDIX

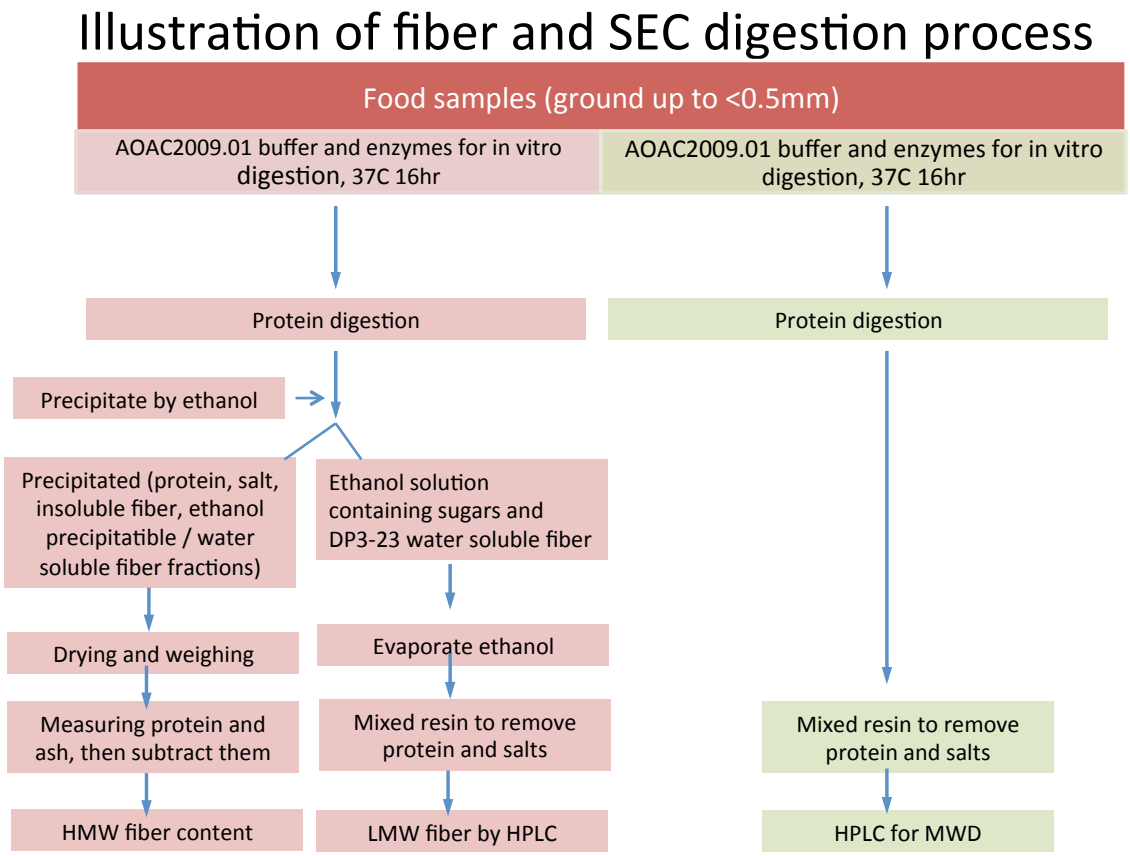


Figure 18. Comparison of the isolation process used to separate the high and low molecular weight fibers from the RTE cereal based on adaptation of the AOAC 2009.01 method for measuring total dietary fiber content. *Solubility in ethanol was used to separate the samples into relatively high and low molecular weight fractions.*

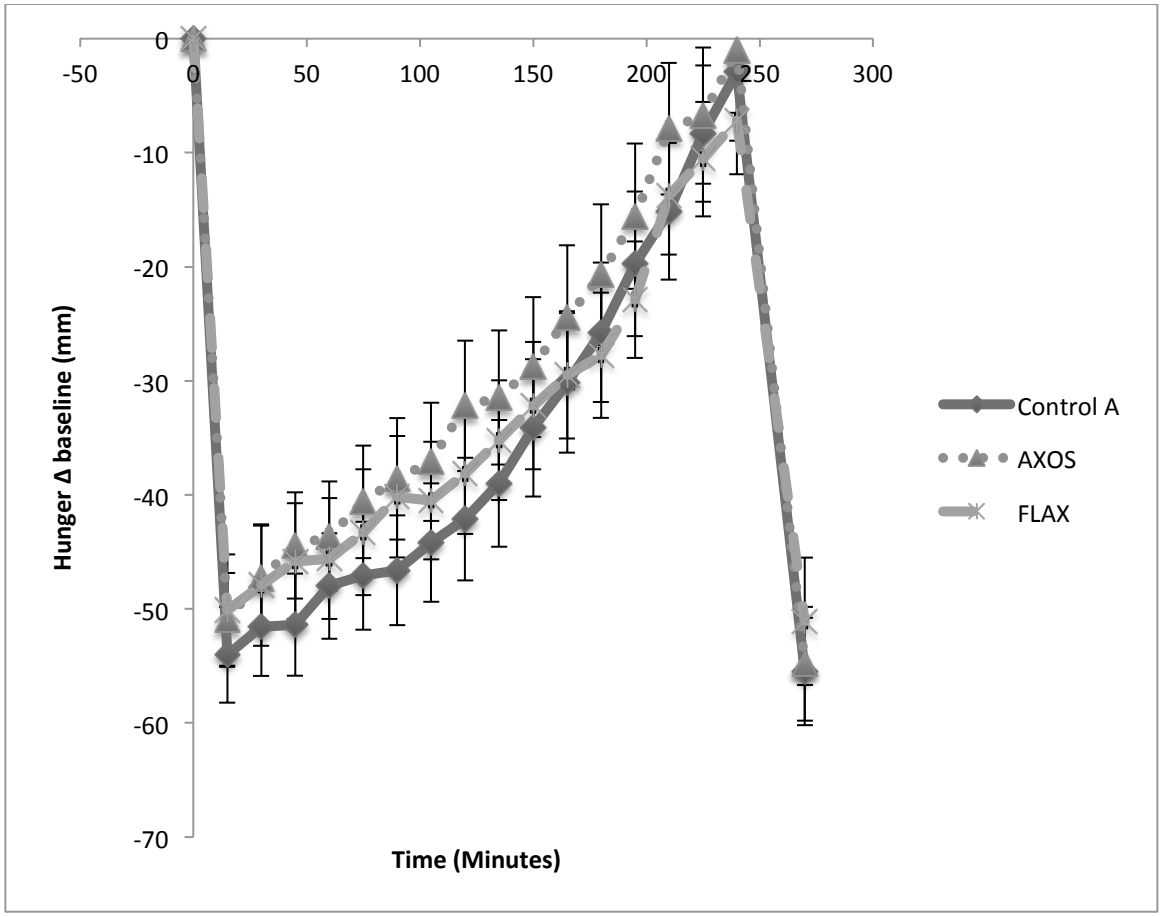


Figure 19. Trial 1 Hunger VAS rating change (Δ) from baseline following consumption of each test breakfast.
Data are presented as mean ± SEM, based on 30 subjects. There were no significant differences between treatments at any time point (p> 0.05).

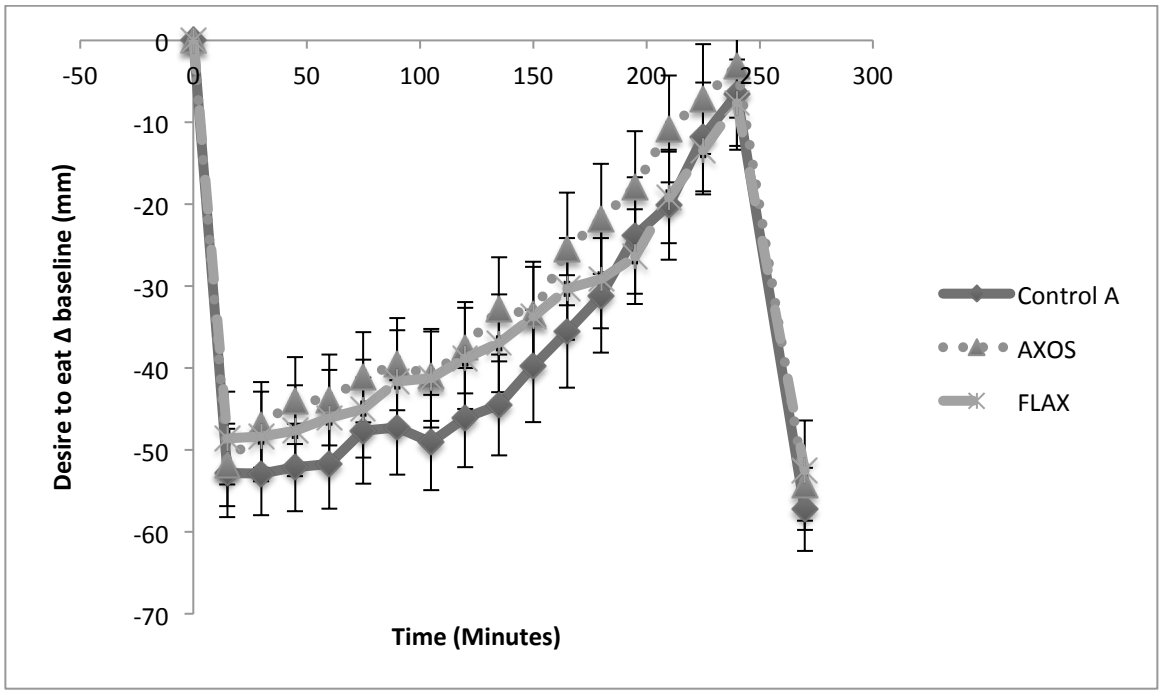


Figure 20. Trial 1 Desire to Eat VAS rating change (Δ) from baseline following consumption of each test breakfast. *Data are presented as mean ± SEM, based on 30 subjects. There were no significant differences between treatments at any time point (p> 0.05).*

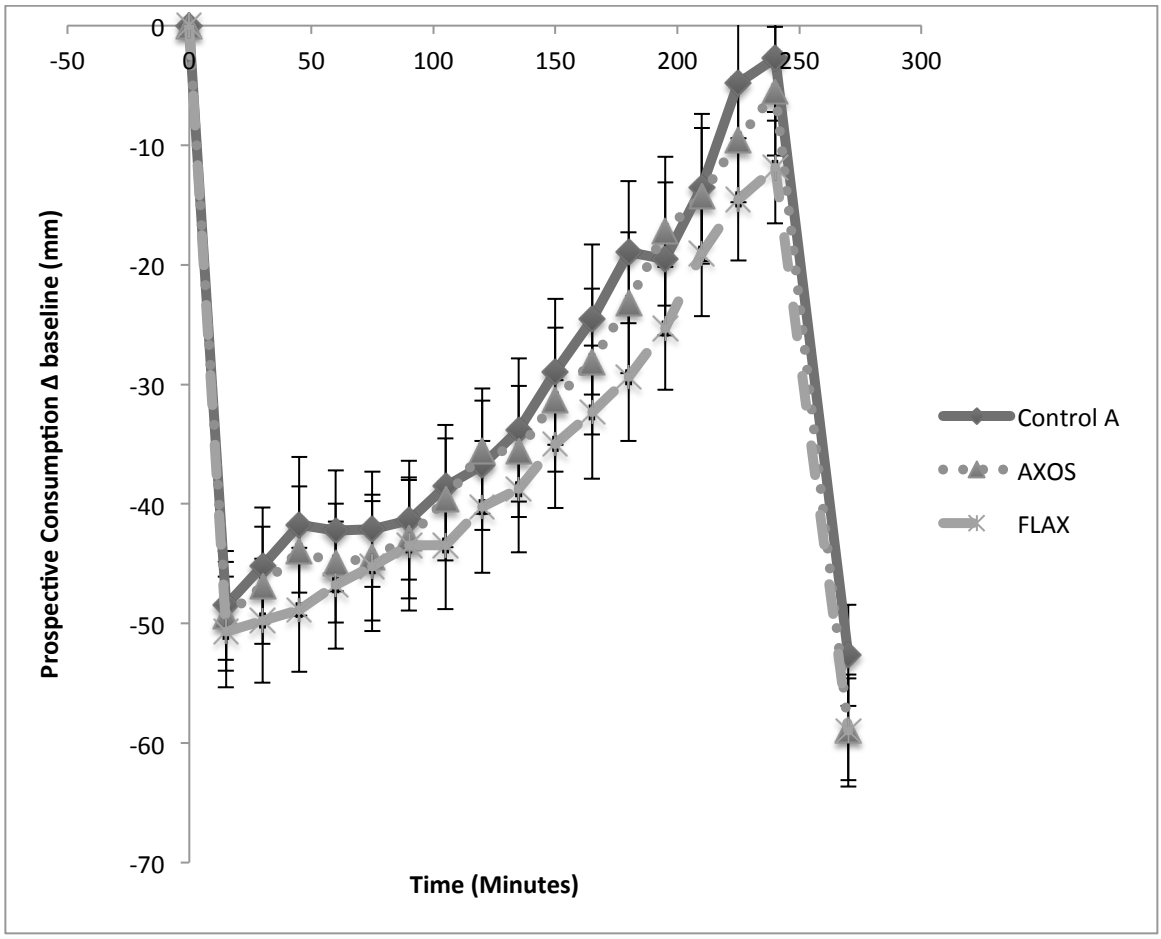


Figure 21. Trial 1 Prospective Consumption VAS rating change (Δ) from baseline following consumption of each test breakfast. *Data are presented as mean ± SEM, based on 30 subjects. There were no significant differences between treatments at any time point (p> 0.05).*

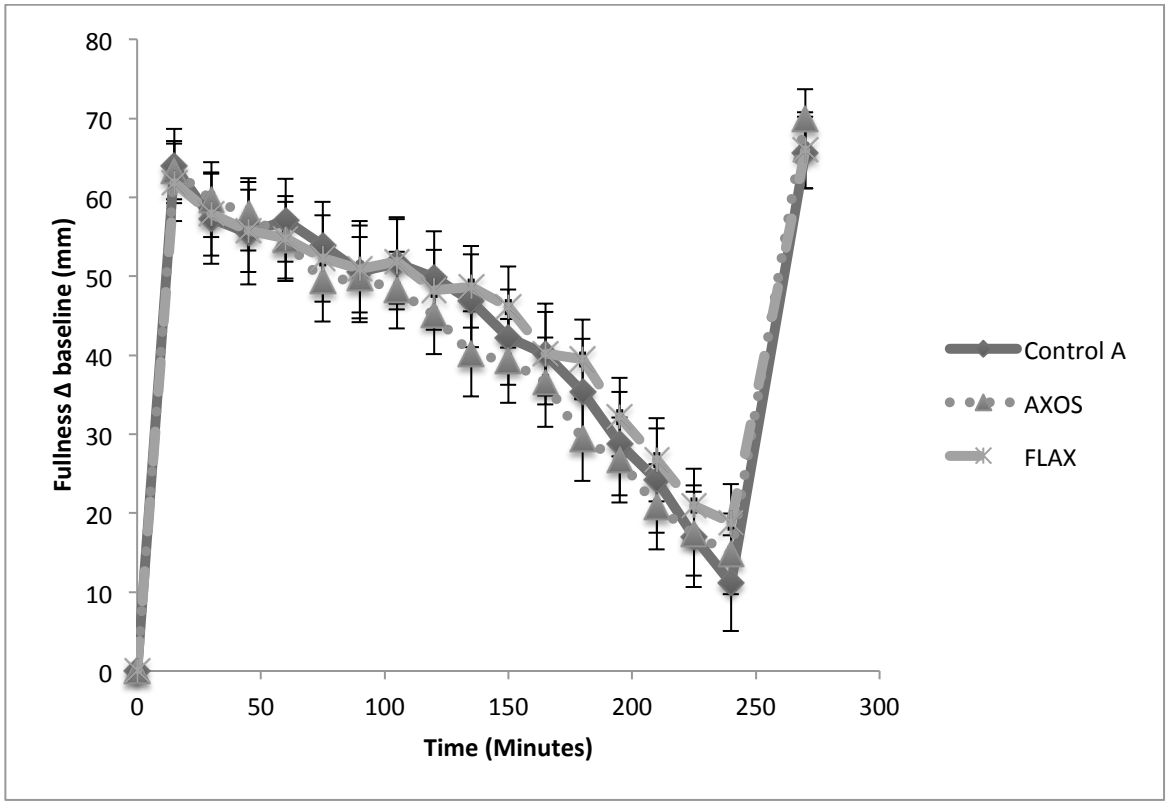


Figure 22. Trial 1 Fullness VAS rating change (Δ) from baseline following consumption of each test breakfast.
Data are presented as mean ± SEM, based on 30 subjects. There were no significant differences between treatments at any time point (p> 0.05).

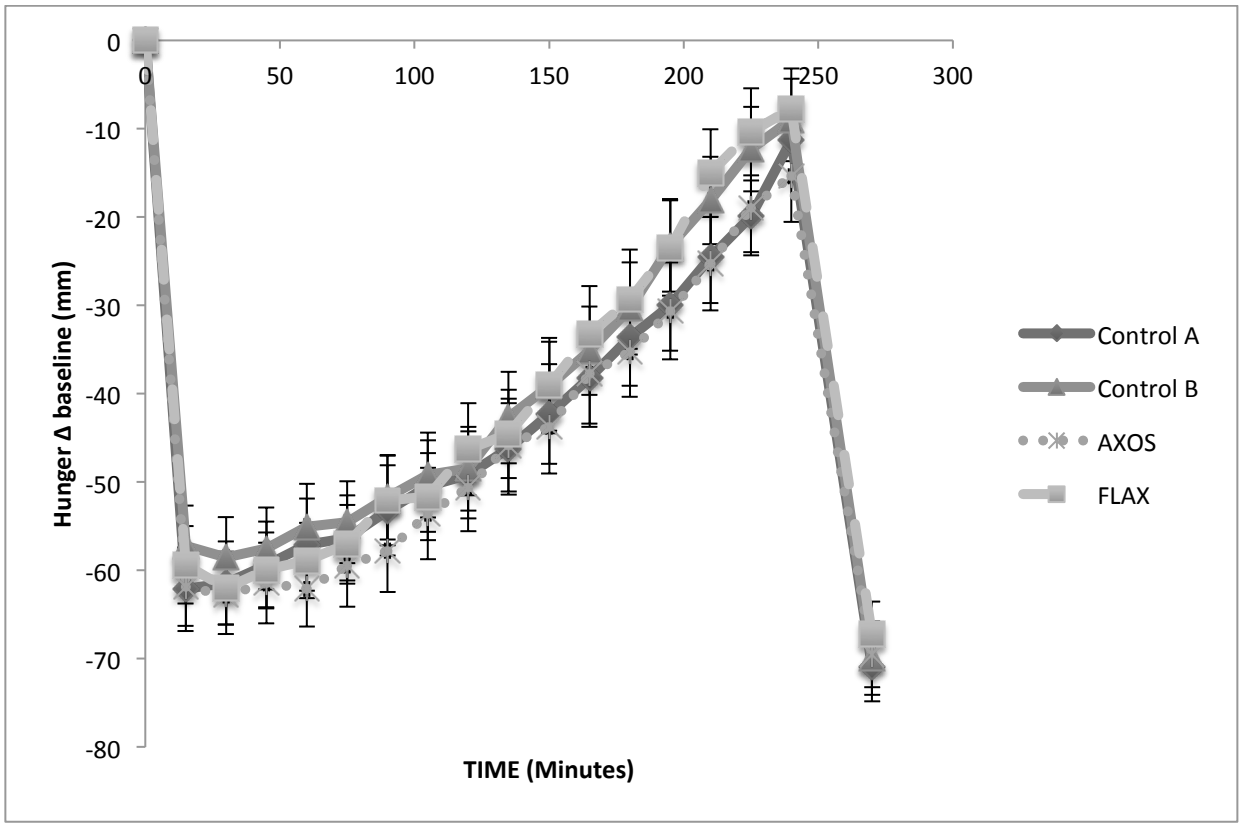


Figure 23. Trial 2 Hunger VAS rating change (Δ) from baseline following consumption of each test breakfast.
Data are presented as mean ± SEM, based on 36 subjects. There were no significant differences between treatments at any time point ($p > 0.05$).

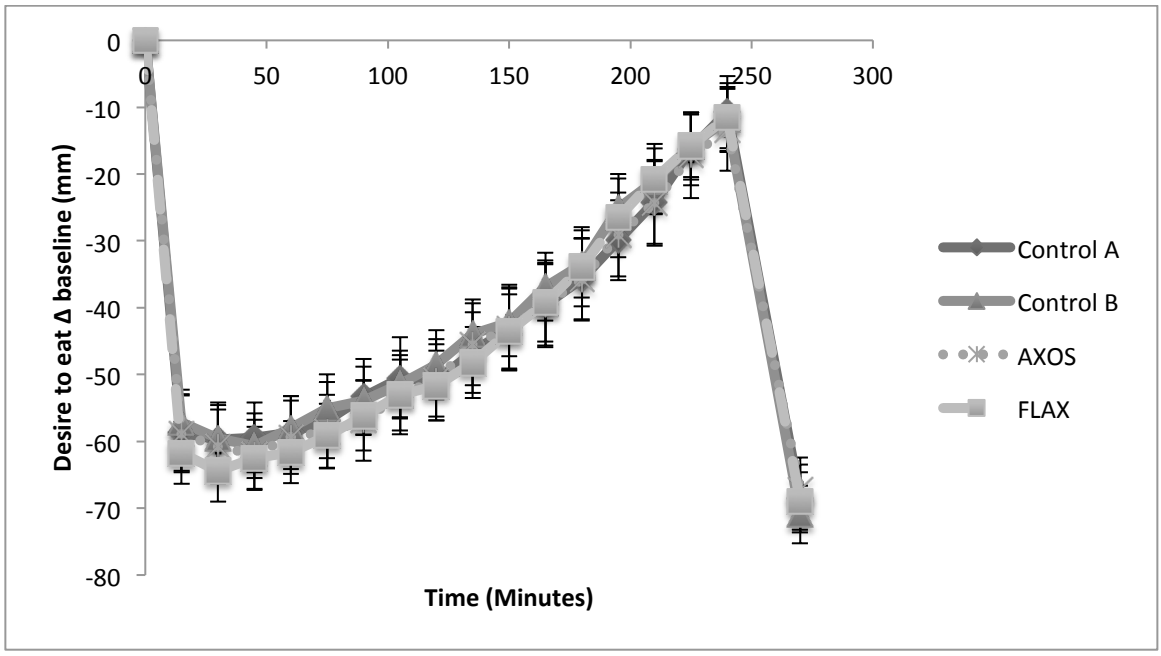


Figure 24*. Trial 2 Desire to Eat VAS rating change (Δ) from baseline following consumption of each test breakfast.* *Data are presented as mean ± SEM, based on 36 subjects. There were no significant differences between treatments at any time point (p > 0.05).*

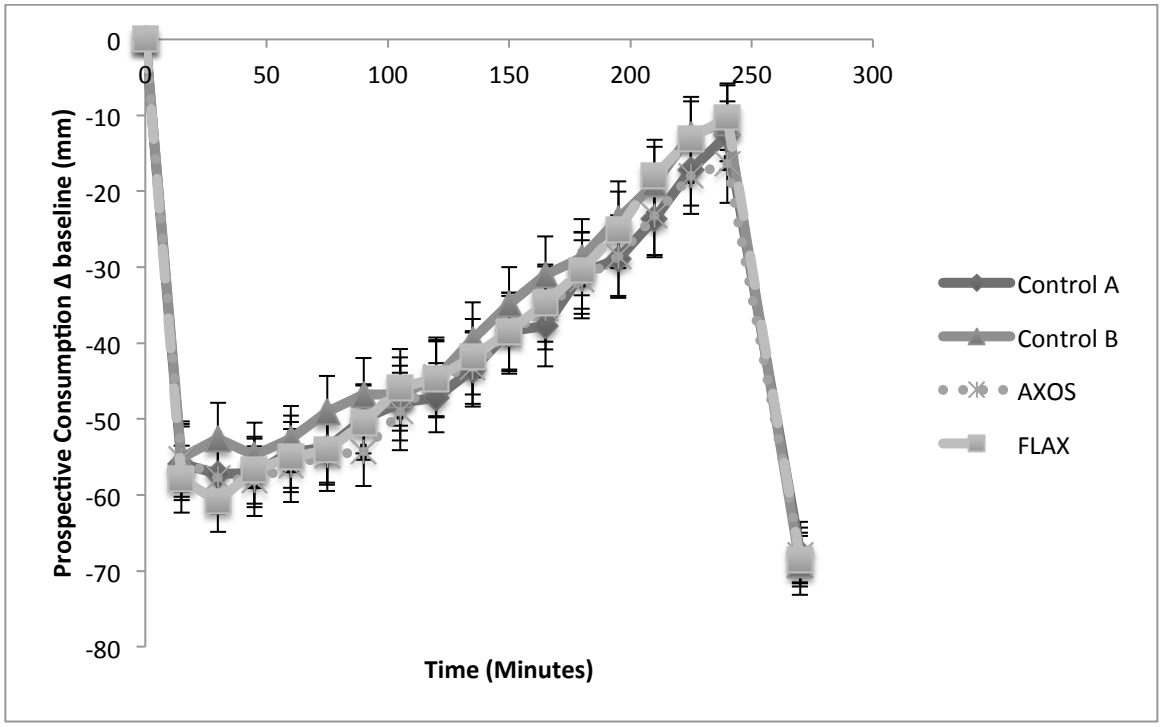


Figure 25. Trial 2 Prospective Consumption VAS rating change (Δ) from baseline following consumption of each test breakfast. *Data are presented as mean ± SEM, based on 36 subjects. There were no significant differences between treatments at any time point (p > 0.05).*

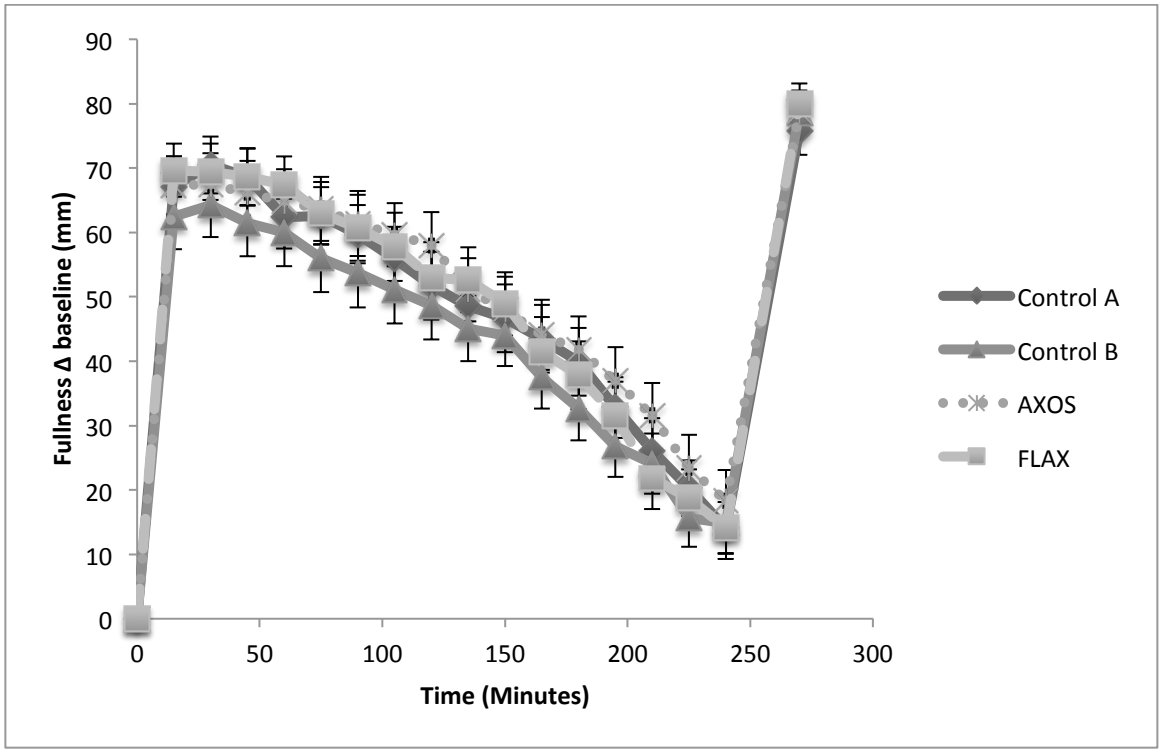


Figure 26. Trial 2 Fullness VAS rating change (Δ) from baseline following consumption of each test breakfast.
Data are presented as mean ± SEM, based on 36 subjects. There were no significant differences between treatments at any time point (p > 0.05).

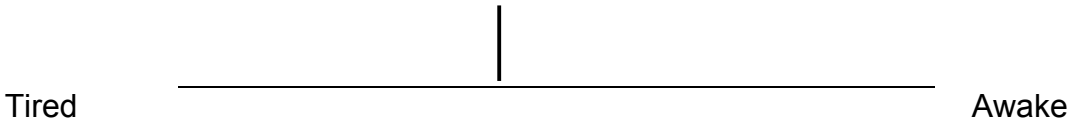
Visual Analog Scale Directions

On the following pages you will be answering several questions. There are no right or wrong responses to the questions.

- Please draw a single, vertical, straight, mark along the line to indicate your response.
- The vertical line must intersect with the horizontal line.
- Please do not circle any words.
- Your response should indicate how you are feeling at this moment.

Example of **correct** mark:

A. How do you feel?



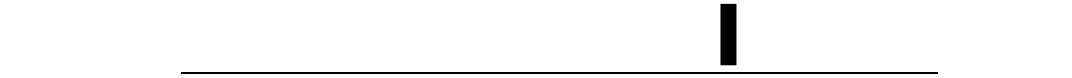
Examples of **incorrect** marks:

A. How do you feel?

Figure 27. Visual analog scale (VAS)

Figure 27 (cont'd)

A. How do you feel?



VISUAL ANALOG SCALE

Visit #: _____ Date: ___/____/____

Subject Initials: _____ Screen #:_____

Start of Study Product Consumption (t = 0): _____:_____ (24-hr clock)

Test Time t = _____ Scheduled Time: _____ :_____(24-hr clock)

Actual Time: _____:_____(24-hr clock)

DIRECTIONS: On the following pages you will be answering several questions. There are no right or wrong responses to the questions. Please draw a single, vertical, straight, mark along the line to indicate your response. The vertical line must intersect with the horizontal line. Please do not circle any words. Your response should indicate how you are feeling at this moment.

Figure 27 (cont'd)

Begin:

1. How strong is your desire to eat?

Very weak _____ Very strong

VAS measurement (mm): _____

2. How hungry do you feel?

Not hungry _____ As hungry as I've ever felt at all

VAS measurement (mm): _____

Figure 27 (cont'd)

Subject initial: _____ Date ____/____/_____

PLEASE CONTINUE ON NEXT PAGE

3. How full do you feel?

Not full _____ Very full

at all

VAS measurement (mm): _____

4. How much food do you think you can eat?

Nothing _____ A large

amount

at all

Figure 27 (cont'd)

VAS measurement (mm): _____

Subject initial: _____ Date ____/____/_____

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