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Effect of Functional Starch in Brown Rice and Grain Sorghum on Plasma Glucose and Insulin Responses in Humans

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Effect of Functional Starch in Brown Rice and Grain Sorghum on Plasma Glucose and Insulin Responses in Humans
Effect of Functional Starch in Brown Rice and Grain Sorghum on Plasma Glucose and Insulin Responses in Humans

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Food Science

by

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University of Arkansas
Bachelor of Science in Food Science, 2011

December 2013
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This thesis is approved for recommendation to the Graduate Council.

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ABSTRACT

Diabetes and obesity are chronic illnesses increasing at dramatic rates in the U.S. and around the world. Research has looked to prevent or control diabetes and obesity through functional ingredients such as healthy-dietary carbohydrates to control blood glucose levels. Starch is a principle carbohydrate, which influences blood glucose levels, and measuring starch digestibility fractions can help predict the glucose response in the body. The objectives of this study were to investigate the functional starch content of parboiled brown rice flour and grain sorghum flour, and measure the effects on postprandial plasma glucose and insulin levels of 14 and 10 healthy men after consuming a parboiled brown rice flour pudding and grain sorghum flour muffin, respectively. For control treatments, a standard 50g glucose solution served as the parboiled brown rice flour pudding control, and a wheat flour muffin was compared to the grain sorghum muffin. Initial flour materials and final food products were analyzed for total starch (TS) content as well as the starch digestibility fractions: rapidly-digestible starch (RDS), slowly-digestible starch (SDS), and resistant starch (RS). Using a randomized-crossover design, male subjects consumed treatments during respective studies within a one-week washout period, and glucose and insulin levels were observed at 15 minutes before and at 0, 15, 30, 45, 60, 75, 90, 120, 180 minutes after consumption in addition the incremental Area Under the Curve (iAUC) was calculated. Significant reductions of the mean glucose responses were observed after the consumption of parboiled brown rice pudding at 6 intervals and mean glucose iAUC responses also significantly lowered (P<0.05). Mean plasma insulin responses also reduced in a similar trend. The grain sorghum study observed mean glucose responses significantly reduced at 5 intervals and the mean insulin responses reduced at 6 intervals (P<0.05). Mean plasma glucose iAUC responses reduced significantly an average of 26% and mean plasma insulin iAUC significantly reduced an average 55% (P<0.05). Results suggest parboiled brown rice flour or grain sorghum flour would be a good functional ingredient to assist in managing blood glucose levels and additional research of slowly-digestible and resistant starches may help in the prevention of diabetes and obesity.
ACKNOWLEDGEMENTS

Special thanks to Dr. Sun-Ok Lee for her constant guidance and direction and without this research would not be possible. Also thanks to Dr. Ya-Jane Wang and Dr. Craig Coon for their support and participation as committee members. Thanks are also due to graduate and undergraduate students of Dr. Lee’s and Dr. Wang’s lab for research assistance and the individuals who participated in research experiment.
DEDICATION

This thesis manuscript is dedicated to Dr. Lee and my family for their help, encouragement, and support.
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LITERATURE REVIEW

Introduction
Carbohydrates known to cause glucose level increases after eating, however, not all are broken down and digested the same. Digestion rates vary, particularly for starches. Based on source, structure, particle size, or interactions with other food matrix components, such as proteins or minerals as phytic acid, starches are broken down and hydrolyzed at different rates. (Englyst et al., 1992, Annison and Topping, 1992). Starch digestion is expressed by three fractions: rapidly-digestible starch (RDS), slowly-digestible starch (SDS), and resistant starch (RS) (Englyst et al., 1992). The third fraction, RS, is the starch which escapes digestion in the small intestine and continues to along the GI and has been an important nutrition topic, mainly for controlling blood glucose levels. Both SDS and RS, also known as functional starch, are linked to reduced glucose and insulin responses in healthy diabetic human participants (Behall et al., 1988; Blaak and Saris, 1995; Panlasigui et al., 1991, 1992, 2006; Parhiraje et al., 2010). Starch digestion also varies based on processing and cooking conditions, and there are a number of potential sources which can be modified to additionally slow digestion. Brown rice is one starch source which may be modified for a slower digestion and grain sorghum is also another starch source which has demonstrated slower digestion rates for a cereal (Newton et al., 2010, Lakshmi and Vimala, 1996). Brown rice has reduced blood glucose levels compared to milled rice in vitro and in vivo (Panlasigui et al., 2006), and investigating processing conditions such as parboiling could increase functional nutritional properties. While previous research has established brown and parboiled rice as good candidates for glucose and insulin control, little or no evidence has been published investigating such effects of parboiled brown rice flour. Grain sorghum has been recognized for indigestible properties and is also a good candidate for a functional starch ingredient (Lakshmi and Vimala, 1996; Dicko et al., 2006; Carciofi et al., 2007; Giuberti et al., 2012). The indigestibility of grain sorghum would be an excellent candidate to control glucose and insulin in the body. The goal of this study is to identify functional starch ingredients which may be used for the control or prevention of diabetes. The hypothesis is both parboiled brown rice and grain sorghum flours will be good starch sources for slow in vitro digestion as well as reduce glucose and insulin responses in humans. The objectives are to measure in vitro starch digestion fractions and to measure the glucose and insulin effects in healthy, male subjects in separate human studies.
**Starch**

Carbohydrates are an important component of the diet mainly for contributing energy and occur in a large variety of forms, namely categorized as mono-, di-, poly-, and oligosaccharides. As the second most consumed component in the diet, starch can be found in nearly every edible food source. Starch is categorized as a polysaccharide and is scientifically known as a glucan. Starch contains monosaccharides which are linked by glycosidic bonds, also defined as a homopolysaccharide or homoglycan. Starch occurs in four main areas of a plant: the seed as for cereals, legumes, and grass; the root, tuber and stem such as potatoes or tapioca; in fruit such as a green banana or plantain; or in leaves such as tobacco (Blaak and Saris, 1995). Representing the main energy reserve for plants, starch is composed of amylose and amylopectin and is formed by a complex biological pathway involving photosynthesis (Annison and Topping, 1994). In starch composition, both amylose and amylopectin are typically present in complementary amounts based on the source. On average, amylopectin represents around 75% and amylose 25% of starch content. Amylose and amylopectin are chemically identical, however, each differ based on structural arrangement. Amylopectin is composed on D-glucose units with α1-4 and α1-6 linkages which form branches and amylose is also composed of D-glucose units, but with only α1-4 linkages which form more a linear pattern as shown in Figure 1 below.

![Figure 1 Amylopectin and Amylose Structures (Annison and Topping, 1994)](image)

Depending upon the starch source, starch content can greatly vary in addition the composition of amylose and amylopectin can exist in different ratios which will consequently influence structure and
physicochemical properties. For example, potato is typically 25-30% and corn or maize about 70% starch, but both starch sources contain about 20-25% amylose (Blaak and Saris, 1995). Also, amylose and amylopectin content can range greatly between varieties or phenotypes of a botanical source; usually the term “waxy” will be used represent a starch that has 10% or less amylose present within the starch matrix. The starch is organized in to what is identified as a granule; it is a unique “thumbprint” by which a starch can be identified from one crop to another or within a crop varietal. Starch granules are both dense and insoluble, and can only hydrate a small amount at room temperature.

While starch amount and composition provides the foundation for starch properties such as granule size and structure, digestibility is another significant result of starch characteristics (Annison and Topping, 1994). It is the disruption of starch granules which starch is broken down into its structural constituents of amylose and amylopectin, and enzymes such as amylases and amylolytic enzymes further break and depolymerize glycosidic linkages to yield glucose for utilization in the small intestine (Englyst et al., 1992).

**Functional Starch Fractions RDS, SDS, RS and Digestibility**

While starch is broken down or hydrolyzed by a large number and complex process of enzymatic attacks, it was Englyst who first quantified and identified the two important enzymatic digestion rates. Englyst identified three separate starch digestion fractions, also known as nutritionally important starch fractions. Since then, researchers have thoroughly investigated various starch sources to understand the variance of digestion rates across different sources (Englyst et al., 1992; Cummings et al., 1997). The nutritionally important starch factions are known as rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS). On average, the RDS faction is hydrolyzed within the first 20 minutes of digestion, and the SDS faction is the starch hydrolyzed after 120 minutes of digestion. The RDS and SDS fractions are broken down beginning with amylase present in saliva through the small intestine. The third fraction, resistant starch, is the starch not digested after 120 minutes. Figure 2 below illustrates the molecular structure of SDS starch fraction from Zhang and authors (2008) demonstrating the basis for physical or chemical entities. The authors describe the ‘other factors’ as representing the food matrix as well as food form, starch treatments, and enzyme inhibitors which all influence the SDS components.
starch fraction. Both SDS and RS starch fractions are important to analyzing functional starch ingredients and digestion rates of various starch sources.

![Figure 2 SDS Starch Fraction Factors (Zhang et al., 2008)](image)

The RS portion was coined by Englyst in 1992 as the fraction that escapes digestion in the small intestine and continues through the GI tract to the large intestine which can act as substrate for fermentation. Based on the starch source, both SDS and RS can vary greatly, and both pose great nutritional benefits. Before the discovery of the nutritional starch fractions, starch was believed to be completely hydrolyzed during digestion, and the breakthrough that proved otherwise changed the perspective of carbohydrate chemists’ and nutritionists’ view of starches and their role in the diet. Since such findings, the role and analysis of starches has evolved greatly and continues to be modified based on different starch types and forms.

**RS Quantification and Analysis of Foods**

The measurement and quantification of functional starch fractions was established by Englyst in 1992 as previously described and additional methods have been developed to quantify all three fractions or to analyze only RS content. Below is the calculation used for RDS, SDS, and RS:

\[
\text{RDS} = (G_{20} - \text{FG}) \times 0.9 \\
\text{SDS} = (G_{120} - G_{20} - \text{FG}) \times 0.9 \\
\text{TS} = (TG - \text{FG}) \times 0.9 \\
\text{RS} = \text{TS} - (\text{RDS} + \text{SDS})
\]

While there are three separate nutritionally important starch fractions, forms of resistant starch vary more depending upon source and structure. In Table 1, the five types of resistant starch with food form description and source are listed depending upon classification from Fuentes-Zargoza et al. (2010).
Different classifications of resistant starch occur mainly due to starch structure, source, or processing methods.

**Table 1. Resistant Starch Classifications and Food Sources**

<table>
<thead>
<tr>
<th>Type</th>
<th>Description</th>
<th>Food Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>RS I</td>
<td>Physically Entrapped</td>
<td>Whole / partly milled grains, seeds, legumes, pasta</td>
</tr>
<tr>
<td>RS II</td>
<td>Native Starch</td>
<td>Raw potatoes, green bananas, some legumes, high amylose starches</td>
</tr>
<tr>
<td>RS III</td>
<td>Retrograded</td>
<td>Cooked &amp; cooled: potatoes, bread, corn flakes</td>
</tr>
<tr>
<td>RS IV</td>
<td>Chemically Modified</td>
<td>Some fiber drinks or foods where modified starches used (cakes &amp; breads)</td>
</tr>
<tr>
<td>RS V</td>
<td>Lipid Complex</td>
<td>Starch chemically modified with lipid molecule</td>
</tr>
</tbody>
</table>

*Table adapted from Fuentes-Zaragoza et al. (2010)*

For in vitro measurement, methodologies can vary for resistant starch fraction determination according to the sample. Typically, resistant starch content is determined by chemical or enzymatic degradation, however, enzymatic is more favored because both SDS and RS can be quantified and RDS by difference of the two fractions. (Englyst et al., 1999; Walter et al., 2005; Perera et al., 2010). Though RS is indigestible by enzymes in the body, the various forms of resistant starch can be evaluated depending upon characteristic type. For type 1, the chemical difference between glucose released by the enzyme digestion of a homogenized food sample and that released from a non-homogenized sample; type 2, is the chemical difference between glucose released by the enzyme digestion of a boiled homogenized food sample and that from an un-boiled, non-homogenized food sample; type 3, known as the most resistant starch fraction, is almost measure chemically with KOH or dimethyl sulphoxide because it is entirely resistant to digestion by pancreatic amylases (Sajilata et al., 2006). For measuring RS content of type 4 and 5, since the resistant starches form different bonds rather than α1-4 linkages due to chemical modification cross-linking, differential scanning calorimetry (DSC) is required for analysis (Fuentes-Zaragoza et al., 2010, Sajilata et al., 2006).

For in vivo measurement, the glucose response is measured and calculated almost identically to the glycemic response. The area under the curve (AUC) is calculated from glucose measurements taken
0 minutes after consumption until 120 minutes for measuring the glycemic index, however, when measuring the glucose response, it is recommended to observe the glucose response up to 180 minutes post consumption to verify levels have returned to baseline or homeostasis levels, but sample collection and overall methodology is nearly identical for measuring the glucose response and glycemic index (Ludwig, 2002).

**Resistant Starch Consumption**

In a study conducted 2003 by Murphy and authors, efforts were made to understand what and how much resistant starch Americans were or were not eating. Since RS does differ from fiber, it can be more difficult to quantify and however, unsurprisingly the cereal-based foods were the top source for American diets. Breads were ranked number one or about 21% for total RS consumption for the total population surveyed and cooked cereals / pastas were number two at about 19% for Americans (≥1y).

Below shows a table adapted from Murphy et al. (2003) showing a complete analysis of persons surveyed and their range and average RS consumption based on age group as well as gender for 12 years and older groups. Male age groups have been observed to consume a higher average of RS starch on a gram per day basis, and authors suggest this may be due to more food intake compared to females.

**Table 2 Average Consumption of Resistant Starch in the U.S.**

<table>
<thead>
<tr>
<th>Age Group</th>
<th>n</th>
<th>Average</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infant/Children</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1y</td>
<td>706</td>
<td>1.9</td>
<td>1.0 - 3.2</td>
</tr>
<tr>
<td>1-5y</td>
<td>2,013</td>
<td>3.7</td>
<td>2.0 - 6.0</td>
</tr>
<tr>
<td>6-11y</td>
<td>2,098</td>
<td>4.2</td>
<td>2.6 - 6.8</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 - 19y</td>
<td>2,261</td>
<td>4.3</td>
<td>2.6 - 6.9</td>
</tr>
<tr>
<td>20 - 49y</td>
<td>2,622</td>
<td>4.4</td>
<td>2.6 - 7.1</td>
</tr>
<tr>
<td>≥50y</td>
<td>2,132</td>
<td>4.2</td>
<td>2.1 - 6.9</td>
</tr>
</tbody>
</table>
Overall, the consumption of resistant starch is lower compared to other countries. Table 2 illustrates the consumption of resistant starch in the U.S. by a gram per day basis, and the average rate between 4-5 grams per day for adults, with 20-49y age categories consuming the average higher amounts, is much lower compared to the average 9 grams per day in Australia and most of Europe (Murphy et al., 2003). Though the data does reflect information from nearly 10 years ago, information regarding the increase of fiber in many food products has been strongly encouraged the past decade, and perhaps some increases across age groups has occurred since last recorded data. New information would be beneficial for the most updated consumption information since many good sources of fiber can also be a good source of RS and/or SDS, additionally.

### SDS and RS Fractions and Nutrition

The ratio of amylose to amylopectin is important to digestibility. The two structures that compose starch can be helpful in predicting the digestibility of the total starch source. For example, higher amylose content is linked with slower or reduced digestion rates, and in turn, the in vitro analysis can demonstrate more SDS and RS starch fractions which explain the difference of digestibility rates. Other equally important qualities which coincide with starch composition are starch granule size, shape, and matrix influence digestibility and starch fractions. The shape and overall distribution of a starch granule is influenced by the amylose and amylopectin, but the even similar compositions of starch sources can demonstrate very different starch morphologies due to other food matrix properties as protein, lipid, or...
mineral content that will be further explained. Additionally, processing techniques involving heat or moisture can disrupt starch granules and followed by a cooling stage, are believed to modify digestibility in the starch source (Annison and Topping, 1994, Niba, 2003). Typically, the long, mostly linear structure amylose retrogrades by heating and cooling process, and by lowering digestibility, can increase SDS and RS starch fractions and reduce the glucose response (Perera et al., 2010). By modifying starch digestibility via processing methods or selecting sources high in SDS and RS, homeostasis glucose levels can be maintained or reduce the occurrence of sharp plasma glucose peaks. Individuals diagnosed with diabetes mellitus would greatly benefit from functional starches such as SDS and RS starch.

**Table 3 Physiological effects of RS on Health (Fuentes-Zaragoza et al., 2010)**

<table>
<thead>
<tr>
<th>Protective Effect</th>
<th>Potential Physiological Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes</td>
<td>Glycemic and insulinemic control</td>
</tr>
<tr>
<td>Colorectal cancer, ulcerative colitis, inflammatory bowel disease, diverticulitis and constipation</td>
<td>Improved bowel health</td>
</tr>
<tr>
<td>Cardiovascular disease, lipid metabolism syndrome, cholesterol and triglycerides</td>
<td>Improved blood lipid profile</td>
</tr>
<tr>
<td>Colonic health</td>
<td>Prebiotic and culture protagonist</td>
</tr>
<tr>
<td>Obesity</td>
<td>Increased satiety, reduced energy intake</td>
</tr>
<tr>
<td>Osteoporosis via enhanced calcium absorption</td>
<td>Increased micronutrient absorption</td>
</tr>
</tbody>
</table>

**Diabetes and Obesity**

In a 2011 report from the CDC, diabetes mellitus affects almost 26 million people, in 2010 alone, nearly 2 million newly diagnosed. Diabetes mellitus is a chronic disease can severely affect the overall health of an individual, however, specifically diabetes mellitus affects glucose metabolism. The increasing trend of type II diabetes occurrence and diagnosis in the U.S., and around the world, has increased at a dramatically high rate within the last two decades (CDC, 2011). Currently, reports by the CDC cite over 8%, or nearly 26 million people, of the U.S. population are diagnosed with diabetes mellitus; the increase represents a doubling since the last 10 years and is expected to rise alongside obesity rates (2011). As shown in Figure 3, 4, and 5, the CDC reports an increasing trend among the U.S. population and particularly those between the ages of 18-64 years with nearly equal proportion for gender.
Medical costs attributed to diabetes have been quantified as $27 billion by the American Diabetes Association (ADA) in a report released in 2007. The ADA noted also severe economic consequences due to chronic complications, a figure totaling $58 billion dollars with every 1 in $5 spent for diagnosed
diabetes and nearly every 1 in $10 spent in health care costs are attributed to diabetes. Chronic illness such as cardiovascular disease (CVD), hypertension, and stroke are all strongly associated with the diabetes mellitus and are also expected to increase with the occurrence of diabetes mellitus. In the report, the ADA also cited additional indirect costs such as problems caused by diabetes estimated at $55.6 billion.

Blood glucose concentration control is imperative for health of diabetic individuals. Regular observation and regulation are extremely important for individuals with pre-diabetes or diabetes, and the role of starches in the control of blood glucose and insulin are essential. As starch structure can vary plant to plant, starch metabolism can also vary individual. However, much work has been carried out to understand and quantify starch digestion in the human body, and by doing so, has demonstrated different plant sources can be broken down after two critical time points postprandially. This information can also explain the metabolic significance of starches to the system. The RDS starch fraction provides a rapidly-available amount of glucose to the system for energy, and while SDS provides a slower release of glucose to the system between 20 and 120 minutes post-consumption. For the RS content of starches, the portions not digested after 120 minutes can offer an array of health benefits, but namely, glucose and insulin control. Starch sources, processing, and structure will be further discussed in the application of functional starch fractions and the affect on glucose and insulin metabolism.

**Human Studies on Glucose Response to Starch**

Beginning in the early 1980’s, interest in studying how the body reacted to certain food sources depending on their blood glucose levels was explored. Ultimately, the Glycemic Index (GI) was created in order to quantify and qualify various food sources and their effects on blood glucose levels, a tool particularly important for assisting individuals with diabetes of what foods to eat. In 1981, Dr. Jenkins created this tool and a human study was published outlining a large range of carbohydrate foods which subjects consumed and blood glucose measurement levels tested. From grain and cereal products to bread, rice, pasta, vegetables, and fruits, food samples of 25 g were consumed and incremental glucose differences were observed and quantified and compared to a standard control. With slight modifications, the glycemic index is still a helpful tool today and is utilized in a number of studies for glucose
measurement. In a review by Chiu et al. published in 2011, there is strong epidemiology evidence that GI is closely linked to long term health such as chronic illnesses as cardio vascular disease (CVD) and macular degeneration.

Beginning with Dr. Jenkins and researchers work in 1981, starch structure and the metabolism responses with respect to glucose and insulin were investigated. In a study conducted in 1988 by Behall and associates, a human study was conducted to view the responses of a study comparing a meal consisting of 70% amylose to a meal containing 70% amylopectin. For the meal containing 70% amylose, glucose responses were significantly lower at the 30 minute interval in additional insulin concentrations were significantly lower at the 30 and 60 minute interval. Overall, Behall (1988) noted a significantly lower insulin response for the whole duration for the amylose meal, strongly linking the influences of starch structure with glucose metabolism. Following the next year, Behall and researchers (1989) looked at a more long-term human study in 1989. The study was again conducted with eight healthy participants, and treatments were again divided by 70% amylose or amylopectin meals. Lower fasting glucose and insulin levels were observed after 5 weeks with the amylose meal and glucose response tests showed higher levels after amylopectin treatment. Work has been continued to understand the influence of starch and digestibility on the glucose and insulin responses in humans. Recently, a study published in 2012 by Eelderink and researchers focused on quantifying the extent to which starch digestibility and the glycemic response effect in a starchy food product. In the study, 10 healthy participants consumed 3 treatment meals: pasta with normal wheat bran, bread with normal wheat bran, and bread with purple wheat bran. Researchers conducted the study to observe the GI responses and compare in vivo starch digestibility using $^{13}$C-isotope enrichment. Interestingly, Eelderink and group found conflicting results with the GI responses and in vivo starch digestibility and proposed intervention studies to reconsider relying solely on the GI score for characterizing products. The lowest RS content meal, the pasta with normal wheat bran (PA), exhibited the lowest GI score for the three meals in addition the lowest readily-available glucose and highest slowly-available glucose according to data presented. Relating the discrepancy between methods as differences in glucose kinetics, authors suggested that for both reliable and repeatable results, researchers should consider additional quantification other than only GI measurement. The in
vivo measurement techniques Elderlink and group using the C-isotope used are relatively new, further studies would confirm if methodology.

**Brown Rice**

Rice is a staple for over 26 countries around the world in Asia and North and South America (FAO, 2004). In 2004, the FAO recognized rice as providing 20% of the world’s dietary energy with wheat following behind at 19% and maize at 5%. In countries such as Thailand, India, Vietnam, and the U.S. are top exporters of rice. In report released by the FAO in July 2013, world rice production is forecasted to expand by 1.9% to 500 million tones. Rice is also known for providing a rich source of vitamins such as thiamine, riboflavin, and niacin, and in addition, unmilled or brown rice is a good source of fiber.

**Table 4. Nutrient Content of Rice Varieties from FAO (2004)**

<table>
<thead>
<tr>
<th>Rice Type</th>
<th>Protein (g/100g)</th>
<th>Iron (mg/100g)</th>
<th>Zinc (mg/100g)</th>
<th>Fiber g/100g</th>
</tr>
</thead>
<tbody>
<tr>
<td>White, polished</td>
<td>6.8</td>
<td>1.2</td>
<td>0.5</td>
<td>0.6</td>
</tr>
<tr>
<td>Brown</td>
<td>7.9</td>
<td>2.2</td>
<td>0.5</td>
<td>2.8</td>
</tr>
<tr>
<td>Red</td>
<td>7.0</td>
<td>5.5</td>
<td>3.3</td>
<td>2.0</td>
</tr>
<tr>
<td>Purple</td>
<td>8.3</td>
<td>3.9</td>
<td>2.2</td>
<td>1.4</td>
</tr>
<tr>
<td>Black</td>
<td>8.5</td>
<td>3.5</td>
<td>-</td>
<td>4.9</td>
</tr>
</tbody>
</table>

Numerous pureline and hybrid rice cultivars are grown and harvested around the world, and most crops depending upon desired finished product, are usually milled. With the removal of the bran though, many beneficial nutrients and fibers are lost in the milling process. The polyphenols, dietary fiber, and lipids found in the bran are reported higher in brown rice compared to milled rice, and are also linked to lower glycemic response (Panlasigui -and Thompson, 2006). The study found that long grain brown rice resists starch hydrolysis in comparison to milled rice. The cooked brown rice in comparison to identical, milled rice batch showed a lower in vitro sugar release in addition significant reductions in glycemic area and glycemic index values for both healthy and diabetic individuals. The role of fiber and protein present in bran have also been noted for slower starch digestion; principally, the interaction and binding of protein and starch. In addition, the physicochemical properties brown rice, and the starch physicochemical
properties have strongly linked to starch digestibility (Reader et al., 2002; Frei et al., 2003; Jung et al., 2009; Fuentes-Zaragoza et al., 2010; Patindol et al., 2010; Newton et al., 2011; Chung et al., 2011). Physicochemical properties are inherent to starch source and structure, however, it is the cooking or processing methods that can greatly influence those properties, and in turn, digestibility.

**Fig 6 Low (A), Intermediate (B), and High Amylose (C) Rice Starch (Zhu, 2011)**

**Parboiling and Storage and Metabolic Glucose Effects**

Parboiling is a good example of a processing technique used to modify starch fractions. Parboiling is a well-known method of soaking rough or brown rice in excess water at room temperature, and then steaming and drying the rice. Parboiling conditions vary according to initial feedstock and the functional properties of the finished products (Newton et al., 2011). Because starch structure can be modified through parboiling conditions, brown rice is a good source to have decreased starch digestibility while also providing additional health benefits. Rice cultivars can also have a relatively wide range of amylose content, which is an important factor in structure and digestibility in addition to physicochemical properties (Shu et al., 2006; Sajilata et al., 2006; Shi et al., 2011).

In a study conducted in Sri Lanka in 2010, traditional and improved parboiled and un-parboiled rice varieties were evaluated for their postprandial glycemic effect. It was found that traditional rice varieties and parboiled samples of same variety had a reduced glycemic response in healthy subjects, one cultivar known as Bg 352 having a 10% reduction (Parhiraje et al., 2010).
Grain Sorghum

Grain sorghum, Sorghum bicolor L. Moench, is the fifth most important cereal crop in production with production figures such as 60 million tons. The cereal crop is attributed to have originated in Ethiopia (Dicko et al., 2006) and Sudan (Kimber et al., 2013). Sorghum is a crop which represents a large spectrum of phenotypes and also exhibits a large diversity of biotic and abiotic-stress resistances (Kimber et al., 2013). Commonly referred to as sorghum in the United States and Australia, it is also known as durra in Africa, jowar in India, and bachanta in Ethiopia (FAO, 2013). Currently, production is predominately in Nigeria, India, Mexico, Argentina and the U.S. as recognized by the FAO in 2010. Sorghum has been cultivated and produced for food, feed, and energy, with cultivars modified and produced for its dense grain, stalk, or forage qualities. Sorghum production is diverse with regard to climate and terrain mainly because of its ability to produce a fibrous, deep-root system that has also been described as a robust, cane-like species similar to maize (Kimber et al., 2013).

Figure 7. Global Production and Cultivation of Sorghum (FAO, 2010)

Four wild and five cultivated sorghum phenotypes have been identified by plant scientists such as Snowden in 1936 and Harlan in 1995, and to this day, the United States hold the largest collection of sorghum germplasm residues which began in the late 1750s. Researchers also recognize with the introduction of sorghum hybrids in 1958, genomic diversity increased from over 13,000 to over 44,000 accessions in the U.S. alone to better understand the phenotypic and genotypic information changes,
which occurred (Kimber et al., 2013). Interestingly, sorghum phenotypes can grow in ranges from sea level to elevations exceeded 2500 m, drought to extremely wet rain conditions, and on good to poor quality soils (Duncan et al., 1991).

Similar to other cereals such as maize, sorghum is composed mainly of starch. Starch content can range from 55 – 76% depending upon cultivar or crop production year (Dicko et al., 2006, Duncan et al., 1991, Gidley et al., 2011, Rooney and Pflugfelder, 1986).

![Grain Sorghum Diagram](Sautier and O'Deye, 1989)

Sorghum has been reported to contain 12-22g/100 FM (fresh matter) amylose and 45-55 g/100 g FM with typically 60 – 75 g/100 g FM total starch content (Dicko et al., 2006, Yijun et al., 2006). Similar to other cereal crops, sorghum can contain varying amounts of amylose and consequently referred to normal, heterowaxy, or waxy sorghum. In a study conducted by Rooney and Pflugfelder (1986), sorghum samples were analyzed for their starch digestibility based on amylose content and endosperm type. In gross compositional analysis, sorghum is most often compared to maize and wheat; however, both differences in protein type and carbohydrate-protein interactions exist which contributes greatly to the digestibility of grain sorghum.

**Grain Sorghum and Starch Digestibility**

The protein, lipid, mineral, and starch content of sorghum compare closely to those of maize (Dicko et al., 2006, Duncan et al., 1991, Kiang et al., 2010, Zhang et al., 2008), however, the starch-protein properties distinguish sorghum from other cereals (Taylor and Emmabux, 2010, Gidley et al., 2011, Taylor et al., 2006, Rooney and Pflugfelder, 1986). As mentioned above, Rooney and Pflugfelder research study focused on the digestibility differences between sorghum and corn. As they discussed, the
higher indigestibility sorghum possess is mainly attributed to the peripheral endosperm region of the sorghum grain. Both corn and sorghum have similar size, shape, and composition with respect to normal starch kernels, it is the difference of protein type and distribution surrounding the endosperm, which accounts for the differences in digestibility of the starch. According to authors, sorghum does have a higher proportion of peripheral corneous, floury areas around the starchy endosperm and most importantly, has a much more pronounced dense, hard structure with discourages water penetration and digestion via enzyme access. Figure 8 illustrates the composition of grain sorghum. It is the prolamine-rich, also known as a cross-linked kafirin, protein matrix that provides physical protection and causes lower starch hydrolysis yields as Dicko et al. (2006) also confirmed in a review of past sorghum research. 

The protease attack on protein bodies allows for the amylase to attack starch granules, and this has also been observed by with waxy starch granules additionally (Rooney and Pflugfelder, 1986). As outlined by Dicko et al. (2006) the protein kafirin exists in three forms, α-, β-, and γ- kafirins, and can compose ~5 – 10 g of the average 7 – 15 g of protein observed in grain sorghum with α-kafirin being the principle form present. Nutritional quality is known to be very poor for kafirin protein types, particularly because they are protease resistant and a wide range of protein content occurs across cultivars (Anglani, 1998, Dicko et al., 2006).

Sang et al. (2008) investigated the relationship between amylose content in raw grain sorghum starch and digestibility. Though authors observed an inversely related relationship between amylose and digestibility with study results, however, they did also observe higher RS contents from raw heterowaxy sorghum starch in comparison to the raw normal sorghum starch despite differences of amylose content, 14 and 24%, respectively. Differences between SDS and RS were ± 5% for respective fractions of starches. The lower digestibility of the heterowaxy was believed to be due to the amylopectin chain-length distribution rather than the amylose content; the amylopectin chain-length distribution was thought to be attributed to the slight differences in digestibility of the two starches (Sang et al., 2008). Researchers also postulated that grain sorghum with amylose content greater than 40% could have a significantly increased RS content in grain sorghum starch or perhaps manipulation of amylopectin structure would perhaps increase SDS content.
Sorghum and Glycemic Response Application

Though grown in many areas throughout the globe, geographically sorghum is not used and consumed in the same manner. Grain sorghum is typically produced and consumed as food stuffs in areas of Africa, Asia, and Latin America, yet in Australia and the U.S., sorghum is utilized mostly for animal feed (Dicko et al., 2006). Sorghum can be used in a wide array of food applications, but most commonly it is used for baking applications such as flat breads and porridges.

Processing methods have also been shown to increase starch digestibility (Taylor et al., 2006, Rooney and Pflugfelder, 1986, Wong et al., 2009). Due to food matrix and indigestibility of grain sorghum, lower digestion rates of starch have been thought to be beneficial for controlling and maintaining blood glucose levels in humans (Shin et al., 2004, Dicko et al., 2006). Investigating the use of grain sorghum flour compared with similar nutritional-content flour, such as wheat, would evaluate the use of grain sorghum in a food matrix and its ability to maintain glucose levels. In nations in Africa such as Nigeria and Sudan rely on sorghum for their main ingredient in many food stuffs, research of sorghum’s functional food properties have been proposed and investigated (Abdelgadir et al., 2004, Lakshmi and Vimala, 1996). While authors such as Taylor and Emmambux (2010) reviewed the glycemic response and questioned the glucose lowering effects, authors (Lakshmi and Vimala, 1996, Taylor and Emmambux, 2010) found that whole grain sorghum has demonstrated glucose-lowering effects compared to control or de-hulled sorghum treatments.

Research has identified sorghum as a potential functional ingredient for food applications (Taylor and Emmabux, 2010, Taylor et al., 2006, Zhang and Hamaker, 2003), and demand for finding alternative and functional ingredients has prompted expanded use in markets. Recently in the U.S., grain sorghum has been used as an alternative grain for gluten-free food products as well as known for its dense protein content. The lower starch content compared with maize, rice, or potato, the starch structure in grain sorghum is unique in the food matrix (Yijun et al., 2008, Zhang et al., 2008). Dietary fiber and tannin content in grain sorghum has highlighted its nutritional potential (Taylor and Emmambux, 2010, Taylor et al., 2006). With increasing demand of both healthier and reduced allergen products in the market place, grain sorghum has great potential for being incorporated in numerous food applications as a healthy, dietary carbohydrate.
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metabolic glucose and insulin responses compared with cooked rice in female college students.” Nutrition Research 29 (7): 457-461.


Parboiled Brown Rice Product Reduces Postprandial Plasma Glucose Response in Men

Abstract
A staple crop such as rice provides an ideal starch source for creating a functional starch ingredient. Functional starch fractions can act as a functional ingredient by controlling glucose and insulin levels with application for glucose control for health in addition diabetes mellitus. The objective of this human study was to investigate the effect of a parboiled brown rice flour pudding on postprandial plasma glucose and insulin levels. Wells brown rice was parboiled at 120°C for 20 min and ground into flour, and in vitro nutritional starch fractions were measured. A randomized-crossover design was used to observe plasma glucose and insulin responses from 14 healthy, male subjects. Compared with the control, significant reductions after consumption of parboiled brown rice pudding in mean glucose levels at 15, 30, 45, 60, 75, and 90 minutes were observed (P < 0.05). Mean glucose incremental Area Under the Curve (iAUC) were also significantly lower (3795 ± 602 mg/dL) than the control solution (5880 ± 658 mg/dL) (P < 0.05). Plasma insulin mean incremental response reduced also from 3066 ± 525 µU/L iAUC to 2219 ± 715 µU/L iAUC of the control and rice pudding treatments, respectively. Results suggest optimal parboiling of brown rice provided in a flour application could assist in managing plasma glucose levels for individuals, and with additional research functional starch fractions may help in the prevention of diabetes and obesity.

Keywords: Postprandial plasma glucose; Plasma insulin; Slowly-digestible starch; Resistant starch; Diabetes
Introduction

Glucose and insulin control is crucial to the health of both healthy and diabetic individuals. Diabetes has increased in the U.S. within the last two decades, and 25.8 million people or 8% of the population suffers from diabetes mellitus, a trend which is expected to continue to rise along with obesity (CDC, 2011). Diet modifications are one of the most effective ways to maintain a healthy weight and also prevent or control diabetes. Starches and sugars are responsible for the sharp increases in blood glucose levels; however, certain carbohydrates can also allow for a slow release of glucose or maintain homeostasis levels. Differences of starch structure or source influence the breakdown, and consequently, those structural variations allow for a controlled release of glucose (Annison and Topping, 1994). Results from a study conducted with maize-based starches and fibers displayed a strong relationship of reduced glycemic and insulinemic responses from both male and female subjects, while other studies have focused strictly on enzymatic digestion (Annison and Topping, 1994, Frei et al., 2003, Jung et al., 2009). Starch digestion has prompted many nutritional applications for a variety of starch types based on structure and digestion composition. Starch digestion is represented by three enzymatic digestion rates and is identified by three fractions: rapidly-digestible starch (RDS), slowly-digestible starch (SDS), and resistant starch (RS) (Englyst et al., 1992). Additionally, structural differences such as amylose and amyllopectin content correlates with digestion rate and starch fractions (Chung et al., 2011, Newton et al., 2011, Cummings et al., 1997, Denardin et al., 2007). Increasing SDS and RS contents or functional starch fractions in starches has been studied in addition improved starch fraction analysis techniques (Chung et al., 2011, Reader et al., 2002, Zhang et al., 2008). Starch fractions have been investigated thoroughly with human and animal studies, particularly focusing on SDS and RS fractions (Shu et al., 2006, Shi et al., 2011, Zhu et al., 2011). Based on the properties and presence in most diets (Fuentes-Zaragoza et al., 2010, Murphy et al., 2008), functional starch fractions can act as functional ingredients. Novel starches containing increased SDS and RS content from various starch sources have been linked to reduced glucose and insulin responses in both healthy human subjects and diabetic subjects (Behall, 1988, Blaak and Saris, 1995, Panlasigui et al., 1992). Overall, the RS starch fraction presents a two-fold nutritional benefit: escaping digestion in the small intestine and providing substrate for fermentation in the
lower intestine (Topping et al., 2008, Perera et al., 2010). There are five distinctive types of RS which also have a biological impact based on starch form (Sajilata et al., 2006, Han et al., 2007).

One of the most widely consumed starch sources that are consumed as a cooked whole grain or in a variety of products is rice. Previous research shows nutritionally important starch fractions such as RDS, SDS, and RS in rice can be modified based on type of cultivar, environmental conditions, or processing steps (Patindol et al., 2010). Parboiling is a major processing method, which has effectively increased functional starch fractions in rice, and storage is also another important factor for increasing retrograded amylose content and decreasing digestibility (Newton et al., 2011). Increasing functional starch fractions by such methods in rice can reduce the starch enzymatic digestibility and thereby providing a controlled glucose delivery, and improving nutritional benefits (Rashmi and Urooj, 2003). Brown rice is a model starch source based on intrinsic starch structure properties and has shown to provide multiple health benefits as blood glucose lowering effects (Panlasigui et al., 1991, Hu et al., 2004). Previously, cooked whole white and brown rice and its effect on glucose levels has been researched, however, no studies have reported the anti-diabetes effect and glucose control of parboiled brown rice. This study aims to investigate the effects on plasma glucose and insulin responses of healthy men after the consumption of parboiled brown rice flour pudding.

Materials and Methods

Treatment Materials

Wells cultivar brown rice grown in Arkansas was provided by the University of Arkansas Rice Processing Program. Autoclaving and milling preparation of the brown rice was carried out by the University of Arkansas Department of Food Science Carbohydrate lab as well as initial material analysis. Materials were selected based on recent research investigating both optimal parboiling conditions and cultivars for increasing functional starch fractions (Newton et al., 2011). Comparing starch digestibility across hybrid and pureline cultivars exposed to three parboiling conditions identified optimal parboiling conditions for cultivars tested. Results indicated the Wells cultivar parboiled at 120°C for 20 min and stored at room temperature for 24 h (cycle 2 treatment) exhibited optimal starch digestibility of in vitro
analysis (Newton et al., 2010). Lemon extract and sucralose used in pudding formula were purchased from a local grocery store.

**Starch Analysis**

Total starch (TS) was quantified using Megazyme Total Starch kit (Megazyme, Inc., Wicklow, Ireland) and means taken for initial rice flour and final pudding product. Total starch amount was analyzed for parboiled-brown rice flour, and flour amount per serving was calculated based on total starch content. Rapidly-digestible starch (RDS), slowly-digestible starch (SDS), and resistant-starch (RS) were analyzed for both parboiled-brown rice flour and pudding using the modification of Englyst et al. (1992). 20 mL of sodium acetate (0.1 M, 5.2 pH) was vortexed with 800 mg of sample, and 5 mL of enzyme solution (450 mg pancreatin, 6 mL amyloglucosidase) was added to each sample tube in addition a blank and 25 mg/mL glucose control. After 20 min and 100 min of enzymatic digestion, 0.5 mL was taken at both time points and added to 20 mL of 80% ethanol. After deactivating enzymes, 0.1 mL of solution was carried out in the glucose assay (GOPOD). The starch fractions RDS and SDS were observed at 20 min and 120 min, respectively. RS was determined by the difference of RDS and SDS. The parboiled brown rice flour had 30.6% amylose content as determined by iodine method after drying overnight and defatting for 5 h with hexane (Juliano, 1971). Pancreatin and amyloglucosidase enzymes from Sigma-Aldrich (St. Louis, MO) were used for in vitro starch analysis. All other reagents used for in vitro analysis were of analytical grade.

**Experimental Design and Sample Preparation**

A human study was approved by the Institute of Research Board (IRB) at the University of Arkansas and conducted to investigate plasma glucose and insulin responses. A randomized-crossover design was implemented and responses were analyzed after two 3 h periods over 2 wks. Fourteen healthy, nonsmoking male subjects with age range of 18-45 y not taking medication were recruited to participate in the study. Healthy male subjects were recruited to minimize metabolism variability in addition all subjects’ fasting blood glucose levels were < 100 mg/dL. Participants were randomly divided into three separate cohorts and each cohort included 4 or 5 subjects. A one-week washout period was conducted between treatments. After fasting 10-12 h, subjects consumed one serving of parboiled brown
rice pudding containing 50 g of starch or one 273 mL bottle of 50 g glucose reference drink, Fisherbrand SUN-DEX® from Fisher Diagnostics, LLC (Middletown, VA) along with 200 mL of water. Subjects were not allowed to drink additional water during testing. The parboiled brown rice pudding product contained 140 g water, 59 g parboiled Wells brown rice flour, 2 g lemon extract, and 1.2 g sucralose-artificial sweetener and ingredients were mixed immediately before consumption. The pudding did not receive any heating treatment. Based on total starch analysis, 59 g of parboiled brown rice flour contained 50 g of available starch. The 50 g glucose reference beverage was chosen because it did not receive cooking treatment and also consumed in the same amount of time and manner as the parboiled brown rice flour pudding.

**Postprandial Plasma-Glucose-and-Insulin Concentration Analysis**

After 10-12 h fasting, ~ 0.4 mL blood sample was collected as a baseline measurement 15 min prior to each treatment as a reference. Subjects consumed treatments within 2 min and blood samples were taken at 0, 15, 30, 45, 60, 75, 90, 120, and 180 min increments. Lancets (Roche Diagnostics, Indianapolis, IN) were used to obtain whole blood samples and collected with Fisherbrand microhemocrit capillary tubes (Middletown, VA). Whole blood samples were collected in 0.6 mL sterile, centrifuge tubes and centrifuged at 7000 rpm for 10 min at 4°C. Plasma was pipetted and transferred to labeled 0.6 mL sterile, centrifuge tubes and stored at -20°C until analysis. Plasma glucose concentrations were measured using ACE® Glucose Reagent from Alfa Wassermann Diagnostic Technologies, LLC with Alfa Wassermann Clinical Analyzer (West Caldwell, NJ). Plasma insulin concentrations were measured using the Human Ultrasensitive Insulin ELISA kit from Mercodia, Inc. (Uppsala, Sweden). Incremental AUC was calculated by the trapezoidal rule (Whittaker and Robinson, 1967) for each individual and averaged for treatment responses from the group.

**Statistical Analysis**

Incremental plasma glucose and insulin changes based on differences after the baseline measurement were averaged and mean in addition incremental AUCs were analyzed using analyses of
variance (ANOVA) with 9.2 SAS (Cary, NC). Mean differences at each time point and iAUC were evaluated by a t-test using Tukey’s adjustment with a significance level at p<0.05.

Results and Discussion

Nutritional Starch Fraction Analysis

Significant findings from previous research indicated parboiling process variables, feedstock, and storage influenced final outcome of brown or milled rice (Newton et al., 2011). Identifying storage treatment as increasing SDS formation was also significant in addition the decrease of RDS for parboiled rice samples (Newton et al., 2011). Findings were consistent with previous research (Newton et al., 2011, Niba, 2003) and showed nutritional starch fractions can be influenced by cultivar and parboiling condition. Also, past research notes more RS content for cooked brown rice in comparison to cooked white rice, in addition cooked parboiled white rice also reports greater RS content compared to cooked white rice (Englyst et al., 1996). Total starch (TS) analysis and RDS, SDS, and RS fractions were consistent for the parboiled brown rice flour and rice pudding as shown in Table 1. Average TS content varied 0.1% between flour and pudding samples. Starch fractions were overall consistent; however, RS content of pudding increased 1.5% compared to flour while 3.9% SDS content increased in the flour. A 2.2% increase of RDS in the pudding samples may be due to the starch granule swelling from water and slight modifications of starch digestibility in the pudding. Overall, rice flour and rice pudding enzymatic starch analysis of RDS, SDS, and RS fractions did not significantly change, and the rice pudding did not receive heat treatment before consumption. The control glucose reference drink provided a good reference of digestion to the pudding because it also did not undergo heat treatment and consumed in a similar manner.

Participant Profile and Postprandial Glucose Responses

Table 2 illustrates the participant profile of the study group. Fourteen participates identified themselves as either: African/African American, Asian/Asian American, or Caucasian, and participates represented either the normal or overweight BMI (Body Mass Index) category. Analysis of incremental glucose responses based on BMI group was observed, however, no significant difference in responses
was determined between the groups (data not shown). Incremental glucose response of the parboiled brown rice pudding was significantly lower at 15, 30, 45, 60, 75, and 90 minutes compared to the control glucose reference drink (P<0.05) (Fig. 1A). Also, while observing the group response, the mean incremental AUC for the control was significantly different at 5880 ± 658 mg/dL compared to the rice pudding incremental AUC of 3795 ± 602 mg/dL as shown in Fig. 2A (P<0.05). Previous research with studying the glucose response rapidly available starch from Englyst and others (1999) demonstrated the reduction in rapidly available starch had a profound impact on postprandial glucose response. Although the SDS and RS fractions were targeted by parboiling the brown rice flour, the indirect decrease of RDS was also an effect. A study conducting by Panlasigui and Thompson (2006) observed a reduction in blood glucose for both normal and diabetic subjects. Brown rice compared with milled rice had a nearly 20% reduction in glycemic area in healthy subjects and 35% less in diabetic subjects. Previous research has indicated brown rice does have a lower starch digestion in addition glycemic response, yet some results report no changes observed as well (Perera et al., 2010, Yijun et al., 2008). Conflicting results may have been due to amylose content as some previous in vitro starch digestion research has noted (Han et al., 2007), but also physiochemical properties such as the degree of gelatinization of the rice starch content from heating conditions influence digestibility (Perera et al., 2010). Perhaps with a larger participant group differences in glucose response would be more pronounced and investigating additional rice cultivars which have shown high SDS and RS contents.

Postprandial Insulin Responses

A reduction in plasma insulin concentrations was also observed in 12 participants for the rice pudding treatment. Due to limited sample volume, only 12 subjects’ insulin samples were analyzed for the control glucose treatment, and the same 10 subjects’ samples were available for the pudding treatment. Although no specific time interval was significantly different (Fig. 1B), mean incremental AUCs for treatments reflected a strong, similar trend as observed in the glucose response for the participant group. Fig. 2B shows incremental AUC response to the control glucose treatment was 3066 ± 525 µU/L compared to 2219 ± 715 µU/L of the parboiled brown rice pudding, an average 28% less response compared to the control treatment. Although the insulin response did not significantly differ at time
intervals as in the glucose response, increasing the size of the group would perhaps show significant differences at time intervals. Also, additional studies investigating long-term effects of regular consumption of starch products with the parboiled brown rice flour or similar starch types may offer benefits for insulinemic control. A wide variety of applications could utilize parboiled brown rice flour for future product use and provide a healthy, safe alternative for products needing to reduce allergens.

**Conclusion**

Functional starch fractions such as resistant starch (RS) and slowly-digestible starch (SDS) content can be influenced by parboiling conditions and the storage treatment of rice. The results of this study show that consumption of the parboiled brown rice pudding reduced the postprandial plasma glucose to 36% and insulin to 28% compared with the control treatment. Our study suggests parboiled brown rice has a potential for use as functional food ingredient to improve human health such as lower blood glucose, decreased insulin release, and weight control.
References


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Table 1 In vitro mean total starch (TS) analysis and rapidly digestible (RDS), slowly digestible (SDS), and resistant (RS) starch fractions for parboiled brown rice flour and pudding product

<table>
<thead>
<tr>
<th>Material</th>
<th>TS (%)</th>
<th>RDS (%)</th>
<th>SDS (%)</th>
<th>RS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flour</td>
<td>82.0 ± 0.5</td>
<td>55.9 ± 2.2</td>
<td>11.8 ± 2.5</td>
<td>14.2 ± 2.9</td>
</tr>
<tr>
<td>Pudding product</td>
<td>81.9 ± 0.1</td>
<td>58.1 ± 1.9</td>
<td>7.9 ± 2.4</td>
<td>15.9 ± 2.2</td>
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*RDS, SDS, RS values represent mean ± SEM percent per total starch content (n=4 per sample)
Table 2 Male participant information including ethnicity, age, body mass index, and screened fasting blood glucose

<table>
<thead>
<tr>
<th>Subject Group Number (n)</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Ethnicity</td>
<td></td>
</tr>
<tr>
<td>African/African American (n=2)</td>
<td></td>
</tr>
<tr>
<td>Asian/Asian American (n=6)</td>
<td></td>
</tr>
<tr>
<td>Caucasian (n=6)</td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>26.8 ± 4.9</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>26.5 ± 3.4</td>
</tr>
<tr>
<td>Fasting Blood Glucose (mg/dL)</td>
<td>83.1 ± 7.9</td>
</tr>
</tbody>
</table>
Figure 1 Mean incremental plasma glucose response (A), 15 min before consumption to 180 min after consumption (Control n=14, Rice Pudding n=14). Mean incremental plasma insulin response (B) from 0 min to 180 min after consumption displays reduced response. (Control n=12, Rice pudding n=10). Each value represents the mean ± SEM. * indicates significant difference P<0.05.
Figure 2 Mean iAUC (incremental Area Under the Curve) plasma glucose response with SEM (A) (Control n=14, Rice Pudding n=14). Mean iAUC plasma insulin response with SEM (B) (Control n=12, Rice pudding n=10). Different letters indicates significant difference P<0.05.
December 10, 2013

To Whom It May Concern:

Nicole Marie Poquette is the first author of the paper titled “Parboiled Brown Rice Product Reduces Postprandial Plasma Glucose Response in Men,” included in this thesis has completed at least 51% of the work for the paper.

Dr. Sun-Ok Lee
Assistant Professor of Human Nutrition
Food Science Department
Grain Sorghum Product Reduces Glucose and Insulin Responses in Healthy Men

Abstract

Diabetes and obesity have sparked interest in identifying healthy, dietary carbohydrates as functional ingredients for controlling blood glucose and insulin levels. Grain sorghum has been known to be a slowly digestible cereal; however, research is limited on its health effects in humans. The objectives of this study were to measure the contents of functional starch fractions, SDS (slowly-digestible starch) and RS (resistant starch), and to investigate the effects of grain sorghum on postprandial plasma glucose and insulin levels in 10 healthy men. A whole-wheat flour muffin (control) was compared with the grain sorghum muffin with both muffins containing 50 g of total starch. Using a randomized-crossover design, male subjects consumed treatments within a one-week washout period, and glucose and insulin levels were observed at 15 minutes before and 0, 15, 30, 45, 60, 75, 90, 120, 180 minutes after consumption. The mean glucose responses reduced after consuming grain sorghum, particularly at 45 – 120 minute intervals, and mean insulin responses reduced at 15 – 90 minute intervals compared to control (P<0.05). The mean incremental area under the curve (iAUC) were significantly lowered for plasma glucose responses an average of 35% from 3863 ± 443 to 2871 ± 163 mg·(~3h)/dL (P<0.05). Insulin responses also reduced significantly from 3029 ± 965 µU·(~3h)/L for wheat to 1357 ± 204 with sorghum (P<0.05). Results suggest that grain sorghum is an excellent functional ingredient to assist in managing glucose and insulin levels in healthy individuals, and potentially diabetes and obesity.

Keywords: grain sorghum, slowly-digestible starch (SDS), resistant starch (RS), diabetes, obesity
Introduction

Maintenance of glucose and insulin is essential for the health of both normal and diabetic individuals. Diabetes mellitus has been steadily increasing in the U.S. the last two decades of which 25.8 million people or 8% of the population has been diagnosed with diabetes mellitus (CDC, 2011). Diet is crucial for maintaining a healthy weight and preventing chronic illness such as diabetes. Starches and sugars are targeted to maintain low glucose levels, and conversely, some starch sources can slow the release of glucose because of structure and/or processing methods. Investigating starch digestion rates has helped identify potential nutritional applications for a variety of starches (Annison and Topping, 1994). Starch digestion is commonly referred to as three fractions based on the hydrolysis rate of starch: rapidly-digestible starch (RDS), slowly-digestible starch (SDS), and resistant starch (RS) (Englyst et al., 1992).

Grain sorghum, Sorghum bicolor L. Moench, is the fifth most produced cereal crop and is grown predominately in the U.S., India, Mexico, Nigeria, and Argentina as reported in 2010 by the FAO (FAO, 2010). Grain sorghum is typically produced and consumed as food stuffs in Africa, Asia, and Latin America; in contrast, grain sorghum is mainly used as animal feed in Australia and the U.S. (Dicko et al., 2006). However, demand for finding alternative and functional ingredients has prompted expanded use in markets. Similar to other cereal grains, sorghum is mostly composed of starch including the pericarp of the grain, and starch content may range from 55 – 76% depending upon crop and cultivar (Dicko et al., 2006, Taylor and Emmambux, 2010, Gidley et al., 2011, Taylor et al., 2006, Rooney and Pflugfelder, 1986). Grain sorghum is comparable to maize in terms of composition and starch structure (Dicko et al., 2006, Taylor et al., 2006, Rooney and Pflugfelder, 1986, Sang et al., 2008, Abdelgadir et al., 2005), however, its protein properties distinguish the grain from other cereals (Dicko et al., 2006, Taylor and Emmambux, 2010, Gidley et al., 2011, Taylor et al., 2006, Rooney and Pflugfelder, 1986). The protein matrix has been identified as being one of the key components to its indigestibility; however, processing methods have also shown to increase digestibility (Taylor et al., 2006, Rooney and Pflugfelder, 1986, Sang et al., 2008). Research has identified grain sorghum as a potential functional ingredient in food applications (Taylor et al., 2006, Lakshmi and Vimala, 1996), however, researchers have also reported conflicting results based on sorghum source and processing conditions (Gidley et al., 2011). Despite conflicting results, studies using whole grain sorghum have shown strong evidence of reduced plasma glucose levels after

When analyzing grain sorghum, the health benefits associated with consumption have been attributed the indigestible properties to its structure and composition. Dietary fiber and tannin content in grain sorghum have been highlighted for nutritional potential (Taylor and Emmambux, 2010, Taylor et al., 2006), and additionally, sorghum is also an excellent alternative for gluten-free applications. With increasing demand of both healthier and allergen-free products in the market place, grain sorghum has a great potential for incorporation in numerous food applications as a healthy, dietary carbohydrate and could also potentially assist in the control of glucose and insulin levels in humans. The objective of this study was to investigate the efficacy of grain sorghum flour as a functional food ingredient by measuring the contents of functional starch fractions and the effects on postprandial plasma glucose and insulin responses in healthy humans.

Materials and Methods

Materials

Whole grain sorghum flours from Bob’s Red Mill (Milwaukie, OR, U.S.A.) and Archer Daniels Midland (Plainview, TX, U.S.A.) and whole wheat flour (Gold Medal, Minneapolis, MN, U.S.A.) were analyzed for starch fractions. All solvents and chemicals for the experiment were purchased from VWR international, Inc. (Suwanee, GA, U.S.A) or Sigma Chemical Co. (St. Louis, Mo., U.S.A). A kit for total starch determination was purchased from Megazyme International Ireland Ltd. (Bray Business Park, Wicklow, Ireland). A kit for plasma insulin determination was purchased from Mercodia (Uppsala, Sweden).

Participant Profile and Study Design

A human study was approved by IRB at University of Arkansas and participants were recruited from University of Arkansas (Fayetteville, AR, USA). Written informed consent was obtained for all study participants. The study used a randomized-crossover design to investigate glucose and insulin responses. For the study, 10 healthy male subjects were selected after a screening session prior to the
experiment to confirm fasting blood glucose levels < 100 mg/dL in addition to not diagnosed with any disease or illness and not taking any medication within the last 6 months. All subjects were non-smokers and did not frequently consume more than two alcohol servings per week. The mean age BMI and fasting blood glucose levels of the participant group were 25.1 ± 4 years, 24.2 ± 2.8 kg/m², and 92.2 ± 6.4 mg/dL, respectively. Nine participants identified themselves as Asian or Asian American and one participant identified himself as Caucasian. All participants consented to experiment protocols and attended two Saturdays with a one-week washout period. Each subject consumed treatments after 10 hour fasting.

Muffin Preparation

After the analyses of grain sorghum flours, the flour high in SDS and RS content was used for muffin product. For each experiment, two muffins which contained a total serving of 50 g of total starch were used. All raw materials were weighed separately for each treatment as shown in Table 1. Differences in water amounts were used to accommodate different amounts of flour. Dry ingredients were mixed first followed by wet ingredients, and then both were combined and mixed together. The batter was then weighed into individually greased-muffin liners to weigh approximate product samples. Muffins were baked at 425°F for 15 minutes. Muffins were cooled for 10 minutes and stored until the following morning of experiment. Batter preparation and baking were conducted consistently each afternoon prior to experiment days. Analysis of total starch (TS) and starch fractions for final muffin product was also measured on each experiment day. Each subject consumed two muffins for per serving for each treatment on respective experiment days. All ingredients were purchased at a local grocery store.

Total Starch Content Determination

The total starch content of raw material flour samples and finished muffin products was determined following the manual (KOH format). Sample size of 100 mg was dissolved in 2 M KOH in a test tube and neutralized with sodium acetate buffer (1.2 M, pH 3.8) followed by 100 µL of thermostable α-amylase and 100 µL of amyloglucosidase were immediately added. After the incubation period, samples were then diluted to 100 mL and 100 µL of the homogenized sample was added to 3 mL of Glucose Determination Reagent (GOPOD) and incubated for 20 minutes at 50°C. After final incubation,
absorbance was immediately read at 510 nm and content was determined according to formula included in the manual.

Starch Fractions Determination

Starch fractions (RDS, SDS, and RS) were determined with Englyst Method (1996) at the same time when subjects consumed muffins. Enzyme solution was prepared by dispersing 450 mg of pancreatin in 20 mL of deionized water with stirring for 10 minutes, and after centrifugation 54 mL of the supernatant was mixed with 6 mL of amyloglucosidase (140 unit/mL). Muffin samples were ground and weighed in 50-mL centrifuge tubes on the basis of starch content, which were calculated to be the same as 800 mg of flour, and 20 mL of sodium acetate buffer (0.5 M, pH 5.2) was added to the tubes and mixed well. After adding with 5 mL prepared enzyme in each tube, all the tubes were incubated horizontally in a water bath at 37°C and 160 stroke/min. At 20 min, an aliquot of 0.5 mL was pipetted from tube and mixed with 20 mL of 80% ethanol for glucose determination (G20). Samples were replaced in water bath in 30s. At 120 min, another aliquot of 0.5 mL was pipetted from the tube to 20 mL of 80% ethanol and mixed well for glucose determination (G120). Glucose standard (20 mL of 25 mg/mL D-glucose in sodium acetate buffer introduced above) and buffer blank (20 mL of sodium acetate buffer) were carried out. A supernatant of 0.1 mL was pipetted into 3 mL of GOPOD reagent and incubated at 50°C for 20 minutes. The absorbance was read at 510 nm. The starch fractions were calculated as described in methods of Englyst et al. (1992).

Protein and lipid determination

Crude protein of sorghum and wheat muffins was determined with Micro Kjeldahl Method (AACC 30-10) using a Kjeltec® 2300 Analyzer (Foss Tecator, Hoganas, Sweden). Muffins were ground and a 0.5 g sample was completely digested in 5mL concentrated sulfuric acid with catalyst tablet. The distillation and titration were automatically conducted by the Kjeldahl system. Crude fat in muffins was determined with a Soxtec Avanti 2055 system (Foss North America, MN, US) (AwadElkareem and Taylor, 2012). The ground muffin samples were weighed into porous thimbles and extracted in ether, and lipid was collected.
in extraction cups. After the evaporation of solvent and drying process, lipid was weighed and the crude fat percentage was calculated.

Plasma Glucose and Insulin Analysis

Blood samples were collected at 15 minutes before and 0, 15, 30, 45, 60, 75, 90, 120, and 180 minutes after ingestion. Blood was drawn with capillary tubes into a centrifuge tube and centrifuged at 5500 xg with Microfuge® 22R Centrifuge (Beckman Coulter, Inc., Brea, CA, U.S.). Plasma was transferred and stored at -20°C. Plasma glucose concentrations were measured with an ACE AleraTM Clinical Analyzer (West Caldwell, NJ, U.S.). Plasma insulin concentrations were measured using an insulin ELISA kit from Mercodia (Uppsala, Sweden).

Statistical Analysis

All statistical analyses were conducted with the Statistical Analysis System (SAS, Release 9.2, SAS Institute Inc, Cary, NC). Values were expressed as means ± standard error of the mean (SEM). Paired value t-test was conducted for experiment's significance. Results were considered significant at P<0.05.

Results and Discussion

Flour Composition and Starch Analysis

For in vitro analysis, grain sorghum flour contained 22.7% ± 0.8, 43.8% ± 0.8, and 33.5% ± 0.1 for RDS, SDS, and RS, respectively. In comparison, wheat flour contained 37.5% ± 0.3, 47.4% ± 0.4, and 15.1% ± 0.1 for RDS, SDS, and RS measurement (Table 2). The raw grain sorghum flour exhibited significantly lower RDS content and significantly higher SDS and RS content in comparison to raw wheat flour (P<0.05). In a study investigating starch digestibility of cereals, researchers reported unprocessed sorghum contained the highest amount of RS (2-3x) and lowest amount of RDS (g/kg DM) compared with wheat, barley, oat, maize, and rice (Giuberti et al., 2012). The present results also demonstrate a similar starch results with grain sorghum exhibiting 2x amount RS and almost half the amount the RDS. The TS content for the two raw material flours measured 74.9% for grain sorghum flour and 64.5% for wheat.
flour. Nearly identical grain sorghum TS content was also described in a study conducted by Austin et al. (2012) for Texas white sorghum of 74% TS and Sudanese grain sorghum flour from Abelgadir et al. (2005). In the present study, amylose content of initial flour material was measured 30.9% ± 1.5 for whole grain sorghum flour and 21.8% ± 1.8 for whole wheat flour. Dicko et al. (2006) reported sorghum grain containing 60-75 g starch per 100 g fresh matter, consistent with current study data, and amylose content of 12-22 g per 100 g of fresh matter. Gaffa et al. (2004) reported two Nigerian sorghum cultivars containing 25.5 and 25.7% amylose content and Sang et al. (2008) reported 24% amylose content in normal grain sorghum.

Muffin Composition and Starch Analysis

Moisture content varied slightly, 35.2% ± 0.3 for sorghum muffins and 39.5% ± 0.3 for wheat muffins. Differences in batter water amount correspond with moisture sample variance as explained in methods and materials. Mean crude fat (± SD) content of sorghum and wheat muffins measured 18.5% ± 0.6 and 16.0% ± 0.8, respectively. Mean crude protein content was 5.2% ± 0.2 for sorghum muffin and 7.8% ± 0.3 for wheat muffin, and ash content was nearly identical for both samples with 2.2% ± 0.1 for sorghum and 2.0% ± 0.1 for wheat. Muffin ingredients were added identically between treatments, as listed in Table 1, except for flour and water amounts. Since more wheat flour was needed to provide 50 g of starch for each treatment, differences in protein content can be explained because of the different flour amount used owing to total starch content of wheat and sorghum flour. As Table 1 indicates, approximately 10.7g more flour was required for wheat muffin treatments. Nutrition label information from material package stated both flour sources as containing identical amounts of protein, fat, sugar, and fiber. Differences of crude fat and protein content for final products can be explained by small differences of initial flour (not shown) when compositional analysis was carried out and results given on a dry-weight basis.

In the whole grain sorghum muffin, RDS contents were lower and SDS and RS contents were significantly higher compared with the control whole wheat muffin (P<0.05) (Table 2). Starch structure, particularly protein-starch interactions, may explain differences of starch digestion-rates for treatments based on past research (Wong et al., 2009, Austin et al., 2012, Serna-Saldivar et al., 1995, Gaffa et al., 2004, Zhang and Hamaker, 1998) or perhaps amylose content. In a study conducted by Sang et al. (2008)
raw isolated grain sorghum starches were investigated for in vitro starch digestibility with varying amylose contents, and authors found that the intermediate (14.0%) amylose content of heterowaxy starch exhibited higher RS content rather than the amylose content of normal (23.7%) starch. The heterowaxy starch with an intermediate amylose content exhibited slightly lower SDS yet highest RS content, suggesting that amylose content is not necessarily responsible for predicting in vitro digestibility.

Researchers also reported similar digestibility results for RDS and RS content compared to grain sorghum flour used in present study. The RDS content (21.5%) of waxy grain sorghum starch and RS content (23.7%) of heterowaxy grain sorghum starch are comparable to present materials analysis (Sang et al., 2008). In contrast, normal, heterowaxy, and waxy raw sorghum starch samples exhibited higher amounts of SDS content (61.7 - 68.5%), and lower amounts of amylose 23.7, 14, and 0%, respectively. Findings from Benmoussa et al. (2006) also confirm variations in starch digestibility for three sorghum cultivars, suggesting that amylose content does not necessarily indicate digestibility rate. Authors investigated different sorghum genotypes with ranging amylose content (19.2 - 22.4%) and found sample starch digestibility was mostly influenced by starch granule characteristics such as channel density which allows enzymes to attack the granule and the sorghum protein matrix, depending upon sorghum genotype, also influences enzyme accessibility to granule (Benmoussa et al., 2006). The protein content of the final product given to participants in present study was relatively low; however, research has highlighted the protein-starch relationship in grain sorghum and its influences on starch digestion. In an article by Duodu et al. (2003), exogenous factors are discussed such as the grain organizational structure, polyphenols, phytic acid, cell wall components, and starch are primary factors that influence protein digestibility, and additional researchers also confirmed (Mokrane, et al., 2010, DeCastro, 2006, Rooney and Pflugelder, 1986, Zhang and Hammaker, 2005) results which explains the coordinated relationship between sorghum starch and protein digestibility. As researchers have discovered, both starch granules and protein bodies are closely associated to each other; enzymatic accessibility is greatly hindered for starch gelatinization and digestibility, which may contribute to poor protein quality of sorghum (Duodu et al., 2003). In a more recent publication from Mokrane et al. (2010) regarding protein, Algerian sorghum cultivars were only evaluated for protein digestibility. Researchers found the protein content in cultivars could provide good quantity and quality of essential amino acids though the digestibility varied
greatly between cultivars and unfortunately starch digestion was not analyzed. The Algerian sorghum flours contained 12.6 – 16.4% crude protein (DB), approximately 4 - 8% higher than grain sorghum flour used in present study.

Incremental Glucose and Insulin Results

Figure 1 and 2 shows incremental changes of plasma glucose and plasma insulin concentrations over experiment duration. With the grain sorghum muffin treatment, the glucose responses from participants were significantly lower at 45, 60, 75, 90, and 120 minute intervals (P<0.05). Mean iAUC responses of glucose were significantly reduced about 26% from 3863 ± 443 to 2871 ± 163 mg·(~3h)/dL as shown in Figure 3 (P<0.05). Incremental insulin concentrations for the sorghum muffin treatment were significantly lower at 15, 30, 45, 60, 75, and 90 minutes (P<0.05) and were also significantly reduced about 55% from 3029 ± 965 to 1357 ± 204 µU·(~3h)/L (Figure 3).

Processing and cooking conditions do influence starch hydrolysis (Lakshmi and Vimala, 1996, Dlamini et al., 2007). In one human study conducted by Lakshmi and Vimala (1996), the study observed glucose responses from six non-insulin dependent diabetic patients, and researchers compared the three sorghum recipes, both with whole grain and dehulled grains, and also compared to either wheat or rice recipe controls, additionally. Recipes were traditional dietary foods in different preparation forms including a shallow pan-fried product, boiled, and a fermented-steamed product. Researchers used larger treatment samples (85-90g carbohydrate), but with all three samples, and whole sorghum food recipes exhibited lowest plasma glucose and insulin responses compared to identical dehulled sorghum recipes and controls. In addition, whole grain sorghum foods showed an average two-fold amount of fiber content compared to dehulled sorghum foods. Researchers investigating Sudanese cereal foods and their glucose and insulin responses have reported wheat and millet as having slightly lower glucose and insulin incremental areas under the curve compared to sorghum (Abdelgadir et al., 2005), however, flours used for study did not provide total starch content for various treatment grains and forms to confirm identical treatment conditions. With the literature available, it is evident that processing conditions such as de-hulling may greatly influence overall glucose and insulin responses as reported by Taylor and Emmambux (2010), so it is important for human studies to test whole grain sorghum for potential
functional food ingredients. Cultivars bred for functional starch properties would be excellent candidates for functional food ingredients, however, the preparation, processing, and cooking conditions of the final product remain central to evaluating effectiveness.

**Conclusion**

Results showed whole grain sorghum as an excellent functional food ingredient for controlling glucose and insulin levels in humans. Compared to wheat, functional starch fractions for sorghum showed increased SDS and RS content and lower RDS content and decreased glucose and insulin responses in healthy humans. Research implies further investigation with pre-diabetic or diabetic individuals would be beneficial for providing more additional analysis of grain sorghum as a functional ingredient. With increasing gluten-intolerance and environmental concerns, grain sorghum will continue to be utilized for future research applications.
References


DeCastro Pallmino Siller, A. In vitro digestibility and estimated glycemic index of sorghum products. 2006; Texas A&M University: College Station, TX.


**Table 1** Ingredients used for muffin preparation

<table>
<thead>
<tr>
<th>Ingredient/muffin</th>
<th>Wheat Muffin (g)</th>
<th>Sorghum Muffin (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flour</td>
<td>77.5</td>
<td>66.8</td>
</tr>
<tr>
<td>Water</td>
<td>46.3</td>
<td>37.7</td>
</tr>
<tr>
<td>Egg</td>
<td>28.8</td>
<td>28.8</td>
</tr>
<tr>
<td>Butter</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Vegetable Oil</td>
<td>7.5</td>
<td>7.5</td>
</tr>
<tr>
<td>Sucralose</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Baking Soda</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>Salt</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Vanilla Extract</td>
<td>1.8</td>
<td>1.8</td>
</tr>
</tbody>
</table>
Table 2 In vitro total starch and starch fraction measurement of wheat and sorghum flours/muffins

<table>
<thead>
<tr>
<th>Starch Composition</th>
<th>Wheat (%)</th>
<th>Sorghum (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flour</td>
<td>Muffin</td>
</tr>
<tr>
<td>TS %</td>
<td>64.5 ± 0.5</td>
<td>43.8 ± 0.5</td>
</tr>
<tr>
<td>RDS %</td>
<td>37.5 ± 0.3</td>
<td>88.2 ± 0.3</td>
</tr>
<tr>
<td>SDS %</td>
<td>47.4 ± 0.4</td>
<td>11.5 ± 0.1</td>
</tr>
<tr>
<td>RS %</td>
<td>15.1 ± 0.1</td>
<td>0.5 ± 0.1</td>
</tr>
</tbody>
</table>

TS% is on the dry basis; RDS%, SDS% and RS% are on the TS basis. Values reflect means ± standard deviation (n=4); *, † indicates p<0.05 compared to respective wheat flour or muffin.
Figure 1 Mean incremental change of plasma glucose in healthy males (n=10) with standard error of mean bars (SEM). * p<0.05 at time interval.
Figure 2 Mean incremental change of plasma insulin in healthy males (n=10) with standard error of mean bars (SEM). * p<0.05 at time interval
Figure 3 Total mean incremental area under the curves (iAUC) for plasma glucose and insulin responses of healthy males (n=10) with standard error of mean (SEM) values. * p<0.05 significance.
Conclusion

The functional starch fractions present in foods have a great potential to play a pivotal role in maintaining health and preventing chronic illness and diseases such as diabetes and obesity. Although in vitro starch fraction analysis is important for the development of functional starch ingredients, particularly for identifying products for the development, thorough examination and analysis with well-designed human studies does present the strongest evidence. In previous research emphasis on starch composition such as amylose and amylopectin ratios was prominent; however, research has shown many additional considerations are required for analyzing starch digestibility. New research and methods for starch digestion analysis will continue to improve and perhaps prompt new forms of resistant starch for the improvement of health.

Results from the present research has shown success of demonstrating lower glucose and insulin effect in health humans with parboiled brown rice flour and grain sorghum flour as functional food ingredients, and additional investigation with pre-diabetic or diabetic individuals would help confirm the present results to continue pursuing optimal functional starch ingredients. As previously summarized, SDS and RS content can be influenced by the parboiling process for rice and the results observed a reduction in the postprandial plasma glucose and insulin responses. For grain sorghum, large reductions in plasma insulin levels and mean glucose responses were observed, suggesting that grain sorghum may be utilized for functional food applications. Food forms and food matrices will need to be taken into consideration; however grain sorghum flour does show to be a successful functional food ingredient to control glucose and insulin levels.

The studies conducted suggest parboiled brown rice flour and grain sorghum flour are helpful for improving human health with respect to lowering blood glucose and insulin levels as well as weight control. Another consideration is for gluten-free food products because both parboiled brown rice and grain sorghum flours would also be excellent gluten-free alternatives. Functional starch ingredients will continue to be an important research area, and with continued research will help identify additional nutritional mechanisms for improving health from parboiled brown rice and grain sorghum.